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ABSTRACT

A whole-tree chamber (WTC) system was installed at Flakaliden in northern Sweden to examine the long-term physiological responses of field-grown 40-year-old Norway spruce trees [*Picea abies* (L.) Karst.] to climate change. The WTCs were designed as large cuvettes to allow the net tree-level CO_2 and water fluxes to be measured on a continuous basis. A total of 12 WTCs were used to impose combinations of atmospheric carbon dioxide concentration, [CO_2], and air temperature treatments. The air inside the ambient and elevated [CO_2] WTCs was maintained at 365 and 700 μmol mol^{-1}, respectively. The air temperature inside the ambient temperature WTCs tracked air temperature outside the WTCs. Elevated temperatures were altered on a monthly time-step and ranged between +2.8 and +5.6 °C above ambient temperature. The system allowed continuous, long-term measurement of whole-tree photosynthesis, night-time respiration and transpiration. The performance of the WTCs was assessed using winter and spring data sets. The ability of the WTC system to measure tree-level physiological responses is demonstrated. All WTCs displayed a high level of control over tracking of air temperatures. The set target of 365 μmol mol^{-1} in the ambient [CO_2] chambers was too low to be maintained during winter because of tree dormancy and the high natural increase in [CO_2] over winter at high latitudes such as the Flakaliden site. Accurate control over [CO_2] in the ambient [CO_2] chambers was restored during the spring and the system maintained the elevated [CO_2] target of 700 μmol mol^{-1} for both measurement periods. Air water vapour deficit (VPD) was accurately tracked in ambient temperature WTCs. However, as water vapour pressure in all 12 WTCs was maintained at the level of non-chambered (reference) air, VPD of elevated temperature WTCs was increased.

Key-words: *Picea abies*; elevated air temperature; elevated CO_2; gas exchange; Norway spruce.

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INTRODUCTION

Global carbon dioxide concentration ([CO_2]) increased from 280 to 365 μmol mol^{-1} between the years 1800 and 2000 (IPCC 2001). The increase in [CO_2] is predicted to continue, with possible concentrations of 700 μmol mol^{-1} by the year 2100 (IPCC 2001). Concomitant with this rise in global [CO_2] is predicted increases in mean surface temperatures in the order of 2–6 °C (Burroughs 2001). The greatest warming is expected to be at northern high latitudes (IPCC 2001). The response of the large tracts of forest at these latitudes to climate change may be pivotal in determining the future extent to which forests act as net global sinks for atmospheric carbon. Accurate measurements of tree photosynthesis, respiration and transpiration under the predicted future climate are invaluable for understanding and modelling forest-ecosystem carbon exchange and storage. Such measurements rely on systems that provide environmental control and are able to mimic global climate change.

Our understanding of plant functioning and response to changing growth conditions has been greatly advanced by the application of environmental engineering in addressing physiological questions. Engineering designs that both control the growth environment and measure tree responses are particularly useful for examining the effects of exposure to atmospheric pollutants. A range of plant exposure systems have been employed over the last two decades, with noted success in shorter crop species (Allen *et al*. 1992). A new set of challenges arises when attempting to expose large trees and forest ecosystems to increased [CO_2]. Approaches have included branch bags (Barton, Lee & Jarvis 1993; Kellomäki & Wang 1997; Saugier *et al*. 1997; Robertnz 1999), open-top chambers (e.g. Whitehead *et al*. 1995; Jach & Ceulemans 1999; Murray *et al*. 2000), open-sided chambers (Liozon *et al*. 2000), glass domes with adjustable windows (Urban *et al*. 2001), free air CO_2 enrichment (FACE) systems (e.g. Hendrey *et al*. 1999; Hamilton, Thomas & DeLucia 2001; Herrick & Thomas 2001; Körner *et al*. 2005) and closed-top chambers (Kellomäki, Wang & Lemettinen 2000). Each of these systems has a particular set of advantages and disadvantages (see review by Saxe, Ellsworth & Heath 1998). Hence, the choice of system design is a critical first step in addressing any questions regarding tree response to climate change. A closed-top whole-tree chamber (WTC) system was considered to be the best approach for examining the medium to long-term effect of climate change on the functioning of boreal forest ecosystems.

The WTC system presents a number of advantages. It is a field-based system that utilizes mature trees. In this respect there are no root constrictions as with pot-based experiments, which with time can restrict root growth and lower the carbon sink strength of the plant. Root constriction has been linked to the observed down-regulation of photosynthetic capacity following [CO_2] enrichment (e.g. Drake, Gonzàlez-Meler & Long 1997). There is, however, conflicting evidence as to whether photosynthetic down-regulation occurs in mature forest trees growing under elevated [CO_2] (Curtis 1996), highlighting the need to examine this phenomenon using field-grown trees (cf. Körner 2003; Luo *et al*. 2004; Norby & Luo 2004). The responses of mature trees to [CO_2] and temperature elevation are also likely to differ with ontogeny (Saxe *et al*. 1998, 2001). By exposing whole trees to elevated [CO_2], the WTC system enables the responses of mature trees to be examined. The design of the WTC system allows for both the (1) application of [CO_2] and temperature treatments and (2) long-term, continuous measurement of tree-level responses (photosynthesis, respiration and transpiration) to those treatments. The uncertainty associated with scaling seedling or leaf-level responses to estimate the response at the mature tree level is thus eliminated. As the WTC system is an enclosed system, precise control of temperature and humidity is possible. This is an advantage over branch bags and open-top chambers (OTCs), which require high volume air flows to counteract uncontrolled temperature increases (Robertnz & Stockfors 1998). This is an important consideration, given that increases in [CO_2] are accompanied by increases in surface air temperatures (IPCC 2001). The ability of the WTC system to control temperature allows the physiological responses of mature trees to elevated [CO_2] and temperature to be examined singly and in combination. There are, however, possible chamber effects on leaf morphology and crown structure resulting from the absence of exposure to rainfall and snowfall, the potential for protection from radiation frosts, and the possible changes in light interception and reflection.

This paper describes a WTC system at the Flakaliden research site in northern Sweden. The system was used in a first experiment to examine interactions between elevated [CO_2] and nutrient and water availability (cf. Ceschia 2001; Fransson, Taylor & Finlay 2001; Kostiainen *et al*. 2004) and subsequently in a second experiment used to impose elevated [CO_2] and temperature treatments on 40-year-old field-grown Norway spruce trees (cf. Comstedt *et al*. 2006; Slaney 2006; Slaney *et al*. in review). In addition to the system description, the ability of the system in providing accurate environmental control is examined and some examples of tree net CO_2 flux in response to environmental variation are presented. For this description, data from the second experiment was used.

MATERIALS AND METHODS

Site description

The WTCs were installed in a 40-year-old Norway spruce [*Picea abies* (L.) Karst.] plantation at the Flakaliden research site in northern Sweden (64°07′N 19°27′E), where a nutrient-optimization experiment had commenced in 1987 (Linder 1995; Bergh *et al*. 1999). Norway spruce is one of the main tree species of the boreal forest regions of northern Europe, which meant that Flakaliden was a suitable location for examining the responses of boreal forest ecosystems to elevated temperature and [CO_2] levels. The Flakaliden research site was also ideal as it had existing infrastructure required for such a project (e.g. main electricity supply, irrigation system), as well as for a long-term monitoring of tree growth in relation to weather and soil nutrient availability.

The annual mean temperature at the site is 2.3 °C and the monthly mean air temperature varies from –7.3 °C in January to 14.6 °C in July (mean for the period 1990–2004). Mean annual rainfall is 600 mm, with approximately one-third falling as snow, which usually covers the frozen ground from mid-October to early May (Stockfors & Linder 1998a). The length of the vegetation period (average temperature ≥ +5 °C) is 135 d (Bergh *et al*. 1999). The soil at Flakaliden is a podzolic, glacial, loamy till with an average depth of approximately 1.2 m and an average humus layer depth of 30–40 mm (Bergh & Linder 1999).

Experimental design

The WTC system has been used in two experiments where 12 WTCs were installed around individual trees. In a third experiment, commencing in 2006, the WTC system will be used to investigate the interactions between soil water availability and elevated [CO_2] in *Eucalyptus* trees (The Hawkesbury Forest Experiment, NSW, Australia). In the first experiment (1997–2000), the WTC system was used to study the effect of elevated [CO_2] in combinations with a fertilizer and irrigation treatment. In the second experiment (2001–2004), which most of this paper is based on, the WTCs were installed in an untreated control plot that had received no previous treatment. A combination of two temperature levels (T_A, ambient and T_E, elevated) and two [CO_2] levels (C_A, ambient 365 μmol mol^{-1} and C_E, elevated 700 μmol mol^{-1}) was used as treatment variables in the WTCs and was arranged in a 2×2 factorial design. Three reference (R) trees without WTCs were also randomly selected and in total 15 trees were assigned to five treatments (T_AC_A, T_EC_A, T_AC_E, T_EC_E, R). This design allowed us to make (1) a two-way analysis of variance (ANOVA) for the chamber treatments, (2) an unpaired Student's *t*-test of the R trees compared with any chamber treatment, or (3) a one-way ANOVA for all five treatments followed by a post hoc test (e.g. Tukey's honestly significant difference test) to identify which of the treatments are statistically different from each other.

Description of the WTC system

Chamber construction

The WTCs were modular in design and consisted of three main sections; the chamber base (soil compartment), the tree chamber (above-ground compartment) and the cooling unit (Fig. 1). The base section was approximately 0.4 m in height. The circular base frame was, like all other chamber sections, constructed from aluminium and was 3.25 m in diameter. The wall of the base section was sealed with a sleeve of 0.4 mm transparent polyvinyl chloride (PVC) film (Renolit AB, Worms, Germany). The base of the sleeve was covered with soil to provide a seal between the base and the ground. The top of the chamber base was sealed with a combination of the 0.4 mm PVC film and transparent Perspex sheets (Röhm Plexiglas, Dusseldorf, Germany) of 5 mm thickness. The base was sealed around the tree stem to prevent air exchange between the soil compartment and the tree chamber. A small Perspex trapdoor (700 × 850 mm) allowed access to the soil under the chamber base. The trapdoor was sealed and securely screwed to the base frame when not in use. Two microsprinklers (Agridor Ltd., Rosh Ha'ayin, Israel), connected to an irrigation system, were installed under the chamber floor on 0.3 m spikes so that the trees could be irrigated with the same amount of water that was measured by rain gauges outside the WTCs. Each sprinkler could deliver 60 L h^{-1}.

