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Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*

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Summary We measured oxygen isotope ratios ($\delta^{18}O$) of xylem sap, phloem sap, leaves, wood and bark of *Eucalyptus globulus* Labill. growing in southwestern Australia. Carbon isotope ratios ($\delta^{13}C$) were measured in the dry matter of phloem sap, leaves and wood. Results were used to test several aspects of a mechanistic model of ^{18}O enrichment and provided insights into post-photosynthetic variations in dry matter $\delta^{13}C$. Xylem water $\delta^{18}O$ varied little within the tree crown, whereas variation at the landscape-level was more pronounced, with plantations near the coast being enriched by up to 3‰ compared with plantations less than 100 km inland. Phloem water was significantly enriched in ^{18}O compared with xylem water in two of three sampling campaigns; mean enrichments were 0.5 and 0.8‰. Phloem sap sugars exported from *E. globulus* leaves closely reflected observed leaf water enrichment when diurnal variation in photosynthesis was taken into account. Photosynthetic rates were higher in the morning than in the afternoon, whereas leaf water ^{18}O enrichment increased to maximum values in the afternoon. A non-steady-state model of leaf water ^{18}O enrichment accurately predicted observed values through a full diel cycle. Mean estimates of the proportion of organic oxygen effectively exchanging with xylem water during cellulose synthesis were close to 0.40 for both leaves and wood. Carbon isotope ratios of nascent xylem tissues did not differ from those of phloem sap sugars collected concurrently, whereas nascent leaf tissues were depleted in ^{13}C by 2‰ compared with phloem sap sugars, suggesting that, in *E. globulus*, ^{13}C enrichment of sink tissues compared with source leaves does not result from an enriching process within the sink tissue.

Keywords: isotope signal, isotopic fractionation, leaf water enrichment, translocation.

Introduction

Oxygen and carbon stable isotope ratios ($\delta^{18}O$ and $\delta^{13}C$, respectively) are valuable tools for investigating plant ecophysiology and ecosystem processes (Yakir and Sternberg 2000, Adams and Grierson 2001, Dawson et al. 2002, Ehleringer et al. 2002, Maguas and Griffiths 2003). Mechanisms underlying variation in carbon isotope discrimination by plants are generally well understood (Farquhar et al. 1989*a*); however, some patterns remain unexplained, such as the frequently observed enrichment in ^{13}C of heterotrophic plant tissues compared with the leaves supplying them with photosynthate (Brugnoli and Farquhar 2000). Oxygen isotope ratios in plant organic material have been measured less often than carbon isotope ratios. Although much progress has been made recently toward establishment of a general synthetic model of oxygen isotope enrichment in plant organic material (Saurer et al. 1997, Farquhar et al. 1998, Barbour and Farquhar 2000, Roden et al. 2000, Helliker and Ehleringer 2002*b*, Barbour et al. 2004), some theoretical aspects remain untested, especially under field conditions.

Theory of oxygen isotope enrichment

Liquid water within leaves becomes enriched in the heavy isotope ^{18}O during transpiration (Gonfiantini et al. 1965). Under steady-state conditions, ^{18}O enrichment at the evaporative sites within leaves ($\Delta^{18}O_e$; symbols listed in Table 1) can be modeled as (Craig and Gordon 1965, Dongmann et al. 1974, Farquhar and Lloyd 1993):

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)\frac{e_a}{e_i} \quad (1)$$

where ε^+ is the equilibrium fractionation between liquid water and vapor at the air–water interfaces, ε_k is the kinetic fractionation that occurs during water vapor diffusion from the leaf intercellular air space to the atmosphere, $\Delta^{18}O_v$ is the isotopic enrichment of vapor in the atmosphere compared with source water, and e_a/e_i is the ratio of ambient to intercellular vapor pressures. The equilibrium fractionation, ε^+, can be calculated from a regression equation relating it to temperature (T; K) (Bottinga and Craig 1969):

Table 1. Symbols used in text.

A	Net photosynthesis rate
α^+	Oxygen isotope effect between liquid water and vapor
α_k	Oxygen isotope effect during water vapor diffusion from leaf intercellular air spaces to the atmosphere
α_{wc}	Oxygen isotope effect between organic molecules and medium water during synthesis
C	Molar concentration of water
c_i	CO_2 concentration in leaf intercellular air spaces
c_a	CO_2 concentration in the atmosphere
D	Diffusivity of $H_2{}^{18}O$ in water
$\Delta^{13}C$	^{13}C discrimination of plant material compared with atmospheric CO_2
$\Delta^{13}C_c$	^{13}C discrimination of plant cellulose compared with atmospheric CO_2
$\Delta^{13}C_p$	^{13}C discrimination of plant dry matter compared with atmospheric CO_2
$\Delta^{13}C_{suc}$	^{13}C discrimination of phloem sap dry matter compared with atmospheric CO_2
$\Delta^{18}O$	^{18}O enrichment compared with source water of any water or dry matter component
$\Delta^{18}O_c$	^{18}O enrichment of plant cellulose compared with source water
$\Delta^{18}O_e$	^{18}O enrichment at evaporative sites in leaves compared with source water
$\Delta^{18}O_L$	^{18}O enrichment of mean lamina leaf water compared with source water
$\Delta^{18}O_{Ln}$	Predicted non-steady state ^{18}O enrichment of mean lamina leaf water
$\Delta^{18}O_p$	^{18}O enrichment of plant dry matter compared with source water
$\Delta^{18}O_{pw}$	^{18}O enrichment of phloem water compared with source water
$\Delta^{18}O_{suc}$	^{18}O enrichment of phloem sap dry matter compared with source water
$\Delta^{18}O_v$	^{18}O enrichment of vapor in the atmosphere compared with source water
$\delta^{13}C$	$^{13}C/^{12}C$ relative to the value of a standard (Pee Dee Belemnite (PDB) in this paper)
$\delta^{13}C_a$	$\delta^{13}C$ of CO_2 in the atmosphere
$\delta^{13}C_p$	$\delta^{13}C$ of plant dry matter
$\delta^{13}C_{suc}$	$\delta^{13}C$ of phloem sap dry matter
$\delta^{18}O$	$^{18}O/^{16}O$ relative to the value of a standard (Vienna Standard Mean Ocean Water (VSMOW) in this paper)
$\delta^{18}O_L$	$\delta^{18}O$ of average lamina leaf water
$\delta^{18}O_p$	$\delta^{18}O$ of plant dry matter
$\delta^{18}O_{sc}$	$\delta^{18}O$ of potato shoot cellulose
$\delta^{18}O_{suc}$	$\delta^{18}O$ of phloem sap dry matter
$\delta^{18}O_{ts}$	$\delta^{18}O$ of potato tuber starch
$\delta^{18}O_{tw+27}$	$\delta^{18}O$ of potato tuber water + 27‰
$\delta^{18}O_w$	$\delta^{18}O$ of medium water in the experiments of Sternberg et al. (2003)
$\delta^{18}O_x$	$\delta^{18}O$ of xylem water
$\delta^{18}O^2$	$\delta^{18}O$ of oxygen attached to the second carbon of glucose moieties in cellulose
E	Transpiration rate
e_a	Ambient vapor pressure
e_i	Vapor pressure in leaf intercellular air spaces
ε^+	Equilibrium ^{18}O fractionation between liquid water and vapor
ε_{cp}	Difference between $\Delta^{18}O_p$ and $\Delta^{18}O_c$
ε_k	Kinetic fractionation for diffusion of $H_2{}^{18}O$ from leaf intercellular air spaces to the atmosphere
ε_{wc}	^{18}O fractionation between organic oxygen and medium water
g	Conductance to H_2O from leaf intercellular air space to atmosphere
L	Scaled effective path length for calculation of the Péclet number ($\wp$)
p_{ex}	Proportion of oxygen atoms exchanging with medium water during cellulose synthesis
p_x	Proportion of unenriched (source) water in tissue where cellulose synthesis is occurring
$\wp$	Péclet number
R	$^{18}O/^{16}O$ of any sample of interest
R_s	$^{18}O/^{16}O$ of source water
r_b	Boundary layer resistance to water vapor diffusion
r_s	Stomatal resistance to water vapor diffusion
ρ	Proportion of water in developing cell coming from phloem rather than xylem
T	Leaf temperature
t	Time
w_i	Mole fraction of water vapor in leaf intercellular air spaces
W	Leaf lamina water concentration
y	Proportion of hexose phosphates cycling to triose phosphates before incorporation into cellulose

$$\varepsilon^{+}(‰) = 2.644 - 3.206\left(\frac{10^3}{T}\right) + 1.534\left(\frac{10^6}{T^2}\right) \quad (2)$$

where T refers to leaf temperature. The kinetic fractionation, ε_k ‰, can be calculated as (Farquhar et al. 1989*b*):

$$\varepsilon_k = \frac{32r_s + 21r_b}{r_s + r_b} \quad (3)$$

where r_s and r_b are the stomatal and boundary layer resistances to water vapor diffusion (m^2 s mol^{-1}), and 32 and 21 are associated fractionation factors scaled to per ml. These fractionation factors have been revised up from values of 28 and 19, respectively, based on new measurements showing the isotope effect for diffusion of $H_2{}^{18}O$ in air to be 1.032 (Cappa et al. 2003), rather than 1.028 (Merlivat 1978). Throughout this paper, we express the oxygen isotope enrichment of any water or dry matter component ($\Delta^{18}O$) relative to source water as $\Delta^{18}O = R/R_s - 1$, where R is $^{18}O/^{16}O$ of the sample of interest and R_s is $^{18}O/^{16}O$ of source water. Equation 1 is a convenient approximation of the mathematically correct form of this particular model, which it underestimates by about 0.1‰ (Farquhar and Lloyd 1993), a bias considered negligible (Cernusak et al. 2003*b*).

The $\Delta^{18}O$ of water in the leaf mesophyll is expected to be less than that at the evaporative sites because of the influx of relatively unfractionated vein water. Farquhar and Lloyd (1993), and more recently Farquhar and Gan (2003), suggested that the $\Delta^{18}O$ of mean lamina mesophyll water ($\Delta^{18}O_L$) is related to $\Delta^{18}O_e$ as:

$$\Delta^{18}O_L = \frac{\Delta^{18}O_e(1 - e^{-\wp})}{\wp} \quad (4)$$

where $\wp$ is a lamina radial Péclet number, defined as EL/CD, where E is transpiration rate (mol m^{-2} s^{-1}), L is a scaled effective path length (m), C is the molar concentration of water (mol m^{-3}), and D is the diffusivity of $H_2{}^{18}O$ in water (m^2 s^{-1}).

Barbour and Farquhar (2000) related the ^{18}O enrichment of plant cellulose ($\Delta^{18}O_c$) to $\Delta^{18}O_L$ as:

$$\Delta^{18}O_c = \Delta^{18}O_L(1 - p_{ex}p_x) + \varepsilon_{wc} \quad (5)$$

where p_{ex} is the proportion of oxygen atoms exchanging with medium water in the developing tissue during cellulose synthesis, p_x is the proportion of unenriched source water in the developing tissue, i.e., water originating from the xylem rather than the leaf mesophyll, and ε_{wc} is the equilibrium fractionation between organic oxygen and medium water, which has a value of +27‰ (Sternberg and DeNiro 1983, Sternberg et al. 1984, Yakir and DeNiro 1990). The value of p_x, which is expected to be less than unity because enriched water can be transferred from the leaf mesophyll to developing cells by translocation in the phloem (Barbour and Farquhar 2000, Cernusak et al. 2002, 2003*b*), can be calculated as:

$$p_x = 1 - \rho\left(\frac{\Delta^{18}O_{pw}}{\Delta^{18}O_L}\right) \quad (6)$$

where ρ is the proportion of water in the developing cell from the phloem as opposed to from the xylem, and $\Delta^{18}O_{pw}$ is phloem water enrichment compared with source water. Thus, p_x measures the extent to which water in the developing cell is xylem-like in its oxygen isotope composition. The term ρ is a measure of the deviation from being completely xylem-like caused by the contribution of phloem water; however, phloem water only has an effect if it is enriched, and $\Delta^{18}O_{pw}/\Delta^{18}O_L$ is a measure of how enriched, or mesophyll-like, it is. Equation 6 assumes that water in the developing cell is not further enriched by evaporation after it has entered the cell. Barbour and Farquhar (2000) suggested that p_{ex} can be modeled as:

$$p_{ex} = 0.2 + y\left(0.6 + \frac{0.2}{2 - y}\right) \quad (7)$$

where y is the proportion of hexose phosphates that cycle through triose phosphates before being incorporated into cellulose. Barbour and Farquhar (2000) further suggested that a term ε_{cp} could be added to the right side of Equation 5 so that it could be applied to $\Delta^{18}O$ of total dry matter ($\Delta^{18}O_p$), in addition to cellulose:

$$\Delta^{18}O_p = \Delta^{18}O_L(1 - p_{ex}p_x) + \varepsilon_{wc} + \varepsilon_{cp} \quad (8)$$

The empirical term ε_{cp} is defined as the difference between $\Delta^{18}O$ for total dry matter and $\Delta^{18}O$ of cellulose extracted from it.

