Whole-tree chambers for elevated atmospheric CO₂ experimentation and tree-scale flux measurements in south-eastern Australia: the Hawkesbury Forest Experiment

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Abstract (277 words)

Resolving ecophysiological processes in elevated atmospheric CO₂ (Cₐ) at scales larger than single leaves poses significant challenges. Here, we describe a field-based experimental system designed to grow trees up to 9 m tall in elevated Cₐ with the capacity to control air temperature and simultaneously measure whole-tree gas exchange. In western Sydney, Australia, we established the Hawkesbury Forest Experiment (HFE) where we built whole-tree chambers (WTC) to measure whole-tree CO₂ and water fluxes of an evergreen broadleaf tree, *Eucalyptus saligna*. A single *E. saligna* tree was grown from seedling to small tree within each of 12 WTCs; six WTCs were maintained at ambient Cₐ and six WTCs were maintained at elevated Cₐ, targeted at ambient Cₐ + 240 μmol mol⁻¹. All 12 WTCs were controlled to track ambient outside air temperature (T_air) and air water vapour deficit (D_air). During the experimental period, T_air, D_air and Cₐ in the WTCs were within 0.5 ºC, 0.3 kPa, and 15 μmol mol⁻¹ of the set-points for 90% of the time, respectively. Diurnal responses of whole-tree CO₂ and water vapour fluxes are analysed, demonstrating the ability of the tree chamber system to measure rapid environmental responses of these fluxes of entire trees. The light response of CO₂ uptake for entire trees showed a clear diurnal hysteresis, attributed to stomatal closure at high D_air. Tree-scale CO₂ fluxes confirm the hypothesised deleterious effect of chilling night-time temperatures on whole-tree carbon gain in this subtropical *Eucalyptus*. The whole-tree chamber flux data add an invaluable scale to measurements in both ambient and elevated Cₐ and allow us to elucidate the mechanisms driving tree productivity responses to elevated Cₐ in interaction with water availability and temperature.
1. Introduction

Atmospheric CO$_2$ concentration ($C_a$) has risen from 280 $\mu$mol mol$^{-1}$ to the current concentration of ca. 390 $\mu$mol mol$^{-1}$ over the last 150 years, and continues to rise at a rate of 15 – 20 $\mu$mol mol$^{-1}$ per decade (Canadell et al., 2007). Exposure to elevated $C_a$ generally stimulates tree growth (Curtis and Wang, 1998; Norby et al., 1999), increases (20 - 80%) leaf level light-saturated photosynthesis ($A_{sat}$; reviewed in Ellsworth et al., 2004; Ainsworth and Rogers, 2007), decreases leaf-level stomatal conductance ($g_s$; Berryman et al., 1994; Medlyn et al., 2001; Ainsworth and Rogers, 2007), and subsequently increases leaf-level water use efficiency (WUE; Field et al., 1995; Wullschleger et al., 2002; Morgan et al., 2004). Although we have excellent techniques for directly measuring gas exchange in single leaves of plants exposed to elevated $C_a$, few experimental systems resolve gas exchange in elevated $C_a$ at larger scales (Wallin et al., 2001; Dore et al., 2003). Ecophysiological schemes for scaling leaf-level behaviour to larger scales can only approximate CO$_2$ and water fluxes at the whole-tree level. In order to validate such models, we require a system to measure whole-tree fluxes of CO$_2$ and water and their response to the environment.

A wide range of experimental systems has been developed to expose plants to elevated $C_a$, including growth chambers, glasshouses, open-top chambers, field- and laboratory-based mesocosms, and most recently field-based free air CO$_2$ enrichment (FACE) facilities (e.g. Drake et al., 1989; Barton et al., 1993; Whitehead et al., 1995; Griffin et al., 1996; Hendrey et al., 1999). Air temperature may also be controlled in all of these experimental systems except FACE. However, larger trees (>2 - 3 m tall) may only be grown in the field in open-top chambers and FACE, and neither facility is capable of simultaneously exposing trees to elevated $C_a$ and measuring whole-tree fluxes of CO$_2$ and water. On the other hand, whole-tree chambers (WTC) can precisely maintain elevated $C_a$ and air temperature ($T_{air}$) for trees up to 9 m tall whilst growing in the field, and can resolve whole-tree CO$_2$ and water vapour fluxes (Medhurst et al., 2006). In addition, the cost of building and maintaining WTCs is substantially less than that of FACE (Saxe et al., 1998).
Here we describe a unique field-based experimental system designed to grow trees up to 9 m tall in elevated $C_a$ with the capacity to control $T_{air}$ and simultaneously measure whole-tree gas exchange. In western Sydney (Australia), we established the Hawkesbury Forest Experiment (HFE) where we installed WTCs to measure whole-tree CO$_2$ and water fluxes in an evergreen broadleaf tree, *Eucalyptus saligna* Sm.. A single *E. saligna* tree was grown from seedling to 6.5 m tall within each of 12 WTCs for more than one year. Six WTCs were maintained at ambient CO$_2$ ($C_a$ tracked outside conditions) and six WTCs were maintained at elevated CO$_2$ (ambient $C_a$ + 240 μmol mol$^{-1}$). All 12 WTCs were controlled to track ambient outside $T_{air}$ and air water vapour deficit ($D_{air}$). Chamber performance characteristics are described in addition to the impact of variation in daily light ($Q$) and $D_{air}$ on whole-tree fluxes of CO$_2$ and water.

