United States Department of Agriculture

Supporting 13 Years of Global Change Research:
The History, Technology, and Methods of the Aspen FACE Experiment

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Abstract

This publication is an additional source of metadata for data stored and publicly available in the U.S. Department of Agriculture, Forest Service Research Data Archive. Here, we document the development, design, management, and operation of the experiment. In 1998, a team of scientists from the U.S. Forest Service, Department of Energy (DOE), Michigan Technological University, and several other institutions initiated the Aspen Free Air CO_2 and Ozone Enrichment (Aspen FACE) Experiment. Using technology developed at DOE's Brookhaven National Laboratory (BNL), the experiment fumigated model aspen forest ecosystems with elevated concentrations of carbon dioxide (CO_2), or ozone, or both in a full factorial design with three replicates. The Aspen FACE Experiment was one of several free-air CO_2 enrichment experiments at the time, but was the only one that incorporated ozone treatment into the BNL design.

The experiment operated for 13 years, involved more than 70 researchers from 9 countries, has produced 126 scientific publications to date, held numerous tours and scientific conferences, and was the subject of many reports in the public news media. Findings from the experiment contributed to the supplement to the U.S. President's 2002 budget, *Our Changing Planet*; and to the 2006 rewriting of the U.S. Environmental Protection Agency's ozone pollution criteria document. Data and archived plant samples from the experiment continue to be used in many ways, including meta analyses, global change modeling, and studies examining tree characteristics affected by the treatment gases.

Cover Photo

Bird's-eye view of the Aspen FACE Experiment site near Rhinelander, WI. Photo by D.F. Karnosky, Michigan Tech, used with permission.

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The History, Technology, and Methods of the Aspen FACE Experiment

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Quality Assurance

This publication conforms to the Northern Research Station's Quality Assurance Implementation Plan which requires technical and policy review for all scientific publications produced or funded by the Station. The process included a blind technical review by at least two reviewers, who were selected by the Assistant Director for Research and unknown to the author. This review policy promotes the Forest Service guiding principles of using the best scientific knowledge, striving for quality and excellence, maintaining high ethical and professional standards, and being responsible and accountable for what we do.

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INTRODUCTION

Reports from the Intergovernmental Panel on Climate Change (IPCC) in 1990 summarized scientific evidence for the increase of global atmospheric carbon dioxide concentration ([CO_2]). Atmospheric [CO_2] had increased to 350 $\mu L\ L^{-1}$ in 1989 from a preindustrial concentration of 270 $\mu L\ L^{-1}$, primarily due to steadily increasing fossil fuel combustion and loss of terrestrial C sinks, mainly in the Tropics. At the same time, oxidized nitrogen (NOx), also from fossil fuel use, and volatile organic compounds (VOC) emissions, had increased background concentrations of tropospheric ozone ([O_3]) by 35 to 70 percent (IPCC 1990). Although tropospheric [O_3] is highly spatially and temporally variable, nearly a quarter of the world's forests were exposed to peak concentrations exceeding 60 $nL\ L^{-1}$ (Fowler et al. 1999).

Carbon dioxide and O_3 directly affect plants in opposite ways. Whereas CO_2 is a substrate for primary production, O_3 is a potent phytotoxin. The direct effects of increasing [CO_2] and [O_3] on plants have been studied in short-term, small-scale experiments in growth chambers and open-topped plastic chambers since the 1970s. However, the long-term effects of these gases on forest community or ecosystem processes were impossible to determine because the responses of immature, individual trees are poor predictors of responses in competitive situations (Saxe et al. 1998).

When the Aspen Free Air CO_2 and Ozone Enrichment (Aspen FACE) Experiment was begun in 1998, few experiments had considered the potential interactions between elevated [CO_2], tropospheric [O_3], and other global change factors. The experiment addressed contemporary research topics that the National Research Council (2001) called the "grand challenges in environmental sciences." Years earlier IPCC (1992) had identified the need for large-scale, ongoing research of mixed forest community response to elevated [CO_2] in natural settings. To address the need for large-scale global change science, a team of researchers at the U.S. Department of Energy's (DOE's) Brookhaven National Laboratory (BNL) developed technology to expose intact forest canopies to controlled concentrations of atmospheric CO_2 (Hendrey et al. 1993, 1999; Lewin et al. 1994; Nagy et al. 1994). The first prototype of a free-air CO_2 enrichment (FACE) system in a forest canopy was constructed in a mature loblolly pine (*Pinus taeda* L.) plantation at Duke University's Duke Forest, Durham, NC, in 1994 under the name Forest-Atmosphere Carbon Transfer and Storage (FACTS).

At about the same time, principal investigators from six institutions (in the tabulation on page ii) received funding to implement the BNL design to study the effects of CO_2 and O_3 on mixed forest communities in northern Wisconsin. Building upon years of experience with open-topped chambers from short-term CO_2 and O_3 experiments, they acquired initial funding primarily from the Joint Program on Terrestrial Ecology and Global Change (1995-2001), administered by the National Science Foundation (NSF), DOE, the National Aeronautics and Space Administration, and the U.S. Department of Agriculture, and from NSF's Academic Research Infrastructure Program (1996). Additional funding and in-kind support came from the National Council for Air and Stream Improvement, Michigan Technological University (Michigan Tech), the U.S. Forest Service's Northern Global Change Research Program, and the U.S. Forest Service's North Central Research Station. After 2001, the experiment was principally funded by DOE's Program for Ecosystem Research, the U.S. Forest Service, and Michigan Tech. Highlights of the history and development of the experiment are shown in Table 1.

This publication describes:

- Methods, equipment, and site operation
- Chronology of major events
- Details of the CO_2 and O_3 treatments
- Methods used for growth and biomass measurements
- Conversion to the Phase II regeneration study
- Site safety protocols

EXPERIMENTAL METHODS

Site Description

The Aspen FACE Experiment site is located on a 32-ha parcel within the Harshaw Research Farm owned by the U.S. Forest Service near Rhinelander, WI (45.6° N, 89.5° W). The legal description of the site is SW80, Sect. 21, T37N, R7E, Cassian Township, Oneida County, WI. The site was cultivated for crops from the 1920s until 1972, when it was purchased by the Forest Service. Between 1972 and 1996, it served as a short-rotation tree crop and forest genetics research facility for the Forest Service. In 1996-1997 the site was cleared of most of the existing plantations, and the FACE experiment was constructed. The site was surrounded by a 3.7-m-tall deer-proof fence with an automatic security gate. The soil at the site is a Pandus series, mixed, frigid, coarse loamy Alfic Haplorthod. It consists of a 30-cm clay loam plow layer that grades into sandy loam above stratified sand and gravel. Average soil texture across the site is 55.6 percent sand, 36.6 percent silt, and 7.8 percent clay. The topography is level to gently rolling. When the experiment was initiated, total soil nitrogen (N) was 0.12 percent, with 13.53 mg g^{-1} of NO_3^- N, and 0.84 mg g^{-1} of NH_4^+ N; C:N was 12.9. Detailed information on site and soil characteristics is presented in Dickson et al. (2000).

CO_2 and O_3 Fumigation Systems

The free-air CO_2 enrichment system was modeled after the BNL prototype constructed for the FACTS project at Duke. For the Aspen FACE Experiment, the CO_2 fumigation system was modified by the BNL development team to accommodate O_3 and CO_2 + O_3 treatments. The free-air CO_2 and O_3 fumigation equipment consisted of three systems: (1) CO_2 and O_3 supply; (2) fan, plenum, and vertical vent pipes; and (3) gas concentration control. These systems are briefly described in the following pages. Details of the FACE technology, its subsystems, and design specifications are presented in Hendrey et al. (1993, 1999), Lewin et al. (1994), and Nagy et al. (1994). Additional details of the CO_2 and O_3 fumigation systems specific to the experiment, including device specifications, are described in Dickson et al. (2000). Specifications on scientific equipment and instruments are provided in Appendix 1.

Table 1.—Milestones in the development and history of the Aspen FACE Experiment[a]

1995	Inaugural grant proposal awarded.
1996	National Environmental Policy Act Categorical Exclusion issued by Argonne National Laboratory.
	Wisconsin Department of Natural Resources air pollution source permit exemption issued.
	Site cleared and construction initiated.
1997	Construction, testing, and planting completed.
1998	First full year of fumigation.
	Lightning strike destroys control shed at ring 1.2.
1999	Hybrid poplar buffer trees planted around each treatment ring.
	Weather monitoring tower erected.
	Meteorological monitoring equipment installed in rings 1.2, 2.1, 3.3, and 3.4.
2000	First partial biomass harvest.
2001	Lightning strike destroys control shed at ring 3.3.
2002	Second partial biomass harvest.
	Installation of canopy access system[b].
2003	Experiment labeled with tracer ^{15}N[c].
2004	Upgrade of ring meteorological monitoring[d, e]:
	a) Above- and below-canopy wind speed and direction.
	b) Above-canopy photosynthetically active radiation, air temperature, and relative humidity in each ring, and below-canopy photosynthetically active radiation, temperature, and relative humidity in each ring section.
2005	Upgrade of ring soil moisture monitoring[f]:
	a) Net radiometers in each ring.
	b) Above-canopy PAR sensors installed in original four rings.
	c) Tipping rain buckets installed in 10 rings.
	d) Soil temperature and thermocouples installed in all ring sections, and reference thermisters installed in all rings.
	e) Soil moisture probes at three depths installed in each ring section.
	Environmental Assessment conducted by Argonne National Laboratory.
2006	Ring expansion (October-November)[g]:
	a) Canopy access system raised.
	b) 10-m vertical vent pipe support and central utility poles replaced with 20-m poles.
	c) Vertical vent pipes upgraded to larger pipe sizes.
	d) Onsite field laboratory constructed[b].
2007	Third partial biomass harvest.
	Cessation of funding for FACE experiments announced by U.S. Department of Energy.
2009	Official dedication of field laboratory as the David F. Karnosky Laboratory.
	U.S. Forest Service's commitment to funding continuation of the experiment in Phase II.
	Complete harvest of the experiment[g]:
	a) Summer: aboveground and belowground harvest of subplots.
	b) Winter: complete removal of remaining buffer and experiment trees.
	c) Reconfiguration of treatment rings for the Phase II regeneration study.
2010	Phase II regeneration study initiated and concluded.
2011	Aspen FACE Experiment decommissioned.

[a] All activities occurred in summer months unless otherwise noted. Refer to Appendix 1 for equipment specifications.
[b] U.S. Forest Service special construction funds
[c] Dilute solution of $^{15}NH4Cl$ (99.98 percent ^{15}N) applied with backpack sprayer at 0.034 L m^{-2}
[d] Meteorological monitoring equipment was installed in four treatment rings in 1999. The upgrades in 2004 and 2005 expanded monitoring to all 12 rings. See the text for details.
[e] U.S. Forest Service Northern Global Change Research Program funds
[f] USDA NRI Grant #2004-36102-14782 to John King
[g] U.S. Department of Energy core project support funds

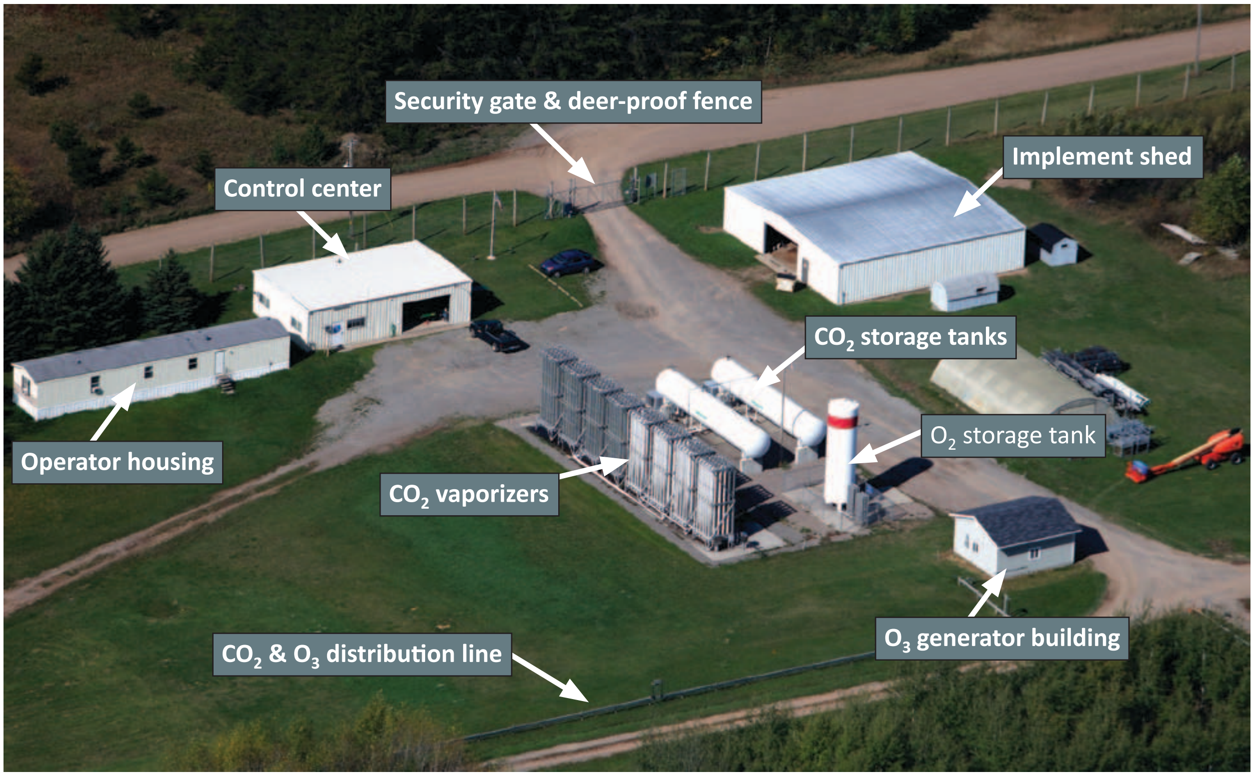

Figure 1.—Overview of Aspen FACE Experiment site near Rhinelander, WI, showing structures and equipment for production and monitoring of CO_2 and O_3 treatments. Photo by D.F. Karnosky, Michigan Tech, used with permission.

CO_2 and O_3 Supply

Liquid CO_2 was delivered to the facility by tanker truck and stored onsite in two 55,000-kg-capacity receivers (Fig. 1). Liquid CO_2 from the receivers was vaporized in a bank of eight passive, ambient-air heat exchangers and the vaporized CO_2 was then distributed to the various treatment rings via high-pressure copper pipe.