Equation 1 predicts evaporative site water enrichment under steady-state conditions. Although leaves are likely to reach isotopic steady state in the early afternoon (Harwood et al. 1998), there will be times when leaf water enrichment is not at steady state. Therefore, non-steady-state variation must be considered when modeling leaf water ^{18}O enrichment in the field. Farquhar and Cernusak (unpublished data) have derived a non-steady-state modification of Equation 4:

$$\Delta^{18}O_{Ln} = \Delta^{18}O_L - \alpha^{+}\alpha_k\left(\frac{1 - e^{-\wp}}{\wp}\right)\left(\frac{\frac{d(W\Delta^{18}O_{Ln})}{dt}}{gw_i}\right) \quad (9)$$

where $\Delta^{18}O_{Ln}$ is the non-steady-state mean lamina leaf water ^{18}O enrichment, α^{+} is defined as $(1 + (\varepsilon^{+}(‰)/1000))$, α_k is defined as $(1 + (\varepsilon_k(‰)/1000))$, W is lamina leaf water concentration (mol m^{-2}), t is time (s), g is total conductance to water vapor of stomata plus boundary layer (mol m^{-2} s^{-1}), and w_i is the mole fraction of water vapor in the leaf intercellular air spaces (mol mol^{-1}). Because the term $\Delta^{18}O_{Ln}$ occurs on both sides of Equation 9, it is simplest to solve the equation iteratively using the solver function in Microsoft Excel or similar software. Equation 9 is similar to the non-steady-state equa-

tion presented for mean lamina leaf water by Cernusak et al. (2002), but differs in the inclusion of the terms $\alpha^+\alpha_k$ and $((1 - e^{-\wp})/\wp)$. The term $\alpha^+\alpha_k$ was omitted from the equation given by Cernusak et al. (2002) because it is close to unity; it typically has a value of ~1.04. Inclusion of the term $((1 - e^{-\wp})/\wp)$ represents a revision in the derivation of Equation 9. To derive the equation presented by Cernusak et al. (2002), we assumed that $\Delta^{18}O_L = \Delta^{18}O_e$. Rather than make this assumption in the derivation of Equation 9, we used Equation 4 to relate $\Delta^{18}O_L$ to $\Delta^{18}O_e$. Use of the non-steady-state equation given by Cernusak et al. (2002) in place of Equation 9 results in only a small bias. For data presented in this paper, differences in predictions of $\Delta^{18}O_{Ln}$ between the two equations ranged from 0.0 to 0.2‰, with a mean of 0.1‰.

Under field conditions, Equation 5 can be modified such that the leaf water enrichment term is weighted by photosynthesis:

$$\Delta^{18}O_c = \frac{\int A\Delta^{18}O_L}{\int A}(1 - p_{ex}p_x) + \varepsilon_{wc} \quad (10)$$

where $\int A\Delta^{18}O_L$ is the daily integral of the product of photosynthesis and leaf water enrichment (‰ mol m^{-2}) and $\int A$ is the daily integral of photosynthesis (mol m^{-2}). This modification of Equation 5 allows for differing diurnal patterns of variation in $\Delta^{18}O_L$ and A. Equation 8 can be similarly modified:

$$\Delta^{18}O_p = \frac{\int A\Delta^{18}O_L}{\int A}(1 - p_{ex}p_x) + \varepsilon_{wc} + \varepsilon_{cp} \quad (11)$$

Hypotheses tested

We measured oxygen isotope ratios in xylem sap, phloem sap, leaves, wood and bark of Tasmanian blue gum, *Eucalyptus globulus* Labill., trees growing in several plantations in southwestern Australia. These measurements were used to field test six hypotheses relating to ^{18}O enrichment in plant organic material. (1) The oxygen isotope composition of xylem water is spatially uniform within the crowns of trees and among plantations across the landscape. (2) Phloem water is enriched in ^{18}O compared with xylem water. (3) The non-steady-state leaf water model summarized in Equation 9 accurately predicts leaf water ^{18}O enrichment over a full diel cycle. (4) Sugars exported from leaves in phloem sap are enriched in ^{18}O by 27‰ compared with mean lamina leaf water, thereby recording the leaf water enrichment signal. (5) The proportion of oxygen atoms exchanging with medium water during cellulose synthesis, p_{ex}, is about 0.40. (6) The difference between $\Delta^{18}O$ of total leaf dry matter and $\Delta^{18}O$ of leaf cellulose, ε_{cp}, does not vary among leaves within a species. In addition, we analyzed $\delta^{13}C$ in phloem sap, leaves and wood of *E. globulus* to test a hypothesis (7) relating to ^{13}C enrichment of heterotrophic plant tissues compared with source leaves. (7) The process causing ^{13}C enrichment of sink tissues takes place within the sink tissue; i.e., sink tissues are enriched in ^{13}C compared with incoming phloem sap sugars.

Materials and methods

Sampling campaign 1

Samples for $\Delta^{18}O$ analysis were collected from *E. globulus* plantations in southwestern Australia on three occasions, as detailed in Table 2. Also given in Table 2 is the sampling campaign during which each plantation was sampled.

The first sampling campaign took place at the Mount Barker plantation on November 3, 2000. Six trees, about 5 m in height, were harvested at 2–3-h intervals between 0500 and 2000 h. Leaves for leaf water extraction were collected separately from the upper and lower canopy. One fully expanded leaf was collected from each of four sides of the tree, and one half of the lamina quickly separated from the midrib. The four lamina halves were sealed together in a glass tube with a rubber stopper embedded in a screw cap. Sampled leaves had areas of about 60 cm^2. Newly emerged leaves at the apex of the main stem were collected for water extraction. The uppermost 3 cm of the apices was collected, which included leaves with areas ranging from 0.1 to 3 cm^2.

Gas exchange was measured on five to 10 fully expanded leaves from the upper and lower canopy under ambient conditions with an LCA 4 portable gas exchange system (ADC Bioscientific, Hoddesdon, Hertfordshire, U.K.). Leaf temper-

Table 2. Location, year of planting and time of sampling for *E. globulus* plantations in southwestern Australia. Also shown is the sampling campaign during which each plantation was sampled.

Plantation	Latitude	Longitude	Year of planting	Time of sampling	Sampling campaign
Denbarker	–34°42′41″ S	117°30′22″ E	1997	December 2001	3A
Denmark	–34°58′45″ S	117°20′06″ E	1999	December 2001	3B
Eulup	–34°36′51″ S	117°33′04″ E	1997	December 2001	3A
Frankland	–34°19′24″ S	116°57′26″ E	1997	December 2001	3A
Kwornicup	–34°33′24″ S	117°24′24″ E	1997	December 2001	3A
Mount Barker	–34°32′28″ S	117°30′24″ E	1999	November 2000, March 2001	1, 2
Peaceful Bay	–35°01′36″ S	116°53′44″ E	2000	December 2001	3C
Redmond	–34°54′19″ S	117°36′39″ E	1997	December 2001	3A
St. Werburghs	–34°39′32″ S	117°34′19″ E	1997	December 2001	3A

ature of five leaves was measured with a 0.13-mm-diameter chromel-constantan thermocouple (Omega Engineering, Stamford, CT). Ambient air temperature and relative humidity were measured with a Vaisala temperature and humidity probe (Vaisala, Helsinki, Finland).

Phloem sap was collected from the upper and lower main stem, as described previously for *E. globulus* (Pate et al. 1998). Phloem sap droplets were attracted into microcapillary tubes immediately upon exudation and expressed into small vials that were then sealed to prevent evaporation. Xylem sap was collected from the upper and lower main stem by a mild-vacuum extraction technique (Jeschke and Pate 1995, Dawson and Pate 1996, Pate and Arthur 2000, Cernusak et al. 2002). All samples were frozen on collection. Leaf and apex water was later extracted by cryogenic distillation.

Sampling campaign 2

The second sampling campaign took place at the Mount Barker plantation on March 13–14, 2001. Five trees, 5 to 6 m tall, were harvested at various times between 0530 and 1500 h. The leaf water sampling scheme was similar to that conducted in the first sampling campaign, except that the canopy was stratified into four levels instead of two. Phloem and xylem sap were collected from four levels of the main stem, as well as from inner and outer branches of canopy level two, where level one was the base of the crown, and level four the top. Xylem wood and bark were also collected from the stem or branch sections from which phloem sap and xylem sap were collected, and quickly sealed in glass tubes. Xylem wood from the stem sections sampled for xylem water by mild-vacuum extraction was later subjected to cryogenic distillation to test whether the immobile xylem water in the stem had a different isotopic signature from the mobile xylem water.

Sampling campaign 3

The third sampling campaign took place December 5–12, 2001. The first component of this sampling campaign, termed 3A, was to examine isotopic fractionation between phloem sap sugar and newly synthesized xylem wood. Phloem sap, newly differentiated xylem tissue and mature xylem tissue were sampled from six trees at each of six plantations of similar age, and distributed over a rainfall and productivity gradient. These plantations were Denbarker, Eulup, Frankland, Kwornicup, Redmond and St. Werburghs. Trees in these plantations ranged from about 10 to 16 m in height. Phloem sap was collected from near breast height as described previously. A square of bark, about 15 × 15 cm, was removed from the same location. The gelatinous layer of recently differentiated xylem tissue was gently scraped off the inner face of the exposed surface of cambium with a clean razor blade and placed in a vial containing ethanol as described by Pate and Arthur (1998). Two to three pieces of mature xylem tissue (about 0.75 cm wide by 3 cm long), penetrating radially to about 0.75 cm depth from the cambial surface, were quickly removed with a chisel and hammer. These tissues were sealed in glass tubes and frozen. Xylem water was later extracted from these tissues by cryogenic distillation.

In the second component of the third sampling campaign, termed 3B, we investigated diel variation in $\Delta^{18}O$ of leaf water, phloem sap and leaf dry matter. Sampling took place at the Denmark plantation on December 7–8, 2001. Samples were collected 10 times at about 3-h intervals between 1800 h on December 7 and 2030 h on December 8. For each sample collection time, three uniform trees of about 10 m height were selected. Fully expanded leaves were collected from the lower-middle canopy as described previously. Phloem sap was collected from the main stem at the same canopy height. Xylem water was collected by mild-vacuum extraction from one branch on each tree at the same canopy height. Atmospheric water vapor in the vicinity of the sampled trees was collected by drawing canopy air through an ethanol–dry ice trap at a flow rate of about 1 l min^{-1}. Three leaves from each sampled tree were collected and quickly sealed and frozen in glass tubes for gravimetric measurements of leaf water concentrations. Gas exchange was measured on five to 10 leaves, and leaf temperature, air temperature and relative humidity were measured as previously described.

