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Oxygen isotope enrichment of organic matter in *Ricinus communis* during the diel course and as affected by assimilate transport

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Summary

• The oxygen isotope composition in leaf water and organic compounds in different plant tissues is useful for assessing the physiological performance of plants in their environment, but more information is needed on $\Delta^{18}O$ variation during a diel course.
• Here, we assessed $\Delta^{18}O$ of the organic matter in leaves, phloem and xylem in stem segments, and fine roots of *Ricinus communis* during a full diel cycle. Enrichment of newly assimilated organic matter in equilibrium with leaf water was calculated by applying a nonsteady-state evaporative enrichment model.
• During the light period, $\Delta^{18}O$ of the water soluble organic matter pool in leaves and phloem could be explained by a 27‰ enrichment compared with leaf water enrichment. Leaf water enrichment influenced $\Delta^{18}O$ of phloem organic matter during the night via daytime starch synthesis and night-time starch remobilization. Phloem transport did not affect $\Delta^{18}O$ of phloem organic matter.
• Diel variation in $\Delta^{18}O$ in organic matter pools can be modeled, and oxygen isotopic information is not biased during transport through the plant. These findings will aid field studies that characterize environmental influences on plant water balance using $\Delta^{18}O$ in phloem organic matter or tree rings.

Key words: evaporative enrichment, oxygen isotopes, phloem-to-xylem exchange, phloem transport, transitory starch, xylem transport.

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Introduction

The determination of the oxygen isotope composition in leaf water and organic compounds is a promising tool for assessing the physiological performance of plants in their environment (Wang & Yakir, 1995; Barbour *et al.*, 2001, 2002; Cernusak *et al.*, 2005). In particular, the isotopic information in organic matter pools with different turn-over times (e.g. short-lived phloem sugars representing recent assimilates; long-lived structural compounds such as tree ring cellulose) is now widely used – often together with $\delta^{13}C$ signatures – to integrate the influence of a range of environmental factors on the water balance of plants (Saurer *et al.*, 1997; Scheidegger *et al.*, 2000; Keitel *et al.*, 2003; Brandes *et al.*, 2006). The oxygen isotope composition of organic matter is mainly influenced by two factors: the $\delta^{18}O$ signature of source water and the evaporative enrichment of mean lamina leaf water, which is the reaction water for the newly produced assimilates (Fehri & Letolle, 1977; Cernusak *et al.*, 2003b). Mean lamina leaf water enrichment depends in turn on the diffusion of ^{18}O enriched water from the sites of evaporation back into the mesophyll cells and the convection of unenriched xylem water via the transpiration stream in the opposite direction (Farquhar & Lloyd, 1993; Barbour *et al.*, 2000a). As evaporative enrichment

is closely linked to environmental conditions (water vapor pressure in the air, air temperature) and physiological traits (stomatal conductance, transpiration) this measure is of central interest for characterizing water relations in plants.

The theory of evaporative enrichment of leaf water and the imprinting of this signal on newly produced assimilates is well described (Barbour *et al.*, 2000b; Farquhar & Cernusak, 2005). However, there is little information on how the $\Delta^{18}O$ signal is altered with time and during transport through the plant. During the day, transitory starch is accumulated in the leaves. At night sugars originating from starch hydrolysis/phosphorolysis (Smith *et al.*, 2003) are loaded into the phloem serving as a carbon source for heterotrophic tissues. It must therefore be assumed that the oxygen isotopic composition of sugars transported in the phloem during the dark period is influenced not only by $\Delta^{18}O$ of leaf water during the preceding day (when the starch was produced) but also potentially during the night when starch is broken down since during starch breakdown oxygen from reaction water is introduced into the hexose molecules produced (Smith *et al.*, 2003; Weise *et al.*, 2004). Insights into such effects are important for studies that use $\Delta^{18}O$ in different organic matter pools in different plant organs such as phloem sugars (Cernusak *et al.*, 2003a, 2005; Brandes *et al.*, 2006; Keitel *et al.*, 2006) to characterize plant reactions to environmental constraints. Since phloem sugars are the main source of organic matter the stem of trees is supplied with, diel variations might also affect $\Delta^{18}O$ of whole wood or cellulose in tree rings (Saurer *et al.*, 1997; Jäggi *et al.*, 2003). Diel variations in the expression of key enzymes of lignin biosynthesis have been described for herbaceous plants (Rogers *et al.*, 2005). There is not much known about a potential circadian control and environmental triggers of cellulose and lignin synthesis in trees but varying carbon fluxes into lignin or cellulose pools during the diel course in combination with variations in $\Delta^{18}O$ of phloem sugars would strongly affect the oxygen isotope composition of tree rings.

