

## Seasonal and diurnal variation of leaf gas exchange in a tall *Eucalyptus delegatensis* forest

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### Data Files:

**TumbarumbaGasex\_ACis\_Medlyn.csv**

**TumbarumbaGasex\_Spot\_Medlyn.csv**

This dataset consists of leaf gas exchange measurements made at the Tumbarumba eddy covariance site (Leuning et al. 2005). Measurements were made during three five-day campaigns in November 2001, February 2002, May 2002. Measurements were made on the overstorey trees (*Eucalyptus delegatensis*) accessed using a 20-m tall cherry picker. Each day we measured about 4 A-Ci / A-PAR curves, interspersed with spot measurements of photosynthesis at ambient conditions. Leaves were tagged during measurements and cut at the end of the day. Specific leaf area, leaf N and P content, and Chl content were determined on leaves used for A-Ci curves. Full methods can be found below.

These data have been used in the following publications about the Tumbarumba site:

- Keith H, Leuning R, Jacobsen KL, Cleugh HA, van Gorsel E, Raison RJ, Medlyn BE, Winters A, Keitel C (2009) Multiple measurements constrain estimates of net carbon exchange by a *Eucalyptus* forest. *Agricultural and Forest Meteorology*. 149:535-548.
- Pepper DA, McMurtrie RE, Medlyn BE, Keith H, Eamus D (2008) Mechanisms linking plant productivity and water status for a temperate *Eucalyptus* forest flux site: analysis over wet and dry years with a simple model. *Functional Plant Biology* 35: 493 – 508.
- Medlyn BE, Pepper DA, O'Grady A, Keith H. (2007) Linking leaf and tree water use with an individual-based model. *Tree Physiology* 27: 1687-1699.

Additionally, the spot gas exchange measurements have been used in the following publications on stomatal conductance:

- **Medlyn BE**, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CVM, de Angelis P, Crous KY, Freeman M, Wingate L (2011) Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* 17: 2134-2144.
- Lin YS, **Medlyn BE**, Duursma RA, Prentice IC, Wang H, Baig S, Eamus D, Resco de Dios V, Mitchell P, Ellsworth DS, Op de Beeck M, Wallin G, Uddling J, Tarvainen L, Linderson M, Cernusak L, Nippert J, Ocheltree T, Tissue DT, Martin-StPaul N, Rogers A, Warren J, De Angelis P, Hikosaka K, Han Q, Onoda Y, Gimeno T, Barton CVM, Bennie J, Bonal D, Bosc A, Löw M, Macinnis-Ng C, Rey A, Rowland L, Setterfield S, Tausz-Posch S, Zaragoza-Castells J, Broadmeadow M, Drake J, Freeman M, Ghannoum O, Hutley L, Kelly J, Kikuzawa K, Kolari P, Koyama K, Limousin J-M, Meir P, Costa A, Mikkelsen T, Salinas N, Sun W, Wingate L (2015) Optimal stomatal behaviour around the world. *Nature Climate Change* 5: 459–464.

## File Layout

### TumbarumbaGasex\_ACis\_Medlyn.csv

Column Title / Unit / Definition

Date	YYYY /MM/DD	Date of measurement
Time	HH:MM	Time the curve was started
Curve	1 – 48	Curve Number
Qflag	0 or 1	0 indicates something wrong with point
Site	1 – 5	We returned to the same 5 sites in each campaign, visiting one site per day. This column indicates which site was used.
Leaf Age	0 or 1	0 = this year's foliage; 1 = previous year's foliage. Note that leaves flushed in February and the young leaves in this campaign were not fully expanded and were slightly reddish in colour
Chl a+b	$\mu\text{mol m}^{-2}$	Leaf chlorophyll content per unit leaf area
Na	$\text{g m}^{-2}$	Leaf nitrogen content per unit leaf area
Pa	$\text{g m}^{-2}$	Leaf phosphorus content per unit leaf area
SLA	$\text{m}^2 \text{kg}^{-1}$	Specific leaf area
Photo	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Assimilation rate
Cond	$\text{mol m}^{-2} \text{s}^{-1}$	Stomatal conductance to water vapour
Ci	$\mu\text{mol mol}^{-1}$	Intercellular CO <sub>2</sub>
Trmmol	$\text{mmol m}^{-2} \text{s}^{-1}$	Transpiration
VpdL	kPa	Leaf to air vapour pressure deficit
Area	$\text{cm}^2$	Area of leaf in cuvette = cuvette size ( $6 \text{ cm}^2$ )
StmRat	-	Stomatal ratio, set to 1
BLCond	$\text{mol m}^{-2} \text{s}^{-1}$	Boundary layer conductance to water vapour
Tair	deg C	Air temperature in the cuvette
Tleaf	deg C	Leaf temperature (deg C)
TBlk	deg C	Temperature of the cuvette
CO2R	$\mu\text{mol mol}^{-1}$	CO <sub>2</sub> concentration of the input gas
CO2S	$\mu\text{mol mol}^{-1}$	CO <sub>2</sub> concentration of the sample gas
H2OR	$\text{mmol mol}^{-1}$	H <sub>2</sub> O concentration of the input gas
H2OS	$\text{mmol mol}^{-1}$	H <sub>2</sub> O concentration of the sample gas
RH_R	%	Relative humidity of the input gas
RH_S	%	Relative humidity of the sample gas