2. Materials and methods

2.1. Site and experiment description

The Hawkesbury Forest Experiment (HFE) site is situated on an alluvial floodplain near the Hawkesbury River in western Sydney (Australia) at 25 m a.s.l. elevation (33°36’40” S, 150°44’26.5” E). The 5 ha HFE was established in a paddock, which had been converted from native pasture grasses more than a decade earlier. In the HFE, 2000 trees of *E. saligna* and 2000 trees of *E. sideroxylon* were planted at a stocking rate of 1000 trees ha$^{-1}$ (2.6 x 3.85 tree spacing) in April 2007. Soils at the HFE are in the Clarendon Formation (Chromosol; Isbell, 1996), an alluvial formation of low-fertility sandy loam soils (top 70 cm) with low organic matter content (0.7%), moderate to low fertility (available P, 8 mg kg$^{-1}$; exchangeable cations K 0.19; Ca 1.0; Mg 0.28 meq 100g$^{-1}$) and low water holding capacity. There is a partially cemented hard layer with numerous manganese nodules (70 - 100 cm), and a clay layer (below 100 cm). Permanent ground water is a minimum of 15 m below the soil surface.

The climate in the region where the experiment was conducted is sub-humid temperate. Mean annual temperature at this location is 17 °C, with a mean maximum temperature of the hottest month of 29 °C, and mean minimum temperature of the coldest month of 3 °C. Frost events occur an average of 13 times annually (Australian Bureau of Meteorology; www.bom.gov.au).
The long-term mean annual rainfall is 801 mm, with 1st and 9th deciles for rainfall of 528 and 1075 mm, respectively. The wettest months are typically in the summer (November and February) and the driest months are in the winter (July and August); however, inter-annual variation is very large. The mean ratio of annual precipitation to potential evapotranspiration (FAO-56; Food and Agriculture Organization of the United Nations) is approximately 0.6.

2.2. Whole-Tree Chambers

Twelve whole-tree chambers (WTCs; Fig. 1), previously used in an elevated $C_a$ experiment in a boreal forest in Sweden (Medhurst et al., 2006), were shipped to Australia and installed at the HFE in July 2006. Within each WTC, one 30 cm tall, six month old seedling of *E. saligna* (provenance Styx River, NSW; seedlot 20752 CMA from the Australian Tree Seed Centre, Clayton South, Vic., Australia) was planted in April 2007 and supplied with an initial fertilisation of 50 g of $(NH_4)_2PO_4$ and 10 mm of water every 3rd day to ensure good establishment. Six WTCs were operated to track ambient $C_a$ and six WTCs were operated at elevated $C_a$ (ambient $C_a + 240 \mu$mol mol$^{-1}$), while all 12 WTCs controlled $T_{air}$ to maintain ambient outside conditions. A treatment target $C_a$ of $+240 \mu$mol mol$^{-1}$ was chosen to be similar to $C_a$ used in recent free-air CO$_2$ enrichment experiments, and is anticipated in *ca.* 50 years (Pacala and Sokolow, 2004; IPCC Special Report 2001 at http://www.grida.no/climate/ipcc/emission/).

In addition, six control plots were established that were identical to the WTC plots, but without WTCs. Comparison of tree growth and leaf level gas exchange measured on these trees with similar measurements on the ambient chambered trees enables the assessment of any potential chamber effects.

We briefly outline the WTC system and highlight modifications to the WTCs, which were originally designed for relatively cool summers in Sweden (mean maximum temperature of the warmest month of 19°C) and required modification to operate under much hotter Australian conditions (> 40°C in the summer; mean maximum temperature of the warmest month of 29°C). The WTCs are made of a cylindrical aluminum framework 3.25 m in diameter topped with a cone. The WTCs are modular in design and additional cylinders 3.25 x 2.5 m can be installed to increase the internal height from 6.5 m to 9 m (Fig. 1). Each section was initially covered with clear PVC (300 $\mu$m thick; Renolit AG, Germany) which transmitted 89% of incident light. The
PVC was replaced in August 2008 with a self-cleaning, ultra-thin Ethylene-Tetrafluoroethylene (ETFE) co-polymer film with a high transmission for UV as well as visible light (93 - 94% when new) (F-Clean, AGC Singapore Chemicals Pty. Ltd.). The wall covering extended into the ground and was buried just to the outside of a root barrier, which was composed of heavy duty polyethylene (300 μm thick) that extended to 1 m and into the deeper clay soil. Therefore, the majority of roots and associated CO\textsubscript{2} fluxes in the top 1 m of soil were associated with the single experimental tree planted at the centre of each WTC.

A clear PVC floor (400 μm thick) was installed 45 cm above the ground surface, creating an under-floor volume of ca. 4 m\textsuperscript{3} (Fig. 1 (A)). The under-floor space was ventilated with a fan blower at the rate of approximately 4 - 8 m\textsuperscript{3} per minute, but the space could be sealed to monitor soil CO\textsubscript{2} efflux. Trees were watered regularly during establishment with 10 mm of water every third day. Seven 90° spray nozzles mounted under the floor delivered a uniform coverage of irrigation water to the 10 m\textsuperscript{2} of soil surface contained within the underfloor space. Irrigation was under control of the central computer and accurate to ±0.1 mm.

The temperature control system consisted of a central refrigeration plant that cooled a glycol/water solution to slightly below (1 - 2 °C) the dew-point temperature of the ambient air. The coolant was delivered to each WTC, where it circulated through a large surface area heat exchanger (2 m x 1 m) mounted in housing on the south side of the WTC. WTC air was continuously circulated through the housing by a frequency controlled fan (Swegon, Kvanum, Sweden) at a rate that could be regulated between 0 and 12,000 m\textsuperscript{3} hr\textsuperscript{-1}. Variable baffles regulated by a microprocessor controller in each WTC diverted a portion of the air through the heat exchanger, where it was cooled to the temperature of the coolant before re-entering the WTC (Fig. 1). Excess moisture in the airstream, resulting from transpiration by the tree, was condensed, and then collected and measured using a small tipping bucket pluviometer with a 5 mL resolution (Rain-o-matic, Pronamic, Denmark).