To allow any soil disturbances to subside, the base frames and bottom sections were installed in September 2000, eight months before the rest of the chamber sections were mounted. Wooden walkways were constructed around and between each base to prevent soil compaction. Fresh air was continuously drawn through the chamber base using a shielded 125 mm air inlet and an extraction fan. Air was circulated around the chamber base by a fan (Model TD-160, Soler & Palau, Barcelona, Spain) at a flow of 160 m^3 h^{-1}. A shielded thermistor was attached to this fan for measurement of air temperature inside the chamber base and another thermistor was placed in the soil at a depth of 100 mm. Soil moisture at a depth of 150 mm was continuously monitored with a single soil moisture sensor (Thetaprobe ML1, Delta-T Devices Ltd, Cambridge, UK) placed under the floor of each WTC.

The frame of the tree chamber section was also covered with transparent PVC film (0.4 mm). The tree chamber was composed of two sections, a 2.5-m-high bottom section and a top section which was conical in shape and had a height of 3.0 m. To keep pace with tree height growth, the modular design allowed for additional cylindrical sections to be added between the bottom and top sections.

A safety ventilation system was designed to circulate fresh air through the chamber in the event of a power failure or refrigeration unit malfunction. Two circular air inlets were installed in the chamber, one at the base of the tree chamber and one at the top of the conical top section. Each air inlet was 650 mm in diameter and was, when not in use, sealed with a metal damper. The dampers could be rotated by 90° around a spindle by a motor (Model SM24, Belimo, Hinwil, Switzerland). In the event of either a power failure or the temperature inside any of the 12 chambers reaching 8 °C above ambient temperature, a relay started a diesel generator (Model YDG 3700E-E, Yanmar, Tokyo, Japan) and the dampers were opened. A ventilation fan, attached to the base vent, then forced fresh air through the chamber, from

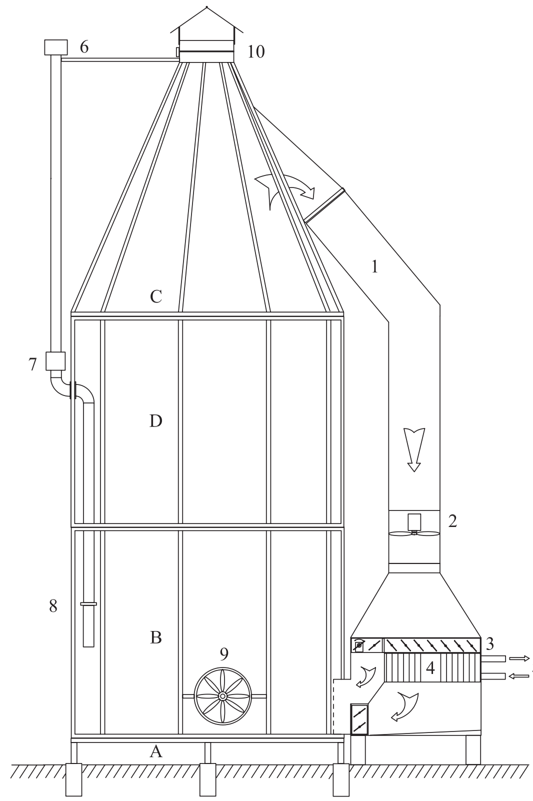

Figure 1. Schematic diagram of a whole-tree chamber (WTC) used in the long term manipulation experiments at Flakaliden. The modular chamber design consisted of three main components: the chamber base (soil compartment), the tree chamber (above-ground compartment) and a cooling unit placed directly outside the chamber. The diameter of the WTC was 3.25 m. The chamber base (A) was approximately 0.4 m high. The tree chamber consisted of a bottom (B) and a top (C) section, with a height of 2.5 m and 3.0 m, respectively. Extra sections (D) could be added to the tree chamber to keep up with tree height growth. Some major components of the system are indicated in the diagram by numbers: (1) pipe (Ø = 630 mm) for circulating chamber air through the cooling unit; (2) frequency-controlled fan (0–12 000 m^3 h^{-1}); (3) dampers to regulate the amount of air going through the cooling battery; (4) large-surface cooling battery; (5) circulating a glycol/water (30/70%) solution maintained at ambient dew point temperature; (6) fresh air inlet; (7) fan for fresh air intake; (8) iris damper for flow control of fresh air intake; (9) safety fan connected to a diesel generator which starts in case of power failure; and a 12-V controlled safety damper working in parallel with a similar damper (10) at the top of the WTC. For further explanations, see text.

the bottom and out via the top vent at a rate of 2300 m^3 h^{-1}. In the event of normal power or temperature regulation being restored, the safety ventilation fan stopped and the safety ventilation dampers were automatically closed.

Air from the top of the chamber was drawn into the cooling unit by a powerful, frequency-controlled fan (Swegon, Kvänum, Sweden) at a rate that could be regulated between 0 and 12 000 m^3 h^{-1}. This fan was positioned on top of the cooling unit at the base of an aluminium circulation pipe (630 mm in diameter). Conditioned air was then returned from the cooling unit to the base of the tree chamber. The incoming air was deflected upwards by a curved aluminium sheet positioned at the mouth of the cooling unit. Two strategically positioned transparent Perspex sheets above the aluminium sheet facilitated the mixing of conditioned air with the remainder of the chamber air. The volume of water condensed from the conditioned air was measured at an outlet pipe at the base of the cooling unit by a tipping bucket rain gauge.

Fresh air was continuously added to the chamber via an inlet pipe of 125 mm diameter. A fan positioned at the midpoint of the inlet pipe drew air from above the canopy. This air passed through a regulating iris (IRIS-125, REC-Indovent AB, Mölndal, Sweden) with an adjustable aperture. The iris was located 400 mm from the end of the fresh air inlet. The rate of airflow into the chamber was calibrated using an inflatable bag of known volume (Mätforum, Nacka, Sweden). Differential pressure measurements across the iris were made by a low pressure transducer (Model LP, Data Instruments, Acton, MA, USA). To reduce the fresh airflow, and hence the amount of CO_2 and heating required to maintain treatment levels during winter, perforated caps were placed over the fresh air inlet during each winter and removed in early spring. The volumetric fresh airflow into the chambers during the winter months was approximately 42 m^3 h^{-1}. At other times of the year the fresh airflow was approximately 54 m^3 h^{-1}. The chamber internal volume (including air circulation pipe and cooling unit) was 56.3 m^3.

CO_2 supply and control

Pure CO_2 was supplied to the WTCs from a set of 12 interconnected tanks, which contained 210 kg of liquefied CO_2 at a pressure of 50 bar (AGA, Sundbyberg, Sweden). During the growing season, one set of tanks would supply CO_2 to the WTCs for approximately 2 weeks. A pressure regulator (Model 8624, Bürket, Ingelfingen, Germany) on the CO_2 line from the tanks maintained a constant CO_2 pressure of 4.0 bar to the chambers. An infrared CO_2 gas analyser in each chamber (SBA-1, PP Systems, Hitchin, UK) was used to measure chamber $[CO_2]$ at 90 s intervals. In order to maintain chamber $[CO_2]$ at the set target level, pure CO_2 was injected into the circulating chamber air through a magnetic valve (Bürket), which opened for a programme-determined period during each 90 s interval. The amount of pure CO_2 added to the chamber was determined from the period of time the magnetic valve was open and the mean CO_2 flow rate measured in the chamber by a mass flow meter (AWM3000 and AWM5000 for C_A and C_E chambers, respectively, Honeywell, Freeport, IL, USA). The mass flow meter was housed in an insulated box that was thermostatically controlled at 20 °C. A copper coil on the CO_2 line before the flow meter allowed the CO_2 gas temperature to reach box temperature before flow measurement in each WTC. Each mass flow meter was calibrated using a piston-driven flow calibrator (DryCal DC-1, Bios International, Butler, NJ, USA).

The $[CO_2]$ of non-chamber air (termed reference air) was continuously measured by two infrared gas analysers (IRGA); a CIRAS-2 DC (PP Systems) and a WBA-2 system (PP Systems). Reference air was drawn continuously from above the canopy using a mast erected in proximity to the WTCs. A quantum sensor (LI-190SA, Li-Cor, Lincoln, NE, USA) to measure photosynthetic photon flux density (PPFD) at 3 min intervals was also installed in this mast.

