

Studying the effects of rising atmospheric CO₂ concentration on the water use efficiency of *Eucalyptus saligna*

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Introduction

Atmospheric CO₂ concentration (C_a) has risen from 280 $\mu\text{mol mol}^{-1}$ to the current concentration of ca. 390 $\mu\text{mol mol}^{-1}$ over the last 150 years, and continues to rise at a rate of 1.5 – 2.0 $\mu\text{mol mol}^{-1}$ per annum (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 CO₂ and water fluxes at the whole-tree level. In order to validate such models, we require a system to measure whole-tree fluxes of CO₂ and water and their response to the environment.

The Hawkesbury Forest Experiment (HFE) established at the University of Western Sydney's Hawkesbury campus sought to test the response of Australian plantation *Eucalyptus* to elevated atmospheric [CO₂] and drought, and parameterise models to predict effects of these factors on net CO₂ assimilation, water use and growth of *Eucalyptus* trees. *Eucalyptus saligna*, a commercial plantation tree of wet sclerophyll forest origin, was successfully exposed to elevated atmospheric [CO₂] whilst ambient temperature and humidity conditions were maintained inside whole-tree chambers (WTC). A single *E. saligna* (Sydney Blue Gum) 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 CO₂ (C_a tracked outside conditions) and six WTCs were maintained at elevated CO₂ (ambient C_a + 240 $\mu\text{mol mol}^{-1}$). 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 elevated C_a on the instantaneous water use efficiency and potential implications for forest water use and growth.

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 (Barton *et al.* 2010). Within each WTC, one seedling of *E. saligna* was planted in April 2007 and supplied with an initial fertilisation of 50 g of (NH₄)₂PO₄ 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 $\mu\text{mol mol}^{-1}$), while all 12 WTCs controlled T_{air} to maintain ambient outside conditions. A treatment target C_a of +240 $\mu\text{mol mol}^{-1}$ was chosen to be similar to C_a used in recent free-air CO₂ enrichment experiments, and is anticipated in ca. 50 years (Pacala and Sokolow 2004).

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 at a rate of approximately 10,000 m³ hr⁻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).

Whole tree carbon and water fluxes

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 50 m³ 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 fresh air while a digital manometer constantly monitored the pressure drop across the orifice, and thus allowed continuous measurement of the airflow. Pure CO₂ 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 IRGA measured the concentration of CO₂ and water vapour in the chamber air and a mass balance calculation then provided an estimate of carbon and water fluxes. A full cycle of measurements, including all 12 WTCs and two reference readings, took 14 minutes; whole-tree CO₂ and H₂O fluxes were calculated every cycle (see Barton *et al.* 2010 for full description of chamber function).

Tree chamber performance

The whole tree chambers maintained the target CO₂ concentrations close to the target values (< 15 ppm deviation from target 90% of the time). Night time respiration by the tree canopy led to slightly higher than target values of CO₂ in ambient chambers as there was no ability to remove excess CO₂ this discrepancy dissipated rapidly on sunrise as photosynthesis commenced. Despite high radiation loads at high ambient temperatures, we were able to control T_{air} within ± 1°C for 90% of the time across a range of temperatures from -2.8 to 43.8°C. 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. A small difference in chamber D_{air} was observed in relation to tree size and transpiration rate. As trees get bigger they intercept a higher proportion of the radiation load on the chamber furthermore rapidly transpiring trees effectively self cool and so require less cooling from the control system. This variable partitioning of the radiation load between sensible and latent heat combined with the common temperature of the coolant among chambers makes it difficult to match the humidity and temperature simultaneously among chambers. It is important to take this into account when analysing the data (Barton *et al.* 2011). Subsequent modifications to the temperature control system allowing different temperatures of cooling coil at each chamber has improved the ability to independently regulate temperature and humidity among trees of varying size and transpiration rates and a new experiment studying the interaction of rising C_a and temperature on *Eucalyptus globulus* is underway.