2.3. Chamber CO\textsubscript{2} and water flux measurements
Each WTC was operated as a hybrid between an open-mode and null-balance gas exchange system (Medhurst et al., 2006). Air volume in the WTC was 30 m\textsuperscript{3} with a continuous supply of
fresh air entering the WTC at a rate of 10 L s\(^{-1}\). A manually adjustable iris orifice allowed
adjustment of the flow of air while a digital manometer constantly monitored the pressure drop
across the orifice, and thus allowed continuous measurement of the airflow. Pure CO\(_2\) was
metered into this air stream to maintain the chamber at its target \(C_a\); hence, the null-balance
aspect of whole-tree gas exchange. Air was continuously sampled from each WTC and from a
reference line mounted 5 m above the ground, and transported through heated tubing to a
manifolded set of 13 three-way solenoid valves, eventually reaching the central infra red gas
analyser (IRGA; Licor 7000, Li-Cor Lincoln, Nebraska) in the control cabin. The fast-response
time of this IRGA allowed the sampling time period for each WTC to be reduced from 90 s
(Medhurst et al., 2006) to 60 s. A full cycle of measurements, including all 12 WTCs and two
reference readings, took 14 minutes; whole-tree CO\(_2\) and H\(_2\)O fluxes were calculated every
cycle.

In addition to a standard meteorological station at the HFE, a separate set of sensors was
connected to the WTC central control system including a tipping bucket rain gauge (RG2,
Monitor Sensors, QLD, Australia), quantum sensor (LI-190SA, Li-Cor, Inc., USA) and
combined air temperature and relative humidity sensor (MP101A, Rotronic Bassersdorf,
Switzerland) contained within a ventilated, shielded housing mounted within the tree stand
surrounding the WTCs to provide a reference temperature for the WTCs. These sensors were
sampled and recorded at one minute intervals.

WTCs were individually instrumented with radiation shielded, ventilated thermistors (10kOhm
NTC Accu-Curve, RTI Electronics, Inc, CA, USA), a soil temperature sensor and a soil moisture
probe (Theta Probe, Delta T Instruments) at 10 cm depth, a neutron probe access tube installed to
4.75 m, and a PVC access tube installed to 2 m depth to house frequency-domain reflectometer
sensors at four depths which were logged every half hour (Sentek EnviroSCAN, Sentek Sensor
Technologies, Stepney, S.A., Australia). In addition, two mini-rhizotron tubes were installed at
45° at two distances from the main tree stem in each WTC and used to track root dynamics in the
upper 80 cm of soil. See Medhurst et al., 2006 for a complete list of parameters measured and
recorded by the system.
2.4 Whole-tree CO$_2$ and H$_2$O flux measurements and calculations

The CO$_2$ flux was calculated from the mass balance of CO$_2$ entering and leaving the chamber as a result of the chamber ventilation, the pure CO$_2$ added to maintain the C$_a$ at the target, and any change in storage of CO$_2$ in the volume of air in the chamber during the measurement cycle.

$$A_{\text{tree}} = I + F - V - \Delta S$$  \hspace{1cm} (1)

where $A_{\text{tree}}$ is the instantaneous net CO$_2$ flux from the tree, $I$ is the injection rate of pure CO$_2$ into the chamber, $F$ is the flow of CO$_2$ carried into the chamber in the fresh air stream, $V$ is the flow of CO$_2$ carried out of the chamber as a result of the fresh air entering, and $\Delta S$ is the change in storage of CO$_2$ in the air mass within the chamber volume during the measurement cycle.

The H$_2$O flux from the tree ($E_{\text{tree}}$) is similarly calculated as the mass balance of water vapour entering and leaving the chamber as a result of the chamber ventilation, taking into account any change in storage of water in the air within the chamber and the amount of water condensed by the cooling system. Calculations for $E_{\text{tree}}$ follow those of Medhurst et al. (2006) apart from the following modifications.

We computed the density of fresh air entering the chamber ($\rho_{\text{ref}}$, kg m$^{-3}$) as

$$\rho_{\text{ref}} = \frac{P_{\text{atm}} - H_{\text{ref}}}{R \cdot T_{\text{ref}}} + \frac{H_{\text{ref}}}{R_v \cdot (T_{\text{ref}})}$$  \hspace{1cm} (2)

where $P_{\text{atm}}$ is the mean atmospheric pressure throughout the measurement cycle (Pa), $R$ the gas constant for dry air (287.05 J K$^{-1}$ kg$^{-1}$), $T_{\text{ref}}$ the mean temperature of the reference air during the measurement cycle (K), $H_{\text{ref}}$ (Pa) the water vapour pressure of the reference air during the measurement cycle and $R_v$ the specific gas constant for water vapour (461.495 J kg$^{-1}$ K$^{-1}$).

The volumetric flow of fresh air entering the chamber ($F_{\text{in}}$, L s$^{-1}$), corrected to standard temperature and pressure was then calculated as
Where ΔP is the pressure drop across the iris valve in the fresh air delivery tube (Pa), \( k \) is the flow coefficient for that iris, and \( P_{ref} \) standard atmospheric pressure (101.3 kPa). The flow coefficients were checked periodically by measuring temperature, atmospheric pressure and pressure drop across the iris and determining the flow rate of fresh air by measuring the time taken to fill a large lightweight bag (900 L) then inverting equation 3 to find \( k \). This parameter was found to be stable over time as long as the iris setting was not changed. To satisfy conservation of mass, the standardised flow of air leaving the chamber (\( F_{out} \), L s\(^{-1}\)) was calculated as the standardised flow of air entering the chamber corrected for any change in moisture content according to:

\[
F_{out} = F_{in} \frac{P_{atm} - H_{ref}}{P_{atm} - H_{wtc}}
\]

where \( H_{wtc} \) is the average water vapour pressure of air in the chamber during the measurement cycle (Pa).

