Methods

## Site description

## Leaf gas exchange, coupled with concurrent measurements of carbon isotope discrimination to estimate mesophyll conductance

Leaf gas exchange measurements were performed between October 22nd - 30th, 2014 at the EucFACE experiment. Measurements were taken on representative fully expanded leaves for the upper and lower canopy of 2 trees in each experimental ring. Small branches, including the measurement leaf, were clipped and re-cut under water prior to gas exchange measurements. Due to an insect outbreak in 2 of the ambient [CO2] control rings during the measurement period some leaves were sampled in the nearest unaffected tree to the border of the experimental ring (rings 3 & 6).

Simultaneous gas exchange and C isotope discrimination measurements were conducted based on methods described in Tazoe *et al.* (2011) and Evans & von Caemmerer (2013). Leaf level gas exchange was measured with a 2 x 3 cm leaf cuvette and red/blue light source using a portable gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA). The gas exchange system was coupled with a tunable diode laser (TDL; TGA100,Campbell Scientific, Inc., Logan, UT, USA) for measurements of 13C isotope discrimination. The CO2 in the reference cell was set at [CO2] that allowed a ambient atmospheric [CO2] (400 ppm) in the sample cell to be achieved. This CO2 set point in the reference cell depends on the actual leaf photosynthetic rate, which was approximately +10-15 ppm CO2. Once these measurements were completed the [CO2] was increased in the reference cell to achieve the elevated atmospheric [CO2] of the EucFACE experiment (550 ppm) in the sample cell. The Leaf temperature were controlled at 25&degC, measurements were made at ambient air humidity and the flow rate was set at 200 mol s-1. Gas exchange data were autologged every 10 s for a total of 12 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) for the same 12 min period as gas exchange. TDL data were averaged over the last 10 s of reference line and sample line gas streams over each loop 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.

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). Here, the CO2 compensation point () and respiration during the day (Rd) parameters originally derived for tobacco plants (von Caemmerer *et al.* 1994) were replaced with parameters derived for *Eucalyptus globulus* from Crous *et al.* (2012) when calculating *g*m. Full descriptions of this method, including carbon isotope discrimination equations with ternary corrections, can be found at (Campany *et al.* 2016).

Once *g*m was calculated, *C*c and the drawdown of CO2 from the intercellular air spaces to the site of carboxylation were estimated from the relationship between *g*m and leaf photosynthesis rate (*A*n) by:

(1)

# References

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