In the third component of the third sampling campaign, termed 3C, we investigated variation in bark and xylem water along the length of branches, and compared leaf water and dry matter ^{18}O enrichment in expanding versus fully expanded leaves. Sampling took place on December 12, 2001 at the Peaceful Bay plantation. Three fully expanded leaves were sampled from the middle canopy of five trees, and half the lamina of each retained for leaf water extraction. Ten expanding leaves were sampled from each of the same trees. These leaves had areas of about 10 cm^2, whereas the fully expanded leaves had areas of about 60 cm^2. The midrib was quickly cut out with a razor blade and both halves of all 10 laminas retained for leaf water extraction. A branch section was cut from each tree close to where the branch joined the main stem. Xylem and bark tissues were separated and quickly sealed and frozen in glass tubes. The procedure was repeated with a branch section from halfway between the branch base and branch tip. The second internode from the branch tip was then collected from eight branches on each tree and quickly separated into bark and xylem, which were sealed in glass tubes and frozen. Water in all these samples was later extracted by cryogenic distillation.

Isotopic analyses

Oxygen isotope ratios of water and dry matter samples were measured as described previously (Farquhar et al. 1997) with an Isochrom mass spectrometer (Micromass, Manchester, U.K.) following pyrolysis in a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy) or a custom-built furnace. Phloem sap dry matter oxygen isotope ratios were measured on samples that were oven-dried overnight at 60 °C. Samples were sealed under argon in tin cups immediately following removal from the drying oven to prevent hydration of sugars (Cernusak et al. 2003*a*). Phloem sap water oxygen isotope ratios were determined as described previously (Cernusak et al. 2002), based on a technique for determination of the water component of a homogeneous mixture of water and dry matter

(Gan et al. 2002). All phloem sap samples were processed for $\delta^{18}O$ analysis of both the water and dry matter components. Oxygen isotope ratios were obtained in delta notation ($\delta^{18}O$) expressed relative to Vienna Standard Mean Ocean Water. Precision of analyses, based on repeated measurements of laboratory sucrose and water standards was ± 0.3‰. The $\delta^{18}O$ values of all components except xylem water are expressed as enrichment above xylem water ($\Delta^{18}O$), calculated as $\Delta^{18}O = (\delta^{18}O - \delta^{18}O_x)/(1 + \delta^{18}O_x)$, where $\delta^{18}O$ refers to the sample of interest and $\delta^{18}O_x$ is the corresponding value for xylem water.

Carbon isotope ratios of dry matter samples were analyzed with an Isochrom mass spectrometer (Micromass) following combustion in a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy). Values were obtained in delta notation ($\delta^{13}C$) relative to the Pee Dee Belemnite standard. The $\delta^{13}C$ values were then converted to carbon isotope discrimination values ($\Delta^{13}C$) calculated as: $\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C)/(1 + \delta^{13}C)$, where $\delta^{13}C$ is the sample of interest and $\delta^{13}C_a$ is the value for CO_2 in air, which was assumed to be –7.8‰.

About half the dry matter samples were subjected to α-cellulose extraction. This included samples from sampling campaigns 1, 2, 3A and 3C. The extraction procedure was as described by Barbour and Farquhar (2000), based on the modified technique of Loader et al. (1997). The α-cellulose samples were analyzed first for $\delta^{18}O$, then for $\delta^{13}C$ if sufficient sample remained; out of 90 samples, 77 had sufficient sample remaining for $\delta^{13}C$ analysis. Samples of recently differentiated xylem tissues were washed three times in 80% ethanol and oven-dried before isotopic analysis (Pate and Arthur 1998). Dry matter was then bulked for the six trees from each plantation to provide sufficient material for α-cellulose extraction.

A subset of 10 phloem sap samples was randomly selected and the ionizable solutes removed with ion exchange resin (Bio-Rad AG 501-X8, Bio-Rad Laboratories, Hercules, CA). The remaining sugar fraction in the sap was oven-dried and analyzed for $\delta^{18}O$. Bulk phloem sap dry matter $\delta^{18}O$ values did not differ from resin-treated sugar $\delta^{18}O$ values ($P = 0.26$, $n = 10$); the mean difference was 0.3‰. A similar result was found for $\delta^{13}C$ of bulk phloem sap dry matter for *E. globulus* versus $\delta^{18}O$ of the purified sugar fraction (Pate and Arthur 1998). Based on these results, we concluded that the isotopic composition of the bulk phloem sap dry matter in our samples was representative of phloem sap sugars. No further attempt was made to separate the sugar and non-sugar fractions for isotopic analyses.

Isotopic calculations

We calculated p_{ex} for the recently differentiated xylem tissue samples and the stem apices with Equation 10 by replacing the term $(\int A\Delta^{18}O_L/\int A)$ with the term $(\Delta^{18}O_{suc} - \varepsilon_{wc})$, where $\Delta^{18}O_{suc}$ is the oxygen isotope enrichment measured in phloem sap dry matter (Barbour et al. 2000, Cernusak et al. 2003*b*). Similarly, p_x was estimated from Equation 6 by replacing $\Delta^{18}O_L$ with $(\Delta^{18}O_{suc} - \varepsilon_{wc})$. Literature estimates of ρ, the proportion of water in developing cells coming from phloem rather than xylem, range from 0.5 to 0.8 (Schmalstig and Cosgrove 1990, Bret-Harte and Silk 1994, Pritchard et al. 2000). We assumed an intermediate value of 0.65 for calculations of p_x.

In sampling campaign 3B (diel leaf water collections), gas exchange was not measured for the nighttime collection times when the ambient vapor pressure was near saturation. For leaf water modeling at these times, stomatal conductance was assumed to be 20 mmol m^{-2} s^{-1}, based on measurements of nighttime stomatal conductance in another *Eucalyptus* species (L. Cernusak, unpublished data). For leaf water modeling, boundary layer conductance was assumed to be 0.9 mol m^{-2} s^{-1}. The scaled effective path length, L, for calculation of the Péclet number in Equations 4 and 9, was estimated from the discrepancy between predicted $\Delta^{18}O_e$ and observed $\Delta^{18}O_L$ in early afternoon samples when $\Delta^{18}O_L$ was assumed to be at steady state.

Variation in isotopic parameters was assessed by analysis of variance, linear regression or paired *t*-test. In cases where pair-wise comparisons were made following analyses of variance, Tukey's method was used. Statistical analyses were performed with SYSTAT 9.0 (SPSS, Chicago, IL).

Results

Xylem water $\delta^{18}O$

The sampled plantations were located across a latitudinal gradient that also corresponds to a rainfall gradient (Figure 1). Total rainfall for 2001 decreased linearly with decreasing south latitude for seven weather stations located in the vicinity of the sampled plantations and covering about the same latitudinal range. The coastline in this part of southwestern Australia runs in an east–west direction, such that distance north from latitude 35° S is effectively a measure of distance from the coast. Locations of the plantations that we sampled thus varied from

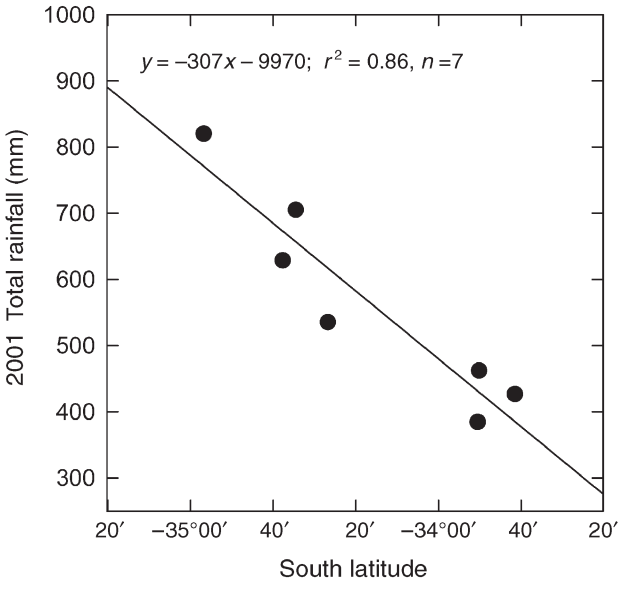

Figure 1. Annual rainfall for 2001 plotted against south latitude for seven weather stations in the vicinity of the sampled plantations and covering about the same latitudinal range.

close to the coast to about 80 km inland. Annual rainfall decreases sharply with distance inland, and there was a corresponding and linearly related variation in xylem water $\delta^{18}O$ (Figure 2). During the December 2001 sampling campaign, plantations nearer the coast had xylem water that was enriched in ^{18}O by as much as 3‰ compared with plantations furthest inland.

There was a tendency for xylem water in upper stems to be enriched in ^{18}O relative to that in lower stems in the trees sampled at the Mount Barker plantation in November 2000. Mean values were –3.6 and –4.0‰, respectively ($P = 0.05$, $n = 6$). A similar tendency, though not statistically significant, was observed at the same plantation in March 2001, with xylem water $\delta^{18}O$ increasing from –3.7‰ at the base of the stem to –3.2‰ at the top of the stem (Table 3). There was a tendency for xylem water sampled from branch tips at the Peaceful Bay plantation to be enriched by about 1‰ compared with xylem water sampled from the middle and bases of branches (Table 4).

The $\delta^{18}O$ of mobile xylem water extracted from stem xylem tissues by mild-vacuum extraction did not differ from that of immobile xylem water extracted later from the same tissues by cryogenic distillation ($P = 0.34$, $n = 20$). Mean values were –3.5 and –3.7‰, respectively. Bark water $\delta^{18}O$ did not differ from xylem water $\delta^{18}O$ ($P = 0.59$, $n = 14$).

Leaf water $\Delta^{18}O$

Observed leaf water ^{18}O enrichment for fully expanded leaves ranged from 6.3 to 19.7‰. It was consistently lower than predicted $\Delta^{18}O_e$ during the day and higher at night (Tables 4, 5 and 6, Figure 3). There was no significant variation in observed $\Delta^{18}O_L$ of fully expanded leaves among different crown positions (Tables 5 and 6). Enrichment of water extracted from stem apices was less than in fully expanded leaves by values ranging from 2.2 to 10.6‰, and showed a dampened diurnal cycle compared with $\Delta^{18}O_L$ for fully expanded leaves (Table 5). Expanding leaves collected at the Peaceful Bay plantation had higher, by 1.7‰, $\delta^{18}O_L$ than fully expanded leaves; however this difference was reduced to 0.7‰ and was no longer significant when leaf water $\delta^{18}O_L$ values were expressed as enrichment above xylem water (Table 4).

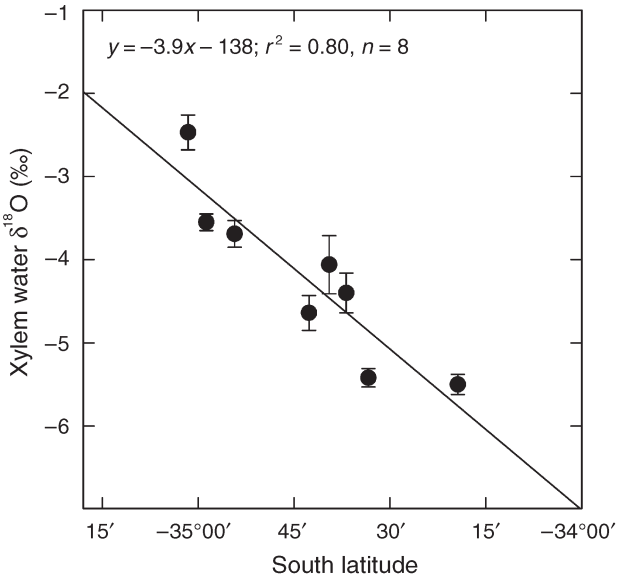

Figure 2. Mean xylem water $\delta^{18}O$ of sampled *E. globulus* plantations plotted against south latitude. At least five trees were sampled within each plantation; samples were collected in December 2001. Error bars represent ± 1 SE.