We operated the WTCs at a positive pressure of ca. 20 Pa in order to minimise gas leaks through gasket-sealed openings (e.g. door, emergency ventilation dampers, condensate tube, floor seal around tree trunk, etc.). However, this positive pressure differential may be insufficient to prevent inward diffusion of specific gases (e.g. CO\(_2\)) when concentration gradients are large. Furthermore, slight errors in the calibration of fresh air-flow, or in mass flow meters used to monitor CO\(_2\) entering the WTC, would lead to errors in the calculation of whole-tree carbon fluxes. In order to assess such errors, the \( C_a \) set point was alternated for each WTC every second night. Ambient WTCs were raised to ambient + 240 μmol mol\(^{-1}\) and the CO\(_2\) supply was turned off to elevated WTCs from 2100 h to 0400 h. Assuming that respiration is insensitive to short term changes in \( C_a \) (Tissue et al., 2002; Gonzales-Meler et al., 2004), any change in the calculated CO\(_2\) flux in response to altering WTC \( C_a \) can be attributed to leaks or errors in the calibration of the fresh air flow or CO\(_2\) mass flow sensors. When \( C_a \) is changed from elevated to
ambient $C_a$, the supply of CO$_2$ is instantaneously turned off and WTC $C_a$ gradually declines by dilution from the fresh air entering the WTC. Thus, a sudden step change in apparent respiration rate would indicate an error in calibration of the mass flow sensor, while a gradual change (proportional to the change in $C_a$ between inside and outside the WTC) indicates either incorrect calibration of the fresh air flow or a diffusion leak, which can be mathematically corrected. In the case of a diffusion leak, the correction is by calculation of a leak rate that is proportional to the $C_a$ concentration gradient between the inside and outside of the chamber:

$$ P_{corr} = P + ([CO_2]_{WTC} - [CO_2]_{Ref}) \cdot l $$  \hspace{1cm} (5)

where $l$ is the chamber specific leak constant (mol s$^{-1}$) and $[CO_2]_{WTC}$ and $[CO_2]_{Ref}$ are the chamber and outside reference CO$_2$ concentrations ($\mu$mol mol$^{-1}$), respectively. Fresh air flows were calibrated on a regular basis and checked, as described above, when an apparent leak/calibration error was observed.

2.5. Analysis of WTC flux data

A brief analysis of the CO$_2$ and water flux data is provided to demonstrate the tractability of the measurements and the potential utility of the whole-tree data. The CO$_2$ fluxes were analysed for two trees growing in ambient $C_a$ over a 160-day period from late February to August 2008, which corresponded to late summer through winter. We analysed responses in terms of the key environmental drivers, which were incident photosynthetically active radiation (400 - 700 nm; $Q_{in}$), $T_{air}$ and $D_{air}$.

Based on leaf-level gas exchange data, we expect that canopy CO$_2$ fluxes should increase and saturate with increasing light levels, but that stomatal closure at high $D_{air}$ reduces the light-saturated CO$_2$ uptake rates (Eamus et al., 1995; Kirschbaum, 2000). To test these responses, we fitted a non-rectangular hyperbola with a $D_{air}$ modifier function to typical diurnal light responses of chamber CO$_2$ flux, using SAS PROC NLIN (SAS Institute Inc., Cary, NC, USA.). The non-rectangular hyperbolic equation fitted was as follows:
\[
A_c = \frac{\alpha \cdot Q_{in} + A_{C_{\text{max}}} - \sqrt{(\alpha \cdot Q_{in} + A_{C_{\text{max}}})^2 - 4 \cdot \alpha \cdot A_{C_{\text{max}}} \cdot Q_{in} \cdot \theta}}{2 \cdot \theta} - R_d
\] (6)

where

\[A_C\] = net assimilation rate of the crown and whole aboveground portion of the tree, \(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ ground s}^{-1}\),

\[Q_{in}\] = incident photosynthetic photon flux density received by the tree, \(\mu\text{mol m}^{-2} \text{ ground s}^{-1}\),

\[A_{C_{\text{max}}}\] = asymptotic or \(Q_{in}\)-saturated net assimilation rate, \(\mu\text{mol m}^{-2} \text{ s}^{-1}\),

\[\alpha\] = initial slope of the response curve, which represents the canopy light utilization efficiency

\[\theta\] = convexity of the response curve (dimensionless, between 0 and 1), and

\[R_d\] = dark respiration rate of the aboveground portion of the tree, \(\mu\text{mol m}^{-2} \text{ s}^{-1}\),

The \(D_{\text{air}}\) modifier function, taken from Lasslop et al. (2010), was as follows:

\[
A_{C_{\text{max}}} = \begin{cases} 
A_{C_{\text{max}0}} \exp(-k(D_{\text{air}} - D_{\text{air0}})), & D_{\text{air}} > D_{\text{air0}} \\
A_{C_{\text{max}0}}, & D_{\text{air}} \leq D_{\text{air0}}
\end{cases}
\] (7)

where \(A_{C_{\text{max}0}}\) is the maximum assimilation rate in the absence of a limitation by \(D_{\text{air}}\), \(D_{\text{air0}}\) is the threshold vapour pressure deficit at which assimilation begins to be affected by \(D_{\text{air}}\) and \(k\) is the exponent. The WTC data support the use of such a functional relationship where a threshold of about 1 kPa was observed before \(A_{C_{\text{max}}}\) started to reduce in a fairly linear way, which was described well using \(k=0.25\) (data not shown).

