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Site-specific responses to short-term environmental variation are reflected in leaf and phloem-sap carbon isotopic abundance of field grown *Eucalyptus globulus*

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The carbon isotopic composition ($\delta^{13}C$) of plant material has been used extensively as an indirect measure of carbon fixation per volume of water used. More recently, the $\delta^{13}C$ of phloem sap ($\delta^{13}C_{phl}$) has been used as a surrogate measure of short-term, canopy scale $\delta^{13}C$. Using a combination of $\delta^{13}C$ physiological, structural and chemical indices from leaves and phloem sap of *Eucalyptus globulus* at sites of contrasting water availability, we sought to identify short-term, canopy scale resource limitations. Results illustrate that $\delta^{13}C_{phl}$ offers valid reflections of short-term, canopy scale values of leaf $\delta^{13}C$ and tree water status. Under conditions limited by water, leaf and phloem sap photoassimilates differ in ^{13}C abundance of a magnitude large enough to significantly influence predictions of water use efficiency. This pattern was not detected among trees with adequate water supply indicating fractionation into heterotrophic tissues that may be sensitive to plant water status. Trees employed a range of physiological, biochemical and structural adaptations to acclimate to resource limitation that differed among sites providing a useful context upon which to interpret patterns in $\delta^{13}C$. Our results highlight that such easily characterized properties are ideal for use as minimally invasive tools to monitor growth and resilience of plants to variations in resource availability.

Introduction

Adjustments of plant processes to variation in resource availability rely on short-term strategies to modulate growth. For trees, these responses gain increased importance across longer life cycles and tree scale variation in resource availability. Short-term temporal and spatial variations in canopy processes are difficult to measure using traditional, leaf scale assessments of plant nutritional and physiological status. Monitoring of plant performance therefore requires the development of tools that encompass quantitative, short-term spatially integrated measures of plant function.

Investigations into short-term responses of *Eucalyptus globulus* to the effects of resource limitation have largely focused on the availability of water. Maintaining cell water content within a narrow operational range is essential for plant metabolism and survival (Galmes et al. 2007), a concept reflected in observed structural, chemical and physiological adaptations of Australian native plants (see Williams and Woinarsky 1997). Stomatal conductance and plant water relations are

Abbreviations – GC, gas chromatography; MCW, methanol–chloroform–water; PAR, photosynthetically active radiation; RWCs, relative water contents.

closely entwined (for review see Buckley 2005) allowing broad categorizations of isohydric and anisohydric behavior (Tardieu and Simonneau 1998). In the genus *Eucalyptus*, both inter- and intra-specific species variation in stomatal sensitivity has been characterized (David et al. 1997, Hatton et al. 1998, White et al. 1999, White et al. 2000a, 2000b, Whitehead and Beadle 2004) correlating with a range of traits governing plant water status. Changes in leaf osmotics, by both the accumulation of solutes (Myers and Neales 1986, Myers and Landsberg 1989, White et al. 2000b, Merchant and Adams 2005, Merchant et al. 2006b) and changes in leaf tissue elasticity (White et al. 2000b, Merchant et al. 2007, Callister et al. 2008) have been shown to have significant influences on plant water relations. Although the combination of trait responses to changes in resource availability is undoubtedly complex, such information assists our capacity to develop quantitative monitoring tools for plant water status and growth.

Naturally occurring carbon isotope abundance ($\delta^{13}C$) has been used extensively to study processes of plant function at the scales of metabolites (Tcherkez et al. 2004, Tcherkez 2006), leaves (Evans et al. 1986, Farquhar et al. 1989), plants (Fan and Blake 1997, Gessler et al. 2007, Cernusak et al. 2009) and ecosystems (West et al. 2006, Bowling et al. 2008). A range of environmental conditions, most notably light, water, temperature and humidity, influences fractionation of carbon isotopes during assimilation. Further fractionation of carbon isotopes may occur during compound synthesis, transport and plant respiratory processes (for reviews see Hobbie and Werner 2004, Cernusak et al. 2009). For larger plants, understanding the processes underpinning plant scale spatial and temporal variation in carbon isotope abundance is crucial for their use in systems biology. The analysis of $\delta^{13}C$ began at the leaf level (Farquhar et al. 1989) and has now grown to encompass a range of tissue types including heterotrophic tissues (Keitel et al. 2003, Gessler et al. 2009). As plants grow larger, carbon fixed in the leaves must move greater distances (primarily through the phloem) to be incorporated into structural material. Fractionation during this process is considered a major gap in our understanding of plant scale distribution of carbon isotope abundance (Cernusak et al. 2009).

Recent investigations into the properties of phloem sap have fostered the development of spatially and temporally integrated tools for the rapid assessment of tree physiological status. We previously used seasonal variation in $\delta^{13}C$ of phloem sap ($\delta^{13}C_{phl}$) as a surrogate for water availability and shown both positive and negative relationships to growth and variation throughout seasonal cycles (Merchant et al. 2010a, 2010b). Under conditions of water limitation, $\delta^{13}C_{phl}$ is negatively related to growth, likely reflecting stomatal limitations to CO_2 diffusion. Conversely, under conditions of high water availability, $\delta^{13}C_{phl}$ is positively related to growth suggesting non-stomatal limitations to carbon assimilation. We have also shown that the carbohydrate composition of phloem sap is an excellent predictor of growth across sites of contrasting water availability (Merchant et al. 2010b). Patterns in phloem chemistry may offer additional surrogate measures of plant water status as well as for the processes of carbon movement out of leaf tissues.

Phloem sap collection is quick, eliminates the need for tissue extraction, is cost effective, avoids analysis of carbon involved in leaf metabolism and may spatially integrate canopy level fractionation. Despite the promise of improved predictions of whole tree physiological function and the dynamic identity of resource limitation, few studies have sought to investigate spatial variation in $\delta^{13}C_{phl}$ and how this reflects spatial variation in leaf level $\delta^{13}C$. To investigate the suitability of phloem sap as a short-term integrated measure of canopy processes, we pursued two objectives: to determine the spatial and short-term (daily) variation of ^{13}C abundance and concentration of primary metabolites obtained from leaves and phloem tissues, and to assess their usefulness as integrated reflections of plant function in the context of short-term physiological, chemical and structural responses during growth of *E. globulus* at sites of contrasting resource limitations.

Materials and methods

Experimental design and site selection

Three plantations of *E. globulus* were chosen to represent sites of contrasting water availability. Sites were located along a rainfall gradient in south Western Australia near the township of Albany (34.95°S; 117.80°E). Sites were chosen from those used in a previous investigation regarding carbon isotopic abundance in phloem sap (Merchant et al. 2010b). Trees were not clonal but originated from parents within same provenance (geographic origin). For this study, we chose two sites characterized in that study that experienced contrasting water availability (with one thought to be accessing a major supply of groundwater) but existed in close proximity thus experience similar light and temperature regimes (sites A and B). Groundwater access was determined indirectly based on the patterns of $\delta^{13}C$ obtained from phloem sap becoming decoupled from seasonal variation (Merchant et al. 2010b). A third site (site C), also used for the previous study was chosen at a lower rainfall approximately

100 km east of these sites. Trees were planted at 3 × 5 spacing (approximately 650 stems per hectare). At each site, five trees were identified for measurement as representative of the modal distribution of tree sizes based upon measurements of stem diameter at 1.3 m in height.