Temperature control – heating and cooling

Temperature control in each WTC was achieved by circulating the WTC air over a heat exchanger inside the cooling unit. The heat exchanger consisted of a battery of tubes (1400 × 960 mm) filled with circulating glycol cooled to dew point temperature by a 150 kW refrigeration unit (Daikin EUV60, Daikin Industries Ltd., Osaka, Japan). The volume of circulating air that passed over the heat exchanger and the volume that bypassed the heat exchanger was controlled by a series of dampers within the cooling unit (cf. Fig. 1). The damper apertures in each WTC were adjusted by a programme-driven actuator (ASU1d15, Staefa Control, Cumming, GA, USA) at 90 s intervals. The adjustments maintained the air temperature within the WTC either (1) at the same air temperature outside the WTCs for T_A WTCs

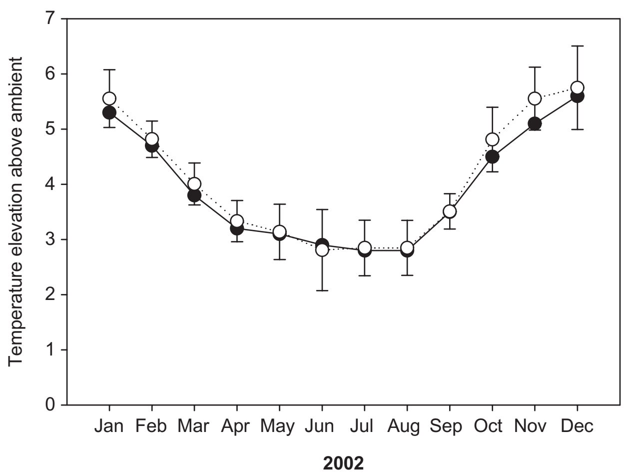

Figure 2. Monthly temperature elevations used at Flakaliden for temperature-elevated (T_E) whole-tree chambers (WTCs). Target temperature elevation values (filled circles) were generated by a meteorological model (SWECLIM) using the latitude of Flakaliden and assuming $[CO_2]$ of 700 μmol mol^{-1}. Actual mean monthly temperature elevations for the T_E WTCs (open circles) are shown for 2002. Means were calculated by pooling all T_E data for each month. Error bars show ± 1 standard deviation. $n = 6$.

or (2) at the set elevation above reference air temperature for T_E WTCs (cf. Fig. 2). The air temperatures inside and outside the WTCs were measured by ventilated and shielded thermistors at a height of approximately 5 m.

In the T_E WTCs, the monthly temperature elevation was based on results from the SWECLIM modelling programme (Christensen *et al*. 2001; Räisänen & Joelsson 2001; Räisänen, Rummukainen & Ullerstig 2001) using the latitude of Flakaliden and a [CO_2] of 700 μmol mol^{-1} (Fig. 2). The highest temperature elevation was during December (+5.6 °C above ambient) and the lowest was during July and August (each +2.8 °C above ambient). Elevated temperature regulation was achieved by a combination of (1) reducing the amount of air passing over the heat exchanger and (2) the use of two heating elements installed in the circulating air pathway in each WTC. The heating elements were regulated by programme-controlled relays using measured incident light intensity. When the light intensity fell below 900 μmol m^{-2} s^{-1}, one element was switched on in each T_E WTC. When light intensity fell further to less than 400 μmol m^{-2} s^{-1}, the second element was switched on. This ensured that the system did not heat the WTC during daytime when incident radiation levels were sufficient to heat the WTCs.

During winter, thick polystyrene sections were placed over the floor inside each WTC to insulate the base, and additional external insulation was placed around each WTC base. This insulation simulated snow cover and prevented deep frost in the soil under the WTCs. The insulation was removed in the spring after complete snowmelt.

System regulation and data acquisition

The programme for controlling the WTC system was developed using 32-bit programming software (Visual Basic 5.0, Microsoft Corp., Redmond, WA, USA). The program was run continuously from a Pentium computer with a Windows NT 4.0 (Microsoft Corp.) operating system. The control of the WTC environment and data acquisition was achieved with an input/output (I/O) module (SIOX S12, Telefrang AB, Göteborg, Sweden). To allow multiple tasks and data acquisition this central module had a combination of analogue and digital input and outputs. Communication between the program and the I/O module was via a K30 RS232C-SIOX converter (Telefrang AB) connected to a RS232 port on the computer. The central I/O module was used to transmit and receive data from the local I/O module (SIOX S12, Telefrang AB) in each WTC, control the signal to the central cooling system, and measure reference climatic variables. Communication between the central and local I/O modules was initiated by the programme every 90 s. The climatic data and WTC data stored by the WTC system are listed in Table 1. Climatic data was stored every 3 min. Data for each WTC was stored on a 42 min cycle after completion of the WTC gas analysis by the central infrared gas analyser (see further discussion).

A differential CO_2/H_2O infrared gas analyser (CIRAS-2 DC, PP Systems), located in a nearby temperature-controlled shelter, measured the concentrations of CO_2 and H_2O in air pumped from the WTCs and the reference mast. Sample gas was drawn continuously from each of the WTCs and the mast using a vacuum pump and a 6 mm high-density polyethylene tubing at a rate of approximately 1.5 L min^{-1} and directed to the analyser in turn using set of 3-way magnetic valves (M-331-c-04, Bürket). The gas samples from each WTC was analysed for 180 s. For each measurement cycle, the analyser first measured samples from the six C_A WTCs followed by a reference air sample, then the six C_E WTCs followed by another reference air sample. The time taken to complete one measurement cycle was approximately 42 min. During each reference gas sample, the analyser was programmed to undergo its auto-calibration sequence where dry, CO_2-free air was directed through the cells. To continuously update the dew point temperature target for the central cooling system, the continuous measurement of water vapour pressure of the reference air was used. The digital data from the analyser was transmitted to the computer via a RS232 port. A 56K data modem and remote access software (pcANYWHERE 7.5, Symantec Corp., Cupertino, CA, USA) enabled remote control of the computer and the WTC system.

WTC performance test

Sample data

Two sets of data were used to assess the effectiveness of the system in controlling the air temperature, water vapour pressure and maintaining the target [CO_2], within the WTCs. All WTCs were used in the evaluation of chamber performance. The first dataset was a 14 d period during the winter (14–28 February 2002). The second dataset was a 14 d period during late spring 2002 (3–17 May) that was characterized by unseasonally high air temperatures (Fig. 3). These time periods were relatively free of disruptions caused by personnel entering the WTCs for routine tree measurements, equipment maintenance or repairs.

Temperature and water vapour pressure control

The air temperatures inside and outside of each WTC, measured at 42 min intervals, were compared throughout each of the winter and spring 14 d periods, which were used to illustrate the performance of the temperature control of the WTCs. Mean temperature differences were calculated for T_A and T_E treatments. In February, the target temperature for the T_E WTCs was +4.7 °C and in May the target was +3.1 °C above ambient temperature, respectively.

The water vapour pressure deficit (VPD) of the WTC and reference air was compared for each WTC using instantaneous measurements of air vapour pressure and temperature. Measurements were made on a 42 min cycle.

[CO_2] control

The [CO_2] inside and outside of each WTC, measured at 42 min intervals, was compared throughout each of the

Table 1. Climatic data and whole-tree chamber (WTC) data automatically stored by the WTC system every 3 and 42 min, respectively

Variable name	Units	Symbol[a]	Notes
Dataset 1 – climatic data (3 min cycle)			
Reference air temperature	°C		
Photosynthetic photon flux density (PPFD)	μmol m^{-2} s^{-1}		Integrated value using a 12 min time interval
Barometric air pressure	Pa		
Reference air CO_2 concentration	μmol mol^{-1}		From reference cell of central IRGA
Reference air vapour pressure	kPa		From reference cell of central IRGA
Reference air vapour pressure deficit	kPa		Calculated from temperature and water vapour pressure
Dataset 2 – Current WTC data (recorded for each WTC on a 42 min cycle)			
WTC air CO_2 concentration	μmol mol^{-1}	c_t & c_{t-1}	From central IRGA
Reference air CO_2 concentration	μmol mol^{-1}		From central IRGA
WTC air vapour pressure	kPa	H_{WTC}	From central IRGA
Reference air vapour pressure	kPa		From central IRGA
WTC air temperature	°C		
Reference air temperature	°C		
PPFD	μmol m^{-2} s^{-1}		Integrated value using a 12 min time interval
Barometric air pressure	Pa		
WTC air CO_2 concentration	μmol mol^{-1}		From WTC CO_2 infra-red gas analyser
CO_2 addition flow rate	mL s^{-1}		Averaged by WTC I/O module per second of flow
CO_2 addition time	s		Total opening time of magnetic valve during cycle
Differential pressure across fresh air iris	Pa		
Condensed water	g		Amount of water condensed from WTC during cycle
Dendrometer	Δ mm		Circumferential stem growth at 1.3 m height
Soil volumetric water content	m^3 m^{-3}		
Soil temperature	°C		At 10 cm depth
Floor air temperature	°C		Air temperature under floor of WTC
Door alarm counter			Number of WTC door openings during cycle
Dataset 3 – Mean WTC data (recorded on 42 min cycle; mean values calculated from 90 s readings)			
Mean reference air CO_2 concentration	μmol mol^{-1}	C_{ref}	From central IRGA
Mean WTC air CO_2 concentration	μmol mol^{-1}	C_{WTC}	From central IRGA
Mean PPFD	μmol m^{-2} s^{-1}		
Mean WTC air temperature	°C	T_{WTC}	
Mean reference air temperature	°C	T_{ref}	
Mean CO_2 addition flow rate when CO_2 valve was open	mL s^{-1}	f	
Total CO_2 injection time	s	t_{CO2}	
Total cycle time	s	t_{tot}	Time of measurement cycle
Mean barometric air pressure	Pa	P_{atm}	
Mean differential pressure across fresh air iris	Pa	ΔP	
Mean reference air vapour pressure	kPa	H_{ref}	From central IRGA

Data acquisition and storage was achieved with the central I/O module (see text for details).
[a]Symbols as used in Eqns 3–10. For further explanations, see text.

winter and spring 14 d periods, and mean concentration differences were calculated for the C_A and C_E treatments.

Radiation quality and quantity

Light transmission through the WTC wall material was measured using a portable spectroradiometer Li-1800 (Li-Cor Inc.). The spectrum runs ranged from 300 to 1100 nm, with a measurement recorded for each nm. The spectrum was measured in direct and diffuse solar radiation during a cloud-free day. Measurements were made on WTC wall material that had been exposed to the elements for 5 years and material that had not been exposed. A total of four scans were done, the first and last being reference measurements and for the two middle scans the spectroradiometer hemispheric dome was covered with old and new WTC wall material, respectively. The four measurements were done in one 5 min sequence. The effect of the WTC structure on PPFD levels inside the WTC was examined by comparing PPFD measurements made at the fifth whorl (from the top) of the R trees ($n = 3$) and the C_A trees ($n = 6$). The PPFD measurements were made as part of a shoot-level gas exchange system (see Wallin *et al*. 2001). Mean daily PPFD for each tree was calculated using measurements made every 30 min. A 6 month period that covered a wide range of sun angles (1 January–30 June 2002) was used for the analysis.