Post-photosynthetic transformation of organic matter also takes place. Depending on the ^{18}O enrichment of medium water within which these transformations occur, $\Delta^{18}O$ of cellulose, lignin, starch and other substances in different organs within the plant will be altered compared with the original organic substrates (Yakir & Deniro, 1990; Farquhar *et al.*, 1998; Roden & Ehleringer, 1999; Roden *et al.*, 2000; Barbour *et al.*, 2004). It is also known that carbohydrate transport in the sieve tubes is highly dynamic and unloading and retrieval of sugars take place in the transport phloem at the same time (van Bel, 2003). In *Phaseolus*, for example, sieve tubes lose 6% of photosynthate per centimeter of stem, of which about two-thirds is retrieved (Minchin & Thorpe, 1987). Sugars unloaded from the transport phloem might undergo metabolic conversion in the unenriched reaction water of stems before they are reloaded into the sieve tubes. As a consequence, $\Delta^{18}O$ of phloem-transported organic matter ($\Delta^{18}O_{phloem\text{-}OM}$; for abbreviations see Table 1) could vary with transport distance and increasingly lose the isotopic information imprinted from leaf water. In addition, Cernusak *et al.* (2005) concluded that refixation of respired CO_2 by photosynthetic bark was responsible for a decrease in $\Delta^{18}O$ in bark organic matter in *Eucalyptus globulus* as bark water within which carbon assimilation was assumed to occur was not subjected to evaporative enrichment. Since *R. communis* stems are also noticeably green, partial exchange of ^{18}O-depleted bark-assimilated sugars with phloem transported organic matter could also cause basipetal $\Delta^{18}O$ gradients within the phloem.

About 15 yr ago Jeschke and Pate (1991) showed that considerable amounts of organic matter (approx. 8% of the net C assimilation) are loaded into the xylem in stems and roots of *R. communis* in order to contribute to the supply of the growing shoot. Depending on the metabolic origin and composition of this organic matter pool it might carry a signature influenced by the evaporative enrichment of leaf water. Basipetal gradients in $\Delta^{18}O$ of phloem- or xylem-transported organic compounds could therefore also influence the isotopic enrichment of structural organic matter in different plant parts.

In order to obtain more precise information on potential variations of $\Delta^{18}O$ in organic matter pools during the diel course and along the plant axis, we assessed the oxygen isotope enrichment of the water soluble ($\Delta^{18}O_{ws}$) and total organic matter fraction in leaves of *R. communis* ($\Delta^{18}O_{total\text{-}leaf}$) during a full diel cycle. In addition, we characterized $\Delta^{18}O$ in total organic matter of stem segments ($\Delta^{18}O_{total\text{-}stem}$) at six different positions and in fine roots and determined $\Delta^{18}O_{phloem\text{-}OM}$ and $\Delta^{18}O$ in xylem-transported organic matter ($\Delta^{18}O_{xylem\text{-}OM}$) at different positions along the plant axis. Barbour *et al.* (2000a) and Cernusak *et al.* (2003b) showed that measured or modeled evaporative enrichment of leaf water in *R. communis* was in good agreement, in the steady state, with phloem organic matter $\Delta^{18}O$ collected from the petiole. We extended this to the nonsteady state, by measuring the oxygen isotopic enrichment of newly assimilated organic matter during the dynamic environmental conditions of a diel course, and comparing this with the enrichment of leaf water as calculated from a new nonsteady-state model (Farquhar & Cernusak, 2005) of leaf water enrichment.

In general, no direct exchange between phloem-transported sucrose and phloem water occurs since the sucrose molecule contains no carbonyl groups. However, there are other processes after equilibration with leaf water in the light that may affect organic matter isotopic enrichment. One is starch breakdown, and another is phloem exchange with stem sugars. Therefore we aimed to explore the following notion:

1 $\Delta^{18}O_{ws}$ and $\Delta^{18}O_{phloem\text{-}OM}$ during the light period can be described with the nonsteady-state model taking a 27‰ enrichment between leaf water and organic matter (Yakir & Deniro, 1990) into account.