Departures from steady-state predictions of $\Delta^{18}O_L$ were apparent at night, whereas non-steady-state predictions using Equation 9 were close to observed $\Delta^{18}O_L$ values throughout a full diel cycle (Figure 3). The $\delta^{18}O$ values for atmospheric water vapor during the diel leaf water sampling at the Denmark plantation ranged from –10.8 to –14.0‰, yielding $\Delta^{18}O_v$ values ranging from –7.3 to –10.5‰. Leaf water concentrations ranged from 10.4 to 13.9 mol m^{-2}. Xylem water $\delta^{18}O$ values did not show a diel trend and averaged –3.5 ± 0.1‰ (mean ± 1 SE). Relative humidity and air temperature ranged from 0.51 to 0.95, and from 6.5 to 20.3 °C, respectively. Leaf temperatures were generally within 1 °C of air temperature. Transpiration ranged from 0 to 1.9 mmol m^{-2} s^{-1} on a projected leaf area basis. The scaled effective path length for *E. globulus* leaves was estimated to be 54 mm, using the revised values for ε_k, or 25 mm using the previously accepted values for ε_k.

Phloem sap $\Delta^{18}O_{pw}$

Phloem water enrichment varied significantly among sampling dates ($P = 0.006$, $n = 98$); the mean value for March 2001 differed from that for November 2000 ($P = 0.02$) and December 2001 ($P = 0.01$), whereas values for November 2000 and December 2001 did not differ ($P = 0.69$). In November 2000, phloem water was significantly enriched compared with xylem water ($P = 0.02$, $n = 11$); mean $\Delta^{18}O_{pw}$ was 0.8 ± 0.4‰ (mean ± 1 SE), and although it was not significantly enriched compared with xylem water ($P = 0.17$, $n = 24$) in March 2001, it was enriched compared with xylem water by a mean of 0.5 ± 0.1‰ ($P = 0.001$, $n = 63$) in December 2001. Phloem water enrichment values for plantations at which recently differentiated xylem tissues were sampled in December 2001 are shown in Table 6. The mean value for the Denmark plantation, at which diel collections were made, was 0.3 ± 0.2‰. Across all phloem sap samples, there was a significant correlation between $\Delta^{18}O_{pw}$ and $\Delta^{13}C_{suc}$ ($r = 0.21$, $P = 0.05$, $n = 91$). Although not strong, the relationship suggests that phloem water ^{18}O enrichment decreased as phloem sap sugar ^{13}C discrimination decreased; this indicates a response of $\Delta^{18}O_{pw}$ to canopy water stress, with $\Delta^{18}O_{pw}$ decreasing as canopy water stress increases. Mean p_x across all phloem sap samples was 0.98 ± 0.01.

Phloem sap $\Delta^{18}O_{suc}$

Phloem sugar ^{18}O enrichment ranged from 34.5 to 44.7‰, with a mean of 40.1‰ ($n = 101$). These enrichments correspond to inferred photosynthetic-rate-weighted leaf water enrichments ranging from 7.5 to 17.7‰. We did not observe a pronounced variation in $\Delta^{18}O_{suc}$ at different canopy heights

Table 3. Variation in isotopic parameters within the crown in *E. globulus* trees sampled at the Mount Barker plantation in March 2001. Values in parentheses are 1 SE ($n = 5$). Values within a row followed by different letters are significantly different at $P < 0.05$. Branches were sampled from the lower middle crown.

Isotopic parameter (‰)	Main stem				Branches	
	Lower	Lower, middle	Upper, middle	Upper	Inner	Outer
Xylem water $\delta^{18}O$	–3.7 (0.3) a	–3.5 (0.1) a	–3.4 (0.1) a	–3.2 (0.2) a	–3.0 (0.4) a	–3.5 (0.2) a
Phloem sap $\Delta^{18}O_{suc}$	43.0 (0.6) a	42.4 (0.5) a	42.3 (0.4) a	41.8 (0.6) a	43.0 (0.8) a	42.0 (0.1) a
Mature xylem $\Delta^{18}O_p$	32.2 (0.7) a	30.7 (0.4) ab	31.0 (0.4) a	30.9 (0.3) a	28.7 (0.5) b	30.5 (0.4) ab
Bark $\Delta^{18}O_p$	30.7 (0.5) a	30.6 (0.2) a	29.8 (0.7) ab	28.2 (0.5) ab	27.4 (0.9) b	28.8 (0.5) ab
Leaf $\Delta^{18}O_p$	33.7 (0.4) a	33.7 (0.3) a	34.1 (0.4) a	33.2 (0.2) a		
Phloem sap $\Delta^{13}C_{suc}$	15.4 (0.3) a	15.2 (0.2) a	15.0 (0.1) a	14.8 (0.1) a		
Mature xylem $\Delta^{13}C_p$	17.3 (0.5) a	17.4 (0.3) a	16.6 (0.5) a	16.0 (0.5) a		
Leaf $\Delta^{13}C_p$	19.6 (0.2) a	19.4 (0.3) ab	18.5 (0.2) b	16.5 (0.3) c		

(Table 3). There was variation in $\Delta^{18}O_{suc}$ among plantations sampled in December 2001 (Table 7). There appeared to be some variation in $\Delta^{18}O_{suc}$ of phloem sap samples collected at different times over the diel cycle at the Denmark plantation, with values increasing by about 1 to 2‰ during the night. There was little variation in $\Delta^{18}O_{suc}$ at the Mount Barker plantation between samples collected in November 2000 and March 2001; mean values were 42.3 and 42.4‰, respectively.

Observed $\Delta^{18}O_{suc}$ values accurately reflected observed $\Delta^{18}O_L$ values when the latter were weighted by photosynthetic rates, taking into account the fractionation between water and organic molecules of 27‰ (Table 8). Photosynthetic rates consistently decreased from morning to late afternoon (Figure 4A), with about 50% of daily photosynthesis occurring before 1100 h. In contrast, leaf water enrichment continued to increase well into the afternoon (Tables 5 and 6, Figures 3 and 4B). As a result, the leaf water signal recorded for the phloem sap sugars was less enriched than the maximum leaf water enrichment achieved in the late afternoon.

Leaf $\Delta^{18}O_p$ and $\Delta^{18}O_c$

Leaf dry matter $\Delta^{18}O$ values for fully expanded leaves ranged from 28.3 to 34.8‰, with a mean of 32.2‰ ($n = 67$). Leaf $\Delta^{18}O_p$ for expanding leaves at the Peaceful Bay plantation was similar to that for fully expanded leaves ($P = 0.77$, $n = 5$); mean values were 29.6 and 29.9‰, respectively. The $\Delta^{18}O_p$ for stem apices at the Mount Barker plantation was less than $\Delta^{18}O_p$ for fully expanded leaves from the same trees ($P < 0.001$, $n = 6$); mean values were 32.1 and 33.9‰, respectively. We did not observe significant variation in leaf $\Delta^{18}O_p$ with crown position in the March 2001 sampling at the Mount Barker plantation (Table 3). In the sampling at the Denmark plantation in December 2001, there appeared to be a diel variation in $\Delta^{18}O_p$ of about 1.5‰ that coincided with observed variation in $\Delta^{18}O_L$. In samples for which phloem sap was collected at the same time as leaves, leaf $\Delta^{18}O_p$ was significantly correlated with phloem sap $\Delta^{18}O_{suc}$ ($r = 0.76$, $P < 0.0001$, $n = 61$; Figure 5).

Leaf $\Delta^{18}O$ for cellulose of fully expanded leaves was more variable than that of the corresponding dry matter; $\Delta^{18}O_c$ values ranged from 25.0 to 38.0‰ (mean = 31.3‰, $n = 17$). The $\Delta^{18}O_c$ values for fully expanded leaves and expanding leaves from the same trees at the Peaceful Bay plantation were correlated ($r = 0.88$, $P = 0.05$, $n = 5$); mean values were 28.4 and 31.0‰, respectively. The $\Delta^{18}O_c$ of fully expanded leaves at the Mount Barker plantation was significantly lower than $\Delta^{18}O_c$ of stem apices of the same trees ($P = 0.01$, $n = 5$); mean values were 32.5 and 36.5‰, respectively. This difference was oppo-

Table 4. Xylem water oxygen isotope ratio ($\delta^{18}O$) in different branch sections of *E. globulus* and observed lamina leaf water $\delta^{18}O$. Values in parentheses are 1 SE ($n = 5$). Values within a row followed by different letters are significantly different at $P < 0.05$. Note that the observed leaf water $\delta^{18}O$ is different between fully expanded leaves on the middle of branches and expanding leaves at the tips; however, the difference disappears when leaf water $\delta^{18}O$ is expressed as enrichment above xylem water ($\Delta^{18}O_L$). Samples were collected between 1300 and 1400 h at the Peaceful Bay plantation in December 2001.

Isotopic composition (‰)	Position on branch		
	Base	Middle	Tip
Xylem water $\delta^{18}O$	–2.5 (0.3) a	–2.4 (0.1) a	–1.5 (0.4) a
Observed leaf water $\delta^{18}O$		10.8 (0.6) a	12.5 (0.5) b
Observed $\Delta^{18}O_L$		13.3 (0.6) a	14.0 (0.6) a
Predicted steady-state $\Delta^{18}O_e$		15.4	15.4

Table 5. Observed leaf water enrichment in samples collected at the Mount Barker plantation in November 2000. Also shown is the predicted steady-state evaporative site water enrichment at the times of collection.

Leaf water ^{18}O enrichment (‰)	Time of day (h)					
	0500	0800	1100	1400	1700	2000
Observed upper canopy $\Delta^{18}O_L$	10.3	9.5	11.1	19.7	16.2	12.5
Observed lower canopy $\Delta^{18}O_L$	9.2	9.1	10.9	16.5	16.6	12.4
Observed stem apices $\Delta^{18}O_L$	6.9	6.9	7.2	9.1	10.3	8.6
Predicted steady-state $\Delta^{18}O_e$	2.1	11.7	22.9	24.0	12.7	0.8

Table 6. Observed lamina leaf water enrichment in samples collected at the Mount Barker plantation in March 2001. Also shown is the predicted steady-state evaporative site water enrichment at the times of collection.

Leaf water ^{18}O enrichment (‰)	Time of day (h)				
	Day 1			Day 2	
	1000	1200	1500	0530	1000
Observed upper canopy $\Delta^{18}O_L$	11.5	13.2	19.6	12.0	16.2
Observed upper, middle canopy $\Delta^{18}O_L$	9.4	13.2	18.8	11.9	15.4
Observed lower, middle canopy $\Delta^{18}O_L$	6.8	13.3	18.4	14.3	14.7
Observed lower canopy $\Delta^{18}O_L$	7.9	14.6	17.3	15.1	14.4
Predicted steady-state $\Delta^{18}O_e$	18.6	21.9	26.7	3.5	23.1

site in sign to the difference in $\Delta^{18}O_p$ between fully expanded leaves and apices.

Parameter ε_{cp}, the difference between leaf $\Delta^{18}O_p$ and leaf $\Delta^{18}O_c$, varied among different developmental stages of leaves. The mean value for fully expanded leaves was 1.4 ± 0.7‰ (mean ± 1 SE, $n = 17$). The mean value for expanding leaves at the Peaceful Bay plantation was –1.4 ± 2.0‰ ($n = 5$). The mean value for stem apices at the Mount Barker plantation was –4.6 ± 0.5‰ ($n = 6$).

Calculation of $p_{ex}p_x$ for the stem apices sampled at the Mount Barker plantation yielded a value of 0.38 ± 0.03 (mean ± 1 SE, $n = 6$). The mean value calculated for p_x for trees from which apices were sampled was 0.95 ± 0.03, such that the mean value for p_{ex} for stem apices was 0.40 ± 0.04.