Tree-level net CO_2 flux calculations

Net CO_2 uptake and release by the trees within the WTCs was calculated using the continuous measurements made

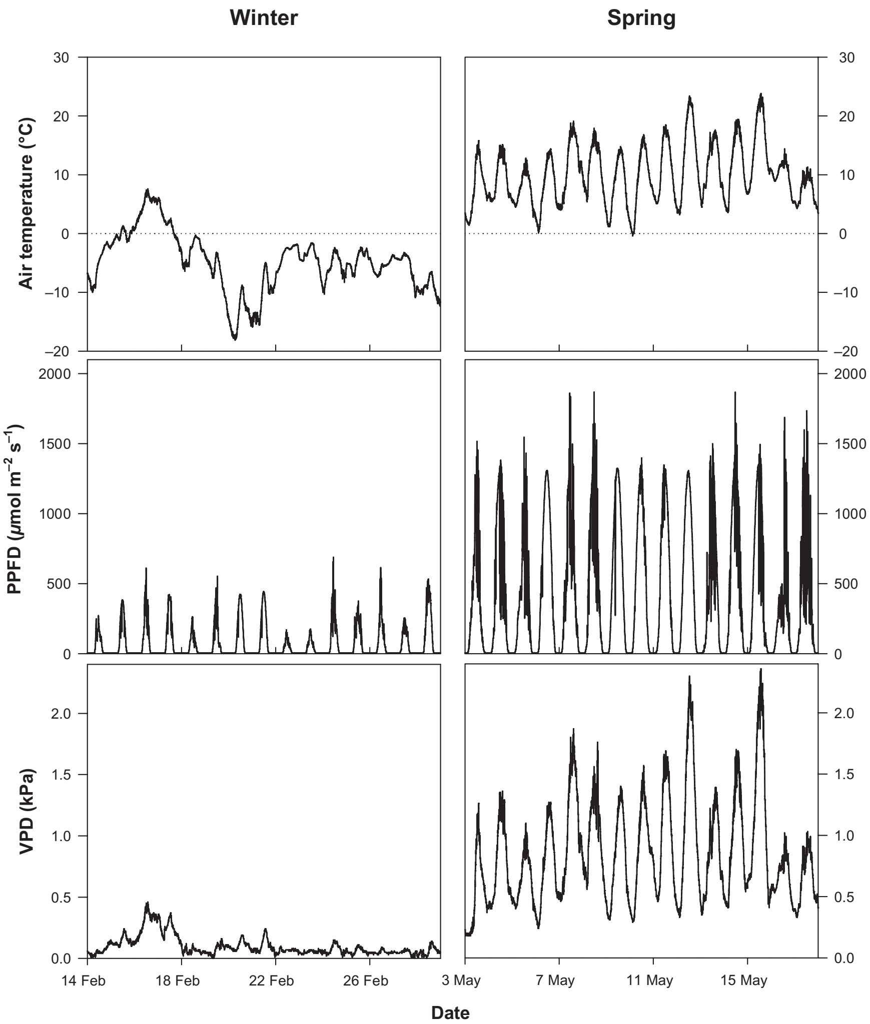

Figure 3. Flakaliden climate [air temperature, photosynthetic photon flux density (PPFD) and water vapour pressure deficit (VPD)] for 14 d in February and 14 d in May 2002. The data shown are measurements made at 3 min intervals. Air temperature was measured at a height of 2.5 m and PPFD was measured at 11 m.

by the WTC system (see Table 1). Fluxes were calculated using a carbon balance approach with influx terms on the left side of the equation, and efflux terms on the right:

$$I + F = V + P + S, \tag{1}$$

where I is the CO_2 injection rate, F is the rate of CO_2 addition to the chamber in the fresh air supply, V is the venting of CO_2 from the chamber, P is net photosynthesis, and S is the change in CO_2 storage in the chamber resulting from changes in $[CO_2]$ in the WTCs over time. Solving for P gives

$$P = I + F - V - \Delta S. \tag{2}$$

Each of these terms is now considered separately.

(a) CO_2 injection rate (I)

The amount of CO_2 injected into the chamber during a measurement cycle (approximately 42 min) was calculated from the total time that the magnetic valve to the CO_2 supply line was open, and the mean flow rate of CO_2 through the mass flow meter. Each CO_2 mass flow meter was calibrated to standard temperature and pressure (0 °C and 101.3 kPa, respectively). Thus, the CO_2 injection rate (mmol CO_2 s^{-1}) throughout each measurement cycle was calculated as

$$I = \frac{\left(\frac{f}{22.4}\right) t_{CO_2}}{t_{tot}} \tag{3}$$

where f was the mean CO_2 flow rate when CO_2 valve was open (mL s^{-1}), t_{CO_2} the total time the CO_2 valve was open during the measurement cycle (s), and t_{tot} the total time of the measurement cycle (s).

(b) CO_2 addition via fresh air supply (F)

The amount of CO_2 added to the chamber via the fresh air supply was calculated from the volume of fresh air entering the chamber and its $[CO_2]$. Before the volumetric flow rate of fresh air could be determined, the density of the fresh air (D_{ref}, g^{-1}) was calculated as

$$D_{ref} = \frac{P_{atm}/RT_{ref}}{(1 - H_{ref}/P_{atm}) * (1 - 0.622)} \tag{4}$$

where P_{atm} was mean atmospheric pressure throughout the measurement cycle (Pa), R the gas constant (287 J K^{-1} kg^{-1}),

T_{ref} the mean temperature of the reference air during the measurement period (Kelvin, K) and H_{ref} the vapour pressure of the reference air during the measurement (Pa). The volumetric flow of fresh air entering the chamber (A_{in}, l s^{-1}), corrected to standard temperature and pressure, was then calculated as

$$A_{in} = \left(k\sqrt{\Delta P \frac{1.2}{D_{ref}}} \right) \frac{273}{T_{ref}} \frac{P_{atm}}{1013} \tag{5}$$

where k was a chamber-specific constant calculated from calibrating the air flow at a set iris position using an inflatable bag of known volume, and ΔP was the mean pressure differential (Pa) across the iris of the fresh air inlet during the measurement cycle. The rate of addition of CO_2 by the fresh air supply (mmol s^{-1}) during the measurement cycle was then calculated as

$$F = \frac{A_{in} C_{ref}}{22\,400} \tag{6}$$

where C_{ref} was the mean [CO_2] (μL l^{-1}) of reference air throughout the measurement cycle. The mean [CO_2] of the reference air was calculated from the CIRAS-DC IRGA measurements made every 3 min.

(c) CO_2 venting from the chamber (V)

The loss of CO_2 from the chamber by venting was calculated using the same method as (b) and replacing mean reference air variables with mean chamber air variables. The mean air density of the chamber during the measurement cycle (D_{WTC}, g l^{-1}) was calculated as

$$D_{WTC} = \frac{P_{atm}/RT_{WTC}}{(1 - H_{WTC}/P_{atm}) * (1 - 0.622)} \tag{7}$$

where T_{WTC} was the mean air temperature (K) in the chamber during the measurement cycle and H_{WTC} was the vapour pressure (Pa) of the chamber air.

The air flow out of the chamber (A_{out}, l s^{-1}) was then calculated as

$$A_{out} = \left(k\sqrt{\Delta P \frac{1.2}{D_{WTC}}} \right) \frac{273}{T_{WTC}} \frac{P_{atm}}{1013} \tag{8}$$

where T_{WTC} was the mean chamber temperature (K) during the measurement cycle. The rate of CO_2 loss from the chamber by venting (V, mmol s^{-1}) during the measurement cycle was then calculated as

$$V = \frac{A_{out} C_{WTC}}{22\,400} \tag{9}$$

where C_{WTC} was the mean [CO_2] (μL l^{-1}) of the chamber air throughout the measurement cycle.

(d) Change in CO_2 storage in the chamber (S)

To calculate the rate of change in storage of CO_2 in the chamber during the measurement cycle (mmol s^{-1}), the chamber [CO_2] measured by the CIRAS-DC gas analyser, c_t (μL l^{-1}) was compared with that measured during the previous measurement cycle, c_{t-1} (μL l^{-1}).

$$S = \frac{(c_t - c_{t-1})v}{22.4} * \frac{1}{T} \tag{10}$$

where v was chamber volume (m^3). The S-value was a small part of the carbon balance equation because of the stable [CO_2] maintained in the chamber by the control system (see Results). Typically the S-value was less than 2% of the CO_2 injection term.

WTC effects and tree-level net CO_2 flux in response to climate

The effect of the WTCs on the functioning of the enclosed trees was examined by comparing a range of structural characteristics and physiological parameters from the trees grown inside ambient temperature and [CO_2] WTCs (T_AC_A) and non-chambered trees (R). The analysis of the structural characteristics of the wood and needles included both the (1) material that was formed prior to the installation of the WTC (i.e. 2001) and (2) material that was formed during the last year of the experiment (2004). The majority of the physiological data used for the analysis was collected in the spring of 2002. Unpaired Student's t-tests were used to test the statistical significance of differences between R and T_AC_A values.

To illustrate the ability of the WTC system to examine tree-level responses to environmental variation, the net CO_2 uptake and release was calculated for one T_AC_A WTC and one T_EC_A WTC from January to December 2003 using Eqns 3–10.

The response of tree-level net CO_2 uptake to air temperature, air water VPD and PPFD was examined by using the 2003 data from the T_EC_A chamber. Daily mean and daily maximum WTC data were used for this exercise. The response of tree-level maximum respiration rate to a 10 °C increase in temperature, Q_{10}, was calculated for the T_EC_A tree by plotting the natural logarithm of mean respiration rate as a function of mean air temperature using data when PPFD was less than 25 μmol m^{-2} s^{-1}:

$$Q_{10} = \exp(10\beta) \tag{11}$$

where β is the slope. The fitted regression was also used to calculate R_0 and R_{15}, the maximum respiration rate at 0 and 15 °C, respectively.