Environmental monitoring

Meteorological and edaphic variables were monitored at each site for 5 days prior to the commencement of measurements and 5 days post-measurement, using a HOBO weather-station (Onset Computer Corp., Pocasset, MA). Photosynthetically active radiation (PAR), air temperature (T, °C), relative humidity (RH), rainfall and wind speed and direction were logged every 15 min. Stations were positioned adjacent to the canopy so as to capture light and environmental conditions as experienced by the upper canopy. Sampling times were coordinated with relatively consistent daytime temperatures and negligible cloud cover for the previous 3 days. The 3-day period was chosen according to previous findings on the time offset for coupling environmental conditions and phloem $\delta^{13}C$ (Keitel et al. 2003, 2006, Cernusak et al. 2005, Merchant et al. 2010b).

Measures of tree water use

Tree water use was determined using the Heat Ratio Method (HRM30, ICT International, Armidale, Australia) as described in Burgess et al. (2001). Heat velocity was recorded in conjunction with meteorological information (every 15 min) and transformed to sap flux density per unit sapwood area (J_s ml cm^{-2} h^{-1}) as per Pfautsch et al. (2010). Data were summed per tree and day before averaged for the period of 27 January 2008 to 3 February 2008. True zero flow measurements were obtained by felling trees above sensor installations on 4 February 2008 and continued recording of data during the following 2 days.

Leaf gas exchange

Stomatal conductance to water vapor for both the upper and lower canopy positions was measured every hour for 24 h using a hand-held leaf porometer (SC-1, Decagon, Pullman, WA). Each measurement was made on a separate young, fully expanded leaf located on the northern aspect of the canopy. Following each measurement, leaves were detached and stored in sealed plastic bags prior to measurement of leaf water potential.

A vs c_i curves and light-saturated rates of photosynthesis (A_{sat400}), stomatal conductance (g_{s400}) and dark respiration rate (R_{d400}) at [CO_2] of 400 ppm were recorded for leaves in the lower and upper canopy for each tree using a portable infrared gas analyzer (LI-6400 with 6 cm^2 chamber and LED light source, Li-Cor, Lincoln, NE). For all measurements, airflow through the chamber was set at 500 μmol s^{-1} and vapor pressure deficit was set to track ambient conditions. For A vs c_i curves, leaves were equilibrated to a PAR of 1500 μmol m^{-2} s^{-1}. A vs c_i curves were generated in a stepwise fashion by lowering [CO_2] from 1500 to 0 μmol mol^{-1}. Maximum rate of assimilation (A_{max}) were taken to be the rate of assimilation at PAR = 1500 μmol m^{-2} s^{-1}. Estimates of J_{max} and V_{cmax} were made using the minimization routine in PHOTOSYN ASSISTANT 1.1 to produce the best fit. For modeled values of assimilation, rates of net assimilation were calculated using the photosynthesis model of Farquhar et al. (1980) combined with a model for CO_2 diffusion into leaves (von Caemmerer and Farquhar 1981) and constrained with hourly values of PAR, stomatal conductance and air temperature measured by the LI-COR 6400. Photosynthetic parameters were adjusted for temperature using response functions given by Bernacchi et al. (2001).

Leaf water relations

Leaf water relations were determined for leaves on a terminal branch of each sample tree. At each site, leaf water potential was measured pre-dawn (ψ_{pdwn}) and hourly for a 24-h period using a Scholander-type pressure chamber (PMS Corvallis, OR). Leaf relative water contents (RWCs) were determined on five leaves from each tree. Each leaf was weighed (fresh weight), then placed in deionized water for 6 h to allow full hydration. Leaf samples were then lightly blotted dry with tissue paper and re-weighed (saturated weight), before drying at 60°C for 48 h and re-weighing (dry weight). Leaf osmotic potential at full turgor (π_{ft}), leaf osmotic potential at turgor loss point (π_{tlp}) and leaf relative water content at turgor loss point (RWC_{tlp}) were determined using pressure volume analysis (Tyree and Hammel 1972, Tyree and Richter 1982, Turner 1988). Sections of branch were cut under water (while keeping leaves dry) and placed in the dark for 3 h at room temperature to re-saturate. Samples were considered to have re-hydrated if their water potentials exceeded −0.3 MPa. Maximum bulk elastic modulus (ε) was calculated according to Turner (1988) as the slope of the relationship between pressure potential and RWC in the positive turgor range. Three pressure volume curves were generated for each tree at each site.

Sample collection for chemical and isotopic analysis

Leaf material was collected predawn and at 14:00 h from each of the sites on the day of gas exchange

measurements. Leaves were immediately microwaved to impede metabolism as per Popp et al. (1996) and then oven dried at 75°C. Samples were then ground to a powder and stored at −80°C awaiting analysis.

Phloem sap was collected using a previously described bleeding technique (Pate and Arthur 1998, 2000, Pate et al. 1998, Cernusak et al. 2003, 2005, Merchant et al. 2010b). In brief, phloem sap was collected using a single sided razor blade. An incision of approximately 5–8 cm was made in the stem of the tree. Samples were collected at three heights at the end of stomatal conductance measurements (at the same time porometry measurements were taken in the upper and lower canopy levels). Samples were collected in a clean, glass disposable pipette and bulked into a single microtube for each tree height. Samples were immediately transferred to 4°C and frozen within 4 h.

Analysis of phloem sap and leaf extracts for soluble carbohydrates

For carbohydrate analysis of leaves, soluble compounds were extracted from approximately 40 mg of dried leaf material with a methanol–chloroform–water (MCW) solution as detailed in Merchant et al. (2006a). An internal standard of 0.1% xylitol was introduced to the water fraction of this MCW mix. Leaf extracts were deionized by the addition of 300 µl of mixed bed resin consisting of one part Dowex 1×8 (anion exchange, Cl^- form) and one part Dowex 50 W (cation exchange, formate form). Samples were agitated for a period of 2 h at room temperature. Following pulse centrifugation, 400 µl of the supernatant was transferred to a clean micro-tube and stored at −80°C. For carbohydrate analysis of phloem sap, 5 µl was diluted with 700 µl of deionized water and deionized using the method described above.