Ozone was produced onsite from liquid O_2 stored in a 23,000-L tank. From 1998 until 2002, O_3 was produced with a 16 kg d^{-1} O_3 generator. In 2002, O_3 generating capacity was upgraded to a maximum production capacity of 38 kg d^{-1} and in 2007 to a maximum production capacity of 154 kg d^{-1}. The O_3-O_2 mixture was delivered to the treatment rings via stainless steel pipe. Excess O_3 delivered to each treatment ring, i.e., O_3 not required to maintain the fumigation set point, was converted back to O_2 by a canister of magnesium dioxide catalyst located at each ring.

Fan, Plenum, and Vertical Vent Pipes

The 12 treatment rings were 30 m in diameter defined by a circle of thirty-two 15.2-cm-diameter polyvinyl chloride (PVC) vertical vent pipes (VVPs) that were supported by wood utility poles. Each pole supported two VVPs. The VVPs were connected to an octagonal plenum made of 38-cm-diameter PVC pipe (Fig. 2). The plenum was connected to a radial fan that delivered 102 m^3 min^{-1} air flow at 2.0 kPa pressure. A computer-regulated mass of pure CO_2 gas, the O_3-O_2 mixture, or a combination thereof was delivered to the outlet of the radial fan, where it mixed with ambient air. The mixture was distributed around the circumference of the treatment plot via the plenum. Horizontal slots (emitters; 2.5 cm × 16 cm) were cut

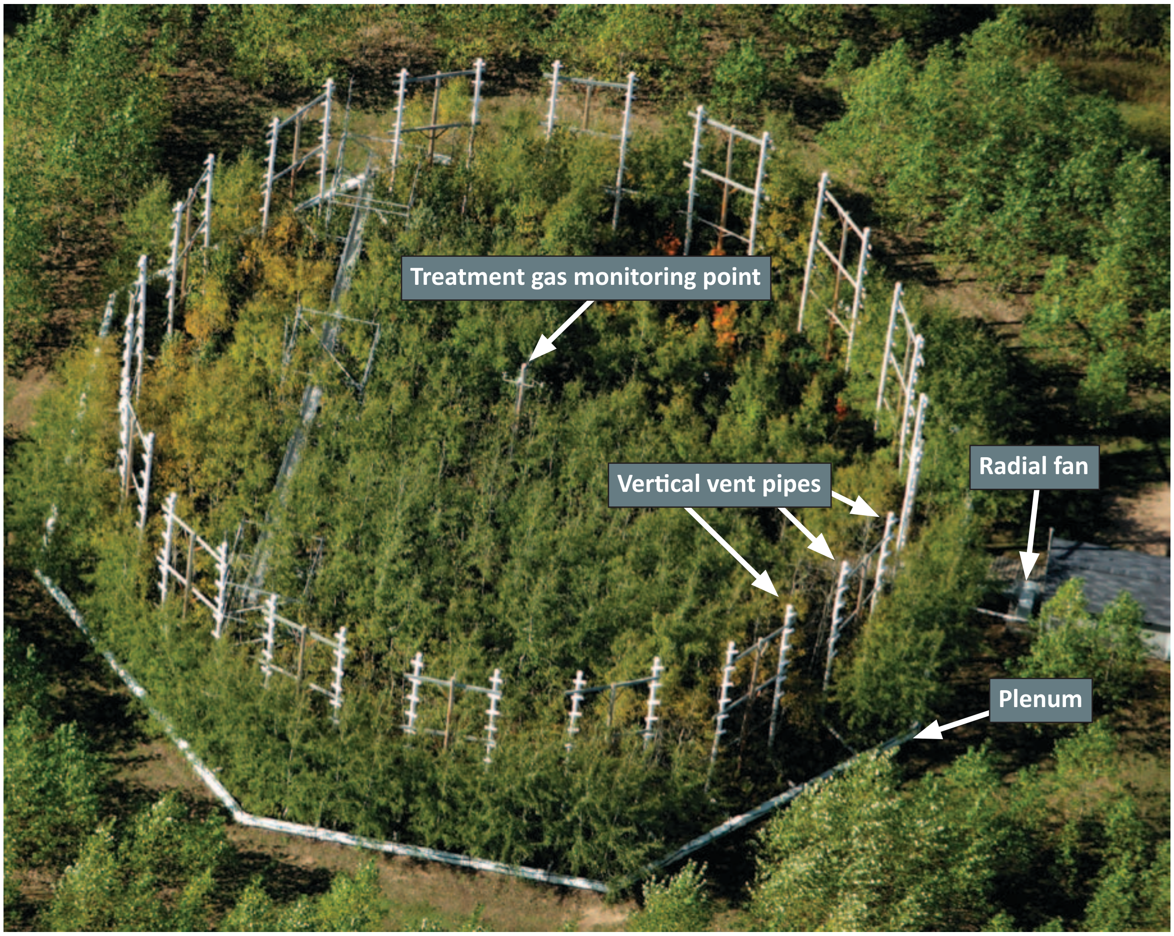

Figure 2.—A typical treatment ring showing the vertical vent pipes, plenum, radial fan, and the location of treatment gas and wind monitoring for control of gas concentration. Photo by D.F. Karnosky, Michigan Tech, used with permission.

into the VVPs and fitted with an aluminum deflector that created more rapid mixing of the treatment gas with ambient air.

Control of Gas Concentration

Treatment gas concentrations were controlled by three subsystems: (1) atmospheric gas concentration and wind monitoring, (2) computer algorithm, and (3) gas delivery control. Additional details of all of these systems are described in Dickson et al. (2000).

Instruments for monitoring wind speed and direction, and inlet ports for CO_2 and O_3 monitors, were mounted just above the tree canopy on a wood utility pole at the center of each treatment ring. In the nine treated rings, elevated [CO_2] or [O_3] was monitored with an infrared gas analyzer (IRGA) or UV absorption gas analyzer (UVAGA), respectively, located in an air-conditioned shed adjacent to each treatment ring (Fig. 2). Filtered, atmospheric air was pulled through about 20 m of 4.3-mm-diameter Teflon® (DuPont, Wilmington, DE) tubing at 3 L min^{-1}. A portion of that air was delivered to the IRGAs and UVAGAs. The 1-sec wind speed and direction and [CO_2] and [O_3] were communicated via optical fiber to the control computers in the control center. The fiber optic data communication network was designed in a complete serial loop topology so that if a connection were broken at any one ring, the remaining rings would continue to communicate with the control computers.

Figure 3.—Portion of a treatment ring showing plenum, vertical vent pipes, aluminum deflectors, and pneumatic butterfly valves. Photo by U.S. Forest Service.

A bank of three personal computers operated the gas control algorithms, which were developed at BNL. Gas concentrations were controlled independently in each of 12 virtual treated rings: [CO_2] in six virtual rings (three elevated CO_2-only and three CO_2 + O_3 rings), and [O_3] in six virtual rings (three elevated O_3-only and three elevated CO_2 + O_3 rings). The control algorithms are described in detail in Hendrey et al. (1999) and Dickson et al. (2000). Using the wind speed and gas concentration information from each ring, the algorithms controlled the mass of CO_2 or O_3 delivered independently to the air stream of each ring.

Information on wind direction was also sent to the control computers, which ordered independent, pneumatically operated butterfly valves on the VVPs to open or close (Fig. 3). The computer algorithm would open 12 VVPs on the windward side of a ring: 10 VVPs adjacent to each other, and 1 additional VVP that was 1 pipe removed from the 10 on each end. A low wind speed threshold of 0.4 m sec^{-1} opened every second VVP in the elevated CO_2 rings, and switched off O_3 fumigation. In the three control rings, every second vertical pipe vented ambient air from the fan. Days of operation of the fumigation systems are shown in Table 2.

Table 2.—Start and stop dates of CO_2 and O_3 fumigation treatments at the Aspen FACE Experiment

Year	Start date	Stop date	Days of operation
1998	1 May	13 Oct	165
1999	10 May	30 Sep	143
2000	15 May	30 Sep	138
2001	11 May	3 Oct	145
2002	28 May	12 Oct	137
2003	21 May	12 Oct	144
2004	21 May	17 Oct	149
2005	23 May	12 Oct	142
2006	14 May	1 Oct	140
2007	17 May	19 Sep	125
2008	23 May	9 Oct	139

Table 3.—Significant interruptions of treatment fumigations during Phase I of the Aspen FACE Experiment

Year	Dates down	Treatment ring	Reason
1998	16 May – 15 July	1.2	Lightning strike and fire in control shed
1998	1 Oct – 13 Oct	1.4, 1.3	Undocumented
1999	25 Aug – 8 Sep	1.4	Undocumented
2001	2 Aug – 15 Sep	3.3	Lightning strike and fire in control shed
2007	16 June – 19 Jul	All x.3 and x.4	Ozone generator chiller failure
2009	15 May – 22 May	All x.3 and x.4	Ozone generator pump failure

Protection from Lightning

Lightning strikes in 1998 and 2001 destroyed control sheds at rings 1.2 and 3.3, respectively (Table 3). After the first lightning strike in 1998, Faraday cages of copper cable and 3.1-m-long grounding rods were buried around each ring and the CO_2 and O_3 supply facilities. Following the second strike, 12-m-long grounding rods were added, as well as lightning rods atop each control shed and, in 2002, on the canopy access scaffolds. The Faraday cages, lightning rods, and grounding rods were bonded together to equalize electrical potential among all the components in each ring. This system provided effective lightning protection for the duration of the experiment.

Experimental Design

The Aspen FACE Experiment consisted of 12 treatment rings: 3 replicates each of control, elevated CO_2, elevated O_3, and elevated CO_2 + O_3 atmospheres (designated as treatments 1-4, respectively). Target elevated concentrations were 560 $\mu L\ L^{-1}$ CO_2, and 1.5 times ambient [O_3] (except in the first 2 years, described below). The treatment rings were dispersed around the 32-ha site at a spacing of about 100 m between rings (Fig. 4; Table 4). The three replicates were blocked roughly into north (replicate 1), central (replicate 2), and south (replicate 3) locations of the site. Each treatment ring was identified by number as R.T, where R is replicate and T is treatment. Blocking was included in the experimental design to account for a roughly north-south gradient in soil N concentration and clay content as shown in Dickson et al. (2000). Blocking also facilitated field measurements; time-consuming measurements could be performed one block at a time, allowing temporal separation between replicates to factor out of experimental error as block effects.

Each treatment ring was divided in half with a north-south wooden walkway. The west half of each ring was again divided into quarters (Fig. 5). The east half was planted with five genotypes of trembling aspen (*Populus tremuloides* Michx.), the northwest quarter was planted with a 1:1 mixture of trembling aspen and sugar maple (*Acer saccharum* Marsh.), and the southwest quarter was planted with a 1:1 mixture of aspen and paper birch (*Betula papyrifera* Marsh.) (justification for the selected plant material is detailed below). All of the planting occurred across a 1 m × 1 m coordinate grid with the north-south coordinate identified by letters (AD, AC, AB, AA, A, ... Z; from north to south), and the east-west coordinate identified by numbers (1-29; from west to east). In addition to being identified by ring number and coordinates, each

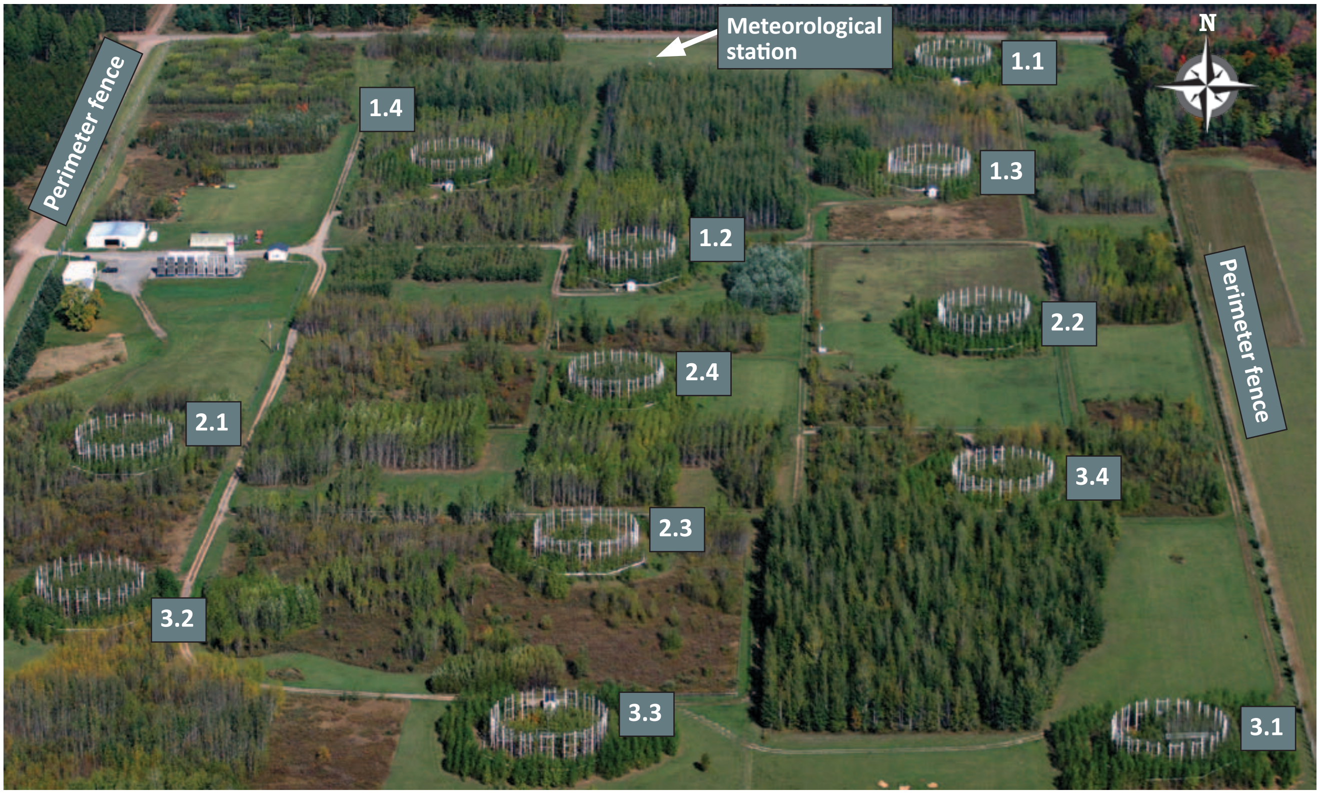

Figure 4.—Overhead view of the Aspen FACE Experiment showing location of the perimeter fence, meteorological station (monitoring tower), and 12 treatment rings. Digit in front of the decimal point of each ring number is the replicate, and the digit after the decimal point is the treatment number: 1 = control, 2 = elevated CO_2, 3 = elevated O_3, 4 = elevated CO_2 + O_3. Photo by D.F. Karnosky, Michigan Tech, used with permission.