RESULTS

[CO_2] control

Excellent [CO_2] control was achieved in the C_E WTCs during both measurement periods (Table 2). The [CO_2] of the C_E WTCs was between 690 and 710 μmol mol^{-1} for 98 and 93% of the time for the winter and spring periods, respectively (Fig. 4). The target [CO_2] of 365 μmol mol^{-1} in the C_A WTCs was, however, not achieved during the winter period:

Table 2. Regulation of $[CO_2]$ in the whole-tree chambers (WTC) by $[CO_2]$ treatment

CO_2 treatment	Target $[CO_2]$ (μmol mol^{-1})	Mean difference from target (μmol mol^{-1})	Standard Deviation	10th percentile (μmol mol^{-1})	90th percentile (μmol mol^{-1})
14–28 February 2002					
C_A	365	+20.1	± 5.0	+15.3	+23.5
C_E	700	+2.4	± 3.3	–0.8	+5.3
3–17 May 2002					
C_A	365	+8.9	± 14.9	–6.5	+25.0
C_E	700	–1.6	± 5.8	–7.4	+3.5

The $[CO_2]$ values measured by the central IRGA on a 42 min cycle from each WTC were pooled by $[CO_2]$ treatment. $n = 6$.
C_A, ambient $[CO_2]$; C_E, elevated $[CO_2]$.

The $[CO_2]$ in the C_A WCTs was between 355 and 375 μmol mol^{-1} for only 5% of the time. This was because of tree dormancy and the naturally high $[CO_2]$ of the fresh ambient air entering the WTCs during this period. During the spring period, the $[CO_2]$ of the C_A WTCs was between 355 and 375 μmol mol^{-1} for 51% of the time.

Temperature control

Temperature regulation displayed a high level of accuracy throughout both measurement periods (Table 3). In the T_E WTCs, the temperature was within ± 0.5 °C of the target temperature for 99% of the time of both measurement periods (Fig. 5). The reduction in cooling capacity during the winter period meant that the temperature of the air in T_A WTCs was within ± 0.5 °C of ambient temperature for 54% of the time (and within ± 1.0 °C for 80% of the time). With full cooling capacity reinstated in the spring, the temperature of the air in T_A WTCs was within ± 0.5 °C of ambient temperature for 89% of the time (and within ± 1.0 °C for 97% of the time).

Humidity control in the T_A WTCs was of high standard

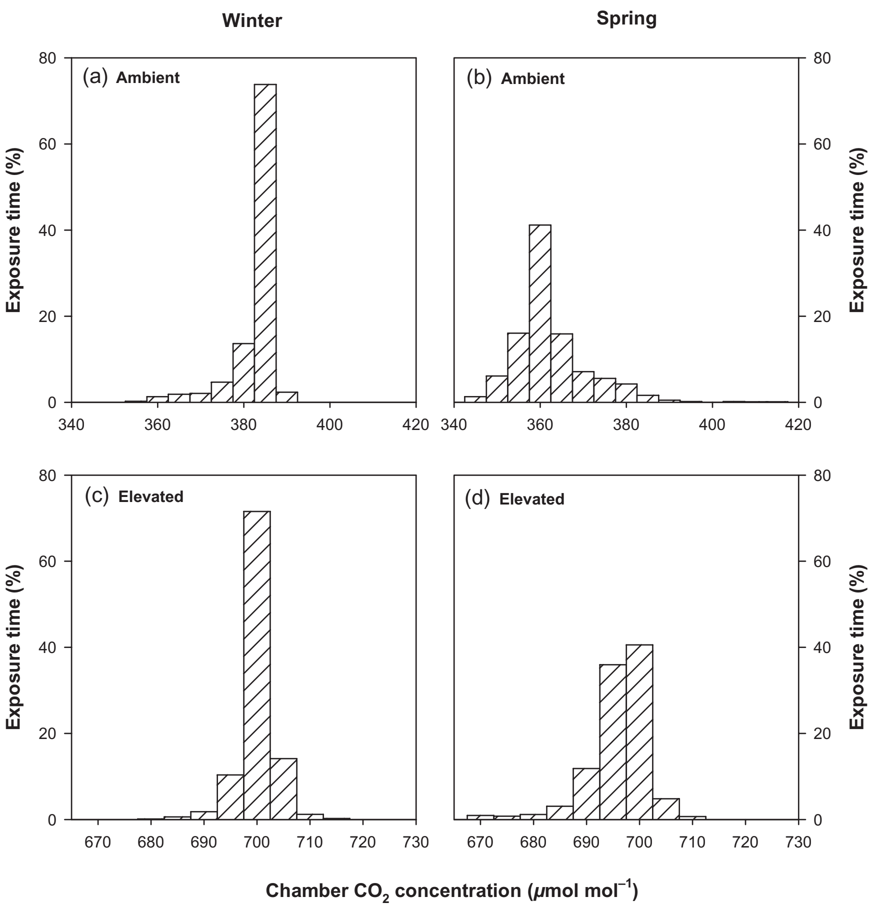

Figure 4. Frequency distributions of $[CO_2]$ in the ambient, C_A (a & b), and elevated, C_E (c & d), chambers for the measurement periods 14–28 February and 3–17 May, 2002. Frequency distributions were calculated from the central IRGA measurements made on a 42 min cycle from the six C_A and six C_E whole-tree chambers, respectively.

Table 3. Whole-tree chamber (WTC) temperature control by temperature treatment

Temperature treatment	Target temperature elevation (°C)	Mean temperature difference from ambient (°C)	Standard Deviation	10th percentile (°C)	90th percentile (°C)
14–28 February 2002					
T_A	0	+0.6	± 0.6	0.0	+1.3
T_E	+4.7	+4.8	± 0.2	+4.6	+5.0
3–17 May 2002					
T_A	0	0.0	± 0.4	–0.3	+0.3
T_E	+3.1	+3.1	± 0.2	+3.0	+3.4

Note that during February the temperature control was deliberately reduced to minimize excessive power consumption over the winter months. Temperatures measured on a 42 min cycle from each WTC were pooled by temperature treatment ($n = 6$).
T_A, ambient temperature; T_E, elevated temperature.

throughout both measurement periods (Fig. 6). In the T_A WTCs, the mean VPD was 0.02 kPa higher than the outside air for the winter measurement period (SD = 0.02). In the spring measurement period, the mean VPD in the T_A WTCs was the same as the outside air (SD = 0.02 kPa). The higher temperatures in the T_E WTCs meant that VPD was significantly higher in these WTCs, relative to the outside air. During the winter and spring measurement periods the maximum VPD of the outside air was 0.44 and 2.28 kPa, respectively. The mean increase in VPD for the T_E WTCs during the same periods was 0.19 and 0.30 kPa, respectively (SD = 0.06 and 0.08).

Light transmittance

The new polyethylene film used in construction of the WTC walls had a high transmittance of visible light (88%) at wavelengths of 400–800 nm. After 5 years of exposure, the old polyethylene film had only a small decrease (4%) in light transmittance in the visible spectral range. This decrease was largely confined to the 400–500 nm range of the spectrum. The amount of light transmitted at wavelengths less than 400 nm decreased sharply for both the new and old film. At 350 nm there was only 4 and 1% light transmission for the new and old film, respectively. High

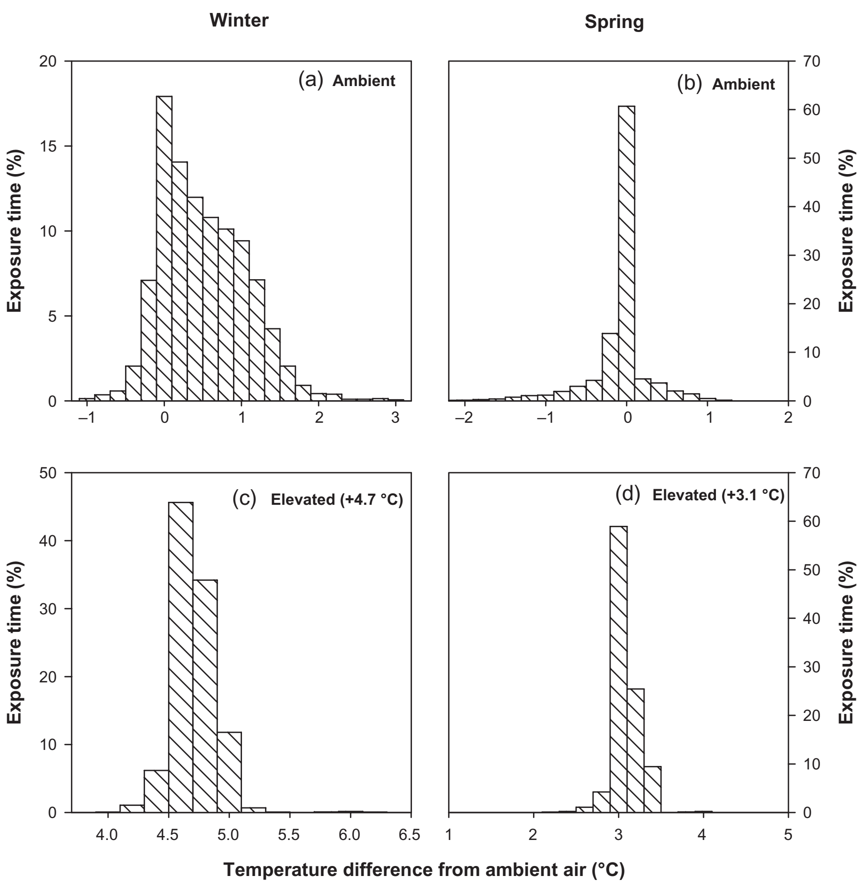

Figure 5. Frequency distributions of temperature in the ambient, T_A (a & b), and elevated, T_E (c & d), chambers for the measurement periods 14–28 February, and 3–17 May, 2002. Frequency distributions were calculated using temperature measurements from the six T_A chambers and the six T_E chambers measured at 42 min intervals. The target temperature elevations in February and May were +4.7 °C and +3.1 °C, respectively.