3. Results

3.1. Chamber environmental control: \(C_{as}, T_{\text{air}}, \text{and } D_{\text{air}}\)
The WTC $C_a$ control algorithm was modified from Medhurst et al. (2006) to track outside ambient $C_a$ rather than using a constant $C_a$ because of a substantial natural diurnal $C_a$ variation at the HFE: daytime outdoor $C_a$ was ca. 380 μmol mol$^{-1}$, but night-time $C_a$ could be > 500 μmol mol$^{-1}$ depending on wind-speed and time of year. The target $C_a$ was maintained ± 15 μmol mol$^{-1}$ of the target $C_a$ 90% of the time (Fig. 2A-B, and Table 1). The narrow peak of the frequency distribution demonstrated that $C_a$ control tracked the fluctuating ambient $C_a$ well. For the elevated $C_a$ treatment, the WTC was at ambient $C_a + 240$ μmol mol$^{-1}$, which produced an average daytime $C_a$ of 620 μmol mol$^{-1}$ and a night-time $C_a$ of 740 μmol mol$^{-1}$ in each WTC. Strict night-time control of $C_a$ in ambient WTCs was not possible because the WTCs do not scrub CO$_2$ from the atmosphere. Increased $C_a$ at night in all WTCs declined rapidly after sunrise with the onset of photosynthesis.

Despite high radiation loads at high ambient temperatures, we were able to control $T_{air}$ within ± 1 °C for 90% of the time (Fig. 2C-D) across a range of temperatures from -2.8 to 43.8 °C (Table 1). $T_{air}$ in the WTCs increased by 1 - 2 °C relative to ambient air in the few minutes after dawn, when $T_{air}$ was close to dew point. This transient increase was due to the maintenance of coolant liquid at or slightly below dew point. Under such conditions, there was no temperature differential between the heat exchanger and the chamber air. In addition, when extremely dry air (dew point temperature of -1°C) and high $T_{air}$ (35 °C) conditions occurred, the cooling unit was unable to chill the coolant to the target value. Although a sufficient temperature reduction was maintained to enable regulation of chamber temperatures, WTC humidity was higher than outside air. Under such extreme conditions, $D_{air}$ was ~ 4 kPa in the WTCs while outside $D_{air}$ was ~ 5 kPa; failure to control humidity during these transient and extreme conditions was rare. In general, daytime WTC $D_{air}$ was slightly lower than outside $D_{air}$ (ca. 0.2 kPa) as a result of the large transpiration flux, while nighttime WTC $D_{air}$ was occasionally slightly higher than outside $D_{air}$ as a result of the coolant being held 1°C below reference dew point (Fig. 2E-F).

3.2. Leak corrections

We developed leak constants that corrected for small gas diffusion leaks between the WTC and outside air during whole-tree gas exchange measurements. In most cases, the sensitivity of
calculated night time flux rates to switching CO\textsubscript{2} control on and off was found to be very small (Fig. 3). There was a slight drift in the uncorrected fluxes when CO\textsubscript{2} injection was turned off and a sudden change when the CO\textsubscript{2} injection was turned on and the C\textsubscript{a} concentration gradient between the WTC and outside air was re-established. Leak corrections greatly improved the relationship between night-time respiration and WTC \textit{T}\textsubscript{air} (Fig. 4). It should be noted that the impact of these corrections on daytime CO\textsubscript{2} fluxes was generally small (< 5%), but these corrections were very important during transition periods (e.g. during dawn and dusk) when trees were near their light compensation point and fluxes were low.

3.3. Chamber gas exchange data

Two ambient WTCs with trees of similar size (basal areas 21 and 23 cm\textsuperscript{2}; 3-4 m tall) were chosen to illustrate how \textit{A}\textsubscript{C} and \textit{E} fluxes of whole-trees responded to variation in irradiance and \textit{D}\textsubscript{air} on a diurnal basis (Fig. 5). The two representative diurnal courses shown are for mild mid-autumn days. The first day was clear in the morning, but had broken cloud cover in the afternoon, while the second day was clear and sunny all day. The diurnal courses of CO\textsubscript{2} and H\textsubscript{2}O fluxes demonstrate the capacity of the WTC system to measure fairly rapid environmental responses of fluxes at the whole-tree scale to fluctuations in light levels. Rainfall occurred during the night preceding the first day shown, explaining why \textit{D}\textsubscript{air} remained close to zero until 1100 h. Because of the low \textit{D}\textsubscript{air}, H\textsubscript{2}O fluxes were very small during that morning. Though watered frequently, the tree crowns were not wetted during outside rain events, so \textit{E} could still be resolved whilst outside trees were wet. For the two trees, integrated daily carbon uptake was 69 and 54 g d\textsuperscript{-1} on the first day and 82 and 68 g d\textsuperscript{-1} on the second day, respectively. Water loss for the two trees was 17 and 12 L on the first day and 26 and 22 L on the second day.

There is also clear evidence of a strong influence of \textit{D}\textsubscript{air} on CO\textsubscript{2} and H\textsubscript{2}O fluxes (Fig. 6). On the second, sunny day, there was a strong hysteresis in the response of \textit{A}\textsubscript{C} to \textit{Q}\textsubscript{in} (Fig. 6A and B). Several lines of evidence suggest that this hysteresis can be attributed to stomatal closure in response to rising \textit{D}\textsubscript{air}. We fitted the non-rectangular hyperbola with \textit{D}\textsubscript{air} modifier (equations 6 and 7) to these data and found a very good fit (Fig. 6A, B). The transpirational flux \textit{E} also shows a hysteresis in response to \textit{D}\textsubscript{air}, with higher rates of transpiration in the morning than in the
afternoon (Fig. 6C, D), which is consistent with stomatal closure. Also, the ratio of $E$ to $A_c$ increased linearly with $D_{air}$, but showed no hysteresis (Fig. 6E, F), indicating a close coupling of transpiration and photosynthesis and therefore stomatal control. Note that there was a rapid rise in the ratio of $E$ to $A_c$ towards evening (Fig. 6E, F). This rise occurred because $A_c$ decreased as $Q_{in}$ decreased towards the compensation point in the early evening, while $D_{air}$ remained fairly high and $E$ continued into the night (Fig. 5B). Night-time $E$ was observed frequently especially early in the evening when $D_{air}$ was still high.