Flow	$\mu\text{mol s}^{-1}$	Flow rate through the cuvette
PARi	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Input PAR
Press	kPa	Pressure

## File Layout

### TumbarumbaGasex\_Spot\_Medlyn.csv

Column Title / Unit / Definition

Date	YYYY /MM/DD	Date of measurement
Time	HH:MM	Time of measurement
Obs	Number, or 'acX'	Some initial measurements from A-Ci response curves were suitable for inclusion in this dataset. These measurements can be identified by 'acX'
Qflag	0 or 1	0 indicates something wrong with point
Site	1 – 5	We returned to the same 5 sites in each campaign, visiting one site per day. This column indicates which site was used.
Tree	1 – 10	There were 2 trees used at each site
Leaf Age	0 or 1	0 = this year's foliage; 1 = previous year's foliage. Note that leaves flushed in February and the young leaves in this campaign were not fully expanded and were slightly reddish in colour
Na	$\text{g m}^{-2}$	Leaf nitrogen content per unit leaf area
Pa	$\text{g m}^{-2}$	Leaf phosphorus content per unit leaf area
SLA	$\text{m}^2 \text{kg}^{-1}$	Specific leaf area
PARi	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Input PAR
Press	kPa	Pressure
Photo	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Assimilation rate
Cond	$\text{mol m}^{-2} \text{s}^{-1}$	Stomatal conductance to water vapour
Ci	$\mu\text{mol mol}^{-1}$	Intercellular CO <sub>2</sub>
Trmmol	$\text{mmol m}^{-2} \text{s}^{-1}$	Transpiration
VpdL	kPa	Leaf to air vapour pressure deficit
StmRat	-	Stomatal ratio, set to 1
BLCond	$\text{mol m}^{-2} \text{s}^{-1}$	Boundary layer conductance to water vapour
Tair	deg C	Air temperature in the cuvette
Tleaf	deg C	Leaf temperature (deg C)
TBlk	deg C	Temperature of the cuvette
CO2R	$\mu\text{mol mol}^{-1}$	CO <sub>2</sub> concentration of the input gas
CO2S	$\mu\text{mol mol}^{-1}$	CO <sub>2</sub> concentration of the sample gas
H2OR	$\text{mmol mol}^{-1}$	H <sub>2</sub> O concentration of the input gas
H2OS	$\text{mmol mol}^{-1}$	H <sub>2</sub> O concentration of the sample gas
RH_R	%	Relative humidity of the input gas
RH_S	%	Relative humidity of the sample gas
Flow	$\mu\text{mol s}^{-1}$	Flow rate through the cuvette

## Methods

### Site

The study site is located in the Bago State Forest near Tumbarumba in temperate south-eastern New South Wales, Australia. Elevation of the site is 1200 m and mean annual precipitation is approximately 1000 mm. *E. delegatensis* is the dominant

species in this wet sclerophyll forest. The forest is tall but relatively open: mean tree height is approximately 40m and leaf area index is approximately  $1.4 \text{ m}^2 \text{ m}^{-2}$ .

Measurements were made at five subsites located within 1 km of the Tumbarumba eddy flux tower ( $35^\circ 39' 20.6'' \text{ S}$ ,  $148^\circ 9' 7.5'' \text{ E}$ , Leuning et al. 2005.). A 20m tall cherry picker was used to access the middle and upper canopy of two 20-30 m tall trees at each sub-site. The position of the leaves measured was determined largely by the reach of the cherry picker and therefore they generally occurred adjacent to openings in the forest canopy. The aspect of these openings varied among the sites as discussed below.

Measurements were made during three field campaigns conducted in spring (14 - 16 November 2001), summer (11 - 15 February 2002) and autumn (6 - 10 May 2002). During each campaign, measurements were made at a different subsite each day. Gas exchange was monitored from approximately 1-2 hours after sunrise until sunset. Daytime temperatures in the canopy ranged from  $13.5 - 26.2^\circ \text{C}$  in spring,  $9.8 - 33.8^\circ \text{C}$  in summer, and  $10.1 - 22.9^\circ \text{C}$  in autumn.