Carbohydrates were separated and quantified using gas chromatography (GC) according to Merchant et al. (2006a). Sixty µl of deionized MCW extracts were dried and resuspended in 400 µl anhydrous pyridine to which 50 µl of trimethylchlorosilane (TMCS)/bis-trimethylsilyl-trifluoroacetamide mix (1:10 v/v, Sigma Aldrich, Sydney, Australia) was added. Samples were incubated for 1 h at 75°C and analyzed by GC within 24 h. GC analysis was performed using a Shimadzu 17A series gas chromatograph using a DB1 (30 m) column. Split injection was made at 300°C with an initial oven temperature program of 60°C for 2 min ramping to 300°C at 10 °C min^{-1} and maintained for 10 min. Column flow rate was maintained at 1.5 ml min^{-1}. Peak integration was made using Class VP analysis software (Shimadzu Corporation Limited, Columbia, MD, USA).

Carbon isotope analysis

For analysis of carbon isotope abundance in the soluble extract of leaves ($\delta^{13}C_{leaf}$), 40 mg of ground leaf material was weighed into a 2 ml micro-tube to which 1 ml of hot, deionized water was added and incubated for 1 h at 75°C. Samples were centrifuged at 11 400 *g* for 3 min and 800 µl of the supernatant transferred to a 2 ml micro-tube to which 300 µl of mixed bed resin had been added. Samples were deionized as described above then 200 µl was progressively transferred into tin cups and dried at 45°C then kept over desiccant awaiting analysis.

Samples were analyzed on an Isochrom mass spectrometer (Micromass, Manchester, UK) coupled to a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy). Samples were dropped from an AS200 auto-sampler and combusted by Dumas-combustion in a furnace kept at 1060°C. Carbon isotope ratios are expressed in delta-notation, where $\delta^{13}C = R_{sample}/R_{standard} - 1$, and R is the ratio of ^{13}C to ^{12}C in a sample and standard (VPDB), respectively. The precision for the standard materials was between 0.06 and 0.11‰.

Inorganic ion analysis

Major cations in leaf extracts were quantified by inductively coupled plasma-optical photoemission spectroscopy (ICP-OES) as per Merchant et al. (2010a). In brief, approximately 40 mg of ground dried leaves were extracted into 1 ml of hot (80°C) deionized water for 1 h and agitated during this time. Samples were cooled and then centrifuged at 11,400 g for 2 min. The supernatant was then removed and placed into a 1.5 ml microtube. 250 µl of this extract was then taken and placed into a 15 ml tube and diluted with 6 ml of deionized water ready for analysis.

Calculations of osmotic potential

Molar concentrations of solutes in phloem sap were converted to osmotic potential (π_s) using the van't Hoff equation:

$$\pi_s = -RTc_s \quad (1)$$

where R is the universal gas constant (8.3145×10^{-3} MPa L mol^{-1} K^{-1}), T is the temperature (K) and c_s (mol L^{-1}) is the concentration of osmotically active solutes in the solution. Concentrations of leaf metabolites in leaf water were calculated based on leaf water content (measured as described above under *Leaf water relations*).

Statistics

Significance of variation in measured parameters was tested using analysis of variance (ANOVA) using STATISTICA

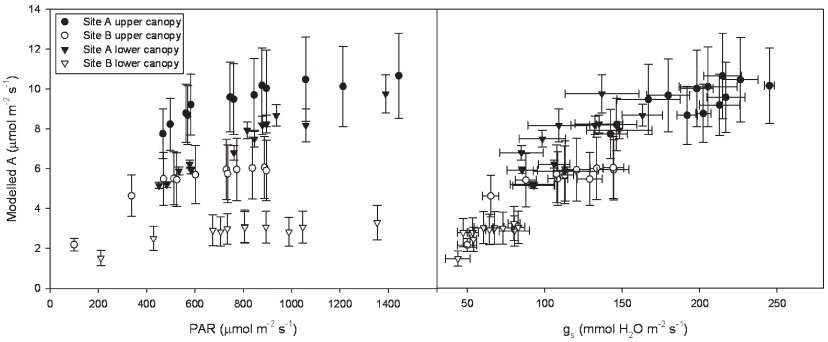

Fig. 1. Modeled photosynthetic rate plotted against ambient PAR and stomatal conductance (g_s) during a diurnal cycle for five *Eucalyptus globulus* trees on sites of contrasting water availability. Each point is modeled from parameters derived from the average of A vs c_i for five trees per site. A single PAR measurement was made using a HOBO™ PAR sensor located adjacent to the plantation outside the effects of canopy shading. Stomatal conductance was measured using a porometer and is expressed as the average g_s of two measures of fully expanded sun leaves in the top of the canopy.

analytical software (version 6, StatSoft, Tulsa, OK). *P* values were calculated using Fisher's least square difference post hoc test.

Results

Assessment of resource limitation among sites

Assessments of resource limitation (light and water) to carbon assimilation were made at sites A and B. Adverse weather conditions (electrical storm) prevented our assessment of canopy scale carbon assimilation rates at site C. Modeled values for net assimilation at site A were strongly influenced by diurnal patterns in PAR (Fig. 1), increasing by up to 6 μmol m^{-2} s^{-1} over the diurnal cycle. Modeled assimilation rates approached measured light saturated rates in both canopy layers at sites A and B (cf. Figs 1 and 2). Site B exhibited significantly lower g_s and A_{sat} in both upper and lower canopy leaves (Fig. 2). Modeled c_i values did not significantly differ between sites irrespective of canopy position, ranging from 245 ± 9 and 286 ± 19 μmol CO_2 mol air^{-1} in the lower canopy and 287 ± 11 and 272 ± 12 in the upper canopy at sites A and B, respectively. Differences in modeled assimilation rate between canopy levels observed at site B were likely influenced by lower stomatal conductance (Fig. 1) while in contrast, little variation in assimilation was observed between canopy levels at site A (Fig. 1). Leaf temperature did not significantly influence modeled assimilation rates (data not shown).

Tree water relations

Trees at site A used approximately 250% more water per square centimeter of sapwood area than trees at sites B and C (Fig. 4). Within the 8-day sample period, J_s ranged from 210 to 255 ml cm^{-2} day^{-1} at site A and from 60–120 and 11–155 ml cm^{-2} day^{-1} at sites B and C, respectively.

Trees at site A exhibited less negative values of predawn water potential than those at sites B and C (Table 1). Leaves at site A exhibited significantly higher bulk elastic modulus (demonstrated by lower values of ε) than leaves at sites B and C. This corresponded with significantly lower values of RWC_{tlp} for site A. Values of π_{ft} and π_{tlp} showed a consistent pattern of increased values at site B and decreased values at site C compared to site A, with only π_{ft} at site B being non-significant.

Leaf and phloem chemistry

For leaves at sites A and B, measured carbohydrate levels reflected patterns in π determined by pressure volume analysis at both π_{FT} and π_{TLP}. Total contributions of measured osmolytes did not reflect that of π values determined by pressure volume analysis due to the overwhelming influence of cations on the calculated contribution to cellular π. Despite higher levels of carbohydrates, the contribution of measured solutes to π was lower at site C owing to significantly lower concentrations of both potassium and sodium. At each site, between 40 and 51% of contributions to π_{ft} were quantified. Most notably, significantly higher levels of potassium (K^+) were detected in leaves from site A (Table 2).