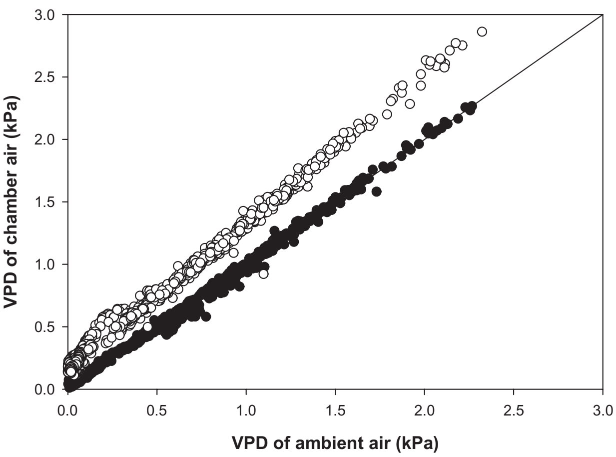

Figure 6. Water vapour pressure deficit (VPD) of an ambient temperature, T_A, chamber (filled symbols) and an elevated temperature, T_E, chamber (open symbols) by reference air VPD. Data from the time periods of 14–28 February and 3–17 May 2002 are pooled for each chamber.

transmittance levels of 88 and 89% were measured in the 800–1100 nm range for the old and new film, respectively.

The daily mean PPFD inside the WTCs was 21% less than that outside the WTCs for a 6 month period from 1 January to 30 June 2002 (Fig. 7).

WTC effects

An analysis of a range of structural characteristics and physiological parameters of trees in T_AC_A WTCs and R trees showed minimal WTC effect on tree performance and function (Table 4). Wood structure and chemistry of the T_AC_A trees were unaffected after 4 years of growth inside the WTCs. No significant differences were detected between the mean light-saturated photosynthetic rate, mean apparent quantum yield and the variable fluorescence of T_AC_A and R shoots during 2002. There were significant differences in phenology between T_AC_A and R trees during spring 2002, with a lower temperature sum required for bud burst for the T_AC_A trees (Table 4). There was a significant difference in the stable carbon isotope composition ($\delta^{13}C$) of the bulk needle material in the T_AC_A trees when compared with the $\delta^{13}C$ of the R trees, but not in starch (Table 4).

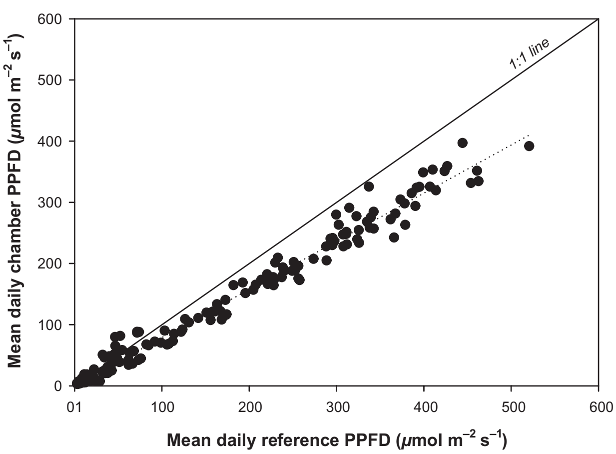

Figure 7. Relationship between the mean daily photosynthetic photon flux density (PPFD) reaching the fifth or sixth whorl from the top of reference and whole-tree chamber (ambient $[CO_2]$ only) trees for the period of 1 January–30 June 2002. The regression line was forced through the origin ($y = 0.79x$; $r^2 = 0.98$; $P < 0.001$).

Annual tree net CO_2 flux and response curves

There was a pronounced seasonal pattern of net CO_2 exchange for both the T_AC_A and T_EC_A trees during 2003 (Fig. 8). The T_EC_A tree showed slightly earlier spring recovery of photosynthetic activity during March–April and a later decline in photosynthetic activity during

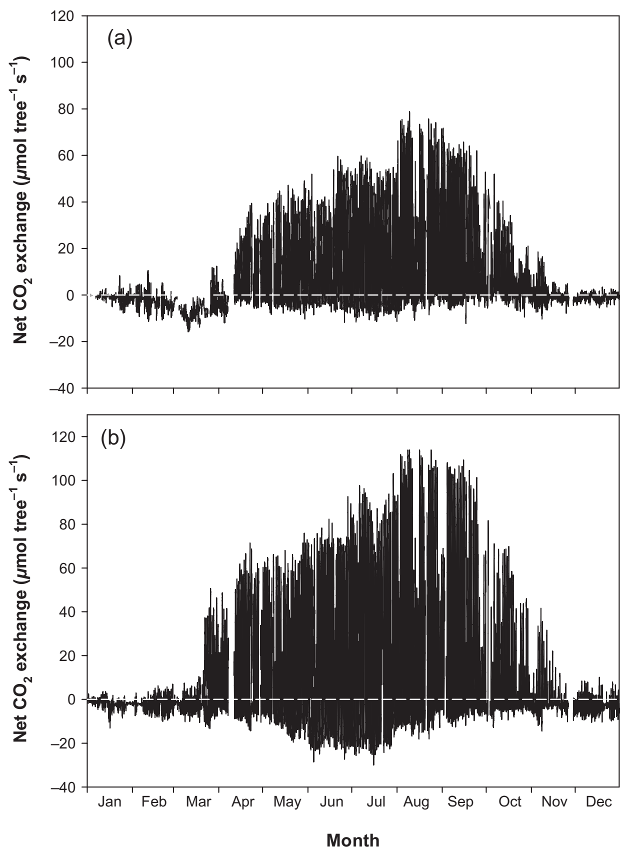

Figure 8. Annual course of net CO_2 exchange of a tree exposed to ambient temperature and $[CO_2]$, T_AC_A (a) and a tree exposed to elevated temperature and ambient $[CO_2]$, T_EC_A (b), throughout 2003. Values shown are the maximum daytime rates of net CO_2 uptake and night-time respiration, respectively. In July 2003 the stem diameter at breast height (1.3 m) was 84 and 117 mm for the T_AC_A tree and T_EC_A tree, respectively. Tree surface needle area was estimated using allometric relationships derived from an earlier destructive harvest at the Flakaliden site (B. Sigurdsson, Agricultural University of Iceland, unpublished results). The estimated needle areas were 13.0 and 23.3 m^2 for the T_AC_A and T_EC_A trees, respectively.

Table 4. Comparison of some structural characteristics and physiological parameters of Norway spruce trees grown in whole-tree chambers (WTC) at ambient [CO_2] and temperature (T_AC_A) and non-chambered reference (R)trees adjacent to the WTCs

Parameter	Unit	R tree	WTC-T_AC_A	*P*-value	Reference	Comment
Wood structure[a]						
Ring density	g cm^{-3}	0.45 ± 0.01	0.50 ± 0.03	Not significant	Kostiainen *et al.* (2004)	Rings from 2000
Wall thickness	μm	2.16 ± 0.13	2.35 ± 0.28	Not significant	Kostiainen *et al.* (2004)	Rings from 2000
Radial lumen	μm	35.43 ± 2.80	33.78 ± 1.39	Not significant	Kostiainen *et al.* (2004)	Rings from 2000
Tracheid length	mm	2.59 ± 0.56	2.73 ± 0.30	Not significant	Kostiainen *et al.* (2004)	Bulk sample 1998–2000
Wood chemistry						
Gravimetric lignin[a]	% of DM	28.44 ± 0.42	27.95 ± 1.28	Not significant	Kostiainen *et al.* (2004)	Rings from 2000
Cellulose[a]	% of DM	48.83 ± 2.27	48.69 ± 1.66	Not significant	Kostiainen *et al.* (2004)	Rings from 2000
Nitrogen[a]	% of DM	0.11 ± 0.02	0.10 ± 0.03	Not significant	Kostiainen *et al.* (2004)	Rings from 2000
$\delta^{13}C$	‰	–26.04 ± –0.70	–25.66 ± –0.84	0.58	J. Marshall (personal communication)	Rings from 2001[2]
$\delta^{13}C$	‰	–26.57 ± –0.61	–27.09 ± –0.33	0.26	J. Marshall (personal communication)	Rings from 2004
Needles						
Specific needle area[c]	cm^2 g^{-1}	26.8 ± 1.6	28.9 ± 0.6	*0.10*	G. Wallin (unpublished results)	Autumn 2001, 1-year-old needles[b]
Specific needle area[c]	cm^2 g^{-1}	30.2 ± 2.0	34.2 ± 4.8	*0.25*	G. Wallin (unpublished results)	Autumn 2004, 1-year-old needles
Needle length	mm	14.1 ± 1.1	13.1 ± 1.3	*0.41*	G. Wallin (unpublished results)	Autumn 2001, 1-year-old needles[b]
Needle length	mm	15.3 ± 2.0	13.8 ± 0.4	*0.25*	G. Wallin (unpublished results)	Autumn 2004, 1-year-old needles
$\delta^{13}C$-bulk material	‰	–26.88 ± –0.34	–27.78 ± –0.38	0.038	Comstedt *et al.* (2006)	August 2002, current-year needles
$\delta^{13}C$-starch	‰	–25.08 ± –0.49	–26.06 ± –0.64	0.10	Comstedt *et al.* (2006)	August 2002, current-year needles
Nitrogen	% of structural DM	1.3 ± 0.06	1.3 ± 0.15	0.70	Slaney (2006)	May 2002
Starch	% of structural DM	23.6 ± 1.8	25.2 ± 1.8	0.33	Slaney (2006)	May 2002
Phenology						
T_{sum} until bud burst	Degree days	408 ± 28	337 ± 16	0.019	Slaney (2006)	Spring 2002
Bud burst	Day of year	149 ± 1.7	142 ± 2.0	0.010	Slaney (2006)	Spring 2002
Shoot length	mm	105 ± 19	116 ± 60	0.77	Slaney (2006)	2002, current-year shoot
Gas exchange (shoots)						
A_{sat}	μmol m^{-2} s^{-1}	11.3 ± 0.6	10.7 ± 0.7	*0.32*	Slaney (2006)	June 2002
Φ	mol CO_2 mol^{-1}	0.053 ± 0.003	0.050 ± 0.001	0.08	Slaney (2006)	June 2002
F_V/F_M		0.83 ± 0.005	0.84 ± 0.006	0.15	Slaney (2006)	June 2002

All values are the mean of three trees ± 1 standard deviation. Unpaired Student's *t*-tests were used to test the statistical significance of differences between R trees and the T_AC_A-WTCs.
[a]From final harvest of first experiment, October 2000; [b]Developed before WTCs were installed; [c]Projected needle area and dry mass.
A_{sat}, light-saturated photosynthetic rate; *F*, mean apparent quantum yield; F_V/F_M, variable fluorescence; $\delta^{13}C$, mean stable carbon isotope composition of wood, needles and starch; T_{sum}, temperature sum (= 0 °C) accumulated from April 1; Bud burst, day of year when bud burst occurred; T_AC_A, ambient temperature and [CO_2].