2 The soluble organic matter transported in the phloem during the night and originating from transitory starch is not

Table 1 Abbreviations and symbols used in text

Abbreviation or symbol	Definition
A	Net assimilation rate
α^+	Oxygen isotope effect for phase transition between liquid water and vapor
α_k	Oxygen isotope effect during water vapor diffusion from leaf intercellular air spaces to the atmosphere
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
$\Delta^{18}O$	^{18}O enrichment of any water or dry matter compared to source water
$\Delta^{18}O_{es}$	Predicted steady-state ^{18}O enrichment of water at evaporative sites (without Péclet effect) in leaves compared with source water
$\Delta^{18}O_{es}^{suc}$	Predicted steady-state ^{18}O enrichment of new photosynthates without Péclet effect ($\Delta^{18}O_{es} + \varepsilon_{wc}$)
$\Delta^{18}O_L$	^{18}O enrichment of mean lamina leaf water compared with source water
$\Delta^{18}O_{Ln}$	Predicted nonsteady-state ^{18}O enrichment of mean lamina leaf water (considering a Péclet effect) compared with source water
$\Delta^{18}O_{Ln}^{suc}$	Predicted nonsteady-state ^{18}O enrichment of new photosynthates ($\Delta^{18}O_{Ln} + \varepsilon_{wc}$)
$\Delta^{18}O_{Ls}$	Predicted steady-state ^{18}O enrichment of mean lamina leaf water (considering a Péclet effect)
$\Delta^{18}O_{Ls}^{suc}$	Predicted steady-state ^{18}O enrichment of new photosynthates ($\Delta^{18}O_{Ls} + \varepsilon_{wc}$)
$\Delta^{18}O_{pw}$	^{18}O enrichment of phloem water compared to source water
$\Delta^{18}O_{phloem\text{-}OM}$	^{18}O enrichment of organic matter transported in the phloem compared to source water
$\Delta^{18}O_{total\text{-}leaf}$	^{18}O enrichment of total leaf organic matter compared to source water
$\Delta^{18}O_{total\text{-}stem}$	^{18}O enrichment of total organic matter in stem sections compared to source water
$\Delta^{18}O_v$	^{18}O enrichment of water vapour compared to source water
$\Delta^{18}O_{ws}$	^{18}O enrichment of water soluble organic matter in leaves compared to source water
$\Delta^{18}O_{xylem\text{-}OM}$	^{18}O enrichment of organic matter transported in the xylem compared to source water
$\delta^{18}O$	$^{18}O/^{16}O$ relative to Vienna standard mean ocean water (VSMOW = 0)
E	Transpiration rate
e_a	Vapor pressure in the atmosphere
e_i	Vapor pressure in leaf intercellular air spaces
ε^+	Equilibrium ^{18}O fractionation between liquid water and vapor
ε_k	Kinetic fractionation for diffusion of $H_2{}^{18}O$ from leaf intercellular spaces to the atmosphere
ε_{wc}	Equilibrium ^{18}O fractionation between organic oxygen and medium water
g	Total leaf conductance (stomata + boundary layer conductance) to H_2O from leaf intercellular air spaces to the atmosphere
g_s	Stomatal conductance to H_2O
L	Scaled effective path length
p_{ex}	Proportion of oxygen atoms exchanging with medium water during cellulose synthesis
$\wp$	Péclet number
R_s	$^{18}O/^{16}O$ of source water
R_{VSMOW}	$^{18}O/^{16}O$ of Vienna standard mean ocean water (VSMOW)
r_b	Boundary layer resistance to water vapour diffusion
r_s	Stomatal resistance to water vapour diffusion
w_i	Mole fraction of water vapour in leaf intercellular air spaces
W	Leaf lamina water concentration

only influenced by the photosynthesis-weighted leaf water enrichment during day but also by the differing leaf water $\Delta^{18}O$ during the night.

3 The dynamic efflux to and retrieval of sugars from the stem may alter $\Delta^{18}O$ of phloem organic matter in the basipetal direction.

Materials and Methods

Plant material

Seeds of *Ricinus communis* L. were germinated in vermiculite moistened with 0.5 mM $CaSO_4$. After 13–15 d the plants were transferred to substrate, one plant in a pot of 5 l. The substrate consisted of two parts commercial potting soil (Floradur; Floragard GmbH, Oldenburg Germany) and one part Perlite (Perligran; G, Deutsche Perlite GmbH, Dortmund, Germany). Every third day the pots were well watered with tap water and after the first month the plants were supplied every second week with a commercial fertilizer (0.3% Hakaphos Blau; Compo GmbH, Münster, Germany).

