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SUONENJOEN TUTKIMUSASEMA
SUONENJOKI RESEARCH STATION

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Open-top Chamber Fumigation of Cloned Silver Birch (*Betula pendula* Roth) Trees to Elevated CO_2 and Ozone: Description of the Fumigation System and the Experimental Site

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Cover photo: Overview from the OTC experiment at
Suonenjoki Research Station in July 2001
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An open-top chamber (OTC) system was designed and constructed at Suonenjoki Research Station, Finland (62°05'N, 27°00'E, 130 m asl) in order to study the responses of silver birch (*Betula pendula* Roth) trees to elevated CO_2 and O_3 alone and in combination. The aim was to provide a facility to study long-term responses of the trees in projected future climate. The facility enabled us to assess the responses on following parameters: growth, allocation, crown and leaf structure, gas exchange, biochemical and chemical properties of the leaves, herbivores and palatability of the leaves, leaf pathogens, and soil systems including root and mycorrhizal growth, soil respiration and litter decomposition. The treatments from 1999 to 2001 were: 1) outside control; 2) chamber control; 3) 2 * background CO_2; 4) 2 * background O_3; and 5) 2 * background ppm CO_2 + 2 * background O_3. Two birch clones with different ozone-sensitivity were included in the study and both clones were represented as four replicates in each treatment. Thus, the experiment included altogether 40 trees, 32 being enclosed in cylindrical open top chambers and 8 serving as outside controls. The experiment was organised as a randomised incomplete blocks -design to eliminate the site differences between the blocks. The mixture of fumigation gases and air was blown at the speed of 0.1–0.6 $m^3 s^{-1}$ at the bottom of the chamber using a computer-controlled blower. The CO_2 and O_3 dispensing and monitoring system was computer-controlled and compared the measured gas concentration to the chosen target concentration and adjusted the gas flow into the chambers. In the present paper we describe the experimental design, site conditions, the OTC system and principles of the measuring/regulation system as well as the measured values for air temperature, light and gas composition in the chambers. The daily mean temperature in the chambers was on an average 1.7, 2.3 and 2.4 °C higher than the daily mean temperature of the ambient air in 1999, 2000 and 2001, respectively. Temperature sums (above +5 °C) were 189 dd (in 1999) to 400 dd (2001) higher in chambers than outside. In 2000 and 2001 the doubled target concentrations of O_3 and CO_2 were reached during nearly most of the fumigation period, while in 1999 the concentrations were slightly lower than the targets. Ozone exposure during the growing seasons 1999, 2000 and 2001, calculated as AOT40-values (accumulated over a threshold of 40 ppb), was 18, 22 and 29 ppm.h greater in the elevated ozone treatments than in ambient air.

Introduction

Atmospheric carbon dioxide (CO_2) and ozone (O_3) are greenhouse gases in the lower atmosphere that contribute to the warming of climate and the concentrations of both gases have increased during the last decades. The concentrations of CO_2 and O_3 in the atmosphere are increasing by 1–2% per year and are expected to double by the year 2100 compared to the end of the last millennium (Runeckles and Krupa 1994, Fowler et al. 1998, IPCC 2001). Almost 30% of the global forests are currently exposed to damaging tropospheric ozone concentrations, and this is predicted to expand to 49% by year 2100 (Broadmeadow 1998, Fowler et al. 1999). The recent agreement in Marrakech, November 2001, to ratify Kyoto-Bonn protocol mean the global cut of emissions by 1.5% of the non-cut alternative (Norhaus 2001). According to various IPCC models (Houghton et al. 2001) large variation and uncertainties exist in the estimation of carbon sinks and therefore the outcome of Kyoto-Bonn protocol to CO_2 levels in 2100 is difficult to predict.

Plants and soils of terrestrial ecosystems are major global carbon pools that can be affected by the increasing CO_2. Carbon dioxide at twice the current atmospheric concentrations has the potential to increase productivity of many agricultural crops and forest trees (Ceulemans & Mousseau 1994, Saxe et al. 1998). In short-term studies a rise in CO_2 concentration has increased photosynthesis and growth while under long-term exposures photosynthesis has either increased or decreased with time (Saxe *et al.* 1998). Increased starch and secondary compound accumulation, and increased root/shoot ratio have been observed in trees grown in elevated CO_2 (Ceulemans & Mousseau 1994). In addition, a reduction in tissue nitrogen/carbon ratio has been often described under elevated CO_2 (Cotrufo et al. 1998).

The plant responses to elevated CO_2 may be influenced by many other environmental factors such as nutrient and water supply, temperature and light. In longer exposures plants may acclimate (decrease photosynthetic rates) in response to elevated CO_2 and this is often associated with accumulation of carbohydrates and decreased N concentration (Stitt & Krapp 1999, Ward & Strain 1999). Decreases in photosynthetic capacity are generally connected to low nutrient supply (Kohen & Mousseau 1994, Laitinen et al. 2000) and thus acclimation has been explained by increased demand for nitrogen under elevated CO_2 (Paul & Driscoll 1997). Photosynthetic acclimation may be related to restricted root growth since no acclimation was found in field growing trees exposed in open-top chambers (Arp 1991, Norby et al. 1996). Recent studies have also shown that low soil fertility clearly limits below ground carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere (Oren et al. 2001).

In Europe, O_3 is estimated to cause *ca.* 10% forest yield losses as a whole (Skärby *et al.* 1998). It has been shown by many authors that birch is sensitive to ozone (Mortensen & Skre 1990, Matyssek et al., 1992, Pääkkönen et al. 1995) but there are clonal variations in the sensitivity (Pääkkönen et al. 1997). Decreased chlorophyll contents and photosynthetic rates, changes in carbon allocation and increased antioxidant activity have often been recorded in ozone exposed trees (Skärby *et al.* 1998). In Europe the current critical levels of ozone are expressed as

AOT40 index (= sum of hourly ozone concentrations above 40 ppb during daytime hours). Significant reductions in growth have been reported for the selected model tree beech at AOT40 of 10 ppm.h (Braun & Flückiger 1995) and at even lower index values of 5–7 ppm.h in European silver birch (*Betula pendula*) and Mountain birch (*Betula pubescens*) (Pääkkönen et al. 1998a, b, Manninen et al. 1999). Moreover, recent data show that the profile of ozone exposure, diurnal fluctuations in stomatal conductance and ozone concentrations, and species/genotype specific recovery time for defence and growth compensations need to be considered in evaluating plant responses to ozone (Oksanen & Holopainen 2001).

The information available on the interactive effects of elevated CO_2 and O_3 on trees is still limited and the recent results have been somewhat contradictory (Polle & Pell 1999, Karnosky et al. 1999, Karnosky et al. 2001). Studies with some species show that exposure to elevated CO_2 may counteract decreases in photosynthesis and growth caused by O_3 (Mortensen 1995, Volin & Reich 1996), while other studies show that elevated CO_2 did not protect the plants against O_3 (Barnes et al. 1995, Kull et al. 1996). Even in cases where added CO_2 partly counteracted the negative impact of O_3, the added O_3 negated increased growth caused by CO_2 (Dickson et al. 1998). Since concentrations of CO_2 and O_3 are predicted to increase simultaneously, it is important to understand their interactive effects on northern forest species. Present growth models predict changes in the proportions of tree species in future climate and propose increasing deciduous forest area by silver birch in southern Finland (Kellomäki et al. 1996). However, only few data exist of silver birch responses to climate change from field experiments (Rey & Jarvis 1998) to support the model predictions. Therefore more data are needed to be able to realistically predict the future production of these forests.

This report describes an open-top chamber (OTC) fumigation system that was constructed in Suonenjoki, Finland to expose young silver birch trees to elevated CO_2 and O_3 and their combination. The 32 OTC and 8 outside control trees of silver birch, that were selected for the experiment, represented two clones from a stand that was planted six years earlier in the research site (Mutikainen et al. 2000). Site characteristics and environmental conditions in the chambers and outside air are reported for the 3-year exposure period. Our aims in the OTC study were to simulate the predicted CO_2 conditions in 2100 (= twice the ambient concentration). Treatments with elevated ozone concentration, alone and in combination with elevated CO_2 were included in the study to provide information of responses of silver birch to multiple environmental stresses.

Materials and methods

Study area, soil characteristics and experimental trees

The study area is located in the experimental field at Suonenjoki Research Station of the Finnish Forest Research Institute (62°05'N, 27°00'E, 130 m asl). The soil, originally composed of sandy clay, was prepared in 1979 for the purpose of bare-root tree seedling production by mixing a 10-cm layer of horticultural peat in the upper layer of the soil. In spring 1993 the field was ploughed and harrowed and the silver birch field experiment was established to the site using 1-year old clonal saplings from 15 different origins in southern and central Finland (Mutikainen et al. 2000). The experiment was divided into ten blocks (Fig. 1) that each contained all the clones in random assignment (for details see Mutikainen et al. 2000).