Table 4.—Global positioning system (GPS) coordinates[a] of the Aspen FACE Experiment recorded in UTM zone 16N and converted to latitude and longitude WGS84

Point description	Northing	Easting	Latitude	Longitude	Elevation (m)
NW site corner	5061992	295128	45.68118365	-89.63077383	497
SW site corner	5061192	295112	45.67398552	-89.63065456	494
SE site corner	5061174	295511	45.67394177	-89.62551667	493
NE-N site corner	5061968	295517	45.68108273	-89.62577385	496
NE-S site corner	5061952	295526	45.68094152	-89.62565167	497
Meteorological station	5061931	295331	45.68069515	-89.62814416	501
Ring 1.1	5061928	295448	45.68070271	-89.62664210	502
Ring 1.2	5061662	295324	45.67827440	-89.62812063	500
Ring 1.3	5061777	295433	45.67934058	-89.62677093	503
Ring 1.4	5061784	295259	45.67935215	-89.62900578	501
Ring 2.1	5061475	295173	45.67654840	-89.62997865	497
Ring 2.2	5061603	295456	45.67778287	-89.62640266	501
Ring 2.3	5061393	295322	45.67585512	-89.62803298	499
Ring 2.4	5061529	295326	45.67707913	-89.62803896	501
Ring 3.1	5061239	295476	45.67451589	-89.62599292	500
Ring 3.2	5061350	295179	45.67542625	-89.62984900	498
Ring 3.3	5061256	295313	45.67462064	-89.62809071	496
Ring 3.4	5061434	295440	45.67625860	-89.62653674	501

[a] Treatment ring coordinates locate the plot center. GPS coordinates were recorded with GPS 76™ (Garmin Ltd., Olathe, KS) devices and converted to latitude and longitude in GPSU (GPS Utility Ltd., Prenton, Merseyside, UK) software. Mean distance between rings is 109 m, calculated in ArcMap 10.1 (Esri, Redlands, CA) based on x-y coordinates.

Figure 5.—Portion of a treatment ring showing initial planting design, walkways, plenum, center pole, and vertical vent pipes. Photo by U.S. Forest Service.

tree was assigned a unique identification number (aspens in the west half were numbered from tree 1 starting in ring 1.1, AD15, through tree 4200 in ring 3.4, Z17; aspen-maple and aspen-birch started with tree 4201 in ring 1.1, AD12, and ended with tree 7920 in ring 3.4, Z14). All grid points encircled by the vertical vent pipes were planted except for coordinates K1 through K14, which were occupied by the east-west walkway. Space for the main north-south wooden walkways was accommodated within the grid by wider spacing between columns 14 and 15.

The experimental design was a fixed effects, split-plot, two-factor, complete block (n=3) with community type as the split-plot factor. If only one community type was considered in analysis, the split-plot factor could be considered as species or clone.

A typical mixed model analysis of variance (ANOVA) for the experiment would be:

$$Y_{ijk} = \mu + R_i + T_k + (RT)_{ik} + S_j + (TS)_{jk} + \epsilon_{ijk}$$

where R_i is the i^{th} block effect, T_k is effect of the k^{th} treatment, $(RT)_{ik}$ is the whole plot error effect of block by treatment interaction, S_j is effect of the j^{th} ring section, $(TS)_{ij}$ is the effect of the ij^{th} ring treatment by ring section interaction, and ϵ_{ijk} is the split-plot or residual error.

Plant Material

Plant material and community types were selected from previous studies conducted by the principal investigators using open-topped chambers. Trembling aspen clones 216, 271, and 259, previously classified as tolerant, intermediate, and sensitive to ozone exposure, respectively, were chosen from elevated [O_3] studies conducted at Michigan Tech's Ford Forestry Center at Alberta, MI (Coleman et al. 1995a, 1995b; Karnosky et al. 1992a, 1992b, 1996; Kull et al. 1996). The aspen clones used in the Alberta experiments were originally collected in 1988 from naturally occurring clones in northern Wisconsin, northern lower Michigan, and northern Ohio and exhibited various degrees of visible ozone injury (Karnosky et al. 1992b). Two additional trembling aspen clones, 8L and 42E, were selected from elevated [CO_2] studies conducted at the University of Michigan Biological Station (UMBS) near Pellston, MI (Curtis et al. 2000, King et al. 1999, Kubiske et al. 1998, Mansfield et al. 1999, Wang et al. 2000). These clones were identified as having late and early autumn leaf abscission times, respectively, in a phenological survey of naturally occurring aspen near Pellston, MI (Barnes 1959). In the UMBS experiments, clones 8L and 42E were found to exhibit relatively greater and lesser growth and photosynthesis responses, respectively, to elevated [CO_2] (Curtis et al. 2000, Kubiske et al. 1998, Wang et al. 2000).

The aspen-birch and aspen-maple mixtures were also chosen based on previous open-topped chamber studies. A 5-year study by Karnosky et al. (1993) demonstrated sugar maple is significantly more tolerant of O_3 than aspen. Experiments using elevated CO_2 revealed differences in growth and physiology among aspen, paper birch, and sugar maple related to the successional status and growth characteristics of those species (King et al. 2001a, 2001b, 2005b; Kubiske and Pregitzer 1996, 1997, 1998; Kubiske et al. 2002), and in defensive chemistry and insect performance (Lindroth and Kinney 1998, Roth et al. 1998). Thus one-quarter of the experiment was designed with a mixed community of two early successional, fast-growing species, aspen and birch; and one-quarter with a mixed community of an early and a late successional species, aspen and maple, respectively.

In March 1997, five clones of trembling aspen were propagated as rooted greenwood cuttings (Karnosky et al. 1996) from selected stock plants, and paper birch and sugar maple seedlings were propagated from open-pollinated seed. Birch and maple seeds were collected below parent trees in Houghton County, MI, in late summer 1996, and in Baraga County, MI, in autumn 1996, respectively. Refer to Dickson et al. (2000) for details on the plant propagation methods.

Cohorts of clones 216 and 271 (referred to as clones E216 and E271, or E clones collectively) were propagated in a CO_2-enriched atmosphere (700 $\mu L\ L^{-1}$) until outplanting. The E clones were planted only in the aspen monoculture sections of the control and elevated CO_2 rings, and made up about 20 percent of all the clone 216 and 271 populations. In the final year of the experiment, there were no statistical differences in height and diameter between the E clones and their counterparts propagated in the ambient-atmosphere greenhouse ($P > 0.25$).

The treatment rings were planted with the three species in summer 1997. The aspen-birch and aspen-maple mixed communities were planted in an alternating pattern throughout the 1 m × 1 m grid. Aspen in the mixed communities was entirely clone 216. In 1999 a 20-m buffer surrounding each ring was planted with hybrid poplar clone NM6 to improve performance of the gas dispensing systems by increasing fetch and reducing turbulence within the rings.

CO_2 and O_3 Treatment Protocols

Elevated CO_2 and O_3 treatments operated only during the leaf-on season, and only during daylight hours (Appendix 2). Fumigation treatments were started each spring when aspen leaves were about one-fourth expanded (Table 2). Fumigation was ended each autumn when aspen leaves had changed color and were just beginning to abscise. Aspen was typically the first species to begin leaf expansion in spring, and maple was typically the last species to senesce leaves in autumn. Fumigation seasons averaged 147 days.

Each of the nine treated rings operated independently but used the same CO_2 and O_3 treatment protocols. The target [CO_2] for the six elevated [CO_2] treatment rings was 560 µL L^{-1}, which was approximately ambient daytime [CO_2] + 200 µL L^{-1} at the beginning of the experiment. It should be noted that ambient [CO_2] increased annually from about 360 µL L^{-1} to about 385 µL L^{-1} during the course of the experiment. This treatment concentration was chosen to approximate the predicted atmospheric [CO_2] for 2060 of 600 ppm reported by the IPCC (1996), and also for consistency with the FACTS project at Duke (Duke University 1995). The CO_2 fumigation system operated automatically each day from dawn to dusk, when the solar elevation exceeded 6^{o} from the horizon. Treatment CO_2 was released every day of the operating season except during brief interruption for system maintenance, in rare instances of equipment failure, or when wind speeds exceeded 5 m sec^{-1}.

The CO_2 used to augment [CO_2] of the ambient air was produced as a byproduct of fertilizer manufacture from methane and atmospheric nitrogen; it had a $\delta^{13}C$ of about -44‰ (Table 5). The ^{13}C content was verified by analysis of periodic samples from the storage tanks on a Finnigan™ DELTAPlus mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA).

Table 5.– Mean (standard deviation) $\delta^{13}C$ (‰) of CO_2 delivered to the Aspen FACE Experiment

Date	$\delta^{13}C$	n[†]
11 June 2002	-43.7 (0.1)	4
25 June 2002	-42.5 (0.2)	4
10 July 2002	-44.3 (0.1)	3
30 July 2002	-44.2 (0.1)	4
26 Aug 2002	-43.1 (0.1)	3
9 Sep 2002	-43.6 (0.2)	3
12 Sep 2002	-43.8 (0.1)	8
17 Sep 2002	-43.9 (0.1)	3
24 Sep 2002	-43.2 (0.4)	3
11 Aug 2003	-44.2	1
18 Aug 2003	-44.1	1
25 Aug 2003	-44.4	1
22 Sep 2003	-41.0	1
1 June 2004	-43.8	1
14 June 2004	-47.6	1
28 June 2004	-47.0	1
20 July 2004	-42.7	1
13 Sep 2004	-46.7	1
28 Sep 2004	-45.4	1
11 Oct 2004	-45.3	1
31 May 2005	-47.1	1
14 June 2005	-41.8	1
28 June 2005	-39.5	1
12 July 2005	-40.1	1
19 July 2005	-45.3	1
9 Aug 2005	-44.4	1
22 May 2006	-39.9 (0.1)	3
19 June 2006	-40.7 (1.3)	3
24 July 2006	-39.3 (1.9)	3
21 Aug 2006	-46.9 (0.4)	3
25 Sep 2006	-44.4 (0.1)	3
2007 and 2008	No data available	

[†] Values with n > 1 are from samples drawn from multiple deliveries of CO_2.

The O_3 fumigation protocol in 1998 and 1999 was designed around diurnal profiles measured at Leelenaw, MI, during summer 1987. The profiles were then modified based on regional 6-year averages (Pinkerton and Lefohn 1987). For that protocol, the site operator selected a daily peak [O_3] based on the weather forecast. The maximum peak [O_3] was 100 nL L^{-1} for hot, sunny days, with a lower maximum, typically 50-60 nL L^{-1}, for cooler, cloudier days.

Beginning in 2000, the O_3 fumigation protocol was modified so that the automated control system would track ambient [O_3] and dose the six treatment rings with 1.5 times the ambient concentration. Throughout the experiment, no O_3 was released when foliage was wet from rain, dew, or fog, or when maximum temperatures were projected to be less than 15 °C. In addition to the high wind speed threshold for system shutdown programmed for the CO_2 treatments, the O_3 fumigation system incorporated a low wind speed cutoff threshold of 0.4 m sec^{-1} for 40 sec. Because the FACE system relies on wind to mix and deliver the treatment gases into the plots, this low threshold helped prevent dangerously high O_3 concentrations from forming near the plot perimeters. Besides the automatic cutoff, the O_3 fumigation system was switched on and off each day by the site operator.

Meteorological Monitoring

In 1999, a 20-m-tall weather monitoring tower was installed on the north end of the site (Table 1). Meteorological variables were recorded electronically: rainfall, photosynthetically active radiation (PAR), net radiation, leaf wetness, and atmospheric pressure at 2 m; and air temperature, relative humidity, and wind speed and direction at 2, 5, 10, 15, and 20 m. Soil temperature was measured at the surface, and at 5, 10, 25, 50, 75, 100, and 200 cm deep. Soil moisture was measured at 0-30, 30-60, and 100-130 cm deep. Wind speed and direction, PAR, and net radiation were measured at 5-sec intervals and the 30-min mean recorded. Air temperature, relative humidity, soil temperature, leaf wetness, and pan evaporation were measured at 5-min intervals and the 30-min mean recorded. Atmospheric pressure was measured and recorded every 30 min. Soil moisture was measured and recorded every 2 hr. Total rainfall (mm) was recorded during rain events.

From 1999 through 2004, meteorological monitoring instruments were deployed in rings 1.2, 2.1, 3.3, and 3.4. Above-canopy PAR, air temperature, and relative humidity were monitored at the ring center according to the measurement protocols described above. Wind speed and direction were monitored above the canopy in the nine treated rings as part of the gas control system. We also monitored below-canopy PAR; air temperature; relative humidity; wind speed and direction; soil temperature at 5, 10, 20, 50, and 100 cm; and soil moisture at 0-30, 30-60, and 100-130 cm in each section of the four rings.

Beginning in 2004, above-canopy PAR, air temperature, relative humidity, and wind speed and direction were monitored at the center of all 12 rings, as were below-canopy PAR, air temperature, relative humidity, and wind speed and direction. Beginning in 2005, rainfall was monitored in 10 rings. In addition, soil moisture at 10-30, 40-60, and 100-120 cm and soil temperature at 5, 10, 20, 50, and 100 cm were monitored in every ring section.

Canopy Access Systems

Canopy access scaffolding and elevated walkways were installed during summer 2002 (Fig. 2, Table 1). Three 3.05-m^2 support scaffolds were erected in each ring. The scaffolds were located in the aspen monoculture, straddling the central ground walkway, and extending into the aspen-birch section. Two 1-m-wide elevated walkways were suspended between each pair of scaffolds, providing access to the upper crowns of aspen and birch trees. Location of the scaffolds and walkways differed among rings. The locations were chosen to provide access to all five aspen clones in each ring. The scaffolds and walkways were raised in 2006 to keep up with tree growth.

ATMOSPHERIC TREATMENTS

Our discussion of the CO_2 and O_3 treatment concentrations is presented to describe the gas concentrations in which the model forest ecosystems developed. For a thorough analysis of the free-air enrichment technology we direct the reader to Hendrey et al. (1993, 1999), Lewin et al. (1994), Nagy et al. (1994), and Dickson et al. (2000).

The [CO_2] and [O_3] reported in Tables 6, 7, 9, and 10 are calculated from hourly mean concentrations during the period from sunrise to sunset, for all days during each fumigation season, irrespective of whether O_3 was actively dispensed on any particular day. The hours of sunrise to sunset were determined to the closest one-half hour for each day of the fumigation.