Phloem sap contained both sucrose and raffinose in concentrations of around 1000 and 300 mmol l^{-1} respectively (Table 3). Sites B and C contained significantly higher sucrose concentrations in phloem sap than site A at 1.3 m and the top of the canopy. In contrast, site C had significantly lower concentrations

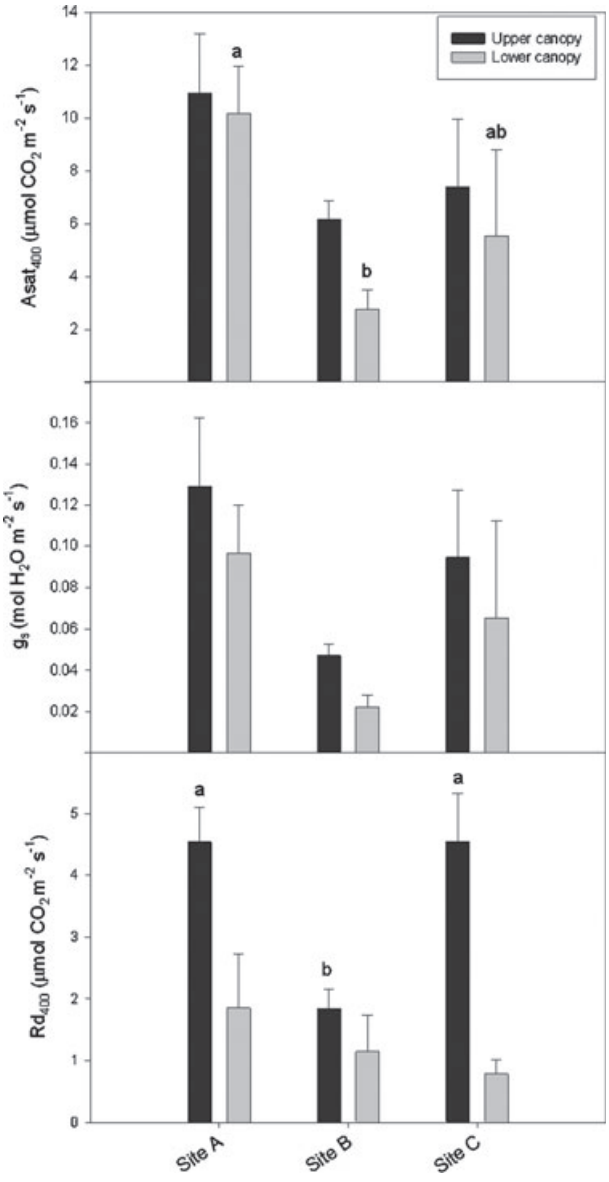

Fig. 2. Light saturated assimilation (A_{sat400}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and dark respiration (R_{d400}, µmol CO_2 m^{-2} s^{-1}) rates measured at 400 ppm atmospheric CO_2 concentration for *Eucalyptus globulus* trees growing on sites of contrasting water availability. Dark and light bars represent measurements taken from leaves in the upper canopy and lower canopy respectively. Error bars represent standard errors of mean values. Differences between sites with constant canopy position are displayed and were determined using Fishers least square difference post hoc test. Significant differences were also tested within canopy position using Fisher's least square difference post hoc test. Only R_d differed within canopy positions at sites A and C (post hoc groupings not shown).

raffinose at all canopy heights. These concentrations resulted in an increasing ratio of sucrose to raffinose in the sequence A<B<C with site C consistently significantly different from site A.

Leaf and phloem carbon isotope abundance

Leaf neutral fraction $\delta^{13}C$ was generally more negative in the lower canopy that in the top of the canopy (Fig. 3) a trend not reflected in $\delta^{13}C_{phl}$. $\delta^{13}C_{phl}$ differed between all sites (Fig. 3, hatching) and did not significantly vary with collection at different canopy heights (post hoc groupings). Site A had the most negative values of $\delta^{13}C_{phl}$ followed by site C and site B.

$\delta^{13}C_{phl}$ collected at sites B and C were significantly enriched in ^{13}C compared to leaf tissues. At site A, $\delta^{13}C_{phl}$ did not significantly differ from leaf $\delta^{13}C$.

$\delta^{13}C_{phl}$ collected at 1.3 m stem height correlated strongly with total sap flux density with the lowest values of δ being collected at the site of lowest water limitation (Fig. 4). $\delta^{13}C_{phl}$ collected at 1.3 m height reflected total sap flux density more strongly than any other carbon pool collected from wither phloem or leaf soluble carbon at any canopy height (Table 4).

Discussion

Resource limitation differed among sites

Patterns of photosynthesis, ψ_{pdawn}, g_s and total daily sap flux density suggest that the magnitude and nature of resource limitation differed between sites. Large differences among sites in total sap flux density likely reflect significant differences in water availability, as evidenced by lower pre-dawn water potentials at sites B and C compared to site A. Trees at sites B and C also exhibited lower values of g_s, suggesting stomatal limitation to CO_2 diffusion is a major limitation to carbon assimilation. Interestingly, reduced A_{sat} values at sites B and C suggest that photosynthetic capacity is also reduced in trees growing under lower water availability, which explains the similarity in modeled c_i values across all trees. Trees at site A, where the biochemical limitation was thought to be strongest, exhibited significantly higher rates of light saturated photosynthesis (Fig. 2), which gave rise to larger increases in modeled photosynthetic rates in response to diurnal changes in PAR (Fig. 1). A limitation to photosynthesis by light availability therefore is a prominent candidate to explain previously obtained positive relationship between $\delta^{13}C$ and growth collected at the seasonal scale (Merchant et al. 2010b).

Patterns in leaf water relation parameters

Maintenance of cell turgor over the diurnal cycle is a significant pre-determinant to the continuation of photosynthesis. Pressure volume curve analysis revealed patterns in both chemical and structural responses

Table 1. Predawn water potential ($\psi_{predawn}$), osmotic potential at full turgor (π_{ft}), osmotic potential at leaf turgor loss point (π_{tlp}), relative water content at leaf turgor loss point (RWC_{tlp}) and maximum bulk elastic modulus of leaf tissue (ε) as measured by pressure volume curve analysis on 5-year-old *Eucalyptus globulus* trees at sites of contrasting water availability. Average values were calculated from the measurement of five trees at each site. Fully expanded leaves were sampled from the upper canopy. Post hoc groupings represent differences between carbon pools using Fishers least squared difference post hoc test ($p < 0.05$). * denotes site with groundwater access.