The plants were cultivated for 35–40 d in a glasshouse (26 ± 5°C) with a 16 h photoperiod provided by natural daylight plus mercury-vapour lamps (Osram HQL 400; Osram, Munich, Germany) supplying the plant with a minimum of 300–500 μmol photons m^{-2} s^{-1}.

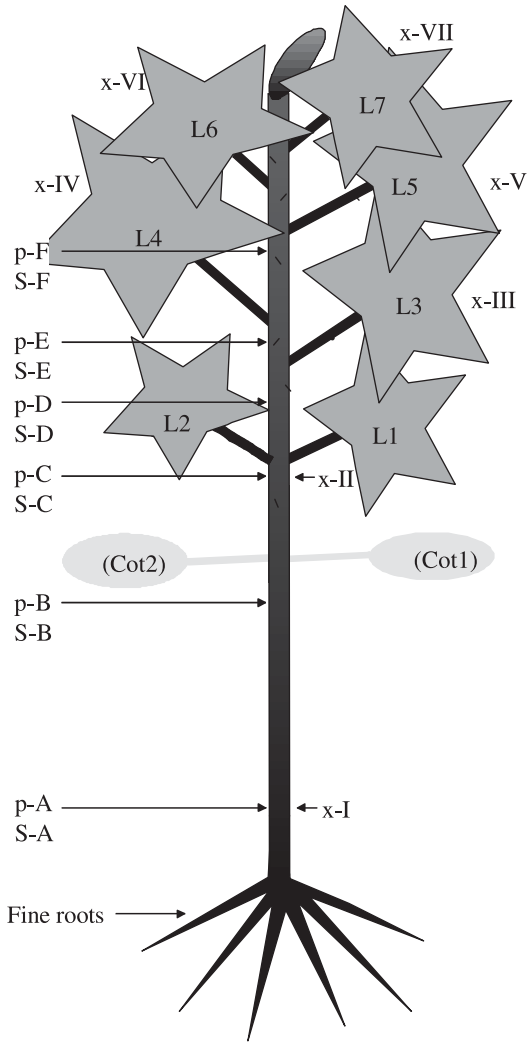

Fig. 1 Scheme of the plants used in the experiments with sampling positions. For the analysis of phloem organic matter, phloem sap was obtained from six positions along the stem (p-A to p-F). From the same position stem sections (S-A to S-F) were harvested after phloem sampling. The cotyledons (Cot1 and Cot2) were already shed at the start of the experiments. Stomatal conductance (g_s), transpiration and oxygen isotope composition were determined for all fully developed leaves (L1–L7). In addition, at all six time-points fine root samples were harvested. Xylem sap was obtained from cut midribs of the leaves (x-III to x-VII) and from tissue flaps of stem internodes (x-I and x-II) at three time points.

Experimental design

The $\Delta^{18}O$ of phloem organic matter was determined at six different positions (p-A to p-F; Fig. 1) along the axis at six time points (four in the light (10 : 30, 12 : 00, 16 : 30, 19 : 00 h (± approx. 1.0 h)) and two in the dark period (24 : 00, 03 : 00 h (± approx. 1.0 h)) during a diel course. At each time-point three to four plants were harvested destructively.

Phloem sap was sampled by cutting the bark with a scalpel according to the procedure described by Jeschke and Pate (1991) transferred with a capillary into a 1.5 ml reaction tube and frozen immediately. The collections of phloem sap from each single plant lasted 45–60 min. After sampling of phloem sap, stem sections with a length of *c.* 3 cm were collected from the same positions (S-A to S-F).

In addition, all seven fully expanded leaves (L1 to L7) and fine roots were harvested and frozen in liquid nitrogen for the assessment of $\Delta^{18}O$ in total and, for leaves only, water soluble organic matter (Fig. 1).

All tissue samples (leaves, stem sections and roots) were homogenized with mortar and pestle in liquid nitrogen. For the extraction of water soluble organic matter from leaves 1.5 ml of deionized water was added to 0.1 g aliquots of plant material. The mixture was agitated for 1 h at 4°C. The extract was heated at 100°C for 1 min to precipitate proteins and centrifuged (12 000 ***g*** for 5 min at 4°C). The supernatant is the water soluble (exportable) organic matter fraction consisting mainly of sugars but with some organic acids and amino acids (Brandes *et al.*, 2006).