CO_2 Concentrations

Hourly mean [CO_2] of the three elevated CO_2 treatment rings was 24 – 58 $\mu L\ L^{-1}$ less than target (which was 560 ml L^{-1}) with a range of 34 $\mu L\ L^{-1}$ among years, and that of the three elevated CO_2 + O_3 rings was 25 – 64 $\mu L\ L^{-1}$ less than target with a range of 39 $\mu L\ L^{-1}$ among years (Table 6). Median (i.e., 50^{th} percentile) [CO_2] of the three elevated CO_2 rings was 4 – 17 $\mu L\ L^{-1}$ less than target, and varied by 13 $\mu L\ L^{-1}$ across years, whereas that of the three elevated CO_2 + O_3 rings was 3 – 23 $\mu L\ L^{-1}$ less than target and varied by 20 $\mu L\ L^{-1}$ across years.

Table 6.—Hourly [CO_2] mean (X) and standard deviation (S)†, and median (50^{th} percentile) for the hours from sunrise to sunset, during the fumigation seasons (Table 2) from 1998 to 2008

	[CO_2] ($\mu L\ L^{-1}$)							
	Background‡		+CO_2		+CO_2 + O_3		All + CO_2	
Year	X (S)	50^{th}	X (S)	50^{th}	X (S)	50^{th}	X (S)	50^{th}
1998			527 (68)	556	533 (62)	556	530 (65)	556
1999	360 (32)	351	536 (60)	556	535 (71)	557	536 (66)	556
2000	350 (18)	346	502 (87)	543	496 (88)	537	498 (87)	540
2001	360 (21)	357	507 (82)	548	502 (83)	544	504 (82)	546
2002	367 (26)	363	527 (64)	552	524 (66)	547	526 (65)	550
2003	370 (24)	364	526 (63)	551	524 (62)	547	525 (63)	549
2004	372 (20)	369	514 (72)	546	514 (68)	541	514 (70)	543
2005	372 (20)	369	522 (66)	548	517 (65)	541	519 (66)	545
2006	386 (30)	378	526 (72)	559	525 (71)	556	526 (72)	558
2007	387 (28)	381	524 (74)	557	495 (85)	541	509 (81)	552
2008	393 (22)	389	530 (68)	558	523 (74)	556	527 (71)	557
All years	372 (24)	367	521 (71)	552	516 (73)	547	518 (72)	550

† 95-percent confidence limits are X±1.96(S).
‡ Current ambient CO_2 concentrations, which are assumed to be consistent among all six ambient CO_2 rings.

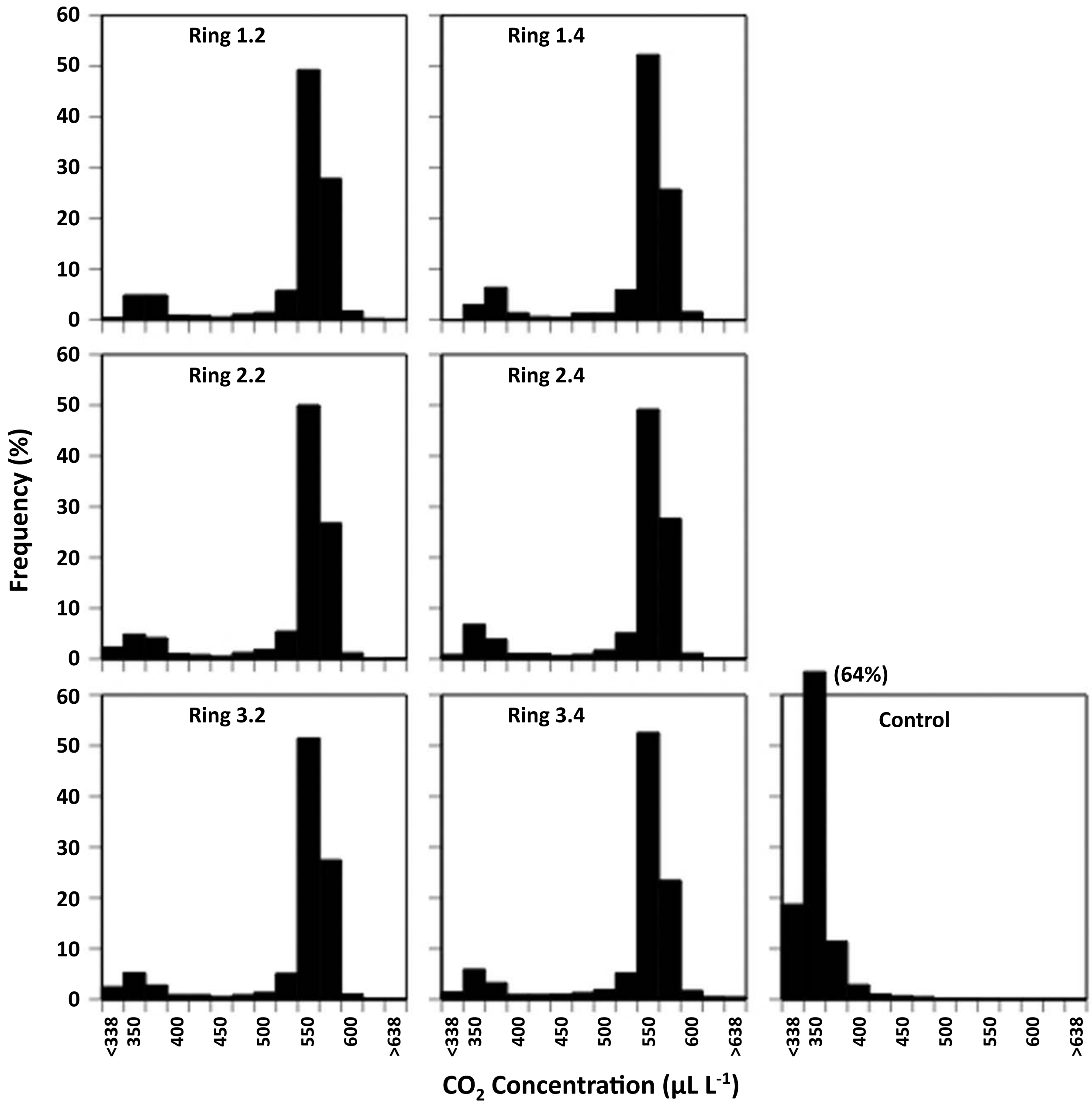

Figure 6.—Frequency distribution of 1-hr [CO_2] during daylight hours (0600 – 2000 CDT; approximately 0500 – 1900 solar time) during the 1999 operating season (Table 2).

There were no χ^2 differences in [CO_2] distributions among the six elevated CO_2 treatment rings (Figs. 6 and 7). Among all six elevated CO_2 rings and months of treatment, the 100th percentile hourly [CO_2] differed from target by 85 – 232 µL L^{-1} (Table 10). In comparison, the 99th percentiles differed from target by 23 – 67 µL L^{-1}, and the 95th percentiles by 9 – 30 µL L^{-1}. Thus, control of treatment [CO_2] was highly consistent among rings and months of treatment with very rare, large deviations of the 1-hr concentrations from the target.

Background [CO_2] increased by 9 percent from the beginning of the experiment to 2008. Target [CO_2] ranged from 156 percent of background in 1998 to 142 percent of background in 2008. Actual treatment [CO_2] ranged from 149 percent of background in 1998 to 134 percent of background in 2008.

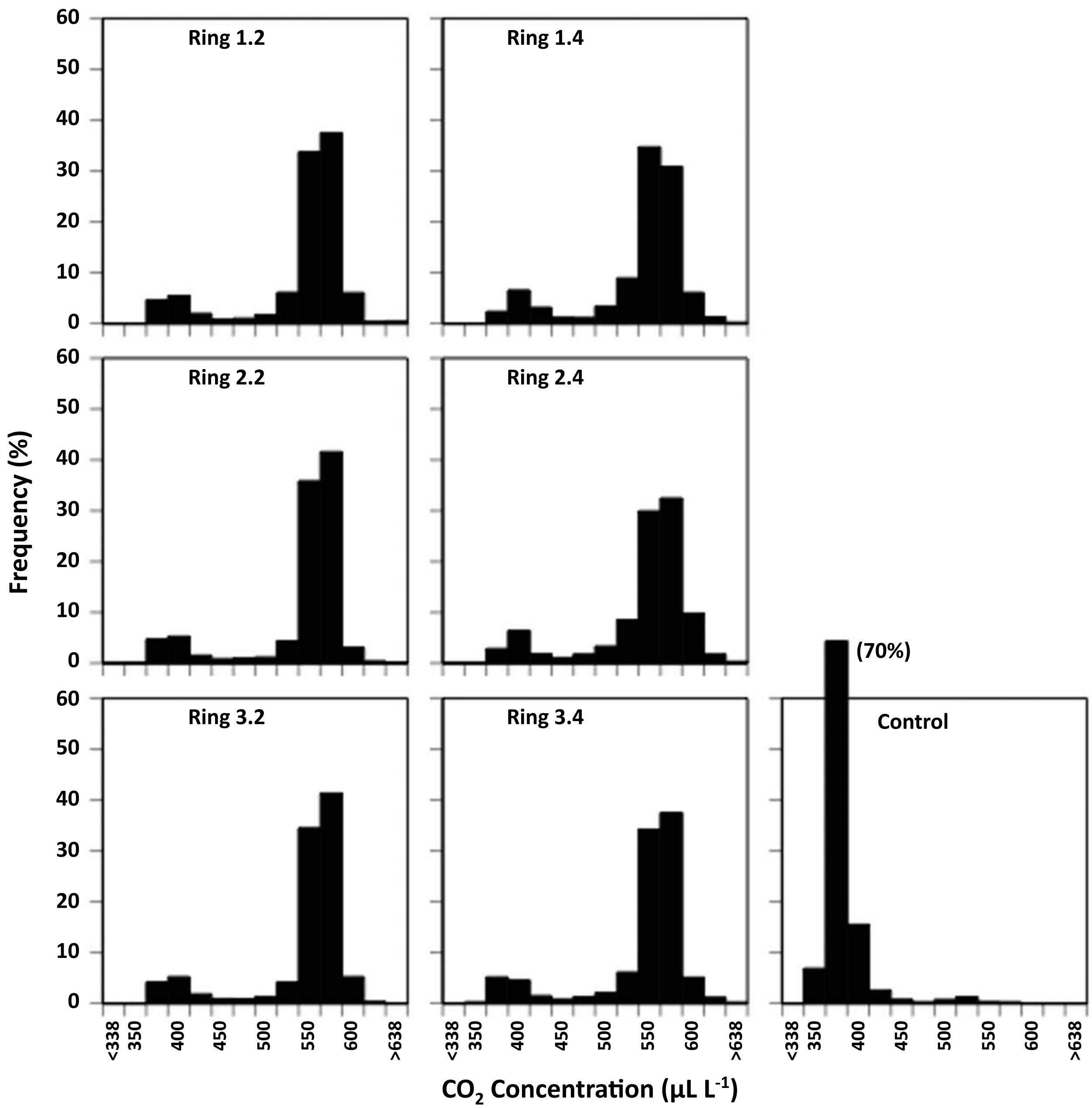

Figure 7.—Frequency distribution of 1-hr [CO_2] during daylight hours (0600 – 2000 CDT; approximately 0500 – 1900 solar time) during the 2006 operating season (Table 2).

Table 7.—Hourly [O_3] mean (X) and standard deviation (S)[†], and median (i.e., 50^{th} percentile) for the hours from sunrise to sunset, during the fumigation seasons (Table 2) from 1998 to 2008

	[O_3] (nL L^{-1}) ‡							
	Background[§]		$+O_3$		$+CO_2 + O_3$		All + [O_3]	
Year	X (S)	50^{th}	X (S)	50^{th}	X (S)	50^{th}	X (S)	50^{th}
1998	35 (13)	34	49 (22)	49	48 (23)	49	49 (23)	49
1999	37 (14)	36	49 (26)	43	50 (26)	46	49 (26)	44
2000	35 (12)	34	45 (21)	40	46 (22)	42	45 (27)	41
2001	37 (12)	35	47 (23)	43	48 (23)	45	48 (23)	44
2002	32 (14)	30	45 (23)	41	45 (23)	41	45 (23)	41
2003	36 (14)	36	45 (21)	44	45 (21)	45	45 (21)	45
2004	34 (13)	33	43 (19)	42	41 (18)	38	42 (18)	40
2005	38 (15)	37	49 (22)	50	50 (22)	51	50 (22)	50
2006	36 (13)	35	40 (17)	39	40 (17)	39	40 (17)	39
2007	35 (14)	34	38 (19)	35	39 (19)	35	39 (19)	35
2008	33 (11)	33	38 (18)	35	39 (18)	36	39 (18)	35
All Years	35 (13)	34	44 (21)	42	45 (21)	42	45 (22)	42

† 95-percent confidence limits are X±1.96(S).
‡ Treatment [O_3] were calculated from all days during the fumigation season, including days on which O_3 was not dispensed.
§ Current ambient O_3 concentrations, which are assumed to be consistent among all six ambient O_3 rings.

O_3 Concentrations

Based on background [O_3] (Table 7), mean hourly target [O_3] (1.5 times background) ranged from 48 nL L^{-1} in 2002 to 55 nL L^{-1} in 1999 and 2001, and the median hourly target [O_3] ranged from 45 nL L^{-1} in 2002 to 48 nL L^{-1} in 1999 and 2003, respectively. Thus, mean hourly [O_3] of the elevated O_3 treatment rings and the elevated CO_2 + O_3 rings was 3 – 10 nL L^{-1} less than target and differed by 9 nL L^{-1} among years. Median hourly [O_3] of the elevated O_3 treatment rings differed from target by -7 to +2 nL L^{-1} with a range of 9 nL L^{-1} among years, and that of the elevated CO_2 + O_3 rings differed from target by -8 to +2 nL L^{-1} with a range of 11 nL L^{-1}.

There were no significant ($P < 0.05$) χ^2 differences in [O_3] distributions among the elevated O_3 rings in any year. However, in 1998 and 1999, when the daily peak [O_3] was set by the site operator, χ^2 tests had their lowest probabilities ($P = 0.09$ and 0.06, respectively; Fig. 8) compared to later years ($0.18 < P < 1.00$; Fig. 9), suggesting greater variability in O_3 dose among treatment rings in 1998 and 1999.