Soil samples were collected from the middle of each block upto the depth of 25 cm after discarding 2–3 cm of the top soil to characterize the soil properties of the site. Soil particle-size distribution was measured using mechanical dry sieving. Organic matter content was estimated as loss in mass on ignition at 550 °C. Water-retention characteristics were measured at desorption using a pressure-plate apparatus (Soilmoisture Corp., USA) and the same undisturbed cylinder samples at successive pressures (matric potentials –0.3, –1, –5, –10 and –100 kPa). Measurement at –1500 kPa was done from disturbed samples and converted into volumetric values using bulk density (BD) (Heiskanen 1993). BD was measured from the cylinder samples as the ratio of dry mass (dried at 105 °C) to volume at –0.3 kPa. Particle density (PD) was measured from disturbed samples using 50 ml water pycnometers with a water bath. Calculated total porosity was estimated as [(PD–BD) / PD].

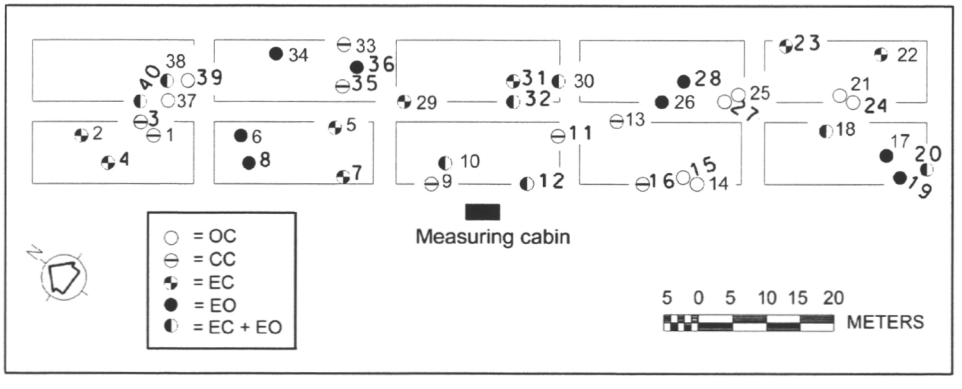

Figure 1. Map of the experimental field showing the positions of the 40 experimental silver birch (*Betula pendula* Roth) trees at the site, numbered 1–40. The trees with normal font denote clone 4 and with bold font denote clone 80. OC = outside control, CC = chamber control, EC = elevated CO_2, EO = elevated O_3 and EC+EO = elevated CO_2 + elevated O_3.

From amongst the 15 clones growing at the site two fast-growing clones, clones 80 and 4 (clone 4= V5952, clone 80= K1659 in the Finnish forest genetic register), were selected for the present experiment (Fig. 1) according to their differences in ozone sensitivity. In a previous survey clone 4 has been classified as an ozone-tolerant genotype while clone 80 represents a more sensitive genotype (Pääkkönen et al. 1997). According to seven years of growth clone 80 was superior over clone 4 in regard to growth rate, gas exchange, nutrient uptake and various biochemical properties of photosynthesis (Riikonen et al., manuscript). The trees, that were selected for the present experiment, were fertilised annually and had received additional fertilisation of 12, 12, 17, 17, 22 and 22 kg N/ha (TyppirikasY-lannos, Kemira Ltd., N-P-K 18-5-10) in 1993–1998, respectively. The fertilisation was given in three to four doses of equal amount during May–July in each year.

In the beginning of the fumigation experiment 40 trees representing clones 4 and 80 (20 trees for each) were assigned to the five different treatments: outside control (OC), chamber control in ambient air (CC), elevated CO_2 (2 * ambient, EC), elevated O_3 (2 * ambient, EO), and elevated CO_2 and elevated O_3 together (both 2 * ambient, EC+EO). The experimental design was randomized incomplete blocks design where two of the above treatments for both clones appeared in each block, and within the whole experiment each treatment appeared once with each other. In the experiment each treatment had four independent replicates for both clones (Fig. 1).

Maintenance of the experimental trees in 1999–2001

To characterise the availability of nutrients by the start of the experiment, three cylindrical soil samples (5.3 cm in diameter, 15–20 cm in length) were collected below each experimental tree in the beginning of June 1999. Each soil cylinder was individually dried at room temperature, then sieved (two sieves on top of each other with grid size of 710 and 425 mm) to remove roots, rocks and debris. Nitrogen and carbon concentrations were determined with a LECO CHN-600 Analyzer (Leco Co., USA). The concentrations of exchangeable mineral elements (P, K, Ca, Mg) were determined from an acidic solution of ammonium acetate (pH 4.65) using plasma emission spectrophotometry (ICP, ARL 3800). In analysing the data, first the mean value of the three replicate samples from each chamber/outside tree were calculated and these mean values were then used in calculating differences between treatments (n=8) and clones (n=20) with ANOVA.

During the three years of fumigation the trees were fertilised yearly and fertilisation was applied in four to six equal doses during May–July at 2–3 week intervals. In 1999 the trees received 22 kg N ha^{-1} (TyppirikasY-lannos, Kemira Ltd., N-P-K 18-5-10), in 2000 33 kg N ha^{-1} (two times with Pellon Y-lannos, Kemira Ltd., N-P-K 20-3-9 and four times with Puutarhan Y-lannos 1 10-7-14, Kemira) and in 2001 41 kg N ha^{-1} (Puutarhan Y-lannos, Kemira Ltd., 1 10-7-14).

The soil water content was measured with Time Domain Reflectometry (TDR, TRIME-FM, IMKO Micromodultechnik GMBH, Germany) twice a week during the three growing seasons and the trees were watered if the soil water content was <10%. In the chambers watering was done using sprinklers that were mounted in the middle of top crown, and in the outside trees the sprinklers were fixed in the ground.

The monitoring of insect herbivores on the experimental trees was done weekly to ensure that the open-top chamber construction would not allow insect infestations that could jeopardise the experiment. E.g. during the first fumigation year the populations of the birch aphid *Euceraphis betulae* (Koch) (Homoptera: Drepanosiphidae) had a very strong population growth. In the sample of 100 buds from ten twigs over 600 over-wintering eggs of *E. betulae* were found in the autumn of 1999. To avoid aphid outbreaks in the chambers during growing season of 2000, trees were sprayed with pirimicarb (0.03% solution of Pirimor) on May 16, 2000 just before the aphid fundatrixes hatched from eggs and started to produce nymphs. Similarly, aphids were sprayed on May 3, 2001. During spraying soil below the trees was covered to avoid penetration of the insecticide into the soil system. Some extra mechanical aphid control was needed during the growing seasons 2000 and 2001. Pirimicarb was selected, because it causes no visual damage to silver birch leaves and is a very specific aphidicide that does not harm other arthropods. This was obvious also in the OTC's, since cicadas living on the same extending birch leaves as aphids, were not reduced after the treatment.

In addition to aphids trees were to some extent damaged by the birch cicada *Oncopsis flavicollis* (L.) (Homoptera: Cicadellidae) which was controlled mechanically crushing and brushing. Occasional small outbreaks of moth larvae were observed. Larvae of *Biston betularius* (L.) (Lepidoptera: Geometridae), *Ennomos autumnarius* (Werneburg) (Lepidoptera: Geometridae), *Lycia hirtaria* (Clerck) (Lepidoptera: Geometridae), *Nymphalis antiopa* L. (Lepidoptera: Nymphalidae), *Rheumaptera hastata* (l.) (Lepidoptera: Geometridae) and *Orgyia antiqua* L. (Lepidoptera Lymantriidae) and some other unidentified geometrid larvae were removed from the trees by hand picking.

