gm methods\_revised

Synchronized gas exchange and C isotope discrimination measurements were made similarly as described in Tazoe et al. (2011) and Evans & von Caemmerer (2013). Leaf level gas exchange was measured with a standard (2 x 3 cm) leaf cuvette using a portable gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA). This system was coupled with a tunable diode laser (TDL; TGA100,Campbell Scientific, Inc., Logan, UT, USA) for concurrent measurements of online C isotope discrimination. The CO2 in the leaf cuvette was set at ambient atmospheric [CO2] (400 ppm) with a flow rate of 200 mol s-1. Two identical gas exchanges systems were run simultaneously, one in each of a randomly chosen WTC for each temperature treatment. Leaf temperatures were controlled at the current AT or ET WTC air temperature. PPFD in the cuvette was set to match the individual light environment of each leaf type (explained above). Periods of high irradiance were simulated for shade leaves by increasing the leaf cuvette PPFD (LI-COR red/blue light source) to match the light environment of the full sun leaf in the same tree. The maximum sunfleck response of shade leaves was then recorded once CO2 and water vapour fluxes re-stabilized in the leaf cuvette (ca. 25 min).

Once CO2 and water vapour flux values were stable for each leaf measurement, the sample and reference gas lines were diverted to the TDL via T-junctions inserted into the reference gas tube and match valve outlet of the LI-6400XT. The gas streams were dried by passing through napion gas dryers in the respective gas lines, and then 12CO2 and 13CO2 concentrations were measured for each gas stream by the TDL. Reference, sample and two calibration gases were run on alternating 80 s loops (20 s each), one for each AT and ET leaf at a matched canopy position, for a total of 12 min. This allowed for 4-5 measurements per leaf and data were averaged over the last 10 s of reference line and samples line gas streams for calculations. The two calibration gases were drawn from compressed air tanks (330 and 740 ppm CO2) in order to correct for gain drift of the TDL on each measurement cycle. Photosynthesis, gs, transpiration, VPD and intercellular [CO2] (Ci) were auto-logged every 15 s for each gas exchange system over the 12 min interval.

Mesophyll conductance was calculated from carbon isotope discrimination with equations and fractionation factors as presented in Evans & von Caemmerer (2013), including the ternary corrections proposed by Farquhar & Cernusak (2012), such that:

(1)

where o is the observed discrimination and i, gm , e and f are the contributions to fractionation if Ci = Cc, gm, respiration and photorespiration, respectively. For this study, the CO2 compensation point () and respiration during the day (Rd) parameters originally derived for tobacco plants (Caemmerer *et al.*, 1994) were replaced with parameters derived for *Eucalyptus globulus* from Crous et al. (2012) when calculating gm. The variation in o between sun and shade leaves and the simulated sunfleck where compared as a function of Ci:Ca. Once gm was caluclated the chloroplast CO2 partial pressure (Cc) and the drawdown of CO2 from the intercellular air spaces to the site of carboxylation were able to be estimated from the relationship between gm to the leaf photosynthesis rate (*An*) by:

(2)

Caemmerer S von**,** Evans JR**,** Hudson GS**,** Andrews TJ. **1994**. The kinetics of ribulose-1, 5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. *Planta* **195**: 88–97.

Crous KY**,** Zaragoza-Castells J**,** Ellsworth DS**,** Duursma RA**,** Loew M**,** Tissue DT**,** Atkin OK. **2012**. Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric CO2 and summer drought. *Plant, cell & environment* **35**: 966–981.

Evans JR**,** Von Caemmerer S. **2013**. Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. *Plant, cell & environment* **36**: 745–756.

Tazoe Y**,** Von Caemmerer S**,** Estavillo GM**,** Evans JR. **2011**. Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO2 diffusion dynamically at different CO2 concentrations. *Plant, Cell & Environment* **34**: 580–591.