For all leaves, leaf temperature, transpiration, net CO_2 assimilation, and stomatal conductance (g_s) were determined at each time point (immediately before harvest) using a portable leaf gas exchange measurement system (LCA 4; ADC BioScientific Ltd, Hoddesdon, UK). The relative humidity (RH) near the lower leaf surface was determined with a temperature and humidity probe (Humicap 113y; Vaisala, Helsinki, Finland). Three to four plants were analysed per time point.

In addition to leaves, stem sections and phloem sap, xylem sap was obtained at three time points during a diel course (09 : 30, 18 : 30, 03 : 00 h (± 2 h)) by applying pneumatic pressure to the root system enclosed in a pressure vessel (Jeschke & Pate, 1991; Peuke, 2000). From each single experimental plant, a series of seven samples of xylem sap was collected first from cut midribs of the leaf and finally from tissue flaps of stem internodes (see Fig. 1, x-VII to x-I). Pressurizing the roots to a level of 0.35 ± 0.04 MPa caused exudation at the various sampling points, enabling sap volumes of sufficient size to be collected for analysis. During the collection procedure sap samples were stored on ice and thereafter kept at −80°C.

Irrigation water on the day of measurement and water vapour (during day and night) of the glasshouse air were collected for oxygen isotope analysis. Water vapour was collected by drawing air though a dry ice–ethanol trap at a flow rate of 1 l min^{-1} (Cernusak *et al.*, 2003b).

Oxygen isotope theory

Steady-state enrichment of water at the evaporative site of the leaf ($\Delta^{18}O_{es}$) can be described according to Dongmann *et al.* (1974)

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

(ε^+ is the equilibrium fractionation between liquid water and water vapour; ε_k the kinetic fractionation during water vapour diffusion; $\Delta^{18}O_v$ is the enrichment of water vapour in the atmosphere above source water; and e_a/e_i the ratio of ambient to intercellular water vapour concentration).

The ε^+ value can be calculated according to Bottinga and Craig (1969) from a regression equation relating it to leaf temperature (T in K):

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

ε_k can be calculated according to Farquhar *et al.* (1989):

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

where r_s and r_b refer to stomatal and boundary layer resistance to water vapour (m^2 s mol^{-1}) and 32‰ and 21‰ are associated fractionation factors (Farquhar & Cernusak, 2005) based on new determinations of the isotopic effect for diffusion of $H_2{}^{18}O$ in air (Cappa *et al.*, 2003).

Average lamina mesophyll water is, however, thought to be less enriched than the water at the evaporative sites because of the influx of xylem water into the leaf (Farquhar & Lloyd, 1993). Steady-state enrichment of mean lamina leaf water ($\Delta^{18}O_{Ls}$) depends on the steady-state enrichment at the evaporative site of the leaf ($\Delta^{18}O_{es}$) and on the lamina radial Péclet number $\wp$ (Farquhar & Gan, 2003).

$$\Delta^{18}O_{Ls} = \frac{\Delta^{18}O_{es}(1 - e^{-\wp})}{\wp} \quad \text{Eqn 4}$$

where $\wp$ is 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}).

Eqn 4 describes mean lamina leaf water enrichment only under steady-state conditions. Although leaves are likely to reach isotopic steady state in the early afternoon, there will be times during the diel course when leaf water enrichment is not at steady state (Wang & Yakir, 1995; Cernusak *et al.*, 2005). Thus, nonsteady-state conditions must be considered in a model that describes leaf water enrichment during the diel course. Nonsteady-state mean lamina leaf water enrichment ($\Delta^{18}O_{Ln}$) can be described according to Farquhar and Cernusak (2005) as follows:

$$\Delta^{18}O_{Ln} = \Delta^{18}O_{Ls} - \alpha^+\alpha_k\left(\frac{1 - e^{-\wp}}{\wp}\right)\left(\frac{d(W\Delta^{18}O_{Ln})}{\frac{dt}{gw_i}}\right) \quad \text{Eqn 5}$$

(α^+ is defined as $(1 + \varepsilon^+)$, α_k is $(1 + \varepsilon_k)$; W is the lamina leaf water concentration (mol m^{-2}); t is time (s), g is total leaf conductance (stomata + boundary layer conductance); and w_i is the mole fraction of water vapour in the air spaces inside the leaf).