OTCs, and the CO_2 and O_3 fumigation system

Chamber construction: The open-top chambers were of cylindrical shape and were composed of two 6-m high metallic poles, and three metallic hoops (2.5 m in diameter) that were positioned one at the ground level, one at the height of three and one at six meters (Fig. 2). After the first year of fumigation (1999) the chambers were extended in the spring 2000 by 1.8 m because of tree growth. The extension had a cut conical shape with the upper diameter of the open top of 1.0 m (Fig. 2). The chambers were covered by polyethene film (Hytilux 4, 0.18 mm in thickness, Hyplast Ltd., Hogstraten, Belgium). Transmittance (Fig. 3) of light by the polyethene film was measured by spectroradiometer (SR9910-PC, Macam Photometrics Ltd., Livingston, Scotland, UK) and by the quantum sensor (Li-Cor Inc., Lincoln, NE, USA). Polyethene film was attached on the surface of the hoops by spanning it with plastic tubing (30 mm in diameter) that was cut longitudinally in one side of the tubing. The door of the chamber (1.8 m * 0.7 m), made of metal frame, was covered in a similar way with the greenhouse plastic. The chambers were anchored in their position in the ground by plastic ropes (6 mm in diameter) in four positions. The ventilation and temperature control was performed by blowing air into the chambers at the flow rate of 0.1–0.6 $m^3\ s^{-1}$ using computer-controlled blowers that were installed in the chamber walls 80 cm above ground (Figs. 2 and 4). The fumigation

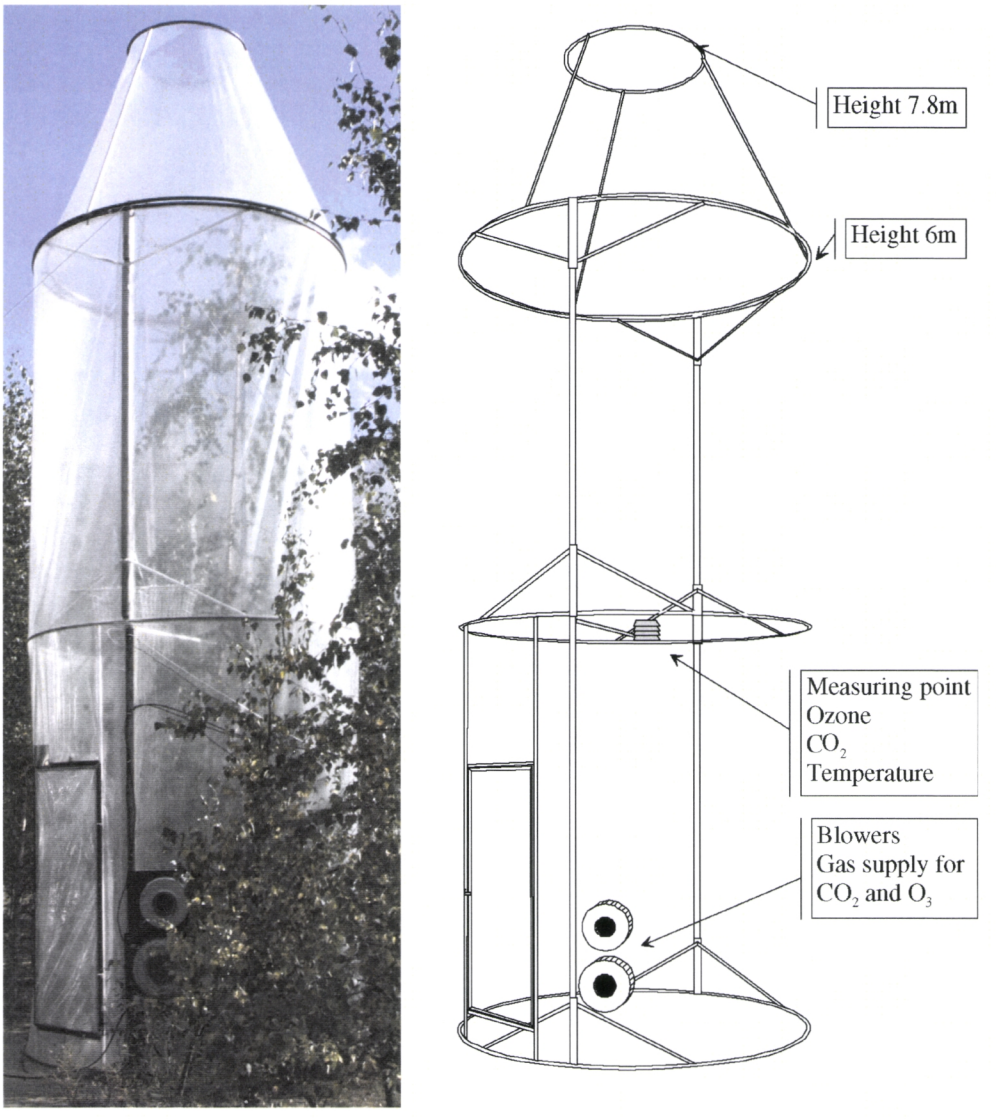

Figure 2. Photograph and construction drawing of an open-top chamber showing the dimensions, blowers and gas supply point, and the measuring point to measure temperature and the concentrations of O_3 and CO_2.

gases were injected into the chambers through the blowers that thus provided proper mixing of the gases into the incoming air.

Fumigation system: The CO_2 and O_3 dispensing and monitoring system was computer -controlled and compared the measured gas concentration to the chosen target concentration and adjusted the gas flow into the chambers (Fig. 4). For fumigations, pure liquid CO_2 was supplied from two 5 m^3 tanks to vaporizers and further through the CO_2 dispensing system to the chambers. The concentrations of CO_2 and H_2O

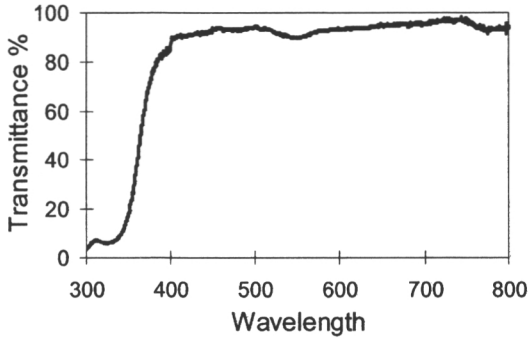

Figure 3. Transmittance spectrum of the polyethene film (Hytilux 4, 0.18 mm in thickness, Hyplast Ltd., Hogstraten, Belgium), measured by a Macam 9910 spectroradiometer.

in the chambers were measured by CO_2–H_2O analyzer (Li-Cor 6262, Li-Cor Inc., Lincoln, NE). Data from the CO_2–H_2O analyzer was transmitted to the computer via RS232 port. Ozone was produced from pure oxygen with an ozone generator (Fisher OG5, VTU Umwelt- und Verfahrenstechnik GmbH, D-53359 Rheinbach, Germany) and was monitored with an ozone analyzer (Thermo Environmental 49C, Thermo Environmental Instruments Inc., Franklin, MA, USA). Data from the ozone analyzer was transmitted in analog mode to the computer where it was transformed to digital mode (A/D transformer). The OTC-program computed the data and accordingly, the controlling cards (144-Bit Paraller Digital I/O Board ACL-7122 and Transistor Board) regulated every seven seconds the proportional opening time of the magnetic valves (ASCO SCG225B5 V/T) in injecting CO_2 and O_3 to the injection tubes. In the fumigation CO_2 was injected at a pressure of 2.5 bar and O_3 gas at a pressure of 0.4 bar.

The OTC -program was using the programming environment for developing data acquisition applications in ANSI C (LabWindows/CVI of National Instrument). The operating system was Windows NT 4.0. The multi-function data acquisition card (ACL-8112) was used to measure analog signals, controlling the thermocouple amplifiers, multiplexer and counting the wind speed sensor. Digital I/O Board (ACL-7122) was used to control all valves, rotation rate of blowers and on/off connection of devises.

All the magnetic valves were of the same type (ASCO SCG225B), but the orifices for measuring valves were 3.2 mm in diameter and 1.2 mm for supply valves. The material of the gasket in ozone gas valves was teflon. The ozone gas valves were equipped with a critical hole to achieve more accurate flow control. The sample gases were pumped by vacuum pumps (Thomas 107 CD 18, Thomas Compressors & Vacuum Pumps, Sheboygan, WI, USA) to the analyzers through the magnetic valves and T-connections. Different pumps were used for O_3 and CO_2, as well as for next-coming fresh gas sample. The diameter of tubes was 6/4 mm. The material of ozone-exposed tubes and connectors was teflon, otherwise polyethene.

The sampling location of air for the measurements of CO_2 and O_3 was in the center of the open-top chambers at the height of 3 m from the ground (Fig. 2). Air sample was continuously withdrawn via 6/4 mm teflon tubing, and the samples from each of the 32 chambers and ambient air were measured by the analysis system for 25 s. With this protocol each chamber was measured every 7.5 min enabling new adjustments to the fumigation system.