Wood and bark $\Delta^{18}O_p$ and $\Delta^{18}O_c$

The $\Delta^{18}O_p$ values for mature xylem wood ranged from 24.6 to 33.8‰ (mean = 30.3‰, $n = 71$). There was variation in xylem wood $\Delta^{18}O_p$ among plantations (Table 7) and among different crown positions within trees (Table 3). Mature xylem $\Delta^{18}O_p$ was correlated with $\Delta^{18}O_c$ for cellulose extracted from the same tissues ($r = 0.84$, $P < 0.0001$, $n = 45$). The equation relating the two was $\Delta^{18}O_p = \Delta^{18}O_c - 5.3‰$. The mean ε_{cp} for mature xylem tissues was –5.2 ± 0.1‰ (mean ± 1 SE, $n = 45$).

Bark $\Delta^{18}O_p$ was significantly correlated with the $\Delta^{18}O_p$ of underlying wood ($r = 0.81$, $P < 0.0001$, $n = 45$; Figure 6). However, mean bark $\Delta^{18}O_p$ was lower than mean wood $\Delta^{18}O_p$ (28.0 versus 29.5‰, $P < 0.001$, $n = 45$). Mean bark $\Delta^{18}O_c$ was also less than mean wood $\Delta^{18}O_c$ (29.2 versus 33.4‰, $P < 0.001$, $n = 10$). Bark ε_{cp} was –3.7 ± 0.7‰ ($n = 10$).

Recently differentiated xylem tissues had $\Delta^{18}O_p$ and $\Delta^{18}O_c$ values that were generally lower than corresponding values for mature xylem tissues (Table 7). Mean ε_{cp} for recently differentiated xylem tissue was –6.3 ± 0.6‰ (± 1 SE, $n = 6$). The $\Delta^{18}O_p$ of recently differentiated xylem tissue was significantly correlated with $\Delta^{18}O_{suc}$ of phloem sap collected at the same time ($r = 0.49$, $P = 0.003$, $n = 36$). Mean p_{ex} calculated for recently differentiated xylem tissue, using Equation 10, but replacing the term $\int A\Delta^{18}O_L / \int A$ with $\Delta^{18}O_{suc} - \varepsilon_{wc}$, was 0.39 ± 0.07 ($n = 6$). Mean p_x for the same trees was 0.95 ± 0.02.

Phloem sap, leaf and wood $\Delta^{13}C$

The $\Delta^{13}C$ of phloem sap sugars ranged from 14.6 to 21.6‰ (mean = 18.7‰, $n = 94$). There was variation in $\Delta^{13}C_{suc}$ among plantations sampled in December 2001 (Table 7) and seasonally between the November and March sampling dates at the Mount Barker plantation (Figure 7).

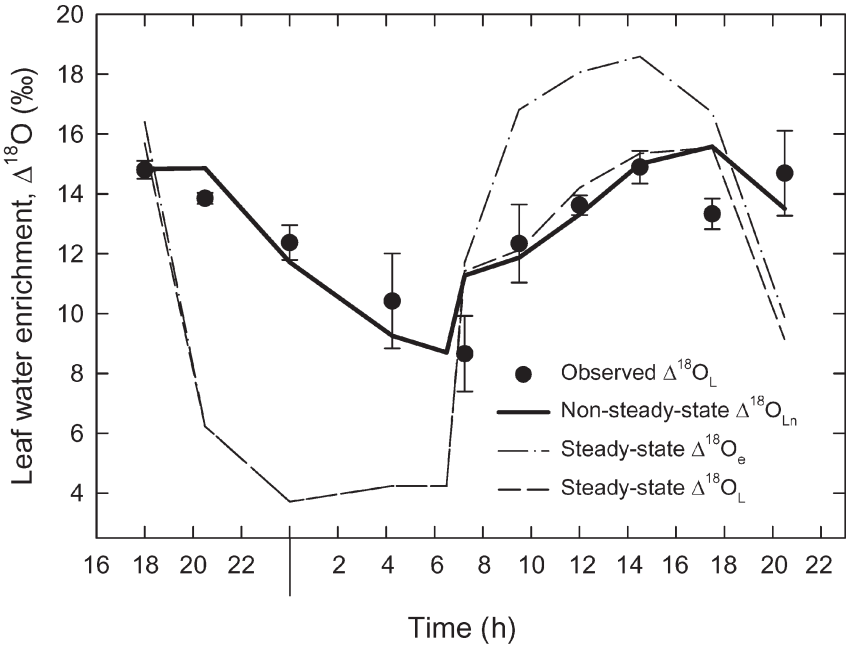

Figure 3. Diel variation in observed and predicted oxygen isotope enrichment of *E. globulus* leaf water. Abbreviations: $\Delta^{18}O_L$ = ^{18}O enrichment of lamina leaf water; $\Delta^{18}O_{Ln}$ = predicted non-steady state ^{18}O enrichment of mean lamina leaf water; and $\Delta^{18}O_e$ = ^{18}O enrichment of evaporative site water. Lines are model predictions, and points are observed values. Error bars represent ± 1 SE. Model formulations and details of input parameters are given in the text. Sampling took place at the Denmark plantation in December 2001.

Table 7. Mean isotopic composition of xylem and phloem sap, new xylem wood and mature xylem wood at six *E. globulus* plantations in southwestern Australia. Samples were collected in December 2001. Values in parentheses are 1 SE ($n = 6$). Values within a row followed by different letters are significantly different at $P < 0.05$. New xylem tissue was bulked for each plantation for cellulose extraction, giving only a single value for $\Delta^{18}O_c$ and $\Delta^{13}C_c$.

Isotopic parameter (‰)	Plantation (sorted in order of increasing latitude)					
	Redmond	Denbarker	St. Werburghs	Eulup	Kwornicup	Frankland
Xylem water $\delta^{18}O$	–3.7 (0.4) a	–4.6 (0.2) bc	–4.1 (0.4) ab	–4.4 (0.2) ab	–5.4 (0.1) c	–5.5 (0.1) c
Phloem sap $\Delta^{18}O_{pw}$	0.9 (0.6) a	0.1 (0.4) a	0.3 (0.2) a	0.8 (0.5) a	2.1 (0.8) a	0.5 (0.3) a
Phloem sap $\Delta^{18}O_{suc}$	38.5 (0.5) a	39.5 (0.8) ab	37.2 (0.7) a	38.7 (0.2) a	37.8 (0.6) a	41.4 (0.8) b
New xylem $\Delta^{18}O_p$	26.7 (0.3) a	28.6 (0.5) b	27.8 (0.3) ab	28.1 (0.4) ab	28.2 (0.4) ab	29.0 (0.2) b
New xylem $\Delta^{18}O_c$	31.7	34.3	34.5	32.7	36.2	36.6
Mature xylem $\Delta^{18}O_p$	29.8 (0.3) a	31.1 (0.4) ab	31.0 (0.3) ab	31.1 (0.4) ab	31.7 (0.5) b	31.6 (0.4) b
Mature xylem $\Delta^{18}O_c$	35.1 (0.2) a	36.3 (0.3) ab	35.8 (0.4) ab	35.2 (0.4) a	37.1 (0.3) b	37.0 (0.4) b
Phloem sap $\Delta^{13}C_{suc}$	20.7 (0.3) a	20.0 (0.3) ab	18.9 (0.3) bc	17.7 (0.3) c	18.5 (0.3) bc	19.1 (0.7) ac
New xylem $\Delta^{13}C_p$	21.4 (0.2) a	19.2 (0.4) b	18.6 (0.2) bc	17.7 (0.2) c	17.9 (0.3) bc	18.8 (0.5) bc
New xylem $\Delta^{13}C_c$	21.0	19.1	19.3	17.8	17.9	18.1
Mature xylem $\Delta^{13}C_p$	22.0 (0.4) a	20.1 (0.4) b	19.5 (0.2) b	18.8 (0.2) b	19.4 (0.1) b	19.9 (0.4) b
Mature xylem $\Delta^{13}C_c$	21.5 (0.5) a	19.7 (0.4) b	19.0 (0.2) b	18.3 (0.2) b	19.0 (0.2) b	19.1 (0.4) b

Leaf $\Delta^{13}C_p$ ranged from 15.4 to 23.6‰ (mean = 20.7‰, n = 72). There was significant variation in leaf $\Delta^{13}C_p$ among canopy positions in the March 2001 sampling at the Mount Barker plantation (Table 3); leaf $\Delta^{13}C_p$ decreased with increasing canopy height. Leaf $\Delta^{13}C_p$ also varied seasonally between the November and March collections at the Mount Barker plantation (Figure 7). Leaf $\Delta^{13}C_p$ was correlated with ($r = 0.87$, $P < 0.0001$, $n = 57$), but consistently higher than, phloem sap $\Delta^{13}C_{suc}$ for samples collected at the same time and from the same height in the canopy (Figure 7). The difference between leaf $\Delta^{13}C_p$ and phloem sap $\Delta^{13}C_{suc}$ ranged from –0.3 to 4.6‰ (mean = 2.3‰, $n = 57$).

Stem apices collected at the Mount Barker plantation in November 2000 had a mean $\Delta^{13}C_p$ of 19.2 ± 0.3‰ ($n = 10$), whereas the $\Delta^{13}C_{suc}$ of phloem sap collected concurrently had a mean of 17.2 ± 0.4‰ ($n = 6$). Thus, stem apices had significantly higher $\Delta^{13}C$ than phloem sap sugars at the time of collection ($P = 0.01$, $n = 6$).

The $\Delta^{13}C_p$ of recently differentiated xylem tissue was not significantly different from the $\Delta^{13}C_{suc}$ of phloem sap collected concurrently ($P = 0.44$, $n = 36$). Variation between the two was closely correlated ($r = 0.85$, $P < 0.0001$, $n = 36$; Figure 8B). In contrast, mature xylem tissue $\Delta^{13}C_p$ was higher than that of $\Delta^{13}C_{suc}$ by 0.8‰ ($P < 0.001$, $n = 36$; Figure 8A), and higher than recently differentiated xylem tissue $\Delta^{13}C_p$ by 1.0‰ ($P < 0.001$, $n = 36$; Figure 8C).

Mature xylem $\Delta^{13}C_p$ and $\Delta^{13}C_c$ were strongly correlated ($r = 0.97$, $P < 0.0001$, $n = 45$). The equation relating the two was $\Delta^{13}C_p = 0.95\Delta^{13}C_c + 1.6‰$. The mean difference between $\Delta^{13}C_p$ and $\Delta^{13}C_c$ for mature xylem wood was 0.5 ± 0.04‰ ($n = 45$). In contrast, the $\Delta^{13}C_c$ for cellulose extracted from recently differentiated xylem tissues did not differ from $\Delta^{13}C_p$ of the same tissue ($P = 0.72$, $n = 6$). Variation among plantations in mature and recently differentiated xylem $\Delta^{13}C_p$ and $\Delta^{13}C_c$ is detailed in Table 7.

Mature xylem $\Delta^{13}C_p$ was consistently less than leaf $\Delta^{13}C_p$ for samples collected from the same canopy position ($P = 0.001$, $n = 24$); the mean difference between the two was 1.0‰. For samples collected at the Mount Barker plantation in March 2001, the difference between leaf $\Delta^{13}C_p$ and stem wood $\Delta^{13}C_p$ appeared to decrease with increasing height in the canopy (Table 3).

Table 8. Observed photosynthesis rate-weighted lamina leaf water ^{18}O enrichment compared with observed phloem sap sugar ^{18}O enrichment. Phloem sap values are the mean of several samples collected throughout the day; values in parentheses are 1 SD. The expected difference between ^{18}O enrichment of phloem sap sugars and mean lamina leaf water is 27.4‰.