Newly produced assimilates are assumed to carry the signature of the leaf water at the time when they were produced with an equilibrium fractionation factor (ε_{wc}) resulting in carbonyl oxygen being *c.* 27‰ more enriched than water (Sternberg & Deniro, 1983; Yakir & Deniro, 1990). Thus the enrichment in sugar produced during photosynthesis should be given by $\Delta^{18}O_{Ln} + \varepsilon_{wc}$.

Isotope measurements and isotopic calculations

Homogenized bulk samples, water extracts, phloem sap and xylem sap were dried at 60°C for 12 h. Samples were combusted in silver capsules (IVA Analysentechnik, Meerbusch, Germany) in a high-temperature conversion/elemental analyser (TC/EA Finnigan MAT GmbH, Bremen, Germany) coupled to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT GmbH) by a Conflo II interface (Finnigan MAT GmbH). The samples contained *c.* 300 μg O. One microliter of liquid phloem sap samples, irrigation water and trapped water vapor were injected manually into the TC/EA using a syringe.

The precision of the measurements as determined by repeated measurements of standards was 0.3‰ (1 SD, $n = 10$). Oxygen isotope ratios ($\delta^{18}O$) are presented relative to Vienna standard mean ocean water (VSMOW).

$$\delta^{18}O = \frac{R_s}{R_{VSMOW}} - 1 \quad \text{Eqn 6}$$

(R_s and R_{VSMOW} are the isotope ratios ($^{18}O/^{16}O$) of the plant material and VSMOW, respectively).

Phloem sap water oxygen isotope ratios were determined as described by 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).

The oxygen isotope composition of organic matter ($\delta^{18}O_{plant\ material}$) in leaves, roots, xylem and phloem was expressed as enrichment ($\Delta^{18}O$) above irrigation water (source water; $\delta^{18}O_{source\ water}$, −6.5‰):

$$\Delta^{18}O = \frac{\delta^{18}O_{plant\ material} - \delta^{18}O_{source\ water}}{1 + \delta^{18}O_{source\ water}} \quad \text{Eqn 7}$$

Nonsteady-state enrichment ($\Delta^{18}O_{Ln}$) of leaf water was calculated according to Eqn 5. From $\Delta^{18}O_{Ln}$, ^{18}O enrichment of newly produced organic matter ($\Delta^{18}O_{Ln}^{suc}$) in isotopic equilibrium with leaf water was calculated assuming $\varepsilon_{wc} = 27‰$. For calculating the Péclet number $\wp$ a scaled effective path length L-value of 11.1 mm according to Cernusak *et al.* (2003b) was used. We also calculated the expected ^{18}O enrichment of newly produced organic matter (1) taking into account a Péclet effect but assuming steady-state

conditions ($\Delta^{18}O_{Ls}^{suc}$) and (2) assuming no Péclet effect and steady-state conditions ($\Delta^{18}O_{es}^{suc}$). The $\Delta^{18}O_{Ls}^{suc}$ was calculated from steady-state enrichment of mean lamina leaf water ($\Delta^{18}O_{Ls}$; Eqn 4) and $\Delta^{18}O_{es}^{suc}$ from steady-state enrichment of water at the evaporative site of the leaf ($\Delta^{18}O_{es}$; Eqn 1) assuming in both cases assimilates to be enriched by 27‰ compared with water.

In order to integrate calculated $\Delta^{18}O_{Ln}^{suc}$, $\Delta^{18}O_{Ls}^{suc}$, $\Delta^{18}O_{es}^{suc}$ over the whole canopy we weighted ^{18}O enrichment for assimilation taking into account leaf area (m^2) and CO_2 assimilation rate (μmol m^{-2}) of leaves L1 to L7. Similarly, observed $\Delta^{18}O$ in leaf water soluble ($\Delta^{18}O_{ws}$) and total organic matter ($\Delta^{18}O_{total\text{-}leaf}$) were weighted by leaf area and oxygen content of the respective organic matter pools per m^2 leaf area.

To calculate mean daytime $\Delta^{18}O$ values, oxygen isotope enrichment was weighted by CO_2 assimilation rate according to Cernusak *et al.* (2005)

$$weighted\ \Delta^{18}O = \frac{\int A \cdot \delta^{18}O \cdot dt}{\int A \cdot dt} \qquad \text{Eqn 8}$$

($\int A\,\Delta^{18}O \cdot dt$ is the daily integral of the product of A and $\Delta^{18}O$ and $\int A \cdot dt$ is the daily integral of photosynthesis).