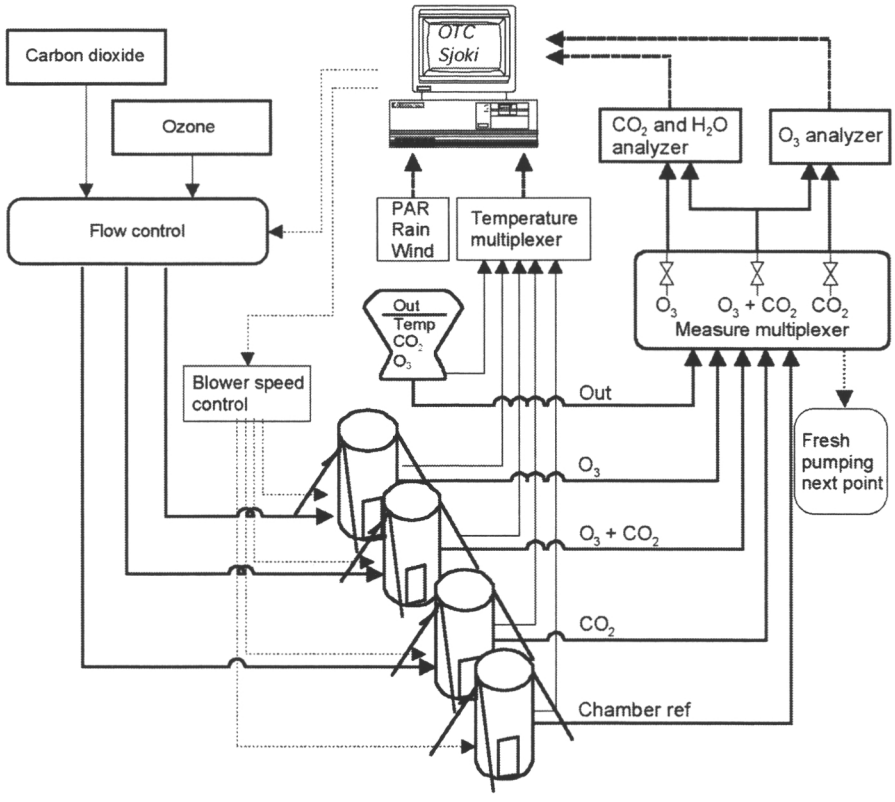

Figure 4. Schematic presentation of the open-top chamber facility in Suonenjoki site. The scheme indicates in the form of a flow chart how the fumigation in the five different treatments was controlled and measured, and also indicates the environmental parameters automatically and continuously measured in the site.

The same computer system monitored also temperature (measured by copper-constantan thermocouples, T-type) and quantum flux density (Li-190SA, LI-COR, inc.) in the chambers and outside air as well as wind speed (Fuess 92B, R. Fuess, Berlin, Germany) and rain (Rain detector, DRD11A, Vaisala, Finland) in outside air. The sensors were connected to the Data Acquisition Card ACL-8112 and the thermocouples were first connected to the Programmable Pre Amplifier & Multiplexer Board ACLD-889.

Duration of the fumigation: In 1999 the fumigation was started on May 25 and was stopped on October 4. In 2000 and 2001 the fumigations were started on May 4 and May 2 and were stopped on September 29 and September 27, respectively. The fumigation with CO_2 operated continuously 24 hours day^{-1}. In 1999 and 2000 the exposure to elevated O_3 was 12 h day^{-1} (between 0800 and 2000) and in 2001 14 h day^{-1} (between 0800 and 2200) whenever the set threshold value of 10 ppb was reached. On rainy days the system also automatically stopped ozone fumigation when the rain detector voltage was <2.5 V.

Results

Site characteristics

The soil was composed mainly of particles 0.06–0.2 mm in size being thus medium fine sandy soil (Table 1). The mean bulk density of the soil was 1.49 g/cm^3 and the mean organic matter content 2.40% (Table 1). There was some variation between the ten blocks, blocks 3, 4 and 8 having somewhat higher organic matter content (on average 3.79%) than the rest of the blocks (1.80%). Samples from the different blocks had similar water-retention characteristics with maximum water retention of 43.6 vol.% at –0.01 kPa, 11.8 vol.% at –10 kPa (field capacity) and 2.7 vol.% at –1500 kPa (wilting point) (Fig. 5).

Table 1. Bulk density, organic matter content and particle size distribution in the soil of the experimental field. BD = bulk density, LOI = organic matter estimated by loss on ignition at 550 °C. Particle fractions were determined by mechanical dry sieving. N=10.

	Mean	SD
BD, g cm^{-3}	1.49	0.05
LOI, mass%	2.40	1.06
>2 mm, mass%	1.1	0.6
2–0.6 mm, mass%	14.9	7.4
0.6–0.2 mm, mass%	57.8	11.9
0.2–0.06 mm, mass%	22.2	9.1
<0.06 mm, mass%	4.1	3.2

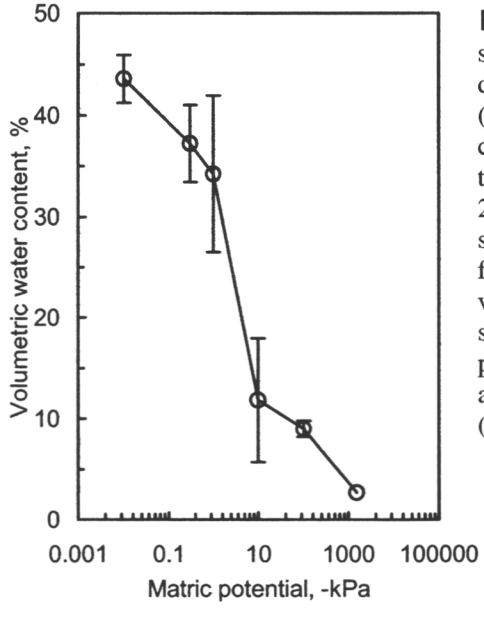

Figure 5. Water-retention characteristics of the soil in the experimental field measured at desorption using a pressure-plate apparatus (Soilmoisture Corp., USA). Undisturbed cylinder samples of soil were collected from the middle of each block upto the depth of 25 cm after discarding 2–3 cm of the top soil. Measurement at –1500 kPa was done from disturbed samples and converted into volumetric values using bulk density and the same measurements were done at successive pressures (matric potentials –0.3, –1, –5, –10 and –100 kPa). Data are the means of 10 (±SD).