Site	$\psi_{predawn}$ (MPa) Ave ± SE	π_{ft} (MPa) Ave ± SE	π_{tlp} (MPa) Ave ± SE	RWC_{tlp} Ave ± SE	ε (Mpa) Ave ± SE
Site A (850 mm rainfall year^{-1})*	-0.39 ± 0.03^{a}	-1.65 ± 0.02^{a}	2.03 ± 0.07^{a}	0.87 ± 0.02^{a}	4.71 ± 1.25^{a}
Site B (850 mm rainfall year^{-1})	-0.86 ± 0.02^{b}	-1.93 ± 0.12^{a}	-2.39 ± 0.06^{b}	0.95 ± 0.01^{b}	12.62 ± 0.97^{b}
Site C (650 mm rainfall year^{-1})	-0.85 ± 0.06^{b}	-1.43 ± 0.05^{b}	-1.80 ± 0.08^{c}	0.92 ± 0.03^{b}	10.85 ± 2.85^{b}

Table 2. Contributions to osmotic potential of major leaf solutes for *Eucalyptus globulus* trees at sites of contrasting water availability. Average values were calculated from the measurement of five trees at each site. Fully expanded, illuminated leaves were collected from the upper canopy. Post hoc groupings represent differences between carbon pools using Fishers least squared difference post hoc test ($p < 0.05$). * denotes site with groundwater access.

Site	Glucose (MPa_{FT})	Fructose (MPa_{FT})	Sucrose (MPa_{FT})	K^+ (MPa_{FT})	Na^+ (MPa_{FT})	Total (MPa_{FT})	% (MPa_{FT})
Site A (850 mm rainfall year^{-1})*	-0.019 ± 0.003^{a}	-0.021 ± 0.004^{a}	-0.002 ± 0.000^{a}	-0.571 ± 0.031^{a}	-0.224 ± 0.017^{a}	-0.83 ± 0.029^{a}	0.507 ± 0.019^{a}
Site B (850 mm rainfall year^{-1})	-0.036 ± 0.003^{b}	-0.036 ± 0.004^{b}	-0.004 ± 0.001^{a}	-0.485 ± 0.086^{ab}	-0.249 ± 0.098^{a}	-0.809 ± 0.178^{a}	0.407 ± 0.07^{a}
Site C (650 mm rainfall year^{-1})	-0.053 ± 0.002^{c}	-0.064 ± 0.007^{c}	-0.003 ± 0.001^{c}	-0.431 ± 0.049^{b}	-0.176 ± 0.016^{a}	-0.728 ± 0.062^{b}	0.514 ± 0.059^{a}

to differences in water availability. The most notable difference in leaf properties among sites was in maximum bulk elastic modulus (ε), which was 2–3 times greater at sites B and C than at site A. It seems unlikely that these large differences in ε among sites can be attributed to difficulty in measurement of ε due to tissue heterogeneity and cellular structure (Tyree and Jarvis 1982) and leaf mass per unit area (Galmes et al. 2007). Increased elasticity (reduced elastic modulus) shifts the turgor loss point to more negative water potentials, increasing the resilience of turgor to reduced water supply. Large values of ε are thought to increase stomatal sensitivity (White et al. 2000b) although contradictory results (Galmes et al. 2007) are often obtained. For eucalypts, several authors have highlighted the importance of elastic adjustment (reduced ε) in regulating turgor under water stress, with comparatively small values of ε reported across a range of species occupying drier environments

Table 3. Concentrations and contributions to osmotic potential of phloem sap sucrose and raffinose for *Eucalyptus globulus* trees at sites of contrasting water availability. Values for the different sampling heights are the average of five trees. Post hoc groupings represent differences between carbon pools using Fishers least squared difference post hoc test ($p < 0.05$). * denotes site with groundwater access.

	$Sucrose_{phloem}$ (mmol l^{-1}) Ave ± SE			$Raffinose_{phloem}$ (mmol l^{-1}) Ave ± SE		
Site	1.3 m height	Base canopy	Top canopy	1.3 m height	Base canopy	Top canopy
Site A (850 mm rainfall year^{-1})*	819 ± 29^{a}	754 ± 113^{a}	844 ± 17^{a}	328 ± 19^{a}	259 ± 47^{a}	338 ± 36^{a}
Site B (850 mm rainfall year^{-1})	1091 ± 39^{b}	1073 ± 49^{a}	1095 ± 41^{b}	317 ± 17^{a}	285 ± 18^{a}	300 ± 8^{a}
Site C (650 mm rainfall year^{-1})	1083 ± 6^{b}	1035 ± 30^{a}	1050 ± 68^{b}	222 ± 10^{b}	175 ± 11^{b}	218 ± 15^{b}
	Ratio (Sucrose:Raffinose) Ave ± SE			Combined osmotic potential (MPa) Ave ± SE		
Site	1.3 m height	Base canopy	Top canopy	1.3 m height	Base canopy	Top canopy
Site A (850 mm rainfall year^{-1})*	2.49 ± 0.47^{a}	2.90 ± 0.76^{a}	2.49 ± 0.15^{a}	-2.57 ± -0.11^{a}	-2.27 ± -0.36^{a}	-2.65 ± -0.12^{a}
Site B (850 mm rainfall year^{-1})	3.43 ± 0.73^{ab}	3.76 ± 0.87^{a}	3.65 ± 1.64^{ab}	-3.16 ± -0.13^{b}	-3.05 ± -0.15^{a}	-3.13 ± -0.11^{a}
Site C (650 mm rainfall year^{-1})	4.87 ± 0.19^{b}	5.91 ± 0.87^{b}	4.81 ± 1.41^{b}	-2.93 ± -0.04^{b}	-2.71 ± -0.09^{a}	-2.85 ± -0.19^{a}

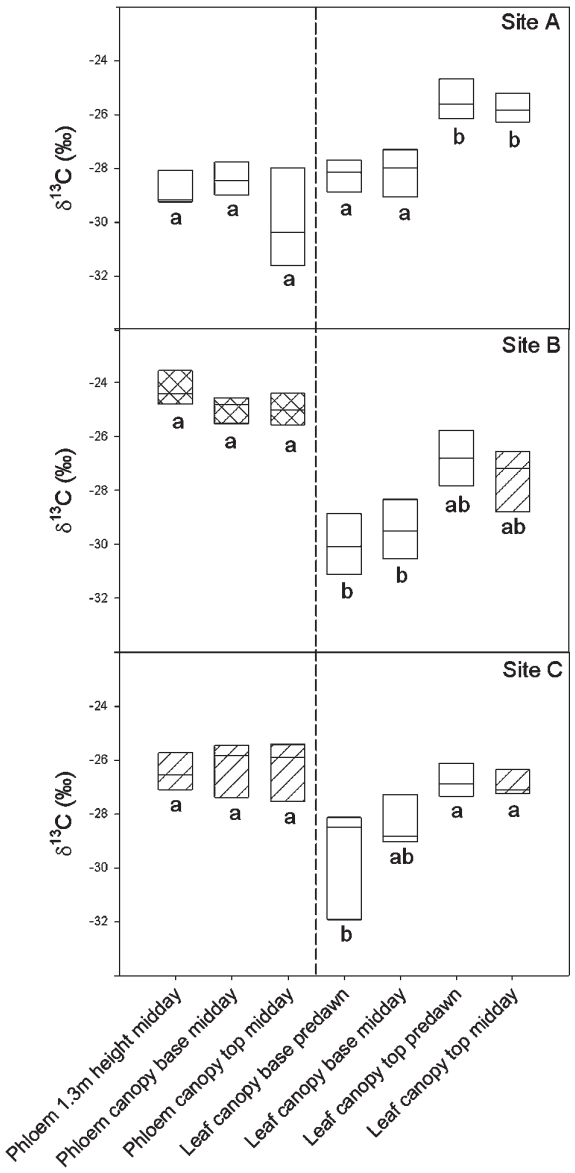

Fig. 3. Average $\delta^{13}C$ of phloem sap and leaf soluble carbon obtained from five *Eucalyptus globulus* trees grown on sites of contrasting water availability. Post hoc groupings represent differences between carbon pools within sites using Fishers least squared difference post hoc test. Hatching represents post hoc groupings of $\delta^{13}C$ of between sites within each carbon pool.