Sampling date	Photosynthesis-weighted $\Delta^{18}O_L$ (‰)	Phloem sap $\Delta^{18}O_{suc}$ (‰)	$\Delta^{18}O_{suc}$ minus $\Delta^{18}O_L$ (‰)
Nov 2000	13.8	42.3 (1.5)	28.5
Mar 2001	14.0	42.4 (1.2)	28.4
Dec 2001	12.5	39.0 (1.1)	26.5
Mean			27.8

Correlations between $\Delta^{18}O$ and $\Delta^{13}C$

Variation in $\Delta^{18}O$ of phloem sap sugars was significantly related to variation in their $\Delta^{13}C$ according to the equation $\Delta^{18}O_{suc} = -0.62\Delta^{13}C_{suc} + 51‰$ ($r^2 = 0.40$, $P < 0.001$, $n = 94$). Leaf $\Delta^{18}O_p$ was related to leaf $\Delta^{13}C_p$ by the equation $\Delta^{18}O_p = -0.61\Delta^{13}C_p + 45‰$ ($r^2 = 0.35$, $P < 0.001$, $n = 72$). Finally, variation in mature xylem wood $\Delta^{18}O_p$ was significantly related to variation in mature xylem wood $\Delta^{13}C_p$ by the equation $\Delta^{18}O_p = -0.38\Delta^{13}C_p + 38‰$ ($r^2 = 0.19$, $P < 0.001$, $n = 64$).

If the oxygen and carbon isotope ratios were expressed with δ notation, the two were still correlated, indicating that correlations between $\Delta^{18}O$ and $\Delta^{13}C$ were not caused by variation in xylem water $\delta^{18}O$. The regression equation describing the relationship between $\delta^{18}O$ and $\delta^{13}C$ of phloem sap sugars was $\delta^{18}O_{suc} = 0.67\delta^{13}C_{suc} + 53‰$ ($r^2 = 0.36$, $P < 0.001$, $n = 94$). For

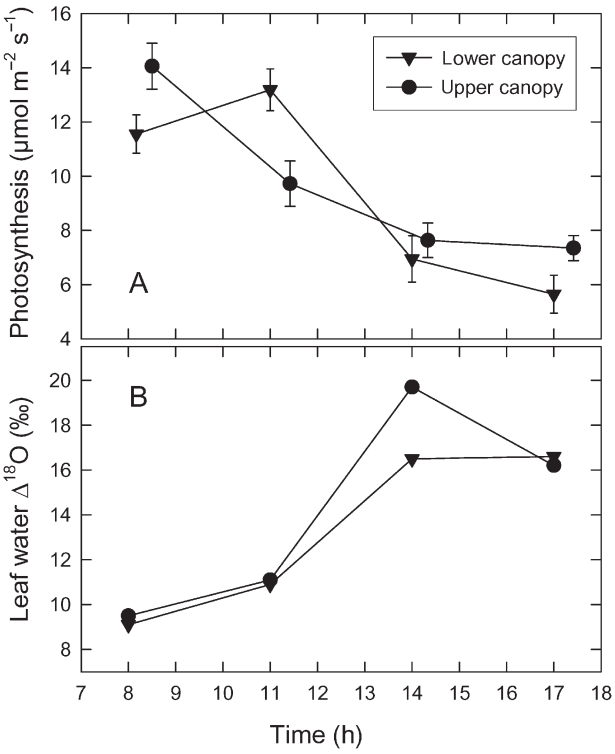

Figure 4. Diurnal variation in (A) photosynthesis and (B) leaf water ^{18}O enrichment ($\Delta^{18}O$) in *E. globulus*. Measurements were made at the Mount Barker plantation in November 2000. Error bars in (A) represent ± 1 SE. Note the differential patterns of diurnal variation between photosynthesis and leaf water $\Delta^{18}O$.

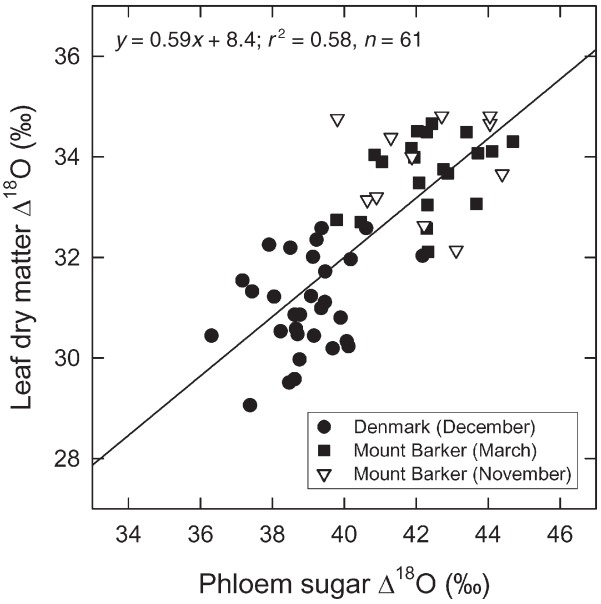

Figure 5. Leaf dry matter ^{18}O enrichment ($\Delta^{18}O$) plotted against that for phloem sap sugars collected from the main stem at the same canopy height as leaves. Samples were collected from the Mount Barker and Denmark plantations.

leaf dry matter, the relationship between $\delta^{18}O$ and $\delta^{13}C$ was $\delta^{18}O_p = 0.62\delta^{13}C_p + 46‰$ ($r^2 = 0.51$, $P < 0.001$, $n = 72$). Finally, for wood dry matter, the relationship between $\delta^{18}O$ and $\delta^{13}C$ was $\delta^{18}O_p = 0.41\delta^{13}C_p + 37‰$ ($r^2 = 0.45$; $P < 0.001$, $n = 64$). The slopes of the regression equations are positive when little delta values are used, because $\delta^{13}C$ and $\Delta^{13}C$ are opposite in sign.

Discussion

Our data provide a means for critically examining several aspects of $\Delta^{18}O$ theory. Responses to the seven hypotheses can be summarized as follows. (1) We observed small variations in xylem water $\delta^{18}O$ within tree crowns that were only marginally significant. In contrast, variation among plantations was more pronounced, with xylem water $\delta^{18}O$ decreasing significantly with increasing distance from the coast. (2) We confirmed that enriched leaf water can be exported from *E. globulus* leaves in the phloem, but found that $\Delta^{18}O_{pw}$ was generally small in this species, yielding p_x estimates close to unity. (3) The non-steady-state leaf water model summarized in Equation 9 performed well in comparison with observed variation in $\Delta^{18}O_L$ over a full diel cycle (Figure 3). (4) We confirmed that sugars exported from leaves in phloem sap are enriched in ^{18}O by about 27‰ compared with average lamina leaf water; importantly, it was the photosynthesis-weighted mean lamina leaf water signal that was recorded in the phloem sugars (Table 8). (5) Observed mean values for the proportion of exchangeable oxygen during cellulose synthesis for both leaf and xylem tissues were close to 0.40. (6) Parameter ε_{cp} was variable among leaves; some of this variation appeared to be related to the developmental stage of the leaf. (7) We found that nascent xylem and leaf tissues were not enriched in ^{13}C compared with phloem sap sugars collected concurrently, suggesting that, in *E. globulus*, ^{13}C enrichment of sink tissues compared with source leaves does not result from an enriching process within the sink tissue.

Xylem water $\delta^{18}O$

We found only weak evidence of appreciable variation in xylem water $\delta^{18}O$ within different parts of the tree crown in *E. globulus*. There was a tendency for xylem water in the upper stem to be slightly enriched in ^{18}O compared with that in the lower stem, and for xylem water in branch tips to be slightly enriched compared with that in the middle and base of branches. Xylem water in branch tips has been previously observed to be enriched in deuterium compared with xylem water in more proximal branch sections (Dawson and Ehleringer 1993). The enrichment was ascribed to evaporative water loss from unsuberized woody tissues. A similar process may also occur in relation to ^{18}O in *E. globulus* branch tips.

We observed a linear decrease in xylem water $\delta^{18}O$ with increasing latitude south from about –35.0° S to about –34.3° S. A corresponding linear decrease in annual precipitation occurred over this latitudinal gradient. This spatial variation in xylem water $\delta^{18}O$ across the landscape might then be ex-

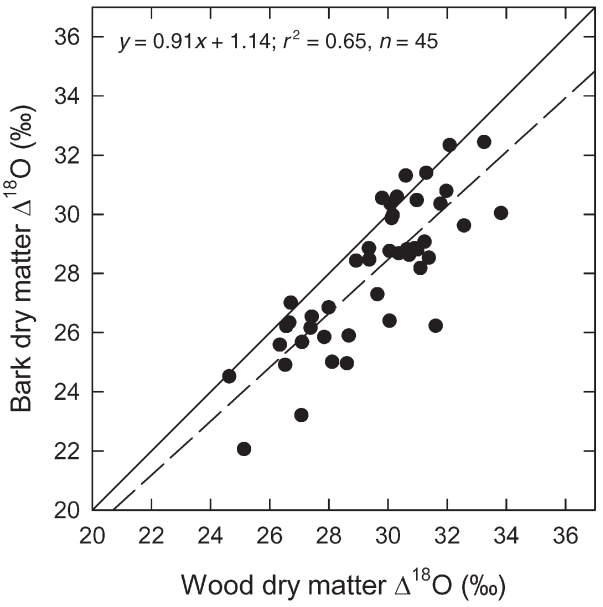

Figure 6. Bark dry matter ^{18}O enrichment ($\Delta^{18}O$) plotted against ^{18}O enrichment of underlying wood. Samples were collected from main stems and branches in the Mount Barker and Peaceful Bay plantations. The solid line is the 1:1 line; the broken line is the regression line.

plained by variation in the isotopic composition of rainfall, caused by the "continental effect" (Rozanski et al. 1993). The continental effect describes a process by which rainfall becomes progressively depleted in heavy isotopes as storms track inland from the coast. This is because heavy isotopes preferentially move from the gaseous to liquid phase, as indicated by positive values for the equilibrium fractionation factor, ε^+. The variation that we observed in xylem water $\delta^{18}O$ of about 3‰ over a transect extending about 80 km inland is similar to variation in xylem water $\delta^{18}O$ recently observed over a similar transect in the northwestern USA (Bowling et al. 2003).

In addition to the possible influence of the continental effect on xylem water $\delta^{18}O$, some of the landscape-level variation that we observed could have been caused by differential use of groundwater relative to soil water in the inland plantations compared with those near the coast. Dawson and Pate (1996) found that ground water in the Mount Barker region of southwestern Australia was depleted in deuterium compared with soil water. Assuming the pattern is the same for $\delta^{18}O$, use of groundwater at the low rainfall end of the transect could have caused lower xylem water $\delta^{18}O$.

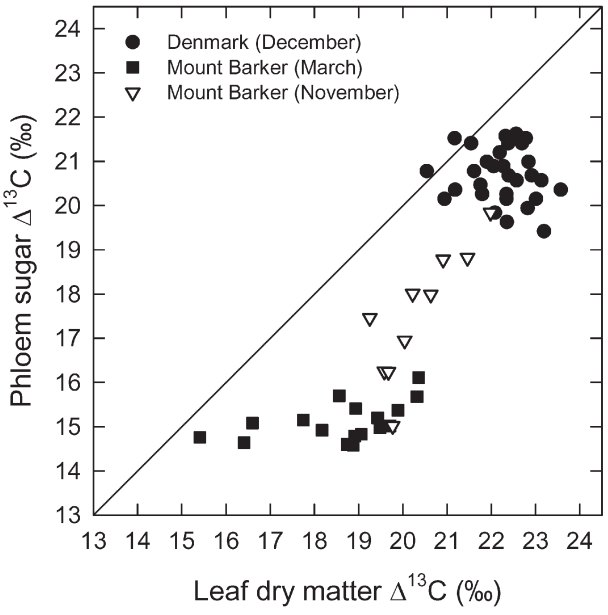

Figure 7. Phloem sugar carbon isotope discrimination ($\Delta^{13}C$) plotted against leaf dry matter $\Delta^{13}C$ from the same canopy height. The solid line is the 1:1 line.