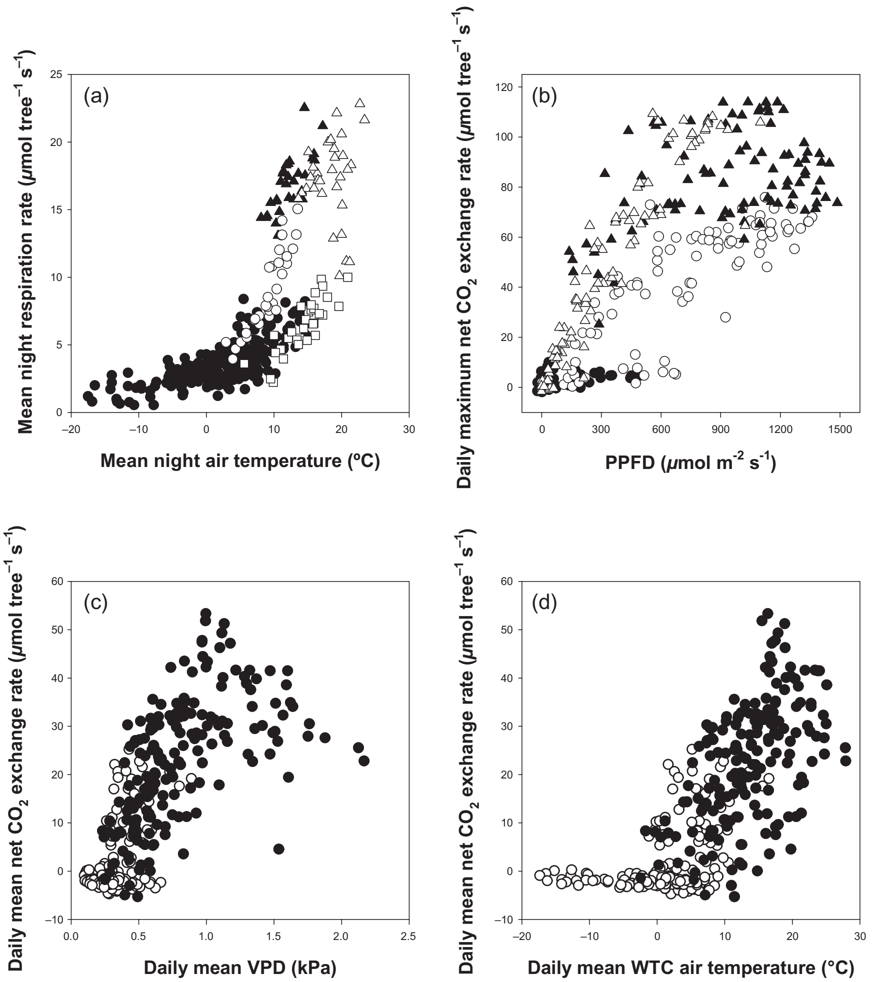

Figure 9. Four examples of tree-level response curves generated from one whole-tree chamber (WTC) with elevated-temperature and ambient [CO_2], T_EC_A, throughout 2003 (n = 362). (a) Mean night-time respiration rate (i.e. net CO_2 exchange when photosynthetic photon flux density (PPFD) < 25 μmol m^{-2} s^{-1}) as a function of mean night-time air temperature inside the WTC (○ = May, ▲ = June, △= July, □= August, ● = remainder of the year); (b) Maximum daily net CO_2 uptake rate as a function of PPFD (● = winter, ○= spring, ▲ = summer, △= autumn); (c) Mean daily net CO_2 uptake rate as a function of mean daily air vapour pressure deficit (VPD) inside the WTC (● = May–October, μ = remainder of the year); (d) mean daily air temperature inside the WTC (● = May–October, ○= remainder of the year). Tree surface needle area was estimated to be 23.3 m^2 (B. Sigurdsson, Agricultural University of Iceland, unpublished results).

October–November, when compared with the T_AC_A tree. The maximum tree net CO_2 uptake rate was higher for the T_AC_A tree (6.1 and 4.9 μmol m^{-2} s^{-1} for T_AC_A and T_EC_A, respectively) and the maximum uptake rates were measured during August night-time respiration rates were generally higher for the T_EC_A tree during the summer months of June and July 2003, as compared with the T_AC_A tree. The maximum respiration rate of the T_AC_A and T_EC_A trees during this period was 1.2 and 1.3 μmol m^2 s^{-1}, respectively. The cumulative respiration for the entire year was 17 and 19% of the cumulative photosynthesis for the T_AC_A and T_EC_A tree, respectively.

The responses of tree-level CO_2 flux to climatic variation throughout 2003 are shown in Fig. 9a–d (T_EC_A tree only). The mean night-time respiration rates increased exponentially with mean night-time air temperature, but there was an obvious seasonal shift in the dependence of respiration on temperature throughout the growing season from May to August (Fig. 9a). The calculated annual Q_{10} and R_0 values for the T_EC_A tree were 2.2 and 3.1 μmol $tree^{-1}$ s^{-1}, respectively. Calculating Q_{10} using data from each month gave a range from 1.5 to 3.3 for months where $r^2 > 0.5$. Calculating R_0 on a monthly time-step throughout the growing season (May–August) showed that respiration varied substantially throughout this period with R_0 values of 2.9, 10.5 and 1.6 μmol $tree^{-1}$ s^{-1} for May, June and August, respectively. There was similar magnitude in the seasonal variation of R_{15} with values ranging between 6.5 and 18.7 μmol $tree^{-1}$ s^{-1} for August and June, respectively. The change in daily maximum net CO_2 uptake with PPFD showed strong seasonal variation with successively increasing daily 'apparent' quantum yields from winter to autumn (Fig. 9b). Daily mean net CO_2 uptake by the T_EC_A tree reached maximal levels at VPDs between 1.0 and 1.5 kPa (Fig. 9c) and air temperatures of 17–18 °C (Fig. 9d).

DISCUSSION

The WTC system demonstrated a good level of control over the growth environment of enclosed trees and an ability to provide tree-level physiological data. The system addresses the pressing need to conduct long-term climate change experiments on mature trees (Saxe *et al.* 1998, 2001; Norby *et al.* 1999). In particular, the WTCs allow the study of large, field-grown trees responses to climate change in the long term. The closed-top nature of the WTCs provides for the continuous measurement of tree gas exchange and precise control over air temperature and

$[CO_2]$. This is a unique feature of the WTC system, as other systems are designed for long-term treatment rather than for combination with measurements of whole-tree gas exchange. As noted by Kellomäki *et al*. (2000), the main drawback of WTCs is the large cost associated with their design, construction and operation. In our opinion there is, however, no alternative to such an investment in terms of equipment and running costs required for long-term controlled manipulation experiments to examine the physiological and growth responses of large trees to climate change.

The WTC system was able to maintain set targets of $[CO_2]$ and air temperature within each chamber on a continuous basis. The energy cost associated with the accurate control of WTC air temperature is high, but an advantage of closed-top WTCs is that most of the air is recirculated, thus reducing the cost of CO_2 additions. The $[CO_2]$ of the C_A WTCs were approximately +20 μmol mol^{-1} above the target of 365 μmol mol^{-1} during the 2-week winter assessment period (Table 2). This was caused by the addition of ambient air to the chambers that had a $[CO_2]$ greater than target value of 365 μmol mol^{-1}. In retrospect it can be concluded that to be able to control the target value of $[CO_2]$ in C_A WTCs during winter, and to compensate for night-time respiration during summer, the target $[CO_2]$ should not have been the current annual mean of 365 μmol mol^{-1}, but 20 μmol mol^{-1} higher, which is close to the annual fluctuations of $[CO_2]$ in high northern latitudes (Keeling, Chin & Whorf 1996). An alternative approach would be to alter the system setup so that the $[CO_2]$ of the C_A WTCs tracks ambient air, rather than being controlled to a fixed $[CO_2]$. We do not consider the deviation of 20 μmol mol^{-1} above the target (< 10%), which occurred during a period of tree dormancy, to carry any significant physiological consequences for the trees inside the C_A WTCs. Control over $[CO_2]$ inside C_A WTCs was improved during the spring assessment period when lower ambient $[CO_2]$ and tree uptake of CO_2 permitted better control by the WTC system. Incursions over the set $[CO_2]$ of 365 μmol mol^{-1} were generally confined to night time when tree respiration rates exceeded tree CO_2 uptake rates. The C_E WTCs displayed precise and accurate control over $[CO_2]$, particularly during the winter assessment period. The ability of the system to maintain tight control over $[CO_2]$ can be attributed to the high frequency of WTC $[CO_2]$ measurement (every 90 s) and the injection of CO_2 directly into the circulating air stream from the cooling unit. This facilitated good mixing of the CO_2 and prevented vertical $[CO_2]$ gradients from developing. The maintenance of set $[CO_2]$ is a vital element of any climate change experiment where CO_2 is included. In this regard, the WTC system has provided reliable and accurate control.