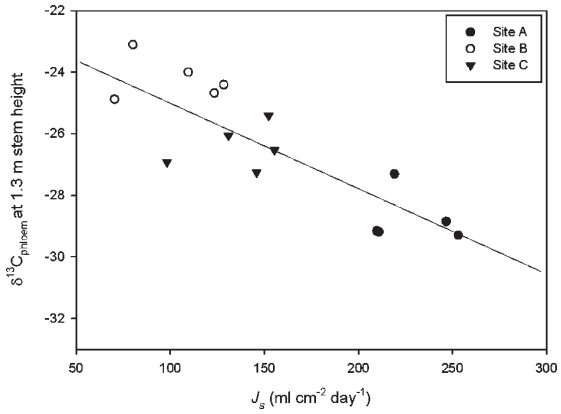

Fig. 4. $\delta^{13}C$ of phloem sap obtained from *Eucalyptus globulus* trees growing on sites of contrasting water availability plotted against average daily sap flux density per cm^2 of sapwood area. Phloem sap was obtained at 1.3 m stem height. Average daily sap flux density was calculated from flux density over the 5 days preceding sampling for measures of $\delta^{13}C$.

Table 4. Regression coefficients of $\delta^{13}C$ and sap flux density (J_s, ml cm^{-2} day^{-1}) by *Eucalyptus globulus* trees growing on sites of contrasting water availability. Average daily J_s was calculated from sap flux density over the 5 days preceding sampling for measures of $\delta^{13}C$. Leaves were sampled at predawn (06:00) and midday (14:00) from both the base and the top of the canopy.

			J_s		Average daily g_s	
Carbon pool	Time	Position	r^2	*P*	r^2	*P*
$\delta^{13}C_{phloem}$	Midday	1.3m height	0.7243	<0.001	0.7859	<0.001
$\delta^{13}C_{phloem}$	Midday	Canopy base	0.6189	0.001	0.7403	0.001
$\delta^{13}C_{phloem}$	Midday	Canopy top	0.4913	0.001	0.4142	0.061
$\delta^{13}C_{leaf}$	Midday	Canopy base	0.2635	0.049	0.4512	0.033
$\delta^{13}C_{leaf}$	Midday	Canopy top	0.2715	0.059	0.1625	0.248
$\delta^{13}C_{leaf}$	Predawn	Canopy base	0.5198	0.003	0.0138	0.747
$\delta^{13}C_{leaf}$	Predawn	Canopy top	0.0722	0.379	0.0281	0.644

(White et al. 2000b, Merchant et al. 2007) and in response to water deficit and/or seasonal cycles (Prior and Eamus 1999, Callister et al. 2008). Mitchell et al. (2008) found increased ε during summer drought in *Eucalyptus salmonophloia* and *Eucalyptus albida* (and no change in *E. capillosa* subsp. *capillosa*). Here we found higher ε (lower elasticity) for trees at the drier sites (B and C), suggesting *Eucalyptus globulus* is more akin to *E. salmonophloia* and *E. albida*, in that it does not appear to maintain turgor via classical elastic adjustment (reduced ε). For our study, trees with greater sap flux density exhibited a characteristic of eucalypt species growing in drier environments suggesting that acclimative strategies work independently in response to varying site conditions. One explanation might be that trees at site A develop tissues with higher levels of elasticity to buffer stomatal sensitivity against short term changes in ψ experienced over the diurnal cycle.

Other options for turgor maintenance are osmotic adjustment (enhanced π) and avoidance of water loss, i.e., reduced transpiration. At site B, π_{tlp} was greater and g_s was lower than at the moister site A – suggesting a degree of both osmotic adjustment and avoidance strategies in site B. The results from site C were somewhat paradoxical; however, π_{tlp} was lower and g_s was similar compared to site A, suggesting no turgor-maintaining adjustments at all at site C. A likely explanation is that

VPD was quite low on the day that g_s was measured at site C, leading to high but non-representative values of g_s.

Patterns in leaf and phloem chemistry

Variation in leaf osmotic potential obtained from both pressure volume curves and leaf chemical composition showed clear delineations between sites. Total contributions of measured leaf osmotica of around 0.8 MPa fit well with overall measures of π_{ft} of around 1.6–1.9 MPa. The largest influence on leaf osmotic potential was that of potassium, contributing up to 0.6 MPa in leaf water. Leaf metabolites showed significant trends albeit at concentrations that had little effect on overall osmotic potential. Despite previous studies showing accumulation of sucrose in *E. globulus* under conditions of water deficit (Merchant et al. 2006b), leaf reducing sugars glucose and fructose significantly differed between sites while leaf sucrose did not. Adjustments in π of leaves via the accumulation of metabolites are therefore not a primary mechanism for osmotic responses of leaves to water availability in the stands of *E. globulus* that we studied.

The most prevalent pattern in plant chemistry was significant differences in phloem sugar composition between sites. *E. globulus* is thought to employ a symplastic mode of phloem loading on account of the high prevalence of sucrose and raffinose found in phloem sap (see Turgeon 1996). Changes in phloem osmotic potential have been suggested as a method of maintaining water potential equilibrium with the xylem (Cernusak et al. 2003). We have previously used measures of phloem osmotic potential as surrogate measures of plant growth (Tausz et al. 2008) and detected changes in phloem composition according to landscape and seasonal variation (Merchant et al. 2010b) as well as the effects of artificially modified water availability (Merchant et al. 2010a). In addition to bulk changes in solute concentration attributable to source/sink relationships and water status, changes in the ratio of sucrose to raffinose will influence π independently attributable to differences in molecular weight. For the trees studied here, the approximate maximum change in sucrose from 800 to 1100 mmol l^{-1} corresponding with an approximate change in raffinose from 300 to 200 mmol l^{-1} would impart a net change of −0.496 MPa – a significant contribution considering their total contribution to phloem sap π of around 3 MPa.