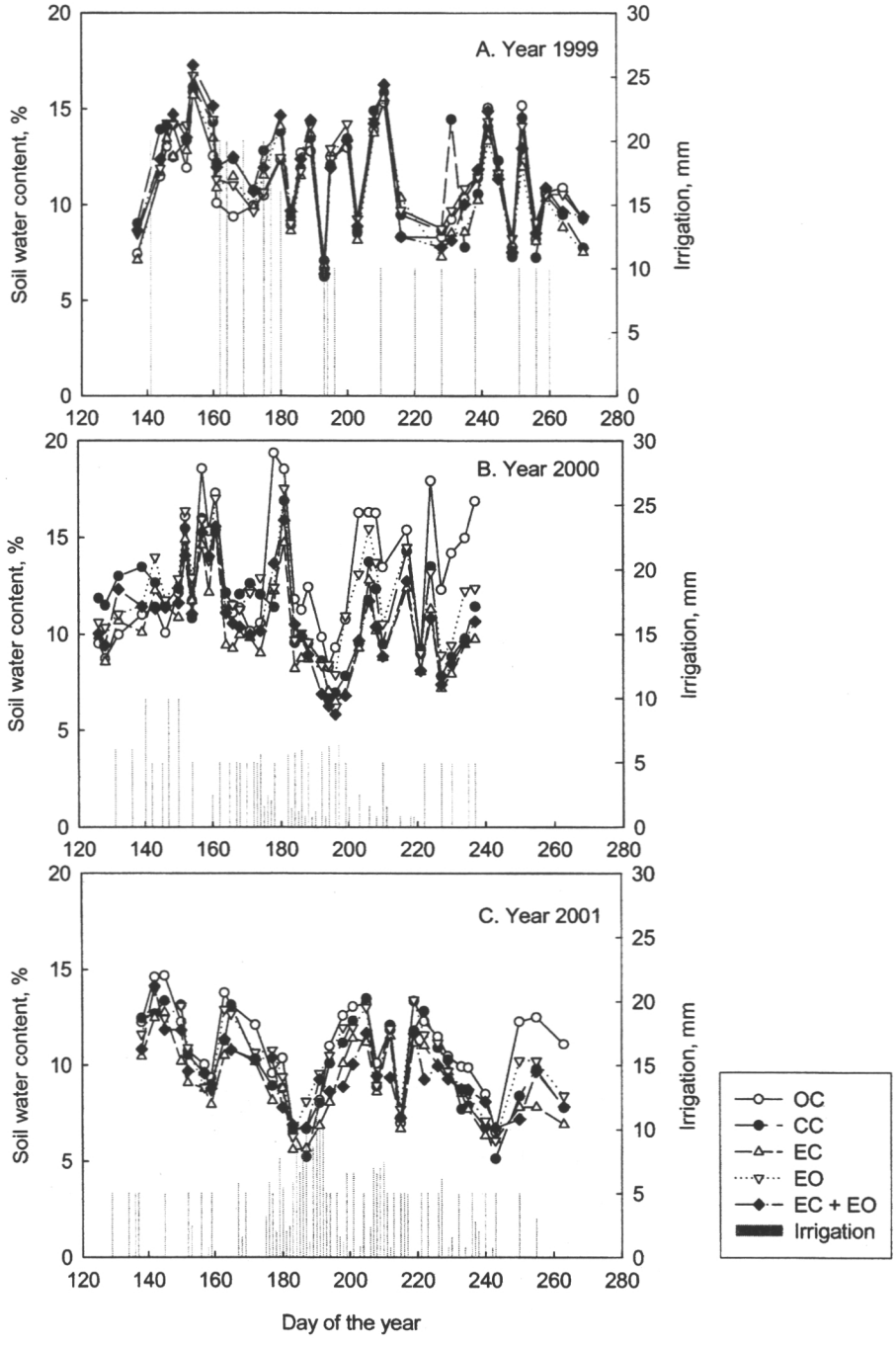

Figure 6. Results of the soil water content measurements and irrigation from the chamber trees and outside controls during the growing seasons 1999 (A), 2000 (B) and 2001 (C). TDR measurements were done twice a week and the results are the means of 32 and 8 replicates from the chambers and outside controls, respectively. OC = outside control, CC = chamber control, EC = 2 * ambient CO_2, EO = 2 * ambient O_3, EC + EO = 2 * ambient CO_2 + 2* ambient O_3.

Table 2. Main nutrient concentrations and C:N-ratio of soil core samples (mean ± SD) in the beginning of June, 1999, by the start of the experiment. Nutrient concentrations are expressed as mg/kg dw of soil and soil C:N-ratio as C%:N%. OC=outside control, CC=chamber control, EC = 2 * ambient CO_2, EO = 2 * ambient O_3, EC+EO = 2 * ambient CO_2 + 2 * ambient O_3. Data are the means ± SD, n=8 for treatment and n=20 for each clone.

	N	P	Ca	K*	Mg	C:N
Treatment						
OC	350± 54	26.8± 8.8	92.0±56.6	20.1±3.7	15.4±11.2	19.8±3.2
CC	500±160	48.6±21.9	107.5±68.6	21.8±3.0	20.4±13.0	21.3±3.6
EC	400±131	44.9±11.9	88.1±56.8	19.8±4.0	14.7±12.2	21.8±3.0
EO	400±177	36.3±18.5	93.1±77.8	25.8±9.8	14.4±11.0	20.9±4.0
EC+EO	525±183	43.9±18.2	120.7±59.8	24.0±5.1	20.4±12.2	21.9±3.8
Clone						
Clone 4	430±168	40.9±17.5	88.6±52.1	22.4±6.9	14.6± 9.8	21.1±4.0
Clone 80	440±147	39.3±18.0	111.9±70.4	22.2±4.8	19.5±13.1	21.2±2.9

* K concentrations tested with Kruskall-Wallis when n = 8.

For measurements of soil nutrient concentrations samples were collected below each experimental tree in early June 1999 before the first fertilisation. Therefore nitrogen concentration was low and varied between 325–575 mg kg^{-1} dw of soil. The concentrations of other major nutrients were similar to the ones previously reported for nursery and forest soils (Luoranen 2000). There were no differences in soil fertility conditions between treatments and clones in any of the examined mineral elements (Table 2). Our aim was to avoid drought in the site and therefore soil moisture content in the chambers and in soils below outside control trees was measured two times per week during the three growing seasons. In 1999 the cylindrical chamber construction allowed rain to enter the chambers and over the 4-month exposure the trees received 239 mm of rain. In addition, the trees received a total of 236 mm as irrigation water. In summers 2000 and 2001 only part of the rain (344 mm and 339 mm between May–September in 2000 and 2001, respectively) could enter due to the cut conical extension to the chambers, and the trees received 209 mm and 323 mm of irrigation water during these years, respectively. Over the 3-year study period the soil water content varied between 5.5–19.3% (Fig. 6) when soil water content was measured before watering. The trees were watered after the measurements whenever soil water content was <10% (Fig. 6).

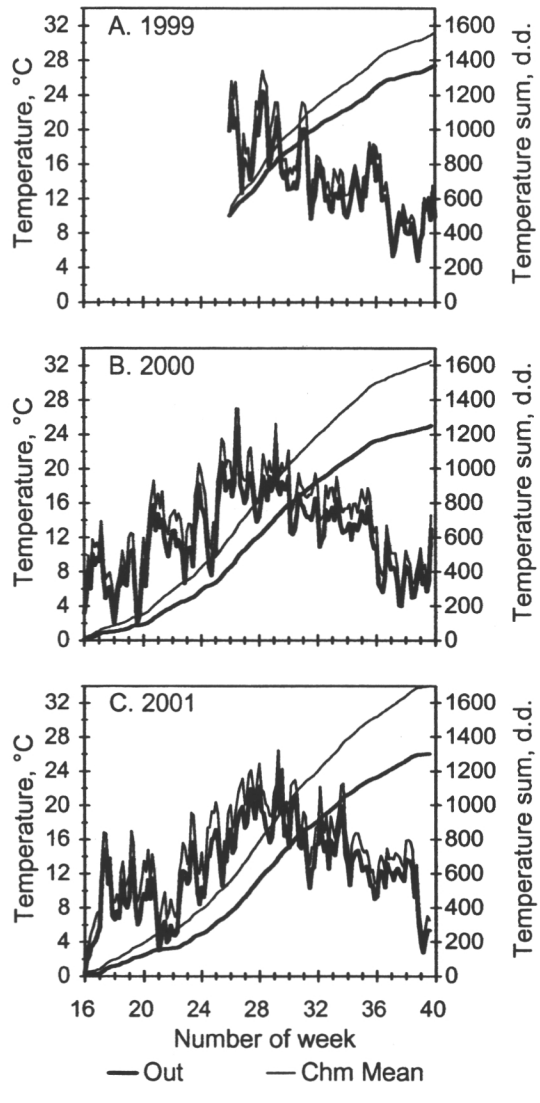

Figure 7. Daily mean temperature and temperature sum (over the threshold of 5 °C) in the chambers (normal line) and ambient air (thick line) in 1999–2001. Results are the means of all chambers (n=32) and ambient air measured in one location within the canopy.

Modification of the growth environment by OTCs

Light

The polyethene film had a high transmittance of light (91%) at wavelengths of 400–800 nm PAR but at wavelengths <400 nm the transmittance decreased sharply, and at 300 nm only 4.3% of light was transmitted through the film (Fig. 3). Due to self-shading of the trees light within the chambers was reduced from the top to the bottom. The light intensity varied between chambers due to differences in size of the trees and their location in the experimental field (data not shown).

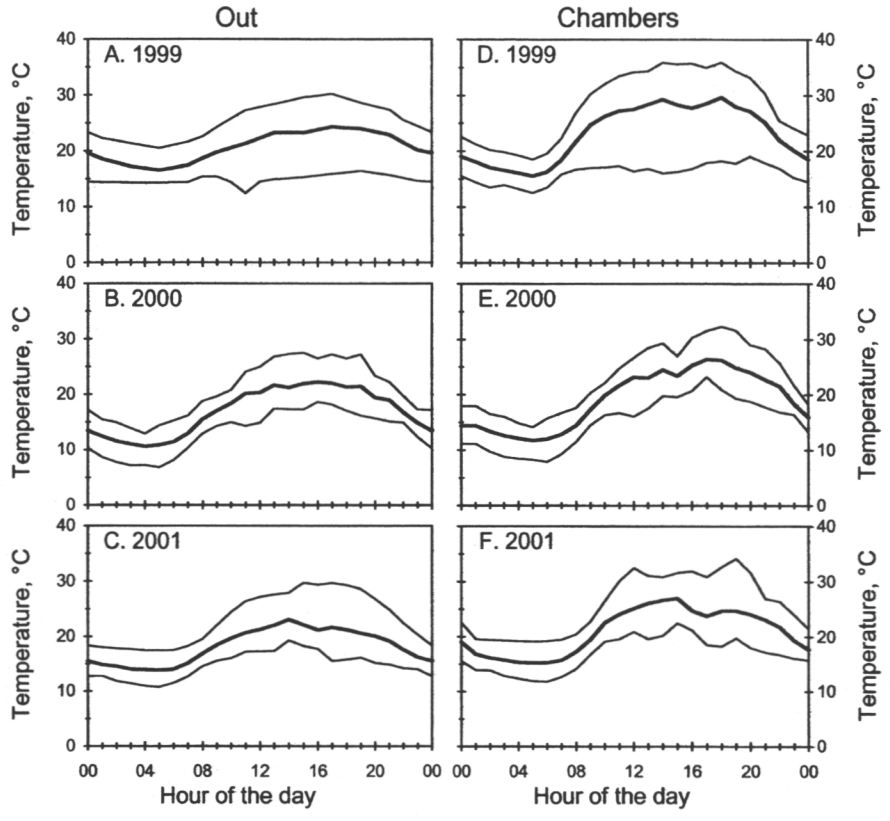

Figure 8. Minimum, maximum and mean temperature in ambient air (A, B, C) and in the chambers (D, E, F) during the week 28 in 1999, 2000 and 2001, respectively. Data show the hourly mean values for all the chambers (n=32). Temperature in ambient air was measured in one location within the canopy.