Statistical analyses

All statistical analyses were performed using NCSS 2004 (Number Cruncher Statistical Software, Kaysville, UT, USA). Differences in $\Delta^{18}O$ between different chemical fractions, time-points or different positions were determined using analysis of variance (GLM-ANOVA). For analysis position was nested within time point. Regression lines between $\Delta^{18}O$ from different organic matter pools were determined by linear regression analysis. Differences between mean $\Delta^{18}O$ during day and night for a given organic matter pool were calculated applying Student's *t*-test. In order to estimate the uncertainties for the different ^{18}O enrichment models we applied the principles of the Gaussian error propagation. For this calculation we included standard deviations of single measured parameters as errors.

Results

Leaf gas exchange

Figure 2 shows mean leaf temperature (T_L) and RH of the air near the leaf of the seven leaves examined as well as transpiration rate (E) and stomatal conductance (g_s). Leaf temperature showed a distinct diurnal course with maximum values of 31°C in the afternoon and minimum values of 28.5°C in the morning and at night. Relative humidity displayed an almost inverse pattern with values close to 95% during the night and a minimum of 53% at 16 : 30 h. Mean leaf area-weighted transpiration followed RH inversely and increased from 1.2 mmol m^{-2} s^{-1} in the morning to 4.5 mmol m^{-2} s^{-1} at 16 : 30 h. Night values were below 0.1 mmol m^{-2} s^{-1}. Mean leaf area-weighted g_s showed a comparable diel pattern with maximum stomatal conductance of approx. 0.25 mol m^{-2} s^{-1} in the afternoon. There was no significant difference in g_s or E among the seven leaves examined.

$\Delta^{18}O$ in leaf organic matter

Observed $\Delta^{18}O$ in leaf water-soluble organic matter ($\Delta^{18}O_{ws}$) did not differ significantly among the seven leaves included in the study (Fig. 3a). Mean canopy $\Delta^{18}O_{ws}$ (Fig. 3a, thin black line) was approximately constant at 38‰ from 10 : 30 to 12 : 00 h. Towards the afternoon $\Delta^{18}O_{ws}$ peaked at > 42‰ and decreased again in the evening. At 24 : 00 h values from the morning were regained and later in the night a diel minimum of 35.7‰ was reached.

We calculated expected nonsteady-state ($\Delta^{18}O_{Ln}^{suc}$) (Fig. 3a, gray bold line) and steady-state ($\Delta^{18}O_{Ls}^{suc}$) (Fig. 3a, black dotted bold line) ^{18}O enrichment of newly produced organic matter in isotopic equilibrium with mean lamina leaf water taking into account a Péclet effect as well as assuming steady-state conditions and no Péclet effect ($\Delta^{18}O_{es}^{suc}$) (Fig. 3a, black dashed bold line).

Assimilation-weighted calculated $\Delta^{18}O_{es}^{suc}$ (the Craig-related enrichment) was consistently higher (up to 2.5‰) than observed $\Delta^{18}O_{ws}$ during the whole light period (Fig. 3a). By contrast, there were only slight differences between the observed $\Delta^{18}O_{ws}$ and predicted nonsteady-state $\Delta^{18}O_{Ln}^{suc}$. Assimilation-weighted predicted steady-state $\Delta^{18}O_{Ls}^{suc}$ was comparable to nonsteady-state $\Delta^{18}O_{Ln}^{suc}$ during the light period. The errors for the model calculations amounted to between 0.2 and 0.3‰.

There was no significant difference in $\Delta^{18}O$ of total foliar organic matter between different leaves or during the diurnal course (Fig. 3b). Mean leaf area-weighted $\Delta^{18}O_{total\text{-}leaf}$ varied in time between approx. 34 and 37‰.

$\Delta^{18}O$ in stem organic matter along the axis

There was no significant spatial variation of the ^{18}O enrichment in stem total organic matter ($\Delta^{18}O_{total\text{-}stem}$) along the plant axis (Fig. 4a). In the morning there was a general trend for $\Delta^{18}O$ to decrease from 10 : 30 h to 12 : 00 h. This tendency was most pronounced in the lowermost segment A. Contrasting with that observation, $\Delta^{18}O_{total\text{-}stem}$ varied thereafter only slightly for the lower segments but a more intensive decrease was observed for the uppermost stem sections at the beginning of the dark period. $\Delta^{18}O_{total}$ in roots was always less than in the lowermost stem section and showed only slight diel variations with a peak-to-peak variation of approx. 2‰. As with $\Delta^{18}O_{total\text{-}stem}$, $\Delta^{18}O_{phloem\text{-}OM}$ (Fig. 4b) did not differ between different collecting positions along the stem. There