Canopy scale patterns in $\delta^{13}C$ obtained from leaves and phloem sap

Given consistent height and timing of collection, $\delta^{13}C_{leaf}$ did not delineate sites. We analyzed the neutral fraction of leaf soluble carbon in comparison with phloem sap, both of which largely consist of carbohydrates and sugar alcohols. The enrichment of $\delta^{13}C_{leaf}$ obtained from leaves collected at the top of the canopy compared to the base agrees with previous studies (Duursma and Marshall 2006) and most likely reflects a combination of both stomatal limitation in the upper canopy and biochemical limitation in the lower canopy imposed by lower light levels.

$\delta^{13}C_{phl}$ did not reflect spatial variation in $\delta^{13}C_{leaf}$ and clearly delineated sites in agreement with measures of ψ, g_s and daily sap flux density. This result is likely to be attributable to the measurement of recently fixed photoassimilates independent of background carbon involved in leaf metabolism and supports previous isotopic patterns (Pate et al. 1998, Keitel et al. 2003, Merchant et al. 2010b) obtained from a range of *E. globulus* plantations of contrasting physiological status. Most notably, at sites where water was limiting, $\delta^{13}C_{phl}$ was enriched compared to $\delta^{13}C_{leaf.}$ Recent reviews have highlighted a general enrichment of phloem sap compared to leaf tissues (Hobbie and Werner 2004, Badeck et al. 2005, Bowling et al. 2008, Cernusak et al. 2009), and the downstream patterns in $\delta^{13}C$ of heterotrophic tissues (Bowling et al. 2008, Cernusak et al. 2009). While the causal relationship for this enrichment is unclear, our data suggest that the degree of enrichment may be sensitive to plant physiological status. Fractionation attributable to the transient storage of carbon as starch (Gessler et al. 2007) and the possibility of fractionation due to alternative loading mechanisms concurrently at play (Voitsekhovskaja et al. 2009) may be sensitive to resource availability. Phloem sap carbohydrate concentrations in *E. globulus* that are sensitive to environmental conditions (Merchant et al. 2010b) and contain compound-specific differences in ^{13}C abundance (Merchant et al. 2011) may serve to explain some of the patterns observed in this study. Concurrent investigations into short-term variation in $\delta^{13}C$ of leaf and phloem metabolites, the dynamics of starch accumulation and degradation, and the inhibition of apoplastic loading mechanisms are required to determine causal relationships of changes in isotopic abundance from leaf to phloem.

$\delta^{13}C_{phl}$ did not vary with the height at which it was collected suggesting negligible fractionation of carbon isotopes during transport. Gessler et al. (2009) highlights contradictory results for within-tree spatial variation of $\delta^{13}C_{phl}$ previously obtained from a range of species by the same author (Gessler et al. 2004, 2007) that may be influenced by seasonal cycles. The transient storage of carbon as starch is one possible explanation to the observed enrichment of carbon with increasing

path length (Gessler et al. 2007), a process likely to be influenced by physiological status. Although our results suggest there is little ^{13}C fractionation with increasing path length during transport in the phloem, our results may be influenced by high degrees of mixing and throughput of carbon at the time of sample collection. It is also not immediately clear why $\delta^{13}C_{phl}$ collected at 1.3 m height provided the strongest correlation with total sap flux density. Previous investigations for both *E. globulus* (Merchant et al. 2010b) and *Fagus sylvatica* (Keitel et al. 2003) have shown that phloem sap collections reflect environmental conditions experienced approximately 2 days prior to collection. One hypothesis may be that $\delta^{13}C_{phl}$ obtained at 1.3 m avoids within canopy redistribution of photoassimilates. Irrespective of the causal relationship, $\delta^{13}C_{phl}$ obtained at 1.3 m offers improved predictions of plant physiological status than any other carbon pool analyzed in this study. This result highlights the usefulness of $\delta^{13}C_{phl}$ as an indicator of canopy scale processes.

The potential for rapid, time and canopy scale integrated measures of plant function obtained by carbon isotope and metabolite abundance in phloem sap offers considerable advantages over more traditional measures of leaf chemistry and physiology. At sites of contrasting resource limitation, the abundance of metabolites and carbon isotopes in phloem sap delineated each site despite an absence of discernible patterns in leaf tissues. Traditional assessments of plant physiological status offered contrasting results, thus emphasizing the need to consider functional traits independently. For example, leaf ε likely plays a role in the regulation of cell turgor but doubt remains over its interaction with other leaf level processes. Providing integrative assessments of plant health and characterizing physical, chemical and structural traits underpinning acclimation to site conditions will form the basis of monitoring and plant improvement programs.

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References

Badeck FW, Tcherkez G, Nogues S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon. Rapid Commun Mass Spectrom 19: 1381–1391

Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant Cell Environ 24: 253–259

Bowling DR, Pataki DE, Randerson JT (2008) Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. New Phytol 178: 24–40

Buckley TN (2005) The control of stomata by water balance. New Phytol 168: 275–291

Burgess SSO, Adams MA, Turner NC, Beverly CR, Ong CK, Khan AAH, Bleby TM (2001) An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree Physiol 21: 589–598

Callister AN, Arndt SK, Ades PK, Merchant A, Rowell D, Adams MA (2008) Leaf osmotic potential of Eucalyptus hybrids responds differently to freezing and drought, with little clonal variation. Tree Physiol 28: 1297–1304

Cernusak LA, Arthur DJ, Pate JS, Farquhar GD (2003) Water relations link carbon and oxygen isotope discrimination to phloem sap sugar concentration in *Eucalyptus globulus*. Plant Physiol 131: 1544–1554

Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. Tree Physiol 25: 129–146

Cernusak LA, Tcherkez G, Keitel C, Cornwell WK, Santiago LS, Knohl A, Barbour MM, Williams DG, Reich PB, Ellsworth DS, Dawson TE, Griffiths HG, Farquhar GD, Wright IJ (2009) Why are non-photosynthetic tissues generally ^{13}C enriched compared with leaves in C_3 plants? Review and synthesis of current hypotheses. Funct Plant Biol 36: 199–213

David TS, Ferreira MI, David JS, Pereira JS (1997) Transpiration from a mature *Eucalyptus globulus* plantation in Portugal during a spring-summer period of progressively higher water deficit. Oecologia 110: 153–159

Duursma RA, Marshall JD (2006) Vertical canopy gradients in d ^{13}C correspond with leaf nitrogen content in a mixed-species conifer forest. Trees Struct Funct 20: 496–506

Evans RD, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust J Plant Physiol 13: 281–292

Fan SH, Blake TJ (1997) Comparison of polyethylene glycol 3350 induced osmotic stress and soil drying for drought simulation in three woody species. Trees Struct Funct 11: 342–348

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149: 78–90

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40: 503–537

Galmes J, Flexas J, Save R, Medrano H (2007) Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant Soil 290: 139–155

Gessler A, Rennenberg H, Keitel C (2004) Stable isotope composition of organic compounds transported in the phloem of European beech – evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport. Plant Biol 6: 721–729