Leaf water $\Delta^{18}O$

The leaf water ^{18}O enrichment that we observed during the day was consistently less than steady-state predictions of evaporative site water enrichment. This is a common feature of many leaf water investigations (Flanagan 1993, Wang et al. 1998). The Péclet effect, summarized in Equation 4, offers a potential explanation for this discrepancy. Application of Equation 4 requires an estimate of L, the scaled effective path length. We estimated this term by assuming that observed leaf water enrichment was at steady state in the afternoon. The L was then fitted to minimize the difference between predicted $\Delta^{18}O_L$ and observed $\Delta^{18}O_L$. The L value that we estimated for *E. globulus* was 54 mm. This value is large compared with previous estimates for some species (Flanagan et al. 1993, 1994, Barbour et al. 2000, Cernusak et al. 2003*b*), but well within the range of 4 to 166 mm estimated for 90 species (Wang et al. 1998). Our estimate of L for *E. globulus* was partly influenced by our use of new coefficients in the calculation of ε_k for prediction of $\Delta^{18}O_e$. If the previously accepted value of 1.028 for the kinetic isotope effect during diffusion of $H_2{}^{18}O$ in air (Merlivat 1978) is used instead of the recently reported value of 1.032 (Cappa et al. 2003), then our estimated L value for *E. globulus* would be 25 mm, which agrees well with some other observations for woody plants (Flanagan et al. 1993).

Nighttime steady-state predictions of $\Delta^{18}O_e$ and $\Delta^{18}O_L$ were consistently less than observed leaf water enrichment. Similar results have been reported for other species (Flanagan and Ehleringer 1991, Cernusak et al. 2002). Application of a non-steady-state modification of the leaf water enrichment model resulted in improved prediction of nighttime leaf water enrichment, both in this and a previous study (Cernusak et al. 2002).

Phloem sap $\Delta^{18}O_{pw}$

We observed small but consistent enrichments in phloem water compared with xylem water for phloem sap samples collected in November 2000 and December 2001, but no phloem water enrichment for samples collected in March 2001. March is normally a time of peak water stress in the Mediterranean-type environment of southwestern Australia. This was

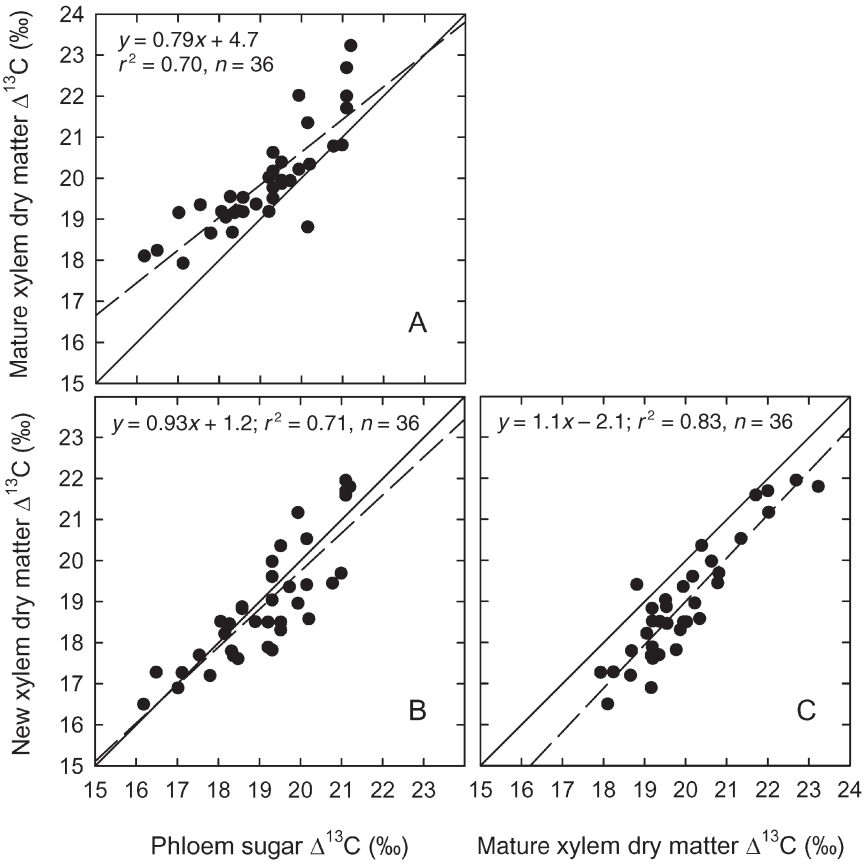

Figure 8. Carbon isotope discrimination ($\Delta^{13}C$) of mature xylem dry matter (A) and newly differentiated xylem dry matter (B) plotted against $\Delta^{13}C$ of phloem sap sugars. Also shown is $\Delta^{13}C$ of newly differentiated xylem dry matter plotted against that of mature xylem dry matter (C). Solid lines are 1:1 lines; broken lines are regression lines. Samples were collected from six trees at each of six *E. globulus* plantations in December 2001.

likely to have been the case in 2001, as indicated by the low $\Delta^{13}C$ that we observed in phloem sap sugars at this time (Figure 7). During times of high water stress, phloem sap flow velocities are likely to be low because of low photosynthetic rates and high phloem sap sugar concentrations (Cernusak et al. 2003*a*). Under such conditions, the residence time of phloem sap in sieve tubes may be long, allowing for extensive mixing between phloem water and xylem water across sieve tube membranes, and consequent dilution of ^{18}O enrichment in phloem water. The same mechanism may explain the generally low $\Delta^{18}O_{pw}$ that we observed in *E. globulus* compared with observations in herbaceous plants (Cernusak et al. 2002, 2003*b*): translocation over longer distances in trees than in herbaceous plants probably results in longer phloem sap residence time, allowing greater mixing between phloem and xylem water, and therefore reducing phloem $\Delta^{18}O_{pw}$.

Phloem sap $\Delta^{18}O_{suc}$

Phloem sap $\Delta^{18}O_{suc}$ reflected leaf water enrichment closely in *E. globulus* when observed leaf water enrichments were weighted by observed photosynthetic rates. The precise relationship between $\Delta^{18}O_{suc}$ and $\Delta^{18}O_L$ was expected to be $\Delta^{18}O_{suc} = 1.027\Delta^{18}O_L + 27‰$ (Cernusak et al. 2003*b*). Applying this relationship to $\Delta^{18}O_L$ data presented in Table 8, a mean difference between $\Delta^{18}O_{suc}$ and $\Delta^{18}O_L$ of 27.4‰ was predicted, which agreed well with the observed mean difference of 27.8‰ (Table 8). A similar result was found for *Ricinus communis* L., where $\Delta^{18}O_{suc}$ of phloem sap sugars accurately reflected mean lamina leaf water enrichment under controlled conditions (Cernusak et al. 2003*b*). For *E. globulus* in the field, we observed that photosynthesis was highest in the morning and decreased through the day, whereas $\Delta^{18}O_L$ was relatively low in the morning and increased to a maximum in the afternoon. The same contrasting diurnal patterns in photosynthesis and leaf water enrichment have been observed in other species under field conditions (Harwood et al. 1998, Cernusak et al. 2002). These results suggest that appropriate weighting by diurnal variation in photosynthetic rates may be important for interpreting organic matter ^{18}O enrichment in terms of leaf water enrichment, and factors affecting leaf water enrichment, such as humidity. Additionally, results presented in Table 8 provide validation of our use of $\Delta^{18}O_{suc} - \varepsilon_{wc}$ in place of $\int A\Delta^{18}O_L / \int A$ in calculations of p_{ex}.

In our analysis, we neglected any possible effect of daytime starch storage followed by nighttime degradation and export on the ^{18}O composition of translocated photosynthate. Further research is necessary to fully understand this process, and to assess its possible effect on the oxygen isotope composition of phloem sap sugars.

Leaf and wood p_{ex}

Table 9 compares our observations of p_{ex} in *E. globulus* with values presented in the literature. There is a surprising consistency among mean p_{ex} estimates over a wide range of species

and growth conditions when the substrate for tissue synthesis is carbohydrate; the mean p_{ex} value among all such entries in Table 9 is 0.42. The mean value that we observed in *E. globulus* for newly emerging leaves was 0.40, and the mean value that we observed for recently differentiated xylem tissue was 0.39.

In reviewing the literature, we found one p_{ex} value that deviated substantially from the overall mean; this was an estimate of 1.0 for sprouting potato shoots (DeNiro and Cooper 1989). A slope of 1.0 was calculated for the relationship between shoot cellulose and tuber water, apparently indicating complete exchange between organic oxygen and medium water during shoot cellulose synthesis. Tuber water $\delta^{18}O$ values ranged from –11 to –3‰. We suggest that this variation in tuber water $\delta^{18}O$ was too small to provide an accurate estimate of p_{ex} from the regression slope. An alternative is to estimate the $\delta^{18}O$ of organic oxygen in equilibrium with tuber water by adding 27‰ to the tuber water $\delta^{18}O$. In doing this, it is apparent that the shoot cellulose $\delta^{18}O$ values in Table 2 of DeNiro and Cooper (1989) generally lie about halfway between those of tuber starch and those expected for organic oxygen in equilibrium with tuber water. The p_{ex} for each potato shoot can then be calculated according to the equation, $p_{ex} = (\delta^{18}O_{ts} - \delta^{18}O_{sc})/(\delta^{18}O_{ts} - \delta^{18}O_{tw+27})$, where $\delta^{18}O_{ts}$ refers to tuber starch, $\delta^{18}O_{sc}$ to shoot cellulose, and $\delta^{18}O_{tw+27}$ to tuber water plus 27‰. One potato sample in Table 2 of DeNiro and Cooper (1989), potato number 21, cannot be analyzed in this way because the difference between $\delta^{18}O_{ts}$ and $\delta^{18}O_{tw+27}$ is only 0.1‰. Among the other 20 samples, this difference ranges from 4.6 to 8.8‰. The mean p_{ex} for these 20 samples is 0.50 ± 0.04. We suggest that the anomalous p_{ex} estimate of 1.0 (DeNiro and Cooper 1989) can thus be satisfactorily reconciled with the other values in Table 9 by considering this alternative approach to calculating p_{ex}.

In our calculations of p_{ex}, we assumed that the fractionation between organic oxygen and water is 27‰, regardless of the intramolecular position of oxygen atoms when they exchange. Sternberg et al. (2003) recently estimated a fractionation factor of 48.8 ± 20‰ for the oxygen atom bound to the second carbon of glucose moieties during heterotrophic cellulose synthesis. The authors suggested that this indicates variation in fractionation factors between organic oxygen and water, depending on where the oxygen atom is located within the organic molecule. However, as noted by Sternberg et al. (2003), the error around the estimate of 48.8‰ is large (1 SE = 20‰). Moreover, the slope of the relationship between $\delta^{18}O$ of oxygen bound to the second carbon of the glucose moieties ($\delta^{18}O^2$) and $\delta^{18}O$ of medium water ($\delta^{18}O_w$) was 1.027. The precise relationship between the two is expected to be of the form $\delta^{18}O^2 = \alpha_{wc}\delta^{18}O_w + \varepsilon_{wc}(‰)$, where α_{wc} is defined as $(1 + (\varepsilon_{wc}(‰)/1000))$. A regression slope of 1.027 therefore indicates an ε_{wc} of exactly 27‰, contradicting the estimate of 48.8‰ indicated by the intercept of the regression. Thus, there is considerable uncertainty associated with the authors' estimated value of 48.8‰ for the fractionation between $\delta^{18}O^2$ and $\delta^{18}O_w$. Nevertheless, if it is shown that ε_{wc} differs depending on

Table 9. Mean $p_{ex}p_x$ and p_{ex} values for analyses in the present study and in the literature. Values from the present study are given as mean ± 1 SE.