Hysteresis in the light response of $A_c$ was observed on most sunny days. For all days with sufficiently high light levels, we calculated the average CO$_2$ uptake rates for $Q_{in}$ between 900 and 1100 $\mu$mol m$^{-2}$ s$^{-1}$ separately for morning and afternoon data, and average $D_{air}$ corresponding to the same time periods. The difference between morning and afternoon CO$_2$ uptake rates was strongly related to the difference between morning and afternoon $D_{air}$ (Fig. 7), again supporting the hypothesis that hysteresis is driven by stomatal closure as $D_{air}$ rises. This raises the possibility that stomatal closure is in direct response to rising $D_{air}$ or caused by hydraulic limitations due to low leaf water potential.

We also investigated whether the initial slope of the $A_c / Q_{in}$ relationship varied throughout the season. To obtain daily values of the initial slope, we fitted a linear regression for each day to the relationship between morning CO$_2$ flux data and $Q_{in}$, when $Q_{in}$ was < 500 $\mu$mol m$^{-2}$ s$^{-1}$, which is typically the linear portion of the $A_c / Q_{in}$ curve. The initial slope values declined during the study period, with average values of around 0.045 mol C mol$^{-1}$ $Q$ in March, declining to 0.03 mol C mol$^{-1}$ $Q$ in July (Fig. 8A). Although leaf area might influence this relationship, there was little change in leaf area between February and August (data not shown). Therefore, the change in slope may be partially attributed to lower night-time temperatures observed in the winter, as minimum night-time temperature decreases below 4 °C (Fig. 8B).

4. Discussion

In this paper, we describe the operation of a unique experimental facility established in south-eastern Australia to study the response of whole-trees to elevated $C_a$ and interactions with other
climate change factors. The experiment uses a sophisticated whole-tree chamber system to
measure whole-tree CO₂ and H₂O fluxes in field conditions. The performance of the WTCs is
clearly demonstrated by their capacity to maintain Cₐ, Tₐir and Dₐir close to the desired target
levels (Table 1; Fig. 2). Despite being designed for boreal conditions in northern Sweden,
climate control by the chambers was excellent even in very hot, dry conditions during the
Australian summer, with Tₐir exceeding 35 ºC. Modifications and improvements to the original
chamber design reported in Medhurst et al. (2006) have enhanced the ability of the chambers to
measure whole-tree fluxes of CO₂ and H₂O at high temporal resolution. The improvements will
enable us to collect a valuable dataset to examine whole-tree flux responses to environmental
drivers.

Numerous experimental approaches, many of which are complementary, may be used to study
the impacts of climate change on plants and ecosystems. For example, the WTC approach is
complementary (Fig. 9) to branch bags (Barton et al., 1993), open top chambers (Drake et al.,
1989; Ceulemans et al., 1995; Whitehead et al., 1995), growth cabinets and glasshouses (Atwell
et al., 2007; Thomas et al., 2007; Ghannoum et al., 2009), mesocosms (Griffin et al., 1996;
Tingey et al., 1996) and Free-Air CO₂ Enrichment (FACE) (Hendrey et al., 1999). Nonetheless,
few of these other systems allow the measurement of whole-tree fluxes in trees ranging from
seedlings to 9 m tall. Measurements of whole-tree fluxes requires enclosing the tree, and
although valuable insight into the response of plants to elevated Cₐ has been obtained using
enclosed systems (e.g. Duff et al., 1994), there has been criticism due to the alteration of the
microenvironment (Drake et al., 1997; Long et al., 2006). However, greatly improved
environmental control in the WTCs was superior to most open-top chambers which relied upon a
rapid turnover of fresh air to keep Tₐir and Dₐir close to ambient levels (e.g. Leadley, 1993;
Whitehead, 1995) whilst sacrificing resolution of gas exchange (Dore et al., 2003). In earlier
versions of open-top chambers, Tₐir in the chambers could be 4 - 5 ºC above ambient Tₐir under
strong radiant loading (Whitehead et al., 1995), and Dₐir varied depending on environmental
conditions and plant size (Piikki et al., 2008). In later designs, Norby et al. (1997) added
temperature control to open-top chambers, which improved temperature control (± 0.4 ºC), but
air was often humidified by the evaporative cooling system. Simultaneous control of both Tₐir
and $D_{air}$ is important as they influence plant physiology and may interact with elevated $C_a$ (Eamus et al., 1995).

In the WTCs, we demonstrated good control of $T_{air}$ and relatively precise control of $D_{air}$ (Fig. 2). Although control was expensive in terms of electricity for the chiller unit, this cost was partly offset by reduced CO$_2$ consumption through recirculation of air through the heat exchangers and a low rate of fresh air exchange. When air temperatures were $> 30$ °C (ca. 2.5% of the total time), the main chiller unit had difficulties keeping the coolant below the dew-point of the ambient air because the air-cooled heat exchanger had to remove the heat. Under these conditions, the chamber humidity rose slightly above the target value. However, this was an infrequent problem, restricted to very high vapour pressure deficits ($D_{air} > 3$ kPa), and unlikely to be of major significance as stomatal closure commenced once the $D_{air}$ was above 1.5 kPa (Barton et al., unpubl.). Modifications to further improve $D_{air}$ control of the WTCs are under consideration.