Temperature and air humidity in the chambers in 1999–2001

Temperature in the chambers and outside air was continuously measured at 7.5 min intervals. From these measurements the hourly mean values were calculated, and this data were used in calculating the daily minimum, maximum and mean temperatures. The daily mean temperature in the chambers was on an average 1.7, 2.3 and 2.4 °C higher than the daily mean temperature of the ambient air during the growing seasons 1999, 2000 and 2001, respectively (Fig. 7). As a consequence of the temperature difference the cumulative temperature sum was considerably higher in OTCs: in 2000 and 2001, when the temperatures in the OTCs were measured throughout the whole growing season, the cumulative temperature sum was 380–400 d.d. higher in the chambers by the end of September when the seasonal fumigation was finished (Fig. 7A–C). The daily maximum air temperatures, examined in detail for a one week period in July (week 28), was 5.6, 5.8 and 5.6 °C higher in the OTCs in 1999, 2000 and 2001, respectively, when compared to the ambient air (Fig. 8A–F). During this period the daily mean temperatures were on

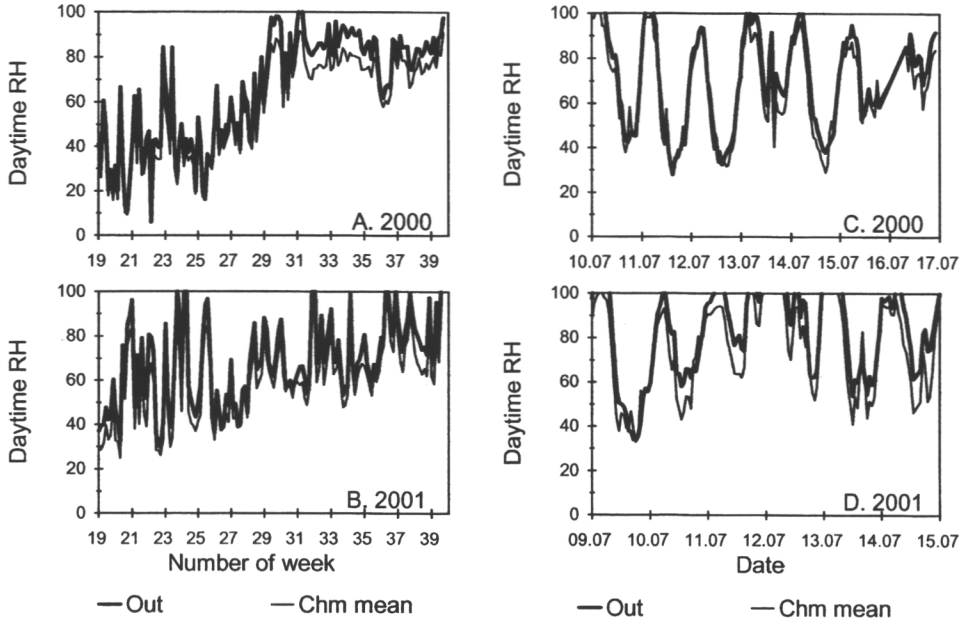

Figure 9. Mean values of relative humidity for the daytime hours (0800–2000 h) in the chambers and outside air in the growing seasons 2000 (A) and 2001 (B), and hourly mean values of relative humidity during week 28 in 2000 (C) and in 2001 (D).

an average 2.5, 2.3 and 2.7 °C higher in OTCs in 1999, 2000 and 2001, respectively, while no differences were found in the daily minimum temperatures between OTCs and ambient air (Fig. 8A–F).

The trends in relative humidity (RH) during daytime hours (0800–2000 h) in the ambient air and chambers followed closely each other (Fig. 9A–B). Relative humidity was constantly higher in the ambient air than in the chambers in both years with maximum RH difference of about 10–15% . This difference was due to higher temperature in the chambers than in outside air. Daily RH values, examined in detail for week 27 in 2000 and 2001 (Fig. 9C–D), varied from low midday values of 35% to 100% at night and on rainy days. The difference in RH between the ambient air and the chambers was similar as was observed in daytime mean RH values.

Control of CO_2 and O_3 concentrations in the chambers

In the present study our aim was to double the concentration of CO_2 in the EC treatments for 24 hours/day, and to double the O_3 concentration in the EO treatments for 12 h/day between 0800–2000 h in 1999 and 2000, and for 14 h/day between 0800–2200 h in 2001. During daytime the target value for the CO_2 concentration was 720 ppm while during the night the target varied and was generally higher due to canopy respiration, particularly with low wind. Due to technical problems during the weeks 22–26 in 1999 CO_2 concentration was considerably lower than the target (Fig. 10A). Therefore CO_2 concentration was at 675–725 ppm during 15% of the

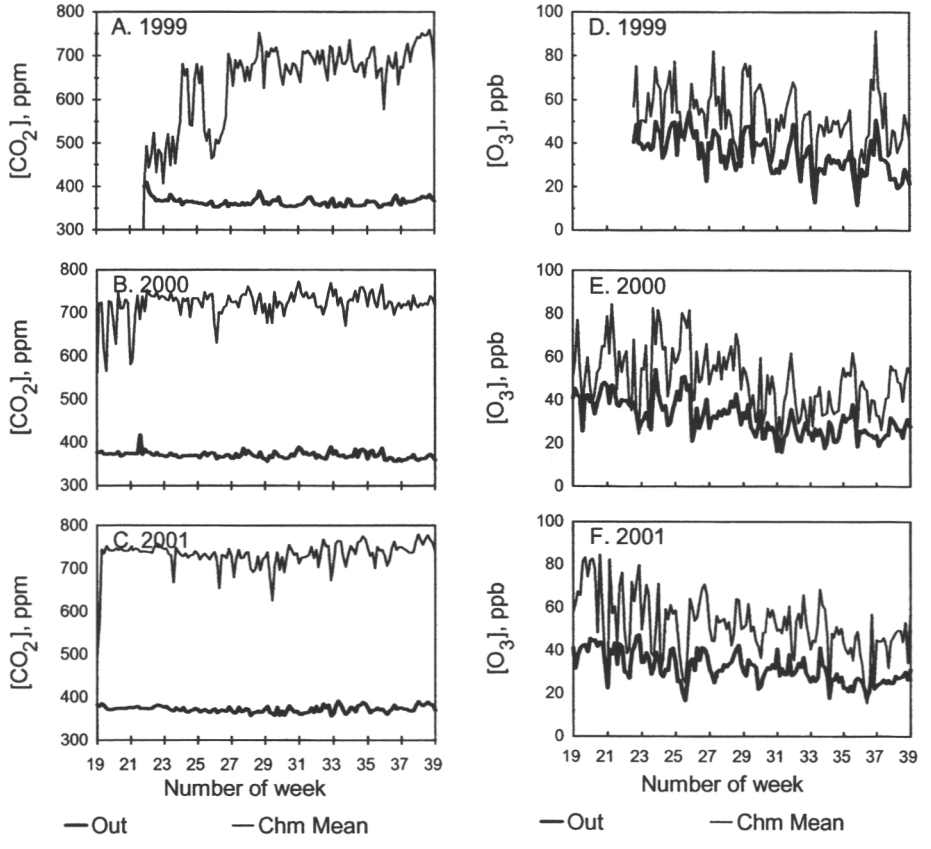

Figure 10. CO_2 and O_3 concentrations in the chambers with ambient air (thick line) and in the chambers exposed to elevated CO_2 and O_3 (thin line). Data show the daily mean values of CO_2 in 1999 (A), 2000 (B) and 2001 (C), and daily mean values of O_3 in 1999 (D), 2000 (E) and 2001 (F). Daily mean values were calculated from hourly mean values; in EC and EO treatments n=16 and in ambient air n=1.

exposure time and for 45% of the time between 625–775 ppm (Figs. 10A and 11A). During the growing seasons 2000 and 2001 the target CO_2 concentrations were achieved (Figs. 10B–10C). In 2000 the CO_2 concentration was between 675–725 ppm for 38% of the exposure time and between 625–775 ppm for 77% of the exposure time (Fig. 11B) and in 2001 between 675–725 ppm for 31% of the exposure time and between 625–775 ppm for 74% of the exposure time (Fig. 11C).