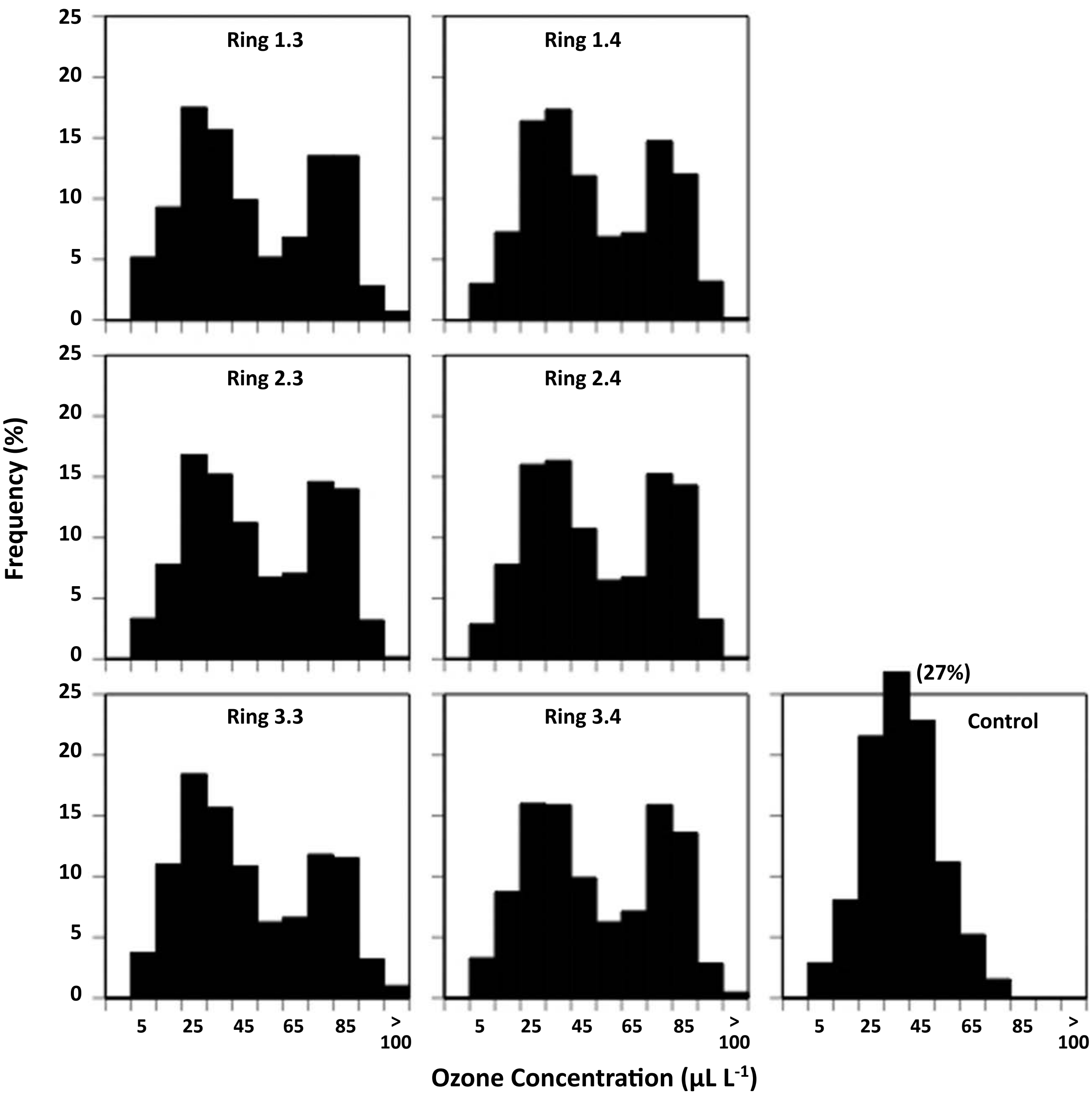

Figure 8.—Frequency distribution of 1-hr [O_3] during daylight hours (0600 – 2000 CDT; approximately 0500 – 1900 solar time) during the 1999 operating season (Table 2).

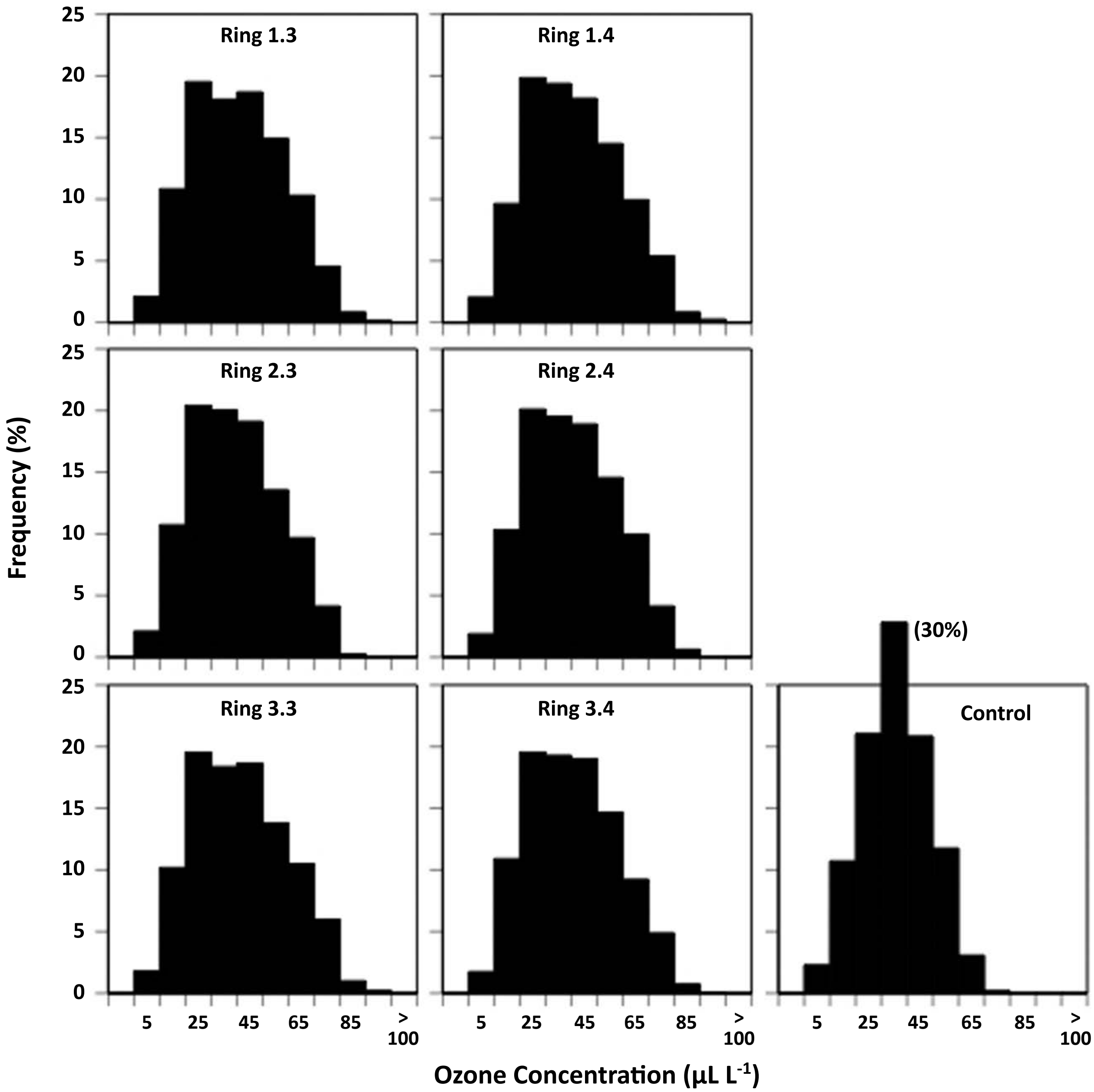

Figure 9.—Frequency distribution of 1-hr [O_3] during daylight hours (0600 – 2000 CDT; approximately 0500 – 1900 solar time) during the 2006 operating season (Table 2).

TREE GROWTH AND BIOMASS MEASUREMENTS

Annual Height and Diameter Measurements

Height and diameter at 3 cm above ground line (d_3) of every tree in the experiment were measured with a meter stick and digital caliper, respectively, immediately after planting in 1997. Height and diameter measurements were repeated following leaf senescence in 1997, and every year thereafter. Following height and diameter measurements in 1999, a buffer zone was identified in each treatment ring composed of the outermost five to six trees, thus defining a circular "core" area within each ring about 20 m in diameter where all subsequent measurements were conducted. Beginning in 2000, heights were measured with telescoping height poles. In later years of the experiment, a crew member served as a spotter to help align the top of the height pole with the tree apex. As diameters and basal swelling increased, diameters were measured at locations farther up the stem. In 2001 both d_3 and d_{10} (diameter at 10 cm above ground line) were measured, and only d_{10} was measured in 2002. In 2003-2005 both d_{10} and diameter at breast height (d.b.h.; 1.37 m above ground line) were measured. Only d.b.h. was measured after 2005. Annual height and diameter measurements are available for download by the public at http://dx.doi.org/10.2737/RDS-2013-0015 (see also Appendix 3).

To compute biomass, allometric equations were developed for each species and aspen clone, by ring, from trees harvested from just outside of the core area in 2000, 2002, and 2007 and from the final analytical harvest in 2009 (described below). Allometric equations developed from harvests in 2000 and 2002 were used to calculate biomass and net primary production by using d_3 as the predictor variable through 2003 (King et al. 2005a). Allometric equations developed from harvests in 2007 and 2009 were used to calculate biomass and net primary production by using d.b.h. as the predictor variable for 2006 through 2008 (Zak et al. 2011). Using the separate allometric equations from different periods of the experiment resulted in large discontinuities in predicted biomass among the different sets of equations based on d_3, d_{10}, and d.b.h. In order to calculate a single allometric equation for each species/clone by treatment combination, d.b.h. was converted to d_{10} by using two sets of regression equations. Equation 1 regressed height (x) vs. d_{10} (y) from 1997 through 1999 in order to determine the d_{10} when height = 1.37 m (d_{10}^*), which was then used as the y-intercept in Equation 2. Equation 2 regressed d.b.h. (x) vs. d_{10} (y) from all years in which both values were measured. Equation 2 was forced through the y-intercept determined as d_{10}^*, and is used to predict d_{10} from d.b.h. Thus, a continuous curve for biomass was created by using d_{10} as the predictor variable for all years of the experiment.

Biomass Harvests

2000 and 2002

Partial harvests were conducted to determine allometry and biomass of species and clones in the experiment. In 2000, one individual of each clone and species from each ring were selected for harvest, for a total of seven trees from each ring. The aspen-maple ring sections were not harvested in 2000. Trees chosen were those located outside of the core measurement area and within ±10 percent of the mean height and diameter of its taxon. Trees were cut by hand at the ground line, carried out of the ring, and dissected by annual height growth increment for leaf area and stem, branch order, shoot type (determinate or indeterminate), and leaf mass. Stumps and coarse roots were sampled with a 25.4-cm coring device centered over the cut stump and driven to a depth of 25 cm. Additional root samples were collected (five in the aspen half, two in the aspen-birch section) with the same coring device to a depth of 25 cm centered between rows and columns of trees. Roots were washed and separated into one of four live size classes, or dead, and the ash-corrected dry mass determined.

A similar harvest occurred in 2002 following the same collection and measurement protocols used previously. In 2002, one individual of each clone and species were selected for harvest from each ring section, including the aspen-maple section, that were within 20 percent of the minimum, 10 percent of the mean, or 20 percent of the maximum height for its taxon. Thus, for each treatment we obtained three trees, one from each replicate, representing smallest, mean, and largest size of each taxon.

2007 and 2009

In 2007 we conducted a partial, aboveground harvest to establish allometric relationships. One tree of each aspen clone and species in each ring section were randomly selected from outside the ring cores. Trees were cut and removed by hand.

We obtained detailed information for total stem mass and branch mass, as well as for leaf mass and area by annual height growth increment for each aspen clone and species. These variables were used to create allometric relationships by using total tree height and d.b.h., which were then used to estimate biomass and leaf area by ring section, and vertical biomass distribution for species and genotype.

On each tree to be harvested, we first labeled the 1.37-m height line and drew an upward pointing arrow on the north aspect. This step was needed to show the upward direction on stem segments used for wood analysis, and to locate the north aspect on the cut trees for photographs. All reachable dead branches were removed before felling. In addition, large lower branches that might be damaged during tree removal were removed but kept with the tree for biomass measurements. Trees were cut at ground level with a chain saw or a bow saw, carefully lowered to the ground by hand, and carried out of the stand by hand. After removal, each tree was placed horizontally on a set of sawhorses. Starting at the top we identified and labeled each annual height growth increment (HGI) based upon bud scars and branching patterns. Annual HGIs were identified until the earliest HGI that contained live branches. Butt diameter, total height, and live crown height were measured. Trees taller than 3 m were cut apart at the bottom of the earliest live crown HGI.

Intact tree crowns were encased within plastic bags and transported to the U.S. Forest Service's Institute of Applied Ecosystem Studies (IAES) on a flatbed trailer, about 7 km away from the experiment. Trees were stored in a walk-in 40C cooler (Model B, Vollrath Company, Sheboygan, WI) until processed. We photographed the live crown of each harvested tree crown from the four cardinal directions, in the order of north, east, south, and west, using a high-resolution digital camera. The tree crowns were held vertically against a dark backdrop calibrated at 2-m intervals.

After the photographing, the tree crowns were taken into the processing room, where they were dissected. We counted and removed all primary branches (branches connecting directly to the main stem) from the main stem by HGI. After all the branches were removed, the main stem was taken to a saw station for cutting into various specific samples (described below).

For each HGI, we removed all intact leaves and current-year shoots. From this sample we removed two subsamples and separated leaves from shoots. From subsample 1 leaves (about 20 leaves), we obtained number of leaves, leaf area, fresh mass, and oven-dry mass. Subsample 2 was frozen for archival purposes (about 150 g). We measured fresh weight of all remaining leaves from each HGI. From subsample 1, we determined the ratio of weight to leaf area on fresh leaves and after air-drying for each HGI, which enabled us to estimate total leaf dry weight and leaf area for each HGI. Subsample 1 leaves were ground to 60 mesh in a Wiley® Mill (Thomas Scientific, Swedesboro, NJ) or small samples to 80 mesh in a mini-amalgamator for C-N analyses.