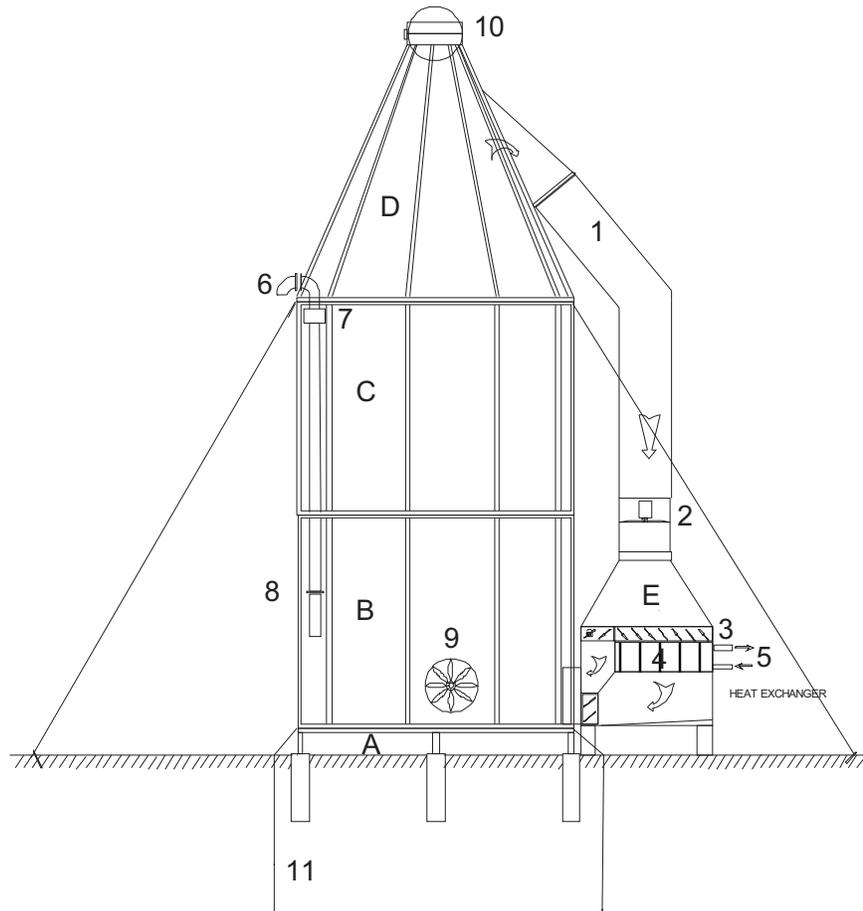


Figure 1 Schematic diagram of a whole-tree chamber. The modular chamber consisted of three main components (A, B-D, and E): the chamber base (soil compartment), the tree chamber (aboveground compartment) and a cooling unit placed directly outside the chamber. The diameter of the WTC was 3.25 m. The chamber 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 ($0 - 12\ 000\ \text{m}^3\ \text{h}^{-1}$); (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 (see Barton *et al.* 2010 for full description).

Instantaneous Transpiration Efficiency

Instantaneous transpiration efficiency is defined as the ratio of carbon uptake per unit water transpired and as such is sensitive to any changes in both photosynthesis and stomatal conductance in response to elevated C_a . While it is well-known that ITE increases with rising C_a (Rogers *et al.* 1983; Eamus 1991; Drake *et al.* 1997), we hypothesised that ITE increases *in proportion to* C_a . The hypothesis that ITE increases in proportion to C_a follows (Medlyn *et al.* 2011) from the original theory of optimal stomatal behaviour proposed by Cowan & Farquhar (1977). Stomatal conductance is subject to a trade-off between carbon uptake and water loss. The theory of Cowan & Farquhar (1977) defines the optimal stomatal conductance as that which maximises daily photosynthetic carbon uptake for a given daily water loss. Data from the whole tree chambers allows us to test this hypothesis at both leaf and whole tree scales.

We increased C_a by 60% and so according to the hypothesis the ITE should also have increased by 60%. We calculated the mean value for ITE for each chamber in 2 hour windows from dawn to dusk for each day between 14th April 2008 and 3rd March 2009 and then calculated the mean value across ambient or elevated CO_2 chambers. Because the vapour pressure deficit was slightly different among chambers and this influences transpiration rate it was necessary to use VPD as a covariate in the analysis. Plotting ITE against VPD and calculating the ratio of elevated to ambient at various values of VPD allowed us to test the hypothesis that ITE increased in proportion to the rise in CO_2 (Figure 2). The ratios are shown at the bottom of the chart and do indeed show that the response of ITE is proportional to the rise in CO_2 (see Barton *et al.* 2011 for a more detailed analysis).