#### *Gas exchange measurements*

A Licor 6400 portable Photosynthesis Measuring System with attached light source (6400-02 LED) was used to take leaf gas exchange measurements. Two sets of measurements were taken. Each day, four leaves (two from each tree) were chosen for detailed measurements of photosynthetic response to  $\text{CO}_2$  concentration and irradiance ( $A - C_i$  and  $A - I$  curves), which were used to estimate parameters of the Farquhar et al. (1982) model of photosynthesis. These measurements were interspersed with spot measurements of photosynthesis and stomatal conductance made at ambient and saturating light. All measurements were made at ambient temperature and relative humidity.

The  $A - C_i$  and  $A - I$  response curves were measured using an automatic programmed procedure that adjusted  $\text{CO}_2$  concentration ( $c_a$ ) and light levels then paused for the leaf gas exchange to stabilise before recording data and calculating the standard Licor 6400 set of photosynthetic variables. The automatic procedure entailed the following. While light was held at  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , the  $c_a$  surrounding the leaf was controlled so that it started at an ambient level ( $\sim 360 \text{ ppm}$ ) and then stepped down in four steps to zero ( $0 \text{ ppm}$ ) before returning to ambient and continuing to step up in three steps to  $1200 \text{ ppm}$ . After each step the gas exchange was allowed to stabilise using the instrument variable, *combined* ( $\text{CO}_2$  &  $\text{H}_2\text{O}$ ) *coefficient of variation* (the threshold was set to  $<1\%$ ). The automatic procedure then held  $c_a$  at  $1200 \text{ ppm}$  and decreased light levels in seven steps down to zero, pausing for stability after each step before recording data.

Spot measurements were made in between the response curves. For these measurements, the ambient light level was measured with a hand-held PAR sensor, and if it was less than  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , an initial spot measurement was made with the light source set to the ambient light level, otherwise  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  was used. Note that the ambient PAR measurements were inexact due to wind in the canopy causing very rapid fluctuations of light levels on shaded leaves.

In the first (spring) campaign, spot measurements were made repeatedly on the same leaves used for the  $A - C_i$  and  $A - I$  response curves, with the intention of monitoring their performance over the course of the day. However, we saw some evidence that repeated measurement of the leaves caused stomata to shut. In the second and third campaigns, therefore, all spot measurements were made on different leaves.

Leaves for measurements were randomly selected, with equal numbers selected from each tree accessible from the cherry picker each day. Two leaf classes were present during each campaign. In spring and autumn, both leaf classes were fully expanded, and were equally represented in measurements. In summer, the current leaf class was not fully expanded and often still reddish in colour. A smaller number of measurements was made on this leaf class. It may be appropriate to omit these data since the leaves were not fully expanded.

#### *Leaf chemistry and morphology measurements*

At the end of the day, all leaves used for gas exchange measurements that day were harvested by cutting the length of twig to which the leaves were attached. Twigs and leaves were placed in black plastic bags and refrigerated at approximately 4°C for the duration of the campaign and the transfer to the laboratory at CSIRO Forestry and Forest Products in Canberra.

Measurements of specific leaf area and leaf nitrogen, phosphorus and chlorophyll a and b content were made on the gas exchange leaves as follows.

*Specific leaf area:* Following each day's measurements, discs of area 2 cm<sup>2</sup> were punched from fresh, green leaves with 5 – 7 replicates per leaf. Leaves that were too small for these discs were scanned and total area determined digitally. Discs and leaves were oven-dried at 60°C for 24 hrs to constant weight, and weight determined to 4 decimal places. These two measurements were combined to obtain specific leaf area (m<sup>2</sup> kg<sup>-1</sup> DW).

*Nitrogen and phosphorus content:* Leaves were oven-dried at 60°C for 24 hrs to constant weight and finely ground. A 0.1 g sample was weighed to 4 decimal places and analysed by semi-micro Kjeldahl digestion and automated colorimetry (Technicon TRAACS 800) (Rayment and Higginson 1992). Data were calculated as % N and P by weight and converted to g m<sup>-2</sup> leaf area.

*Chlorophyll content:* Following each day's measurements, five small 5 mm diameter discs were punched from each leaf used for response curves, and placed immediately in approximately 5 ml dimethylformamide solution. These sub-samples then remained in solution stored at 4°C for < 1 week. Leaf material was removed from solution and adjusted to a final volume of 10 ml. Absorbance was determined (Perkin Elmer UV/VIS Spectrometer Lambda 2), at the peak in the spectrum for the solvent, of 660.8 nm for Chlorophyll a and 642.5nm for Chlorophyll b (Porra et al. 1989).