We dissected the primary branches into annual growth segments numbering them as first-, second-, third-, etc., order branches, counting from distal to proximal ends, so that the current-year, leaf-bearing shoots were first-order branches, the previous year's leaf-bearing shoots were second-order branches, and so on. We measured the fresh weight of each branch order for each HGI; any first-order branches exhibiting indeterminate growth were weighed separately. After weighing each branch order by HGI, we combined HGIs for each order, homogenized them, and removed a subsample of at least 10 percent of the branches from each pooled branch order. We obtained fresh and oven-dry mass for each branch subsample, which allowed us to calculate the dry weight of each branch order by HGI.

Paper birch branch morphology is somewhat different from the other two species as leaves occur on long shoots and spur shoots. The spurs were treated the same as first-order branches. Any flowers or flower buds were collected from the branches and frozen by HGI for later analysis, which could include germination rate, mass, and chemistry.

For analysis of wood properties, wood samples were cut from the lower main stem: two 15-cm-long sections located at the 1.4-m line and at 40 percent of total height. These sections were frozen. We also cut two 3-cm-long sections at 1-m increments along the lower main stem (a frozen section for the archive, and one for stem dry weight determination described below, which was left to air-dry). Similarly, we cut two 3-cm-long sections at the midpoint of each HGI in the live crown. If the cut sections overlapped, the 15-cm-long wood analysis sections had priority and the 3-cm-long dry mass and archive samples were cut as close as possible to their intended positions.

To determine total stem mass, we used one set of the 3-cm-long stem sections. Sections were oven dried at 65 ^{o}C for 48 hr. We plotted dry mass cm^{-1} length of each stem section (*M*) vs. height above ground (*h*), and integrated *M* with respect to *h* from 0 to total tree height (*ht*) to obtain total stem dry mass:

$$\textit{Total stem mass} = \int_0^{ht} M \frac{dm}{dh}$$

In 2009 we conducted a larger-scale, complete-tree analytical harvest in each ring section, which provided total aboveground and belowground biomass, biomass of individual plant organs by annual HGI, and total leaf area. All variables were determined by species and aspen genotype with the exception of roots from the aspen monoculture. We also sampled forest floor and mineral soil, enabling us to compile C and N budgets to a depth of 1 m.

In preparation for the 2009 biomass harvest, we located rectangular subplots in each ring section (referred to as the biomass plots). From these plots we measured all aboveground biomass, and biomass belowground to a depth of 1 m, which provided us with unbiased estimates of total accumulated biomass over the 11-year span of the experiment. The biomass plots were located without bias by first locating them on ring maps that did not indicate mortality or aspen clones. The biomass plots were 2 m × 5 m and 2 m × 3 m in the aspen-only and mixed-species sections, respectively, with the plot sides running parallel to the planting rows and midway between the coordinate grid points that identified planting locations. For the aspen-only and aspen-maple ring section, the plots were located at the same coordinates across all the rings, under the assumption that clones and tree mortality were randomly distributed within each ring section. In the aspen-birch quadrants, the biomass plots were located at one of three different coordinates to avoid the elevated canopy-access walkway, the position of which varied among rings. The grid coordinates contained within the biomass plots were H-I, 17-21 for the aspen-only sections, and H-I, 10-12 for the aspen-maple sections. For the aspen-birch sections, the coordinates were: M-N, 9-11 in rings 1.1, 2.1, 2.4, 3.2, 3.3, and 3.4; Q-R, 9-11 in rings 1.2, 1.4, 2.2, 2.3, and 3.1; and R-S, 9-11 in ring 1.3.

We also identified more trees for aboveground harvesting which would bring the number of each species and aspen genotype to three per ring for calculating aboveground allometry. This selection of additional trees favored the largest trees in each ring as smaller trees were harvested previously in 2007.

Beginning with leaf-out in 2009, we fumigated each ring with the treatment gases until it was harvested. The harvest began on 11 June and progressed ring by ring until the last ring section was completed on 12 August. The harvest sequence was randomized among blocks, and then again among rings within blocks (Table 8). We coordinated four general phases of harvest in each ring, which consisted of, in sequence: (1) preharvest (collecting forest floor, ground cover, and soil bulk density samples, measuring d.b.h., and turning off the fumigation system and cutting access through the plenum), (2) aboveground harvest, (3) belowground harvest, and (4) cleanup (backfilling the soil pit and relocating supplies and equipment). Both preharvest and cleanup took less than 1 day in each treatment ring. We were able to accomplish the aboveground harvest in one to two ring sections per day, and belowground harvest in one ring section per day. Because of weather and other variables, our average pace was one complete ring harvested every 3.75 days. A total of 296 trees were harvested.

The procedures used for dissection and analysis of aerial tree parts followed those established in 2007, with the exception that dissections occurred at the Karnosky Laboratory (Fig. 10), located onsite, rather than at the IAES.

Table 8.—Sequence and timing of harvest activities in 2009, the final harvest of Phase I

				Aspen-only section		Aspen-maple section		Aspen-birch section	
Treatment ring	Plenum cut[†]	FF BD[‡]	d.b.h.[§]	Aboveground harvest	Belowground harvest	Aboveground harvest	Belowground harvest	Aboveground harvest	Belowground harvest
2.1	7 Jun	5 Jun	5 Jun	9 Jun	11 Jun	8 Jun	9 Jun	8 Jun	10 Jun
2.3	9 Jun	8 Jun	9 Jun	12 Jun	15 Jun	12 Jun	17 Jun	11 Jun	12 Jun
2.4	13 Jun	8 Jun	15 Jun	17 Jun	18 Jun	17-18 Jun	23 Jun	18 Jun	22 Jun
2.2	21 Jun	8 Jun	19 Jun	22 Jun	24 Jun	23 Jun	26 Jun	23 Jun	25 Jun
1.3	27 Jun	19 Jun	19 Jun	29 Jun	1 Jul	26 Jun	29 Jun	25 Jun	30 Jun
1.2	30 Jun	19 Jun	30 Jun	2&7 Jul	8 Jul	1 Jul	2 Jul	1 Jul	7 Jul
1.4	6 Jul	7 Jul	30 Jun	8-9 Jul	15 Jul	7 Jul	10 Jul	8 Jul	13 Jul
1.1	13 Jul	9 Jul	9 Jul	15 Jul	21 Jul	13 Jul	20 Jul	13 Jul	16 Jul
3.4	15 Jul	18 Jul	15 Jul	21-22 Jul	27-28 Jul*	16 Jul	22-23 Jul*	21 Jul	24 Jul
3.3	22 Jul	22 Jul	20 Jul	27 Jul	30 Jul	24 Jul	29 Jul	24 Jul	31 Jul
3.2	28 Jul	29 Jul	28 Jul	3-4 Aug	6 Aug	29 Jul	3 Aug	30 Jul	4 Aug
3.1	28 Jul	31 Jul	31 Jul	7 Aug	10 Aug	6 Aug	7 Aug	6 Aug	11 Aug

[†] The date on which the treatment was discontinued and sections of the plenum cut to allow access to the biomass plots.
[‡] The date on which forest floor and soil bulk density samples were collected.
[§] The date on which diameter at breast height was measured.
* Belowground harvest suspended because of equipment failure.

Figure 10.—David F. Karnosky Laboratory at Harshaw Research Farm, near Rhinelander, WI. Photo by A.R. Foss, U.S. Forest Service.

After removing the aboveground biomass in a ring section, we clipped all the understory vegetation at ground level in each biomass plot, sorted the biomass by species, and dried and weighed the understory biomass following the protocols detailed in Bandeff et al. (2006).

To determine coarse and fine root biomass and their distribution by depth, a volumetric pit 1 m deep was excavated in each harvest plot: 2 m × 5 m in the aspen-only sections and 2 m × 3 m in the mixed-species sections. The cut stump and coarse roots of any tree that lay within the surface dimension of the pit were removed by hand, fresh weight was determined, and a 100-g subsample was removed for dry weight determination. The remaining soil with belowground plant material was removed with a mini-excavator. Soil was transported to an area just outside the ring and passed through a gasoline-powered, 1-cm^2 mesh landscaping sieve to recover coarse roots. Coarse roots were washed and oven dried for biomass.

After excavation of the pit, we extracted cores 10 cm in diameter × 30 cm long from the pit face to determine mass and depth distribution of fine roots. Cores were taken at 10-cm increments from the surface of the mineral soil to the bottom of the pit (1.0 m) from all four faces of each pit. Roots were sorted by hand into two size classes, <1.0 mm and >1.0 mm. Roots were oven dried at 65 ^{o}C for 48 hr and ash-weight corrected. In the aspen-birch and aspen-maple ring sections, we were able to identify coarse roots by tree species based on morphology.

Post-harvest Site Reclamation

The site was backfilled by a tracked front-end loader that first filled in all subsoil and then overtopped it with material from the A-horizon. Because the pits are now permanent soil discontinuities at the site, we marked their locations with steel reinforcing rods buried just under the soil surface at the pit corners. These rods will be easily located with a metal detector should it prove necessary for future studies at the site.

ASPEN FACE EXPERIMENT, PHASE II

U.S. Forest Service funding allowed conversion of the Aspen FACE Experiment to a regeneration study. We removed the aboveground biomass of all remaining trees from the experiment during winter 2009-2010. Chainsaw operators cut the trees to ground level and removed them from the rings. The whole trees were then chipped and discarded onsite.

New sampling plots were established within each ring section: three sampling plots in the aspen monoculture, each consisting of three 1 m × 1 m subplots; and one sampling plot in each of the aspen-birch and aspen-maple sections, each sampling plot consisting of four 1 m × 1 m subplots. As the first sprouts began to emerge in June, we re-initiated fumigation of the rings with the same treatments used previously.

In phase II, we used a CO_2 source with a $\delta^{13}C$ signature near that of current ambient atmosphere (-8 $^{o}/_{oo}$), which was very different from the $\delta^{13}C$ of CO_2 used in the previous experiment (-40 $^{o}/_{oo}$), to monitor plant and soil C as the ^{13}C /^{12}C ratio changed over time. Coarse root samples were collected for baseline $\delta^{13}C$ before any sprouts emerged. Beginning with newly emerging sprouts and suckers, we collected growing shoot tips (leaf plastochron index 0-3) four times throughout the summer for $\delta^{13}C$ analysis. We also measured soil respiration every 4 weeks from May to October.

Twice-weekly counts of regenerated sprouts within the subplots were made in June and early July 2010, followed by weekly counts in which basal sprouts, sprout clusters, and single sprouts were counted separately. At each count, sprouts were rated as 1 (emerged), 2 (shoot elongated but leaves not expanded), 3 (leaves expanding but pigmented red), and 4 (leaves expanded and green). In autumn, we labeled each sprout in the subplots with a unique identification number on a stamped aluminum tag.

In spring 2011, we collected leaf samples from one randomly chosen 1 m × 1 m subplot in the aspen monoculture of each ring (ca. 500 samples) for DNA fingerprinting to determine regeneration success of each aspen clone.

SITE SAFETY PROTOCOLS

All users and visitors to the experiment were required to sign in at the control center. A sign (Fig. 11) located at the control center notified all users and visitors that by entering the grounds of the Aspen FACE facility they tacitly agreed to conduct themselves in a professional manner, maintain a safe and comfortable working environment for everyone according to Federal Occupational Safety and Health Administration (OSHA) and Equal Employment Opportunity Commission (EEOC) standards, protect the integrity of the work of other researchers and the experiment as a whole, and respect the local citizens' property and the peaceful surroundings. In addition, all users of the experiment were required to sign the acknowledgment and agreement form in the back of the Aspen FACE safety manual before site access would be issued. The site safety manual was available for download at the Aspen FACE Web site: http://aspenface.mtu.edu/protocol.htm.

Figure 11.—Safety guidelines sign at Harshaw Research Farm. Photo by A.R. Foss, U.S. Forest Service.

Key elements of the safety manual were:

- Use of a hardhat in the presence of an overhead hazard, when heavy equipment or a boom was in operation, when working below an elevated walkway in use, and where use of a hardhat was required by posting
- Use of hearing protection within 30 feet (9 m) of operating generators or when noise surveys indicated more than 85 dB
- Use of ozone mask/particulate respirators while working in ozone rings during fumigations. This required medical clearance for use of a respirator by an occupational health professional according to OSHA regulations.
- Users of the canopy access scaffolding and walkways were required to be trained in the use of a fall-arrest device for ascending and descending the scaffold ladders, and in the use of a safety harness and lanyard while working on the elevated walkways.

There were no serious injuries during the life of the experiment.

CONCLUSIONS

The Aspen FACE Experiment was a highly successful science platform. It was host to more than 70 researchers from 9 countries and held many tours and scientific conferences. Research activity at the experiment was a frequent news feature in the popular media. Findings from the experiment helped to inform *Our Changing Planet*, the supplement to the U.S. President's 2002 budget, as well as the 2006 rewriting of the U.S. Environmental Protection Agency's ozone pollution criteria document.

Thirteen years of research contributed complex information on tree-, stand-, and ecosystem-level responses to the CO_2 and O_3 treatments. As of this writing, 126 peer-reviewed publications report primary data collected at the experiment (Appendix 4). A detailed archive of plant material from the experiment is maintained at the U.S. Forest Service Research Laboratory in Rhinelander, WI, and a number of datasets, including both raw and processed data, and site and ring schematics, are archived at the U.S. Forest Service Research Data Archive (http://www.fs.usda.gov/rds/archive/). In addition, plots of the aspen genotypes are maintained at the site to provide the same genetic material for followup studies.

The direct effects of increasing atmospheric [CO_2] and [O_3] on forests are real and dynamic. In our experiment, elevated [CO_2] increased net primary productivity by 39 percent across all community types, and elevated [O_3] decreased it by 10 percent (Talhelm et al. 2014). These changes in productivity were driven by similar changes in leaf-level photosynthesis rates (Kets et al. 2010) that cascade through the ecosystem to drive other ecosystem processes. Increased or decreased flux of organic C below ground (in elevated [CO_2] or [O_3], respectively) resulted in more or less C deposited in near-surface mineral soil (Talhelm et al. 2014). These changes in soil C content were accompanied by positive feedbacks to soil N cycling, resulting in more N in the forest canopies under elevated [CO_2] or less N in the canopies under elevated [O_3] (Zak et al. 2012).

Limitations in soil N are considered to be a factor that can eliminate growth stimulation by elevated [CO_2]; we found no evidence for this relationship in our experiment (Zak et al. 2012). Rather, annual variations in growth responses to elevated [CO_2] and [O_3] were significantly controlled by prevailing weather patterns, particularly the integrated amount of solar radiation (Kubiske et al. 2006).

Results from the Aspen FACE Experiment increased our understanding of intraspecific and interspecific differences in responses to elevated [CO_2] and [O_3], thereby improving tree- and stand-level modeling of forest responses to those important greenhouse gases. A clear understanding of these effects is needed to manage forests today in a manner that will ensure their continued health and productivity in a future, more polluted atmosphere.