A lack of temperature control has been a major limitation of many approaches used for exposure of plants to elevated $[CO_2]$ (cf. Saxe *et al*. 1998). Approaches such as branch bags (e.g. Barton *et al*. 1993; Kellomäki & Wang 1997) and open-top chambers (e.g. Whitehead *et al*. 1995; Vapaavuori *et al*. 2002) have been hampered by uncontrolled temperature increases and the confounding effect they have on the tree responses to the primary variable of interest, for example $[CO_2]$. The design of the WTC system overcomes this problem by providing a high degree of temperature control. In addition, this system offers controlled temperature elevation, allowing important insights into the interactive effects of $[CO_2]$ and temperature on tree functioning. At Flakaliden, a high level of temperature control in the spring assessment period was demonstrated with target temperatures inside both the ambient and T_E WTCs within ± 0.5 °C for > 89% of the time. This level of control is an improvement on other closed-top chamber systems (Kellomäki *et al*. 2000; Urban *et al*. 2001). Temperature control was reduced during the winter because of a deliberate reduction in the cooling capacity of the refrigeration unit. The Flakaliden site is characterized by extremely low winter temperatures (below –30 °C). The accompanying lack of air humidity would have caused an excessive workload and possible damage to the refrigeration unit if full capacity had been maintained during this period. The reduction in cooling capacity during the winter season would not be necessary in warmer climates. Despite the reduction in capacity, the WTC temperatures during the winter assessment period were within ± 2 and ± 1 °C of the target temperature in the ambient and T_E WTCs, respectively. Short day lengths and low incident light levels during the winter at Flakaliden also minimized radiation warming inside the WTCs. The precise nature of the temperature control for the WTC system gives confidence in the ability of the system to reproduce the temperature conditions outside the WTCs and provides the basis for a realistic assessment of the responses of trees to climate change.

Any air humidity changes caused by tree chambers can affect the stomatal function and transpiration patterns of an enclosed conifer tree more than changes in $[CO_2]$ (cf. Medlyn *et al*. 2001). The WTC system was able to respond quickly to changes in reference air vapour pressure and this fast response time, coupled with the large capacity of the cooling system meant that the vapour pressure of the WTC air was generally the same as that of the reference air (cf. Fig. 6). This meant that the moisture generated by the transpiring tree inside each WTC was condensed from the system rapidly enough so as not to cause feedback influences on transpiration rates. By virtue of the fact that all 12 WTCs were maintained at the same vapour pressure as reference air, the VPD of T_E WTCs was greater than that of the reference air and the T_A WTCs. The decision to maintain all WTCs at reference air VPD was made for two reasons. Firstly, to run the ambient and T_E WTCs at identical VPD would have required two separate cooling systems. For logistical and financial reasons, this was not a feasible option. Secondly, the possibility of increased surface temperatures and potential evaporation not being matched by increased precipitation in some regions has been shown with some global climate change models (Cubasch *et al*. 2001). We acknowledge, however, that greater VPD in the elevated WTCs may influence the interactions between

$[CO_2]$ and temperature for some physiological processes. It is worth noting that a study on the stable $\delta^{13}C$ of the WTC trees at Flakaliden found no difference in the $\delta^{13}C$ of needle soluble sugars, starch and bulk material of T_AC_A and T_EC_A trees (Comstedt *et al*. 2006). This result suggests that the VPD disparity between T_A and T_E treatments had no major effect on stomatal behaviour. This is supported by the similar $\delta^{13}C$ of T_AC_A and T_EC_A wood rings laid down during the course of the experiment (Marshall J., University of Idaho, unpublished results).

The transmittance of radiation through the plastic material of the WTC walls was similar to that reported for other chamber systems (e.g. Whitehead *et al*. 1995; Kellomäki *et al*. 2000; Vapaavuori *et al*. 2002). The decline in radiation transmission with age of plastic was less than that observed by Whitehead *et al*. (1995), despite a greater plastic age and a harsher climate, in terms of temperature fluctuations in the present study. The reduction in PPFD entering the WTCs was, on average, 21% throughout a 6 month period. The difference between the measured reduction in transmittance of the plastic (16%) and the PPFD measured reduction was probably a result of the reflectance and shade from the frame of the WTCs. The aluminium structural components of the WTCs would be expected to cause a degree of shading inside the WTC. The shading effect of each WTC frame changed throughout the course of the day and within season with changing solar angles. The cooling units of each WTC were positioned to minimize shading of both the tree inside the WTC and other nearby WTCs.

There was minimal effect of the WTC structure on the behaviour of the enclosed trees for a range of physiological parameters and structural characteristics (cf. Table 4). There were significant differences in timing of bud burst between R and T_AC_A trees in 2002 and 2004, but not in 2003 (Slaney *et al*. in review). The difference between WTCs and R trees in timing of bud burst was attributed to the WTCs reducing the risk of radiation frosts each year, both as an effect of the plastic cover and the continuous air movement in the WTCs (approximately 0.5 m s^{-1}). The variation in WTC effect on bud break between years has been attributed to the differences in the number and intensity of radiation frosts each year (Slaney *et al*. in review). The reduction in PPFD levels (21%) inside the WTC is likely to reduce the level of photoinhibitory damage to the photosynthetic apparatus sustained by the needles during the period of spring photosynthetic recovery. The $\delta^{13}C$ of wood rings provides a long-term indication of treatment-driven changes in stomatal conductance and/or intercellular $[CO_2]$. The similar $\delta^{13}C$ of wood rings in the T_AC_A and R trees (Table 4) suggests that the WTCs did not alter needle anatomy or physiology. The observed difference in $\delta^{13}C$ of needle bulk material between T_AC_A and R trees was probably a result of the fact that the isotopic composition of the source CO_2 of the C_A chambers (bottled CO_2) was depleted (−10.4‰) compared with air outside the chambers (−8‰) (Comstedt *et al*. 2006). Kellomäki *et al*. (2000) reported significant chamber effects on some physiological parameters for their closed CO_2 and temperature-controlled chamber system used on field-grown Scots pine (*Pinus sylvestris* L.). They attributed these differences to the 34–45% reduction in PPFD levels in the chambers (Kellomäki *et al*. 2000). The PPFD reduction in the present study was less than this range (21% averaged across 6 months), which may explain why significant differences were not observed.

The measurement of the gases entering and leaving the WTCs and their closed-top nature allows the WTCs to be treated as large cuvettes and the tree-level fluxes of CO_2 and water to be calculated. To the best of our knowledge, this paper presents the first results of the annual time course of CO_2 fluxes for the above-grown portion of a field-grown tree. Drawing conclusions from the physiological data presented in the present paper is beyond the scope of the discussion as data is presented to illustrate the performance and potential use of the WTC system rather than drawing definitive conclusions regarding the impact of elevated temperature and $[CO_2]$ on boreal Norway spruce trees. Moreover it serves to illustrate the ability of the WTC system to quantify the CO_2 flux over an annual time course and overcome the difficulties involved in scaling from discontinuous measurements to tree-level and/or stand estimates (cf. Wallin *et al*. 2001; Damesin *et al*. 2002).

There is potential for applying the data from the WTCs to the development and validation of predictive models. In particular, the data has great utility in validating the scaling of shoot-level flux measurements to estimates of tree-level fluxes. Tree-level measurements provide an integration of measurements of individual tree organs such as shoots and stems. For example, the *P. abies* tree-level Q_{10} value calculated in the present paper was greater than mean values reported for *P. abies* stems at Flakaliden (Ceschia 2001) and less than that of *P. abies* needles during winter at Flakaliden (Stockfors & Linder 1998b). The ability to continuously measure gas fluxes of mature trees is an advantage of the WTC system. The data also allows the physiological behaviour of trees to be examined on a range of temporal scales in response to climatic variation and the calculation of additional useful parameters such as above-ground net primary production.

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APPENDIX I

Component	Description	Manufacturer
Chamber wall	0.4 mm transparent PVC film	Renolit AG, Germany, <http://www.renolit.com>
Chamber base circulation fan	Model TD-160	Soler & Palau, Spain, <soler-palau.com>
Soil moisture sensor	Thetaprobe ML1	Delta-T Devices Ltd. UK, <http://www.delta-t.co.uk>
Safety ventilation system motor	Model SM24	Belimo, Switzerland, <http://www.belimo.com>
Safety ventilation system diesel generator	Model YDG 3700E-E	Yanmar, Japan, <http://www.yanmar.co.jp>
Cooling unit circulating fan	LPMA 6–060	Swegon, Sweden, <http://www.swegon.com>
Regulating iris for fresh air inlet	IRIS-125	REC-Indovent AB, Sweden, <http://www.rec-indovent.se>
Differential air pressure sensor	Model LP	Honeywell, Data Instruments, USA, <http://www.honeywell.com>
Main CO_2 line pressure regulator	Model 8624	Bürkert, Germany, <http://www.burkert.com>
Chamber infrared CO_2 gas analyser	SBA-1	PP Systems, UK, <http://www.ppsystems.com>
Chamber CO_2 injection magnetic valve	Model 6011	Bürkert, Germany, <http://www.burkert.com>
Chamber CO_2 mass flow meter	AWM3000; AWM5000	Honeywell, USA, <http://www.honeywell.com>
Flow calibrator	DryCal DC-1	Bios International, USA, <http://www.drycal.com>
Central infrared CO_2 gas analysers	CIRAS-2 DC; WBA-2	PP Systems, UK, <http://www.ppsystems.com>
Quantum sensor	LI-190SA	Li-Cor Inc., USA, <http://www.licor.com>
Cooling unit damper actuator	*ASU1d15*	Staefa Control, USA, <http://www.staefacontrol.com>
I/O modules & converter	SIOX S12; K30 RS232C-SIOX	Telefrang AB, Sweden, <http://www.telefrang.se>
Sample gas magnetic valve control system	M-331-c-04	Bürkert, Germany, <http://www.burkert.com>