The Suonenjoki site has low background levels of ozone and for 35–46% of the daytime hours in 1999–2001 the ozone concentration of the ambient air was about 30 ppb (Figs. 10D–F and 11D–F). Maximum values of 45–55 ppb were occasionally recorded on sunny days during late afternoon hours (Fig. 10D–F). In 1999 the measurements of ozone concentration and also the EO treatment started on week 22 while in 2000 and 2001 the exposure to EO started on week 19. In the EO treatments during all years the ozone concentration was about 50 ppb during 20–21% of the exposure time, and high values of 100 ppb were recorded only during 1.5% of the

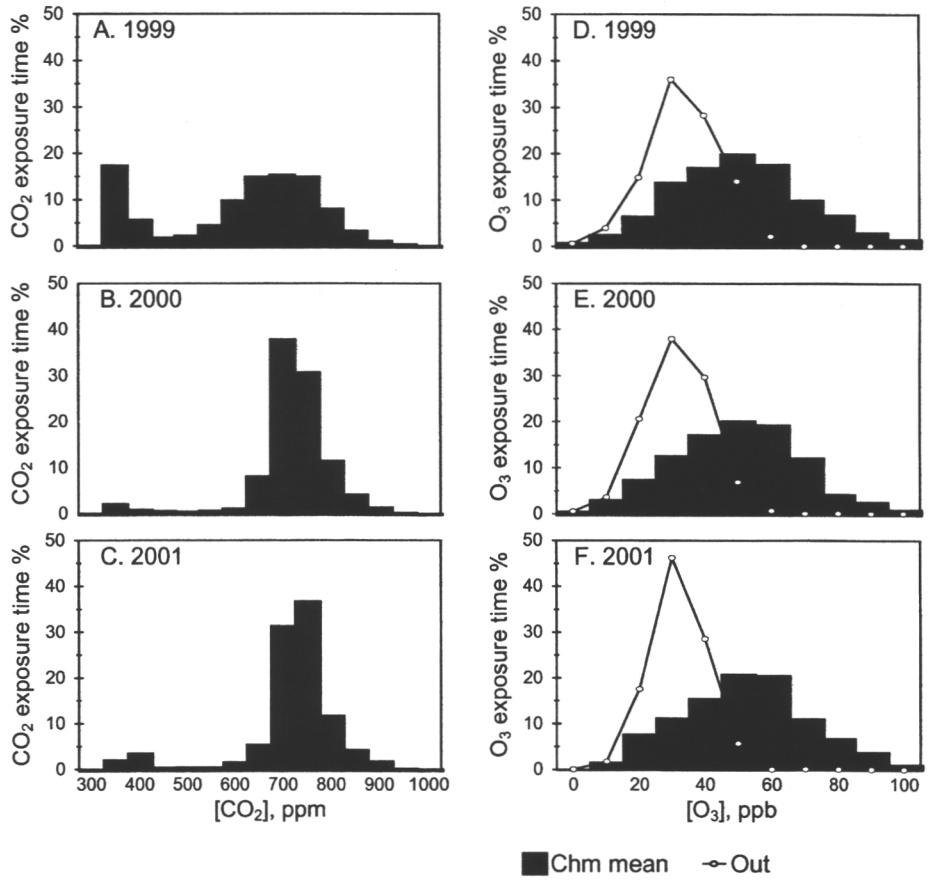

Figure 11. Frequency distribution (% exposure time) of CO_2 and O_3 concentrations in the chambers with elevated CO_2 (A. 1999, B. 2000, C. 2001) and O_3 (D. 1999, E. 2000, F. 2001). Line in Figs. 10D–F indicates the frequency distribution of O_3 concentration in ambient air. Data are based on hourly mean values; in EC and EO treatments n=16 and in ambient air n=1.

exposure time (Fig. 11). Due to delayed start of the EO exposure in 1999 the AOT00 and AOT40 values were lower than in 2000 and 2001 (Fig. 12). In 1999 the AOT40 was 18 ppm.h greater in the EO treatment when compared to ambient air. In 2000 and 2001, due to longer exposure period and successful control of the fumigation, and due to longer daily exposure time in 2001, the AOT40 values were 22 ppm.h and 29 ppm.h greater in the EO treatment than in ambient air (Fig. 12). Altogether the cumulative AOT40-exposure over the 3-year exposure period was 69 ppm.h in the EO-treatments which was almost nine times higher than in ambient air (8 ppm.h).

The vertical measurements between the supply point of the fumigation gases at 0.8 m and at 6.0 m from the ground level showed that in 1999 the concentrations decreased by about 21% for ozone (data not shown). In 2000 and 2001 the exposure conditions were more even with the taller 7.8-m chambers where the cut conical shape of the chamber top reduced turbulence caused by wind, and thus the vertical decrease within the chambers was 14% for CO_2 and 7% for O_3.

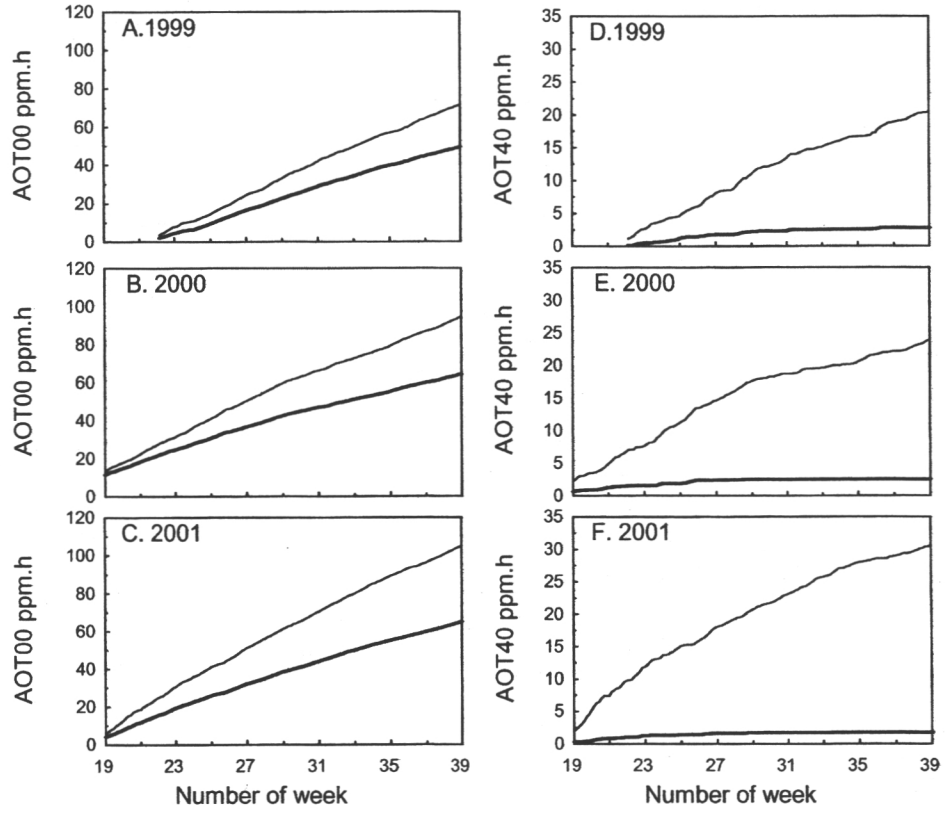

Figure 12. Ozone exposure of the experimental trees in 1999–2001, calculated as accumulated over a threshold of 0 ppb (A–C) and of 40 ppb (D–F). Thick line indicates the values for the ambient air and thin line the values for elevated ozone treatment. Data are based on hourly mean values; in EO treatments n=16 and in ambient air n=1.