Open-top chambers and FACE systems are prone to $C_a$ fluctuations due to incursions of parcels of ambient air, especially during windy periods, which can lead to variation in $C_a$ both spatially and temporally (Whitehead et al., 1995; Hendrey et al., 1999; Mikkelsen et al., 2008). In contrast, $C_a$ in the WTCs can be well-regulated both in ambient and elevated treatments, with 1-minute $C_a$ within 3% of the target over 90% of the time (Fig. 3); in comparison, $C_a$ is within 20% of the target 90% of the time for some forest FACE experiments (e.g. Duke Forest FACE). The precise control of $C_a$ in the HFE is largely due to the closed nature of the chambers and the fast response time of the CO$_2$ regulation cycle. While night-time $C_a$ in ambient chambers exceeded the reference concentration (on average by 25 μmol mol$^{-1}$), due to the slow rate of fresh air exchange and absence of a CO$_2$ scrubber, the excess CO$_2$ rapidly disappeared in the morning. Hence, the higher night-time $C_a$ in the ambient chambers is unlikely to have had a significant influence on tree physiology or net carbon exchange.

Apart from control of $C_a$, $D_{air}$ and $T_{air}$, chambered systems may also affect the light and wind environment of plants. The chambers were constructed with plastic that transmitted light across a wide frequency without substantially increasing the diffuse light component. However, longwave radiation was altered because the outer canopy leaves were radiatively coupled to the chamber.
wall, not to the sky. As a result, leaf temperature in the WTC trees was somewhat higher during
clear sky nights. This temperature difference was probably small because the longwave radiation
balance for a majority of leaves in the canopy was determined by neighbouring leaves rather than
the chamber walls. Furthermore, chamber mixing fans maintained air movement within the WTC
to minimize leaf boundary layer. This approach kept leaf temperatures in the WTC close to those
of outside control trees (data not shown); in the future, we plan to install permanent infrared
sensors to monitor canopy temperature.

Due to the large size of the WTCs, it was vital to test for leaks that might have affected
measurements of CO₂ and H₂O fluxes. We introduced a new method to test for leaks by
reversing the Cₐ between CO₂ treatments for a few hours at night. We assumed that respiration
was insensitive to Cₐ (Tissue et al., 2002; Gonzales-Meler et al., 2004). This method enabled us
to detect leaks or errors in calibration of the CO₂ mass flow and flow of fresh air. Without this
test, systematic errors may undermine the validity of the data, especially given small night-time
carbon fluxes because of the large concentration gradient between the inside and outside of the
chamber. Currently, resolving night-time respiration of trees and ecosystems remains an active
area of research (Reichstein et al., 2005; Aubinet 2008; van Gorsel et al., 2009) but subject to a
variety of problems in open-air measurement systems that are circumvented in the closed WTCs
described here.

One of the main features of the WTCs is the ability to continuously measure whole-tree fluxes of
carbon and water in trees grown under controlled environmental conditions. These measurements
allow us to study in detail the whole-tree integrated response to environmental variation at high
temporal resolution. Previously, only automated shoot cuvette systems yielded similarly high
resolution data at the shoot scale (e.g. Hari et al., 1999; Kolari et al., 2007) while mesocosms
have yielded data from combined plant and soil systems up to 1.5 m tall (Tingey et al., 1996;
2007). The current WTC experiment differs from the WTC experiment in Sweden with 40-year-
old Norway spruce trees (Medhurst et al., 2006) in that E. saligna was grown in our experiment
under the treatment conditions from seedling until harvest at two years old. This fast-growing
Eucalyptus species obtained a height of 9 m within two years allowing us to scale water use and
carbon uptake of trees of different heights at intervals during their growth.
Models that scale from leaf gas exchange measurements to the tree canopy based on simulation of the within-canopy light gradients can now be tested. These models are widely-used, but typically have only been tested with eddy-covariance data (e.g. Amthor et al., 2001; Kramer et al., 2002; Hanson et al., 2005). The main limitation of the eddy-covariance technique is that the measured CO$_2$ fluxes include autotrophic and heterotrophic respiration, which is often difficult to resolve (Reichstein et al., 2005). The advantage of the WTCs is that their sealed floors allow measurement of net canopy carbon uptake (gross photosynthesis minus aboveground autotrophic respiration). Eddy covariance fluxes are more “noisy” than tree chambers because the measurement area is large (several hectares), often hilly, and variable depending on wind direction and strength. In contrast, the inherent stability of the WTCs over the short term permitted measurements that were markedly less noisy. For example, Lasslop et al. (2010) recently investigated whether the light response of net ecosystem exchange measured by eddy covariance was affected by $D_{\text{air}}$. A marked hysteresis was found, but the hysteresis could be accounted for either by a $D_{\text{air}}$ effect on photosynthetic rate or by a strong temperature response of ecosystem respiration. In this experiment fluxes of carbon from the soil are excluded and so the reduction in carbon uptake by the tree canopy as $D_{\text{air}}$ increases is most probably the result of stomatal closure. The strength of the limitation was remarkable, particularly because the trees were well-watered throughout the experimental period.