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APPENDIX 1

Equipment and Instruments at the Aspen FACE Experiment

Carbon dioxide monitoring

- Gas control system for elevated CO_2 within rings
  - ♦ LiCor Model LI-6252 infrared gas analyzer (LiCor Biosciences, Lincoln, NE)

Meteorological monitoring

- Within rings
  - ♦ Wind above canopy in each ring (part of the gas control system) and below canopy in each ring
    - o Wind Sentry 03002-L wind vane and anemometer (R.M. Young Company, Traverse City, MI)
  - ♦ Photosynthetically active radiation (PAR) above canopy at each ring, and below canopy in each ring section
    - o LiCor Model LI-190 quantum sensor (LiCor Biosciences, Lincoln, NE)
  - ♦ Net radiation above canopy
    - o REBS Q7.1 net radiometer (Radiation and Energy Systems, Inc, Seattle, WA)
  - ♦ Air temperature and humidity above canopy at each ring and below canopy in each ring section
    - o Campbell Scientific CS500 probes (Campbell Scientific, Logan, UT)
  - ♦ Soil temperature at -5, -10, -20, -50 and -100 cm
    - o 24-gauge copper-constantan thermocouple
      Reference temperature: Fenwal UUT51J1 thermister
      (Fenwal Electronics, Milford, MA)
  - ♦ Soil moisture at -5, -50, and -100 cm at two locations in each ring quadrant and three locations in each ring half
    - o 1999-2004: CS616 water content reflectometer probe (Campbell Scientific, Logan, UT)
    - o 2005-2008: EC-20 $ECH_2O^{®}$ dielectric aquameter (Decagon Devices, Inc., Pullman, WA)
  - ♦ Rainfall above canopy at each ring
    - o TE5525 tipping bucket rain gauge (Texas Electronics, Inc., Dallas, TX)
- Meteorological station
  - ♦ Wind speed and direction

- ♦ Soil temperature

- ♦ Soil moisture
  - o EC-20 $ECH_2O^{®}$ dielectric aquameter
    (Decagon Devices, Inc., Pullman ,WA)

- ♦ Rainfall
  - o TE5525 tipping bucket rain gauge
    (Texas Electronics, Inc., Dallas, TX)

- ♦ Air temperature and relative humidity
  - o CS500 temperature and relative humidity probe
    (Campbell Scientific, Inc., Logan, UT)

- ♦ Photosynthetically active radiation
  - o LI190SBN Quantum Sensor
    (LiCor Biosciences, Lincoln, NE)

- ♦ Pan evaporation

- ♦ Net radiation

Ozone generation from pure O_2

- 1998 – 2002: Unizone Model MZ18X
  (Praxair-Trailigaz Ozone Co., Cincinnati, OH)
  - ♦ Capacity: 16 kg O_3 d^{-1}
  - ♦ Maximum conversion efficiency of pure O_2: 6%

- 2002 – 2006: TrailigazConcept Model OZC1016
  (Praxair-Trailigaz Ozone Co., Cincinnati, OH)
  - ♦ Capacity: 38 kg O_3 d^{-1}
  - ♦ Maximum conversion efficiency of pure O_2: 6%

- 2007 – 2009: PCI-Wedeco Model HT 100
  (PCI-Wedeco Environmental Technologies, West Caldwell, NJ)
  - ♦ Capacity: 154 kg O_3 d^{-1}
  - ♦ Maximum conversion efficiency of pure O_2: 3%

Ozone monitoring

- Gas control system for elevated O_3
  - ♦ Thermo Environmental Instruments Models 49 and 49C UV absorption gas analyzers
    (Thermo Environmental Instruments, Inc., Franklin, MA)

- Control building (i.e., west fence and Wisconsin Department of Natural Resources regional O_3 monitor)

- Passive ozone monitors
  - ♦ Can Oxy Plate™ gel blot paper loaded with indigo dye

APPENDIX 2

$[CO_2]$ and $[O_3]$ Statistics by Month and Treatment Ring

Table 9.—Mean (and standard deviation) of hourly $[CO_2]$ and $[O_3]$ at the Aspen FACE Experiment for the hours from sunrise to sunset[a] during the fumigation season (Table 2), from 1998 to 2006

	$[CO_2]$ (μL L^{-1})							$[O_3]$ (nL L^{-1})						
	Backgrnd[b]	Ring 1.2[c]	Ring 2.2	Ring 3.2	Ring 1.4	Ring 2.4	Ring 3.4	Backgrnd[b]	Ring 1.3[c]	Ring 2.3	Ring 3.3	Ring 1.4	Ring 2.4	Ring 3.4
1998														
May		519 (76)	504 (87)	497 (92)	513 (81)	508 (86)	495 (92)	41 (14)	47 (18)	49 (19)	49 (20)	49 (21)	48 (19)	45 (19)
June			551 (27)	549 (40)	528 (71)	552 (25)	552 (27)	33 (10)	48 (22)	51 (20)	49 (22)	50 (21)	51 (21)	48 (22)
July		520 (74)	553 (30)	546 (44)	528 (71)	546 (44)	552 (30)	29 (10)	50 (25)	51 (23)	49 (24)	49 (23)	50 (24)	50 (24)
Aug		529 (61)	546 (41)	537 (57)	541 (49)	538 (52)	544 (42)	39 (13)	50 (22)	53 (22)	50 (22)	54 (22)	53 (22)	49 (23)
Sep		529 (60)	537 (51)	528 (67)	529 (59)	530 (59)	536 (53)	36 (16)	53 (25)	54 (25)	52 (26)	51 (26)	54 (25)	50 (26)
Oct		460 (91)	454 (99)	446 (105)		456 (95)		26 (11)	32 (16)	34 (16)	32 (17)	30 (17)	33 (16)	26 (18)
1999														
May	360 (16)	544 (43)	541 (45)	542 (45)	544 (50)	525 (72)	537 (51)	44 (13)	53 (22)	55 (21)	53 (21)	54 (21)	54 (21)	53 (22)
June	353 (21)	555 (22)	554 (22)	555 (22)	554 (19)	555 (19)	554 (74)	39 (15)	55 (27)	57 (27)	54 (28)	56 (27)	56 (27)	56 (28)
July	378 (49)	542 (55)	531 (72)	541 (59)	541 (52)	535 (115)	541 (93)	37 (12)	45 (27)	52 (25)	44 (30)	48 (23)	53 (26)	54 (28)
Aug	347 (21)	524 (73)	522 (76)	523 (76)	520 (74)	524 (74)	521 (77)	32 (13)	47 (27)	46 (26)	46 (27)	48 (26)	48 (26)	48 (27)
Sep	356 (21)	523 (70)	520 (74)	521 (75)	519 (73)	521 (74)	521 (72)	32 (17)	42 (28)	41 (25)	41 (23)	42 (25)	42 (24)	41 (25)
Oct														
2000														
May	360 (13)	489 (89)	492 (85)	498 (85)	491 (86)	475 (92)	469 (92)	42 (9)	46 (16)	48 (15)	41 (17)	48 (16)	46 (17)	46 (15)
June	353 (18)	490 (90)	495 (91)	500 (88)	488 (91)	482 (92)	483 (91)	35 (11)	44 (20)	45 (19)	42 (19)	45 (19)	43 (20)	44 (20)
July	346 (19)	495 (94)	500 (93)	505 (89)	495 (91)	496 (92)	492 (92)	33 (11)	42 (19)	43 (18)	41 (19)	46 (25)	41 (19)	42 (18)
Aug	345 (19)	523 (74)	521 (74)	508 (87)	519 (78)	517 (77)	516 (77)	34 (11)	50 (25)	49 (24)	49 (24)	52 (25)	49 (25)	52 (24)
Sep	349 (13)	507 (80)	502 (82)	504 (84)	498 (82)	498 (84)	488 (85)	31 (12)	45 (24)	45 (24)	44 (24)	46 (25)	44 (24)	45 (25)
Oct					449 (95)									
2001														
May	359 (13)	505 (80)	510 (76)	510 (79)	497 (83)	490 (83)	502 (81)	42 (12)	50 (21)	49 (19)	43 (22)	51 (20)	51 (20)	52 (20)
June	357 (21)	504 (83)	508 (78)	509 (82)	495 (86)	495 (86)	502 (83)	39 (13)	53 (23)	56 (23)	55 (25)	55 (23)	54 (23)	55 (23)
July	367 (26)	518 (75)	509 (81)	515 (79)	514 (76)	517 (76)	512 (78)	38 (13)	53 (24)	53 (23)	54 (26)	54 (23)	54 (28)	52 (24)
Aug	347 (18)	513 (80)	510 (83)	503 (86)	511 (78)	513 (79)	507 (84)	34 (11)	45 (21)	46 (20)	50 (9)	47 (20)	44 (23)	47 (21)
Sep	368 (15)	498 (87)	491 (88)	493 (90)	486 (85)	494 (86)	480 (91)	32 (10)	31 (20)	37 (19)	32 (15)	37 (19)	37 (19)	36 (20)
Oct	385 (29)	509 (78)	512 (74)	511 (77)	512 (74)	512 (74)	480 (91)	32 (11)	29 (17)	32 (16)	32 (16)	38 (15)	34 (17)	32 (17)

continued

Table 9.—continued

	$[CO_2]$ (µL L^{-1})							$[O_3]$ (nL L^{-1})						
	Backgrnd[b]	Ring 1.2[c]	Ring 2.2	Ring 3.2	Ring 1.4	Ring 2.4	Ring 3.4	Backgrnd[b]	Ring 1.3[c]	Ring 2.3	Ring 3.3	Ring 1.4	Ring 2.4	Ring 3.4
2002														
May	382 (14)	498 (79)	483 (83)	518 (67)	513 (64)	483 (80)	511 (68)	52 (11)	58 (15)	57 (14)	56 (14)	60 (15)	58 (14)	53 (14)
June	368 (22)	533 (62)	530 (63)	532 (64)	509 (80)	530 (62)	531 (64)	36 (13)	48 (21)	48 (21)	49 (21)	50 (23)	49 (21)	46 (22)
July	361 (25)	534 (58)	534 (57)	529 (65)	528 (63)	528 (61)	529 (64)	32 (12)	48 (22)	48 (21)	48 (22)	48 (22)	48 (21)	47 (22)
Aug	362 (33)	538 (52)	537 (51)	520 (73)	534 (54)	537 (54)	538 (52)	30 (13)	49 (24)	48 (23)	48 (23)	49 (23)	48 (23)	47 (24)
Sep	371 (27)	525 (62)	524 (60)	520 (70)	517 (67)	517 (69)	518 (70)	29 (13)	40 (25)	40 (24)	40 (23)	41 (23)	40 (23)	38 (23)
Oct	369 (9)	503 (79)	502 (77)	508 (79)	498 (74)	501 (76)	501 (80)	21 (9)	20 (9)	22 (8)	23 (8)	23 (8)	23 (8)	24 (8)
2003														
May	373 (16)	529 (58)	524 (63)	528 (62)	518 (56)	527 (58)	526 (63)	41 (12)	54 (20)	53 (22)	53 (22)	56 (19)	55 (21)	54 (21)
June	366 (20)	536 (53)	534 (59)	532 (61)	534 (56)	533 (59)	538 (56)	40 (13)	51 (22)	50 (23)	49 (23)	53 (21)	51 (22)	50 (22)
July	369 (29)	533 (56)	530 (57)	518 (73)	526 (64)	524 (62)	533 (57)	33 (12)	44 (18)	43 (19)	43 (19)	43 (19)	43 (18)	42 (19)
Aug	369 (32)	541 (44)	546 (36)	539 (49)	541 (45)	531 (49)	540 (45)	34 (13)	47 (20)	46 (21)	45 (20)	46 (21)	46 (20)	46 (20)
Sep	371 (16)	512 (73)	509 (69)	514 (72)	518 (67)	505 (72)	507 (72)	34 (17)	41 (22)	40 (23)	42 (23)	41 (23)	41 (23)	41 (22)
Oct	378 (9)	490 (76)	470 (73)	477 (75)	485 (75)	472 (70)	471 (71)	33 (13)	38 (17)	37 (17)	37 (17)	37 (17)	37 (17)	38 (17)
2004														
May	378 (8)	521 (68)	520 (68)	520 (67)	524 (63)	507 (76)	496 (76)	40 (5)	43 (13)	40 (13)	39 (13)	37 (13)	40 (13)	39 (13)
June	377 (17)	530 (59)	515 (71)	521 (66)	517 (68)	518 (67)	510 (71)	32 (11)	50 (19)	47 (20)	48 (21)	35 (11)	47 (20)	46 (20)
July	366 (26)	538 (54)	536 (56)	537 (56)	534 (56)	535 (58)	525 (60)	33 (12)	43 (20)	44 (19)	44 (19)	42 (19)	44 (18)	43 (18)
Aug	365 (20)	536 (48)	528 (62)	516 (71)	534 (52)	532 (55)	521 (62)	29 (10)	40 (18)	39 (19)	40 (19)	40 (19)	39 (18)	39 (18)
Sep	374 (18)	506 (75)	497 (79)	496 (78)	503 (72)	500 (72)	484 (70)	41 (16)	46 (18)	45 (19)	46 (19)	45 (19)	46 (19)	46 (19)
Oct	378 (16)	378 (16)	463 (79)	482 (76)	485 (73)	477 (75)	465 (72)	33 (11)	34 (13)	33 (13)	33 (14)	33 (14)	29 (14)	33 (13)
2005														
May	379 (10)	542 (44)	541 (50)	539 (46)	538 (49)	532 (48)	529 (54)	36 (10)	44 (20)	46 (18)	48 (20)	49 (20)	45 (21)	47 (20)
June	376 (20)	520 (69)	521 (70)	517 (68)	517 (69)	511 (67)	513 (68)	43 (14)	52 (20)	51 (19)	53 (20)	53 (20)	54 (19)	51 (20)
July	366 (25)	518 (71)	525 (68)	514 (70)	520 (68)	512 (65)	512 (68)	38 (16)	51 (22)	50 (22)	52 (22)	52 (22)	53 (22)	51 (22)
Aug	366 (19)	527 (63)	530 (61)	524 (61)	526 (62)	517 (58)	524 (62)	35 (14)	49 (21)	48 (21)	50 (21)	50 (21)	51 (20)	49 (21)
Sep	377 (16)	527 (57)	528 (61)	522 (57)	532 (54)	516 (59)	517 (59)	37 (15)	49 (24)	48 (23)	50 (24)	51 (24)	50 (23)	48 (23)
Oct	381 (13)	491 (85)	499 (83)	597 (80)	495 (76)	495 (79)	492 (80)	30 (12)	34 (21)	35 (20)	35 (20)	43 (25)	35 (21)	33 (21)