Discussion

Silver birch is a pioneer species and occupies a large range of different habitats in Europe and northern Asia. For high productivity of silver birch soil fertility is important (Raulo 1977, 1981) and best growth is obtained in sandy moraine soils that contain high proportion of organic matter (Niemistö et al. 1997). These soils are mellow but at the same time provide good availability of water and nutrients. In contrast, dense soils with high water-retention capacity and low oxygen are deleterious for birch roots (Raulo 1981) and may retard growth of silver birch (Wall & Heiskanen 1995). In the present study, the experimental site was composed of sandy soil with low organic matter content. Such soils provide good aeration and availability of oxygen to the root system but have low nutrient- and water-retention capacity. The soil properties were taken into consideration during the study by

fertilising and irrigating the trees regularly in order to keep good availability of nutrients and to avoid water deficit. Fertilisation was given in doses to avoid leakage from the soil and fertilisation was increased from 22 kg N ha^{-1} y^{-1} in 1999 to 41 kg N ha^{-1} y^{-1} to compensate for the size increment of the trees during the study. The concentrations of the nutrients in the soil were comparable to forest and nursery soils (Luoranen 2000) except for nitrogen that was low in our study in early June 1999 when the experiment was started. Since the sampling for soil nutrients was done before the first fertilisation the data presented in Table 2 does not provide true picture of nutrient status of the soil. Our data of the nutrient concentrations in the leaves, measured four times during each growing season, showed no symptoms of nitrogen deficiency over the 3-year study (data not shown).

According to our measurements the growth conditions in the field site were relatively even for the establishment of the open-top chamber (OTC) experiment when compared with natural forests. The open-top chamber system has been widely used in climate change studies (Drake et al. 1989, Wang et al. 1995) because the construction costs of the OTC systems are low in comparison to closed-top chambers (CTC) and free-air CO_2 enrichment systems (FACE). Also the running costs of the system are small when compared with FACE systems. Both the OTC and CTC systems provide relatively good means to study the long-term responses of trees to climate change (Wang et al. 1995, Palomäki et al. 1998, Kellomäki et al. 2000) with even exposure conditions for the individual trees that are enclosed in the chambers.

In regard to CO_2 exposure our OTC system operated reliably, and in particular during the second and third year the CO_2 exposure conditions were very accurate. In our study the target CO_2 concentration was 2 * ambient which is around 720 ppm during the daytime hours while in the nighttime the ambient CO_2 concentration could be >400 ppm due to canopy respiration and correspondingly the elevated CO_2 concentration was higher. Interruptions for maintenance of the system were short and the system operated most of the exposure time at the set target values. In this respect our data are comparable to the performance reported for other chamber experiments (Jäger and Weigel 1993, Vourlitis and Ouchel 1993, Kellomäki et al. 2000) and the system simulated well the projected CO_2 conditions in 2100.

In Suonenjoki the daily mean concentration of ozone in ambient air varied between 10 to 60 ppb during the three growing seasons. The declining trend of the ozone concentrations from early to late growing season was observable during each study year. During the growing seasons 2000 and 2001, when the measuring period was almost the same, the AOT00-value was 66 ppm.h and AOT 40 value was about 2–3 ppm.h in the ambient air. In 1999 and 2000 the exposure to elevated O_3 was 12 h day^{-1} and in 2001 14 h day^{-1} whenever the set threshold value of 10 ppb was reached. On rainy days our system also automatically stopped ozone fumigation. Both the AOT00- and AOT40-values increased markedly under the ozone exposure. The simulated doubling of the current O_3 concentrations during 12–14 hours per day resulted in AOT40 values that were 20–30 ppm.h per growing season and 70 ppm.h when calculated as a cumulated AOT40-value over the three growing seasons. Therefore the annual AOT40 values for silver birch were 2–3 times higher than the currently set critical dose of 10 ppm.h, that is based on the observed 10% growth decline of beech (Braun & Fluckiger 1995, Fuhrer et al. 1997) in open-top chambers. In the present study the cumulative ozone exposure over three growing seasons

provides significant and valuable background information for assessments of critical dose in silver birch, since the same trees were exposed continuously throughout the experiment. In the ozone responses of trees the actual flux into the leaves determines the final responses at the cellular level. Some approximation of fluxes could be calculated whenever stomatal conductance and ozone concentration have been simultaneously recorded.

In spite of providing reliable conditions for the exposure to elevated CO_2 and O_3 the chamber constructions, however, change the growth conditions when compared to the conditions of outside air. In our OTC system the chambers were covered with polyethene film that transmitted >90% of light at wavelengths of 380–800 nm. This means that the photon flux density of the photosynthetically active light (400–750 nm) was close to that of ambient air with only small shading by the chamber walls. However, the differences in the light conditions within and between chambers were not measured and compared with that of the ambient air to verify the theoretically similar PAR values. The transmittance of the polyethene film drops rapidly at wavelengths <380 nm. At 300 nm only 3.4% of the radiation could penetrate to the chambers and therefore most of the UV-B radiation was filtered off. Recent studies show that UV-B serves as an environmental signal causing the synthesis of secondary metabolites (Lavola 1998, Lavola et al. 1997) with high UV-B-absorbing properties (Lavola et al. 1997, Tegelberg et al 2001, Tegelberg & Julkunen-Tiitto, 2001) that can prevent UV-B-induced DNA damage (Kootstra 1994). Our OTC conditions prevented the UV-B signalling cascade for synthesis of phenolics in the leaves and focus only on responses of the trees to elevated CO_2 and elevated O_3. Thus our experimental setup will give true information of e.g. changes in the palatability of leaves under elevated CO_2 and elevated O_3 but excludes the consequences of UV-B to the chemical composition of the leaves.

Temperature is a major factor in regulation of growth and phenology of forest trees. Due to the kinetic properties of Rubisco the temperature optimum for assimilation increases with increasing CO_2, and thus carbon assimilation should become greater when air temperature increases (Long 1991). Data to support this hypothesis are not unanimous and recent data on Douglas fir show that changes in the short-term temperature optimum can reduce the positive effect of elevated temperature on photosynthesis under elevated CO_2 (Lewis et al. 2001). In this study we aimed to create the growing conditions for the studied trees that are projected to prevail in the year 2100 (IPCC 2001). Although we did not specially simulate temperature increase, the greenhouse effect resulted in 2.3 to 2.7 °C increases in the daily mean temperatures in the OTC chambers compared to outside conditions. The effect of the chambers on the cumulative temperature sum was marked and increased by 380–400 d.d. during 2000–2001 when temperature records are available over the whole growing season. Such increase of temperature is well within the limits (2 to 4.5 °C) projected by most of the recent climate change models (IPCC 2001) for the year 2100. In our study the differences between chambers and ambient air varied within a day and thus do not fully simulate the projected future temperature conditions. The differences were greatest during the daytime hours on hot and sunny days (during week 28 the maximum temperature difference of 5.7 °C) even though highest ventilation of 0.6 $m^3\ s^{-1}$ was used in carrying the heat load. During the nighttime hours (2200 h to 0600 h) low ventilation of 0.1 $m^3\ s^{-1}$ was used. Therefore the temperature difference between the OTCs and ambient air was not

fully disappearing but was smaller (on an average 1.4 °C) than during the daytime hours.

Vapor pressure of the air has significant effects on stomatal conductance and transpiration, and therefore also on the response of photosynthesis to the growth environment (Schulze 1986). This will cause changes not only in growth but also in the structure of the leaves. In the present experimental setup vapor pressure of the chamber air could not be controlled. Continuous ventilation by blowers at the bottom of the chambers supplied 0.1–0.6 m^3 s^{-1} of fresh air to the chambers. During daytime hours when high flow rates of 0.6 m^3 s^{-1} were used the chamber air was exchanged once a minute and during night with low flow rate of 0.1 m^3 s^{-1} once in six minutes. Even though humidity in the chambers could not be controlled, our experimental setup enabled us to measure vapor pressure of the air continuously in 16 OTCs during the growing seasons 2000 and 2001. Our data show that the relative humidity was constantly higher in the ambient air than in chambers. This was due to higher air temperature in the chambers since the absolute water vapour content in chambers and outside air were similar. There were differences in relative humidity between the growing seasons 2000 and 2001; the growing season 2001 was more humid and the differences were particularly marked in May–June. Irrigation of the trees did not increase the daily RH values in chambers, since RH in the chambers followed closely the trends in the outside air, and ambient air hardly is affected by spotlike irrigation procedures.

In conclusion, we established an open-top chamber experiment to expose two birch genotypes to elevated CO_2 and O_3 and were able to run the experiment successfully over three growing seasons 1999–2001. The climatic conditions in the chambers simulated the doubling in CO_2 and O_3 concentrations that are predicted to prevail in 2100. Compared to the outside controls the chamber trees experienced also a chamber effect in the form of increased temperature (2–3 °C), slightly declined light and relative humidity. However, these changes in the natural environment are close to those predicted to occur along the climate change. The root growth of our naturally growing experimental trees was not limited, which is important when interpreting the results for future forest growth and performance. The OTC system provided a good facility where we were able to study without major technical problems the long-term performance of silver birch in future climatic conditions.

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