The chamber data also clearly demonstrated the effects of cool night-time temperatures on canopy photosynthesis. Effects of frost on photosynthetic parameters are well known from automated cuvette systems in the boreal zone (e.g. Troeng and Linder, 1982; Hari et al., 1999; Kolari et al., 2007), but very limited information exists for temperate evergreen species such as *Eucalyptus*. King and Ball (1998) developed a model of frost effects on leaf photosynthesis based on early studies of frost hardening in snowgum (*E. pauciflora*) (Harwood, 1980, 1981). Their approach has been adopted by process-based models of eucalypt plantation productivity, using empirical parameter tuning to match productivity in cool environments (Sands and Landsberg, 2002; Battaglia et al., 2004). The data presented here will allow us to quantify cold-temperature impacts on *Eucalyptus* photosynthesis at the whole-tree scale for the first time. In conjunction with ancillary leaf-level photosynthesis and chlorophyll fluorescence data, the data
will allow us to identify the mechanisms underlying temperature effects on whole-tree CO$_2$
uptake. These two examples indicate the quality of the whole-tree chamber data and demonstrate
their utility in identifying mechanisms of tree response to environmental drivers. This
mechanistic information is critical in up-scaling leaf-level measurements and understanding
individual tree photosynthesis and transpiration. Whole-tree chamber fluxes can also be
compared with whole-system carbon mass-balance (obtained from growth, litterfall, root
turnover and soil respiration measurements) and whole-system water balance (obtained from
water inputs and soil moisture measurements) to identify and quantify the major mechanisms
driving the $C_a$ responses of broadleaved evergreen plantation trees.

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**FIGURE CAPTIONS**

**Fig. 1.** Schematic diagram of a whole-tree chamber modified from Medhurst et al. (2006) to highlight new or modified components. The modular chamber consisted of three main components (A, B-D, and E): the chamber base (soil compartment), the tree chamber (aboveground 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 0.45 m high. The tree chamber consisted of a bottom (B) and top (D) section with a height of 2.5 m and 3.0 m, respectively. An extra section (C), with a height of 2.65 m was added as the trees grew. Major components of the system are indicated in the diagram with numbers: (1) pipe for circulating the chamber air through the cooling unit; a cooling unit (E) consisting of: (2) frequency-controlled fan (0 - 12 000 m$^3$ h$^{-1}$); (3) dampers to regulate the amount of air going through the cooling unit; (4) large-surface area heat exchanger; (5) circulating a glycol/water solution maintained at ambient dew point temperature; and (6) fresh air inlet; (7) fan for fresh air; (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 (10) a 12-V controlled safety damper working in parallel with a similar damper at the top of the WTC; (11) root barrier to depth of 1 m.

**Fig. 2.** Frequency of deviations from target values for air temperature ($A; T_{air}$), chamber CO$_2$ concentration ($B; C_a$) and air vapour pressure deficit ($C; D_{air}$) for ambient and elevated chambers during daytime and night-time for the period 20$^{th}$ February (summer) to 6$^{th}$ August (winter).

**Fig. 3.** Leak corrections for an elevated $C_a$ chamber over the course of five days and nights. (A) reference and chamber $C_a$ on three nights when CO$_2$ supply was turned off for a several hours. #
indicates the commencement of CO$_2$ injection into the chambers and the cessation of CO$_2$ injection; (B) air temperature in the chamber; and, (C) uncorrected and corrected flux of CO$_2$.

**Fig. 4.** Uncorrected (A) and corrected night-time CO$_2$ fluxes (B) plotted against chamber temperature for an elevated chamber during the five successive nights shown in Fig. 3. Closed symbols are data when the CO$_2$ supply was turned off, and open symbols show when CO$_2$ supply was on. Values in panel A exceeding zero erroneously suggest a positive CO$_2$ flux into the plant at night at the coolest temperatures prior to applying the appropriate corrections (eq. 5).

**Fig. 5.** Diurnal course CO$_2$ flux (A) (μmol m$^{-2}$ ground s$^{-1}$) and H$_2$O flux (B) (mmol m$^{-2}$ ground s$^{-1}$) over two consecutive days representing contrasting conditions. Photosynthetically active photon flux density ($Q$; panel C) and vapour pressure deficit of chamber air ($D_{\text{air}}$; panel D) are shown. Grey shading indicates the night period.

**Fig. 6.** Carbon and water fluxes for two ambient trees on 30$^{\text{th}}$ March (autumn). Whole-tree net CO$_2$ assimilation per unit ground area *versus* photosynthetically active photon flux density ($Q_{\text{in}}$, μmol m$^{-2}$ s$^{-1}$) is shown with fitted values using the function in Eq. 6 and 7 shown as joined line (A and B). H$_2$O flux per unit ground area as a function of $D_{\text{air}}$ (C and D). Ratio of H$_2$O flux to CO$_2$ flux as a transpiration efficiency, plotted against $D_{\text{air}}$ (E and F). Data prior to noon are shown with solid circles and after noon as open circles. Note the sudden departure from the strong linear relationship at low light levels early in the morning and late in the afternoon as CO$_2$ flux diminishes to zero while H$_2$O flux remains at low levels.
Fig. 7. Difference between whole-tree maximum assimilation rate in the afternoon \( (A_{1000\text{pm}}) \) and the morning \( (A_{1000\text{am}}) \), both measured when PPFD was between 900 and 1100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), as a function of the difference in \( D_{\text{air}} \) measured at the same times.

Fig. 8. Initial slope of the relationship between whole-tree assimilation and incident quantum flux of light \( (\mu\text{mol CO}_2 / \mu\text{mol quanta of PAR}) \) for two chambers between February (summer) and August 2008 (winter) as a function of time (A) and minimum temperature of the night preceding the measurement (B) and the minimum daily temperature on each day (C). The line in (A) is the Loess fit, while the lines in (B) are hand-drawn to indicate the general trends in the data and a breakpoint at 4°C.

Fig. 9. Schematic diagram indicating applicability of different ecophysiological techniques for experimental treatments and measurements at a variety of scales. FACE denotes free-air CO\(_2\) enrichment.
Table 1: Absolute deviations of environmental conditions within chambers ($C_a$, $T_{air}$ and $D_{air}$) from target values based on outside ambient air conditions during the period 20.02.2008 to 06.08.2008. Mean, median, and 5th and 95th percentiles are shown.

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Spatial scale

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