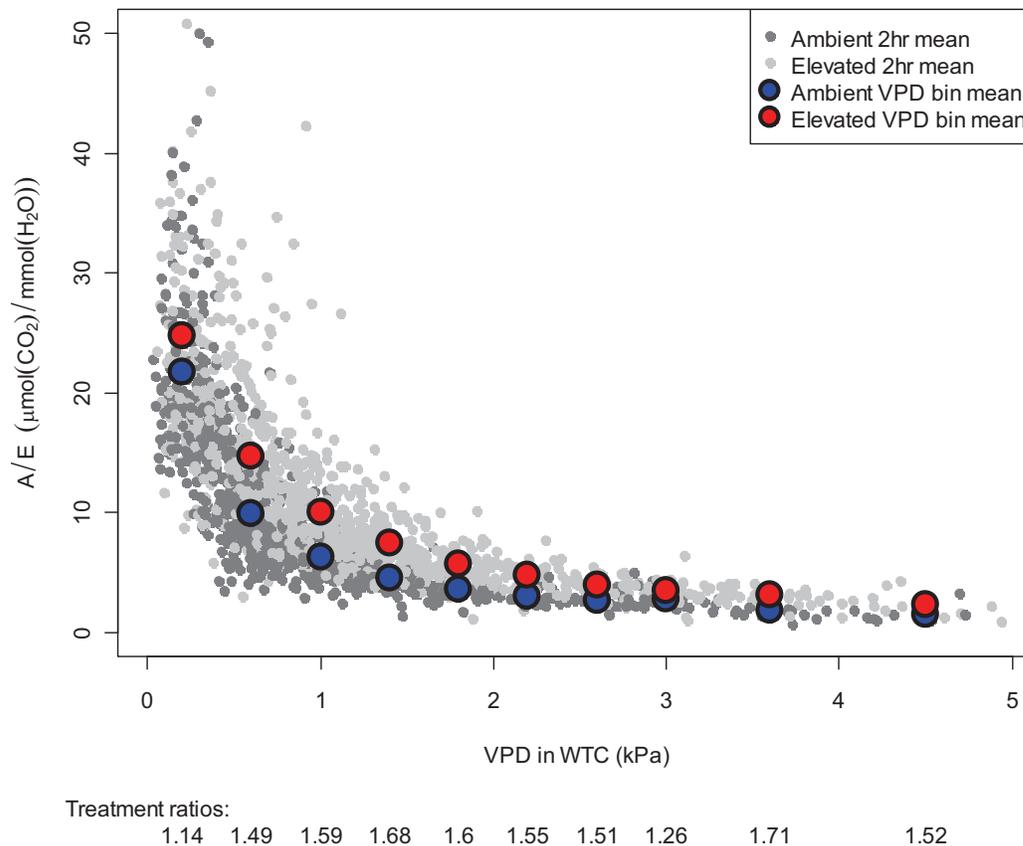


Figure 2 Instantaneous Transpiration efficiency (A/E) plotted against chamber vapor pressure deficit for ambient and elevated chambers. Each point is the mean of three chambers during a 2 hour window from dawn to dusk when light (PPFD) was $>600 \mu\text{mol m}^{-2} \text{s}^{-1}$. The numbers at the bottom of the chart are the ratio of ITE in elevated chambers to that in ambient chambers at a range of values of VPD.

Conclusions

The ratio of CO₂ uptake (A) to transpiration rate (E), the instantaneous transpiration efficiency (ITE), is important because it reflects efficiency of resource use by plants and canopies. At larger scales, canopy ITE affects trade-offs between carbon sequestration and water availability (Jackson *et al.* 2005).

We found that ITE is strongly dependent on vapour pressure deficit (D), showing that D needs to be carefully monitored and used in analyses of ITE. When differences in D were taken into account, ITE was directly proportional to the atmospheric CO₂ concentration (C_a) at both leaf and canopy scales in *Eucalyptus saligna*; literature data appears to support this general conclusion. Importantly, these results allow us to predict the effect of elevated C_a on E , where effects on A are known. In our study, we found that A was more enhanced by C_a at higher D , and from this finding were able to explain the C_a effect on E and its interaction with D .

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