continued

Table 9.—continued

	$[CO_2]$ (μL L^{-1})							$[O_3]$ (nL L^{-1})						
	Backgrnd[b]	Ring 1.2[c]	Ring 2.2	Ring 3.2	Ring 1.4	Ring 2.4	Ring 3.4	Backgrnd[b]	Ring 1.3[c]	Ring 2.3	Ring 3.3	Ring 1.4	Ring 2.4	Ring 3.4
2006														
May	382 (9)	497 (83)	499 (83)	501 (82)	493 (79)	499 (82)	495 (81)	40 (12)	50 (14)	49 (12)	50 (14)	51 (14)	49 (13)	49 (12)
June	377 (18)	532 (71)	530 (71)	532 (70)	526 (70)	534 (71)	531 (72)	41 (14)	45 (18)	45 (17)	46 (18)	45 (18)	45 (17)	45 (17)
July	383 (23)	532 (67)	534 (66)	533 (67)	531 (66)	534 (67)	534 (67)	37 (14)	43 (17)	41 (16)	44 (18)	44 (18)	42 (17)	43 (17)
Aug	401 (54)	535 (65)	535 (64)	535 (65)	538 (59)	536 (63)	534 (66)	32 (11)	37 (17)	37 (17)	38 (16)	38 (17)	37 (16)	38 (17)
Sep	384 (13)	521 (75)	521 (73)	525 (72)	522 (69)	520 (71)	524 (73)	28 (11)	32 (14)	29 (12)	31 (15)	32 (15)	32 (15)	31 (13)
Oct														
2007														
May	397 (13)	535 (70)	528 (64)	534 (66)	516 (68)	530 (63)	532 (62)	47 (12)	43 (13)	40 (13)	39 (13)	37 (13)	40 (13)	39 (13)
June	397 (26)	534 (71)	530 (64)	533 (66)	486 (83)	491 (83)	492 (87)	41 (15)	50 (19)	47 (20)	48 (21)	35 (11)	47 (20)	46 (20)
July	387 (30	505 (85)	529 (66)	532 (70)	449 (76)	450 (87)	449 (89)	30 (11)	43 (20)	44 (19)	44 (19)	42 (19)	44 (18)	43 (18)
Aug	383 (29)	513 (85)	530 (62)	535 (68)	517 (68)	536 (67)	545 (69)	32 (11)	40 (18)	39 (19)	40 (19)	40 (19)	39 (18)	39 (18)
Sep	362 (10)	503 (94)	497 (84)	499 (88)	496 (83)	500 (87)	503 (85)	32 (12)	46 (18)	45 (19)	46 (19)	45 (19)	46 (19)	46 (19)
Oct														
2008														
May	402 (12)	515 (74)	520 (70)	514 (73)	525 (67)	521 (70)	526 (71)	41 (7)	44 (14)	43 (15)	46 (14)	43 (16)	50 (13)	45 (15)
June	405 (20)	542 (61)	531 (63)	540 (59)	535 (65)	539 (61)	539 (63)	37 (10)	40 (17)	40 (17)	41 (18)	41 (18)	42 (17)	42 (19)
July	395 (27)	534 (72)	526 (68)	528 (69)	530 (68)	530 (68)	536 (67)	33 (11)	38 (17	36 (18)	38 (17)	39 (17)	41 (17)	39 (17)
Aug	382 (19)	531 (72)	527 (65)	532 (68)	532 (70)	527 (65)	541 (68)	32 (11)	41 (20)	39 (19)	41 (19)	41 (19)	41 (19)	41 (19)
Sep	387 (12)	534 (70)	526 (66)	533 (68)	388 (16)	531 (66)	531 (70)	30 (13)	35 (19)	32 (19)	36 (19)	34 (20)	35 (18)	35 (19)
Oct	393 (9)	517 (77)	506 (74)	526 (72)	519 (77)	512 (78)	525 (74)	28 (7)	26 (7)	26 (7)	30 (7)	27 (7)	28 (7)	28 (7)

[a] The hours of sunrise and sunset were based on daily calculations according to the U.S. Naval Observatory, Astronomical Applications Department. $[CO_2]$ and $[O_3]$ values reported here were calculated from the tables of hourly means posted on DOE's Carbon Dioxide Information and Analysis Center (CDIAC) Web site, to the nearest 1/2 hour within sunrise and sunset.

[b] Background refers to the current ambient $[CO_2]$.

[c] Treatment rings are designated as R.T, where R is the replicate and T is the treatment (2= elevated CO_2, 3= elevated O_3, 4= elevated $CO_2 + O_3$).

Table 10.—Percentile distributions of hourly [CO_2] and [O_3] at the Aspen FACE Experiment for the hours from sunrise to sunset[a] during the fumigation season (Table 2), from 1998 to 2006

Year		[CO_2] (µL L^{-1})							[O_3] (nL L^{-1})						
	Percentile	Backgrnd[b]	Ring 1.2[c]	Ring 2.2	Ring 3.2	Ring 1.4	Ring 2.4	Ring 3.4	Backgrnd[b]	Ring 1.3[c]	Ring 2.3	Ring 3.3	Ring 1.4	Ring 2.4	Ring 3.4
1998															
	100		651	697	651	618	792	713	89	119	133	119	153	138	107
	99		585	584	584	585	583	586	71	99	99	98	100	99	99
	95		574	569	570	570	568	570	59	88	89	88	89	88	88
	90		568	569	570	570	568	570	53	77	78	77	78	78	76
	75		562	563	563	563	562	564	43	64	65	64	64	65	63
	50		555	557	556	556	557	556	34	48	51	48	49	50	47
1999															
	100	598	673	679	748	747	768	738	77	160	101	181	116	124	292
	99	489	598	590	593	593	593	612	71	96	95	100	95	95	96
	95	426	580	578	578	577	578	581	62	89	89	90	89	89	89
	90	392	573	572	573	572	572	572	45	85	85	85	84	85	85
	75	363	565	564	565	564	565	563	46	74	75	72	74	75	75
	50	351	556	556	556	556	556	558	36	42	46	41	45	46	46
2000															
	100	479	675	684	695	659	669	681	79	89	93	94	140	87	92
	99	422	609	618	619	627	614	625	63	83	82	85	91	82	82
	95	379	586	589	588	591	585	589	55	80	80	80	81	80	80
	90	369	576	577	577	578	576	578	50	78	78	75	79	78	78
	75	356	563	562	563	561	562	559	42	64	64	61	67	64	64
	50	346	544	541	544	538	541	531	34	41	42	38	44	40	42
2001															
	100	547	709	663	668	693	628	645	75	149	91	141	137	274	95
	99	435	599	594	605	608	599	598	69	85	85	95	85	86	85
	95	398	579	579	585	584	582	580	58	81	81	84	81	81	81
	90	384	572	573	576	573	573	573	53	79	79	81	79	79	79
	75	368	562	561	563	560	561	561	45	69	69	74	70	69	70
	50	357	549	547	547	541	546	545	35	42	45	42	46	44	45

continued

Table 10.—continued

Year		$[CO_2]$ (µL L^{-1})							$[O_3]$ (nL L^{-1})						
	Percentile	Backgrnd[b]	Ring 1.2[c]	Ring 2.2	Ring 3.2	Ring 1.4	Ring 2.4	Ring 3.4	Backgrnd[b]	Ring 1.3[c]	Ring 2.3	Ring 3.3	Ring 1.4	Ring 2.4	Ring 3.4
2002															
	100	556	625	635	668	682	648	659	78	91	91	93	148	104	93
	99	464	595	596	609	614	609	606	65	85	85	85	86	85	85
	95	417	582	579	586	590	587	587	57	79	78	79	79	78	78
	90	396	574	573	578	580	578	578	52	75	75	75	75	75	75
	75	375	564	564	565	562	564	564	41	69	68	67	68	68	66
	50	363	553	553	551	545	547	550	30	42	41	41	43	41	38
2003															
	100	543	633	616	645	676	655	635	81	92	98	94	102	97	92
	99	473	598	593	601	613	606	601	67	85	87	85	86	86	84
	95	415	580	580	585	588	587	583	59	79	79	80	79	79	79
	90	392	573	574	577	578	576	575	54	73	74	74	74	74	73
	75	374	563	562	564	563	562	563	45	63	63	63	64	62	62
	50	364	551	551	550	547	544	549	36	46	43	43	46	44	44
2004															
	100	517	633	654	650	651	728	669	79	82	82	82	81	82	83
	99	457	596	596	604	605	603	602	70	78	78	79	74	79	78
	95	409	582	582	585	585	584	580	57	70	70	71	69	70	70
	90	389	574	574	576	576	575	571	49	68	68	69	64	68	67
	75	377	561	561	561	560	559	555	41	59	59	60	54	59	58
	50	369	546	546	544	544	543	533	33	43	41	41	36	40	40
2005															
	100	521	727	687	653	635	653	653	92	96	96	97	99	100	97
	99	453	604	599	600	606	608	599	75	93	92	93	94	93	93
	95	412	582	582	583	586	580	582	64	84	82	84	85	84	82
	90	394	574	575	575	578	570	572	58	78	77	79	80	79	77
	75	379	562	564	559	562	553	558	47	66	65	68	68	67	66
	50	369	548	552	543	547	536	541	37	50	79	51	53	52	49

continued

Table 10.—continued

Year	$[CO_2]$ (μL L^{-1})								$[O_3]$ (nL L^{-1})						
	Percentile	Backgrnd[b]	Ring 1.2[c]	Ring 2.2	Ring 3.2	Ring 1.4	Ring 2.4	Ring 3.4	Backgrnd[b]	Ring 1.3[c]	Ring 2.3	Ring 3.3	Ring 1.4	Ring 2.4	Ring 3.4
2006															
	100	667	708	699	663	651	677	661	72	96	83	93	96	86	93
	99	527	610	601	603	615	623	617	66	79	77	82	81	79	79
	95	429	590	584	588	593	599	591	58	70	69	72	72	69	71
	90	409	581	579	581	583	589	581	54	65	63	66	65	63	64
	75	389	570	659	570	569	574	570	44	53	51	54	53	52	52
	50	378	558	559	560	554	555	558	35	39	38	40	39	39	39
2007															
	100	553	674	636	658	661	669	680	90	108	102	102	103	104	128
	99	473	634	604	618	617	616	640	73	98	97	97	97	97	97
	95	442	602	582	596	589	591	600	57	75	75	75	76	75	74
	90	427	591	575	586	578	581	586	52	6	67	67	67	66	66
	75	398	575	565	571	561	567	567	43	49	49	49	49	49	49
	50	381	556	556	559	515	546	549	34	35	35	35	35	35	35
2008															
	100	591	780	630	730	451	662	780	64	101	94	97	100	97	201
	99	470	623	592	620	616	616	633	59	87	84	86	88	86	87
	95	433	598	579	598	591	594	603	52	72	70	72	73	72	73
	90	418	589	573	586	582	585	588	47	66	64	66	66	66	66
	75	402	576	565	572	569	570	573	41	50	49	50	50	51	50
	50	389	562	556	556	551	556	560	33	35	33	36	35	37	36

[a] The hours of sunrise and sunset were based on daily calculations according to the U.S. Naval Observatory, Astronomical Applications Department. $[CO_2]$ and $[O_3]$ values reported here were calculated from the tables of hourly means posted on DOE's Carbon Dioxide Information and Analysis Center (CDIAC) Web site, to the nearest 1/2 hour within sunrise and sunset.

[b] Background refers to the current ambient $[CO_2]$.

[c] Treatment rings are designated as R.T, where R is the replicate and T is the treatment (2 = elevated CO_2, 3 = elevated O_3, 4 = elevated $CO_2 + O_3$).

APPENDIX 3

Data and Other Materials from the Aspen FACE Experiment on File at the U.S. Forest Service Research Data Archive (http://www.fs.usda.gov/rds/archive/)

1. Hourly CO_2 and O_3 concentrations.
2. Hourly meteorological data from the treatment rings.
3. Hourly meteorological data from the weather monitoring tower.
4. Treatment ring maps showing grid coordinates of aspen clones and species, and tree ID numbers.
5. Annual height and diameter measurements of every tree.
6. Year 2000 partial harvest data: aboveground biomass by tissue type and annual height growth increment, leaf area by annual height growth increment, main stem length and diameter by annual height growth increment.
7. Year 2002 partial harvest data: aboveground biomass by tissue type and annual height growth increment, leaf area by annual height growth increment, main stem length and diameter by annual height growth increment.
8. Year 2007 partial harvest data: aboveground biomass by tissue type and annual height growth increment, leaf area by annual height growth increment, main stem length and diameter by annual height growth increment.
9. Year 2009 final harvest data: aboveground biomass by tissue type and annual height growth increment, leaf area by annual height growth increment, main stem length and diameter by annual height growth increment.

APPENDIX 4

Aspen FACE Experiment Bibliography Through 2014

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This publication is an additional source of metadata for data stored and publicly available in the U.S. Department of Agriculture, Forest Service Research Data Archive. Here, we document the development, design, management, and operation of the experiment. In 1998, a team of scientists from the U.S. Forest Service, Department of Energy (DOE), Michigan Technological University, and several other institutions initiated the Aspen Free Air CO_2 and Ozone Enrichment (Aspen FACE) Experiment. Using technology developed at DOE's Brookhaven National Laboratory (BNL), the experiment fumigated model aspen forest ecosystems with elevated concentrations of carbon dioxide (CO_2), or ozone, or both in a full factorial design with three replicates. The Aspen FACE Experiment was one of several free-air CO_2 enrichment experiments at the time, but was the only one that incorporated ozone treatment into the BNL design.

The experiment operated for 13 years, involved more than 70 researchers from 9 countries, has produced 126 scientific publications to date, held numerous tours and scientific conferences, and was the subject of many reports in the public news media. Findings from the experiment contributed to the supplement to the U.S. President's 2002 budget, *Our Changing Planet*; and to the 2006 rewriting of the U.S. Environmental Protection Agency's ozone pollution criteria document. Data and archived plant samples from the experiment continue to be used in many ways, including meta analyses, global change modeling, and studies examining tree characteristics affected by the treatment gases.

KEY WORDS: climate change, carbon dioxide, ozone, trembling aspen, paper birch, sugar maple, tropospheric ozone, carbon sequestration, carbon-nitrogen cycles, biogeochemical cycles, insect-disease interactions, northern hardwood ecosystems

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