Species	Growth environment	Substrate	Mean $p_{ex}p_x$	Mean p_{ex}	Reference
Arachis hypogaea L.	Dark	Lipid		0.72	Luo and Sternberg 1992
Ricinus communis L.	Dark	Lipid		0.69	Luo and Sternberg 1992
Ricinus communis	Dark	Lipid		0.76	Sternberg et al. 1986
Daucus carota L.	Dark	Glycerol		0.77	Sternberg et al. 1986
Daucus carota	Dark	Sucrose		0.47	Sternberg et al. 1986
Hordeum vulgare L.	Dark	Starch		0.38	Luo and Sternberg 1992
Lemna gibba L.	Dark	Sucrose		0.38	Yakir and Deniro 1990
Solanum tuberosum L.	Dark or light	Starch		0.50 ± 0.04 [1]	DeNiro and Cooper 1989
Triticum aestivum L.	Dark	Starch		0.31	Luo and Sternberg 1992
Triticum aestivum	Dark	Starch		0.43	Sternberg et al. 2003
Quercus robur L.	4-h incubation of stem tissue	Glucose		0.53	Hill et al. 1995
Ten grass species	Normal day/night	Sucrose	0.25	~0.40 [2]	Helliker and Ehleringer 2002*a*
Lolium multiflorum Lam.	Normal day/night	Sucrose	0.22	0.35	Helliker and Ehleringer 2002*b*
Gossypium hirsutum L.	Normal day/night	Sucrose	0.38	~0.44 [3]	Barbour and Farquhar 2000
Alnus sp., *Betula occidentalis* Hook, *Populus* sp.	Normal day/night	Sucrose	0.42	~0.43 [4]	Roden et al. 2000
Eucalyptus globulus (stem wood)	Normal day/night	Sucrose/raffinose	0.38 ± 0.07	0.39 ± 0.07	This study
Eucalyptus globulus (leaves)	Normal day/night	Sucrose/raffinose	0.38 ± 0.03	0.40 ± 0.04	This study

[1] Values are based on a reanalysis of data presented in DeNiro and Cooper (1989).
[2] Calculated assuming $p_x = 0.62$, based on data presented in Helliker and Ehleringer (2002*b*).
[3] Calculated with $p_x = 0.87$, assuming $\Delta^{18}O_{pw} = 4.0‰$ (Cernusak et al. 2003*b*) and mean $\Delta^{18}O_L = 20‰$.
[4] Calculated assuming $p_x = 0.98$, based on our $\Delta^{18}O_{pw}$ measurements.

the intramolecular position of oxygen atoms, theory relating to p_{ex} will have to be reformulated.

Variation among leaves in ε_{cp}

We observed large variation in ε_{cp}, the difference between leaf $\Delta^{18}O_p$ and $\Delta^{18}O_c$, among different developmental stages of leaves. Among the youngest leaves, those emerging from the stem apices, mean ε_{cp} was –4.6‰. In expanding leaves, mean ε_{cp} increased to –1.4‰ and the mean value for fully expanded leaves was 1.4‰. This may reflect an increase in storage of nonstructural carbohydrates in leaves as they develop; such compounds would be expected to have higher $\Delta^{18}O$ than leaf cellulose because none of their oxygen atoms would have had an opportunity to exchange with unenriched source water. The ε_{cp} values that we observed in *E. globulus* can be compared with a mean ε_{cp} of –6.8‰ for a range of species (Cernusak et al. 2004). The smallest absolute value of ε_{cp} observed in the study by Cernusak et al. (2004) was for *Eucalyptus wandoo* Blakely, which had a mean ε_{cp} of –2.8‰ for fully expanded leaves.

In addition to the apparent variation in ε_{cp} with leaf developmental stage, we observed large variation in ε_{cp} among fully expanded leaves that may partly reflect differences in environmental conditions during leaf expansion. Because xylem water $\delta^{18}O$ is probably lower in the winter than in the summer, owing to precipitation being relatively depleted in ^{18}O (Rozanski et al. 1993), and leaf water $\Delta^{18}O$ is probably also lower, owing to humidity being relatively high, leaves expanding during winter could have lower cellulose $\delta^{18}O$ values than those expanding during summer. The cellulose $\delta^{18}O$ signal is not expected to change after cellulose synthesis. In contrast, some components of leaf dry matter, such as soluble carbohydrates, turn over regularly, and are therefore expected to reflect current leaf water $\delta^{18}O$ values. The diurnal variation in leaf $\Delta^{18}O_p$, in correspondence with variation in leaf water $\Delta^{18}O$, in this and a previous study (Cernusak et al. 2002), provides evidence in support of this concept. Thus, we suggest that there is some fraction of leaf dry matter that progressively tracks and incorporates the current leaf water isotopic signal. By this reasoning, a leaf that expanded during winter, but was sampled during summer, would be expected to have a smaller absolute difference between leaf $\Delta^{18}O_p$ and $\Delta^{18}O_c$ than one that expanded and was sampled during summer.

Wood and bark $\Delta^{18}O_p$ *and* $\Delta^{18}O_c$

We observed that bark $\Delta^{18}O_p$ was consistently less than wood $\Delta^{18}O_p$ in *E. globulus* (Figure 6). Similar results were observed for cellulose, with bark $\Delta^{18}O_c$ being consistently less than wood $\Delta^{18}O_c$ by an average of 4.2‰. We suggest that this pattern reflects the influence of refixation, a process by which some of the CO_2 respired by woody tissues is re-assimilated by photosynthetic cells in the bark (Cernusak and Marshall 2000, Pfanz et al. 2002, Damesin 2003). The bark cortex of *E. globulus* is noticeably green when the outermost layer of the bark is peeled away. In contrast to leaf water, we observed no enrichment in *E. globulus* bark water relative to xylem water. Photosynthate produced by chlorophyllous cells in the bark would therefore be expected to have a $\Delta^{18}O$ of 27‰, whereas that produced in leaves had $\Delta^{18}O$ values ranging from 35 to 45‰. It was previously observed that refixation by bark could have a significant effect on the carbon isotope signature of underlying wood (Cernusak et al. 2001). We suggest that the oxygen isotope signature of wood is similarly influenced by refixation, with the extent of influence depending on the proportion of refixed photosynthate being incorporated into the wood. Differential refixation rates in different parts of the crown might reduce wood $\Delta^{18}O$ by varying degrees, and this might explain the apparently coordinated variation in mature xylem wood and bark $\Delta^{18}O_p$ within the crown of *E. globulus* (Table 3).

We observed a small variation in ε_{cp} between recently differentiated xylem tissues and mature xylem tissues; values were –6.3 and –5.3‰, respectively. This variation was much smaller than that observed between recently differentiated leaf tissue and mature leaf tissue. The ε_{cp} for mature xylem wood of *E. globulus* can be compared with mean values of –3.6 and –3.9‰ for many *Quercus* and *Pinus* sp., respectively (Barbour et al. 2001).

Phloem sap, leaf and wood $\Delta^{13}C$

Our carbon isotope data generally confirmed and reinforced the results presented previously for *E. globulus* in southwestern Australia (Pate and Arthur 1998). The observation that $\Delta^{13}C$ of newly synthesized wood dry matter closely matches that of phloem sap sugars agrees with previous results (Pate and Arthur 1998). We observed that $\Delta^{13}C$ of cellulose extracted from recently differentiated xylem tissues also matched that of simultaneously collected phloem sap sugars. This suggests that the consistent difference in $\Delta^{13}C$ between cellulose and wood dry matter for mature xylem tissues (Figure 8) results from lignification during xylem maturation (Loader et al. 2003), because lignin is known to be depleted in ^{13}C compared with cellulose (Wilson and Grinsted 1977). Our observation that phloem sap sugar $\Delta^{13}C$ is consistently less than that of dry matter of mature leaves is also consistent with the previous report (Pate and Arthur 1998), as is the observation that leaf dry matter $\Delta^{13}C$ is consistently greater than wood dry matter $\Delta^{13}C$.

Wood dry matter $\Delta^{13}C$ is less than leaf dry matter $\Delta^{13}C$ in many tree species (Craig 1953, Leavitt and Long 1982, Francey et al. 1985, Guehl et al. 1998, Miller et al. 2001). This finding fits a more general pattern of ^{13}C enrichment in heterotrophic plant tissues relative to the leaves supplying them with photosynthate (Brugnoli and Farquhar 2000, Hobbie et al. 2002, Hobbie and Werner 2004, Cernusak et al. 2004). It was recently suggested that emerging leaves in two neotropical tree species have lower $\Delta^{13}C$ than mature leaves as a result of a greater proportional carbon fixation by PEP-carboxylase in emerging leaves than in mature source leaves (Terwilliger et al. 2001). If we extend this hypothesis to sink tissues generally, we would expect that the process causing heterotrophic tissues to be ^{13}C-enriched compared with source leaves would take place within the sink tissue. One would then expect that the sink tissue would be ^{13}C-enriched compared with the carbon

delivered to it in the phloem sap. Present and previous observations on *E. globulus* (Pate and Arthur 1998) and *Lupinus angustifolius* L. (Cernusak et al. 2002) tended not to support this hypothesis. Sink tissues in these species generally closely reflect the $\Delta^{13}C$ of carbon delivered to them in phloem sap.

An exception to this general pattern in our study was that $\Delta^{13}C$ for stem apices at the Mount Barker plantation was greater than incoming phloem sap $\Delta^{13}C$. However, this trend is in the opposite direction to the hypothesis described above. It suggests, if anything, a depletion in ^{13}C of carbon in new leaves compared with incoming phloem sap carbon. This could reflect a carbon contribution to the new leaf tissue from photosynthesis in the new leaves taking place at relatively high c_i/c_a, thereby producing sugars with higher $\Delta^{13}C$ than incoming phloem sap sugars. This would be consistent with the observations of Terwilliger et al. (2001) concerning c_i/c_a in emerging leaves. We suggest that the consistent differences in $\Delta^{13}C$ between source leaves and heterotrophic tissues reflect a fractionation in the partitioning of carbon in the source leaf between that which remains in the source leaf and that which is exported in the phloem. We acknowledge, however, that multiple processes may be operating simultaneously, such that a single unifying mechanism causing the widespread ^{13}C enrichment of heterotrophic tissues compared with source leaves may not exist. For example, Hobbie and Werner (2004) recently suggested that leaf dry matter becomes depleted in ^{13}C relative to dry matter in roots as a result of lignin and lipid synthesis in leaves. Damesin and Lelarge (2003) suggested that fractionation during both carbohydrate export and tissue synthesis caused enrichment in stem tissues relative to leaves in *Fagus sylvatica*. More experiments along the lines of those conducted by Terwilliger et al. (2001) and Damesin and Lelarge (2003), but including analyses of phloem sap $\Delta^{13}C$, would help resolve these discrepancies.

Correlations between $\Delta^{18}O$ and $\Delta^{13}C$

We observed that $\Delta^{18}O$ was negatively correlated with $\Delta^{13}C$ in phloem sap sugars, leaf dry matter and wood dry matter of *E. globulus* growing in southwestern Australia. Farquhar et al. (1994) and Yakir and Israeli (1995) suggested that the combination of $\Delta^{13}C$ and $\Delta^{18}O$ analyses allows interpretation of $\Delta^{13}C$ in terms of effects on c_i/c_a caused by variation in either photosynthetic capacity or stomatal conductance. If variation in $\Delta^{13}C$ is caused by variation in photosynthetic capacity, no relationship is expected between $\Delta^{13}C$ and $\Delta^{18}O$. In contrast, if $\Delta^{13}C$ varies as a function of variation in stomatal conductance, $\Delta^{13}C$ and $\Delta^{18}O$ should be negatively correlated. The negative correlations that we observed between $\Delta^{13}C$ and $\Delta^{18}O$ of phloem sap sugars, leaf tissue and wood in *E. globulus* suggest that variation in $\Delta^{13}C$ among trees and plantations was at least partly caused by variation in stomatal conductance. This is consistent with measurements of stomatal conductance and $\Delta^{13}C$ in the Mount Barker and Denmark plantations (Cernusak et al. 2003*a*) and accords with the large variation in annual rainfall among the plantations that we sampled.

Acknowledgments

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