Gessler A, Keitel C, Kodama N, Weston C, Winters AJ, Keith H, Grice K, Leuning R, Farquhar GD (2007) $d^{13}C$ of organic matter transported from the leaves to the roots in *Eucalyptus delegatensis*: short-term variations and relation to respired CO_2. Funct Plant Biol 34: 692–706

Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard RL (2009) Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. Plant Cell Environ 32: 780–795

Hatton T, Reece P, Taylor P, McEwan K (1998) Does leaf water efficiency vary among eucalypts in water-limited environments? Tree Physiol 18: 529–536

Hobbie EA, Werner RA (2004) Intramolecular, compound-specific, and bulk carbon isotope patterns in C-3 and C-4 plants: a review and synthesis. New Phytol 161: 371–385

Keitel C, Adams MA, Holst T, Matzarakis A, Mayer H, Rennenberg H, Gessler A (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). Plant Cell Environ 26: 1157–1168

Keitel C, Matzarakis A, Rennenberg H, Gessler A (2006) Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. Plant Cell Environ 29: 1492–1507

Merchant A, Adams MA (2005) Stable osmotica in *Eucalyptus spathulata*–responses to salt and water deficit stress. Funct Plant Biol 32: 797–805

Merchant A, Adams MA, Richter A, Popp M (2006a) Targeted metabolite profiling provides a functional link among eucalypt taxonomy, physiology and evolution. Phytochemistry 67: 402–408

Merchant A, Tausz M, Arndt SK, Adams MA (2006b) Cyclitols and carbohydrates in leaves and roots of 13 *Eucalyptus* species suggest contrasting physiological responses to water deficit. Plant Cell Environ 29: 2017–2029

Merchant A, Arndt SK, Callister AN, Adams MA (2007) Contrasting physiological responses of six *Eucalyptus* species to water deficit. Ann Bot 100: 1507–1515

Merchant A, Peuke AD, Keitel C, Macfarlane C, Warren C, Adams MA (2010a) Phloem sap and leaf $\delta^{13}C$, carbohydrates and amino acid concentrations in *Eucalyptus globulus* change systematically according to flooding and water deficit treatment. J Exp Bot 61: 1785–1793

Merchant A, Tausz M, Keitel C, Adams MA (2010b) Relations of sugar composition and $\delta^{13}C$ in phloem sap to growth and physiological performance of *Eucalyptus globulus* (Labill) Plant Cell Environ 33: 1361–1368

Merchant A, Wild B, Richter A, Bellot S, Adams MA, Dreyer E (2011) Compound-specific differences in (13)C of soluble carbohydrates in leaves and phloem of 6-month-old *Eucalyptus globulus* (Labill). Plant Cell Environ 34: 1599–1608

Mitchell PJ, Veneklaas EJ, Lambers H, Burgess SSO (2008) Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. Plant Cell Environ 31: 1791–1802

Myers BA, Neales TF (1986) Osmotic adjustment, induced by drought, in seedlings of three *Eucalyptus* species. Aust J Plant Physiol 13: 597–603

Myers BJ, Landsberg JJ (1989) Water stress and seedling growth of two eucalypt species from contrasting habitats. Tree Physiol 5: 207–218

Pate J, Arthur D (1998) Delta ^{13}C analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globulus*. Oecologia 117: 301–311

Pate JS, Arthur DJ (2000) Uptake, partitioning and utilization of carbon and nitrogen in the phloem bleeding tree, Tasmanian blue gum (*Eucalyptus globulus*). Aust J Plant Physiol 27: 869–884

Pate J, Shedley E, Arthur D, Adams M (1998) Spatial and temporal variations in phloem sap composition of plantation-grown *Eucalyptus globulus*. Oecologia 117: 312–322

Pfautsch S, Bleby TM, Rennenberg H, Adams MA (2010) Sap flow measurements reveal influence of temperature and stand structure on water use of *Eucalyptus regnans* forests. For Ecol Manage 259: 1190–1199

Popp M, Lied W, Meyer AJ, Richter A, Schiller P, Schwitte H (1996) Sample preservation for determination of organic compounds: microwave vs freeze drying. J Exp Bot 47: 1469–1473

Prior LD, Eamus D (1999) Seasonal changes in leaf water characteristics of *Eucalyptus tetrodonta* and *Terminalia ferdinandiana* saplings in a northern Australian savanna. Aust J Bot 47: 587–599
Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49: 419–432
Tausz M, Merchant A, Kruse J, Samsa G, Adams MA (2008) Estimation of drought-related limitations to mid-rotation aged plantation grown *Eucalyptus globulus* by phloem sap analysis. For Ecol Manage 256: 844–848
Tcherkez G (2006) How large is the carbon isotope fractionation of the photorespiratory enzyme glycine decarboxylase? Funct Plant Biol 33: 911–920
Tcherkez G, Farquhar G, Badeck F, Ghashghaie J (2004) Theoretical considerations about carbon isotope distribution in glucose of C-3 plants. Funct Plant Biol 31: 857–877
Turgeon R (1996) Phloem loading and plasmodesmata. Trends Plant Sci 1: 418–423
Turner NC (1988) Measurement of plant water status by the pressure chamber technique. Irrigation Sci 9: 289–308
Tyree MT, Hammel HT (1972) Measurement of turgor pressure and water relations of plants by pressure-bomb technique. J Exp Bot 23: 267–272
Tyree MT, Jarvis PG (1982) Water in tissues and cells. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Encyclopedia of Plant Physiology. Springer Verlag, Berlin, pp 36–77
Tyree MT, Richter H (1982) Alternative methods of analysing water potential isotherms: some cautions and clarifications. II. Curvilinearity in water potential isotherms. Can J Bot 60: 911–916
Voitsekhovskaja OV, Rudashevskaya EL, Demchenko KN, Pakhomova MV, Batashev DR, Gamalei YV, Lohaus G, Pawlowski K (2009) Evidence for functional heterogeneity of sieve element-companion cell complexes in minor vein phloem of *Alonsoa meridionalis*. J Exp Bot 60: 1873–1883
von Caemmerer SV, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376–387
West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders. Trends Ecol Evol 21: 408–414
White DA, Beadle CL, Sands PJ, Worledge D, Honeysett JL (1999) Quantifying the effect of cumulative water stress on stomatal conductance of *Eucalyptus globulus* and *Eucalyptus nitens*: a phenomenological approach. Aust J Plant Physiol 26: 17–27
White DA, Beadle CL, Worledge D (2000a) Control of transpiration in an irrigated *Eucalyptus globulus* Labill. plantation. Plant Cell Environ 23: 123–134
White DA, Turner NC, Galbraith JH (2000b) Leaf water relations and stomatal behavior of four allopatric *Eucalyptus* species planted in Mediterranean southwestern Australia. Tree Physiol 20: 1157–1165
Whitehead D, Beadle CL (2004) Physiological regulation of productivity and water use in *Eucalyptus*: a review. For Ecol Manage 193: 113–140
Williams JE, Woinarsky JCZ (1997) Eucalypt Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

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