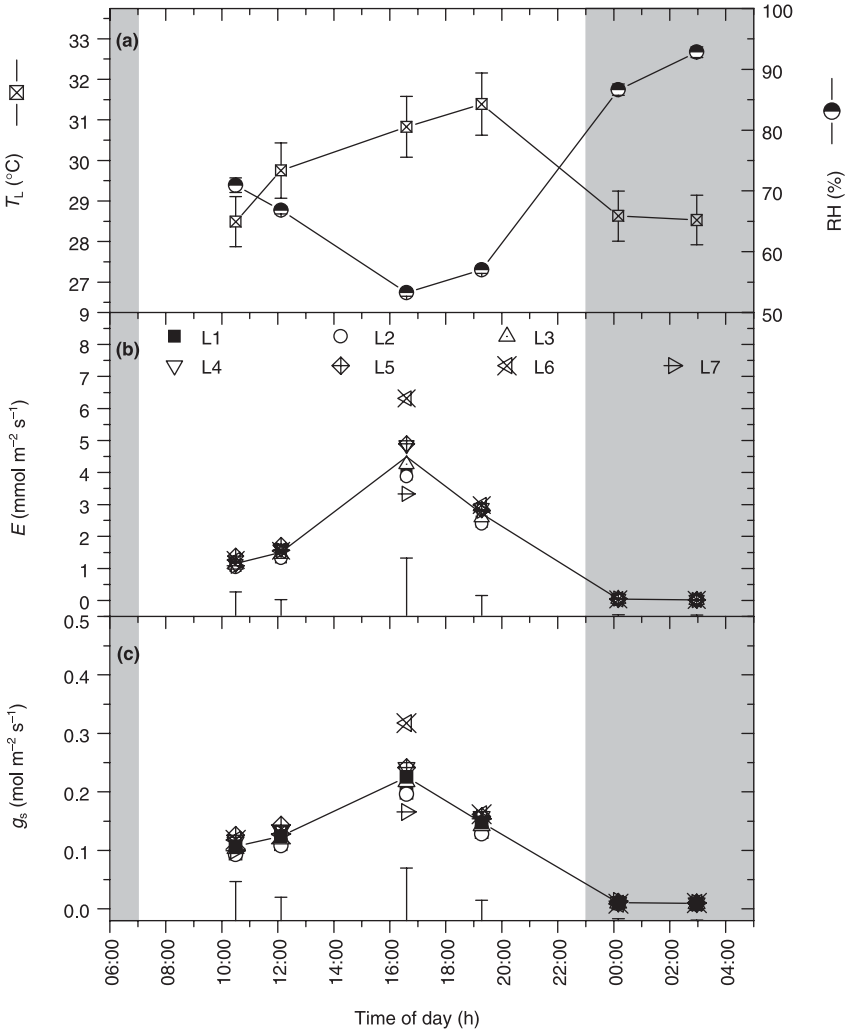

Fig. 2 (a) Mean leaf temperature and mean relative humidity (RH) in the air close to the lower leaf surface; (b) transpiration rates E and (c) stomatal conductance (g_s) of all seven leaves of the *Ricinus communis* plants examined during the diel course. Data shown in (b) and (c) are mean values of three to four plants. In addition, the average standard deviation for the mean values is given for each time-point. The bold lines in (b) and (c) refer to the leaf area weighted canopy mean values. The shaded areas denote the dark period. L1 to L7, leaf numbers as indicated in Fig. 1.

was a temporal trend comparable with $\Delta^{18}O_{total}$ of stem sections during the light period with a decrease in $\Delta^{18}O$ from morning to midday and a diel maximum in the afternoon/evening. In contrast to stem total organic matter, a strong decline in $\Delta^{18}O_{phloem\text{-}OM}$ was observed in the second part of the night. The maximum variation was up to 7‰. As in phloem organic matter there was no variation in $\Delta^{18}O$ of phloem sap water ($\Delta^{18}O_{pw}$) between sampling sites (Fig. 4c). Phloem sap water was slightly enriched compared with source water and showed a diel maximum at 19 : 00 h when calculated leaf water enrichment ($\Delta^{18}O_{Ln}$) was close to its maximum.

The $\Delta^{18}O_{xylem\text{-}OM}$ varied between approx. 34.5 and 42‰ and was thus within the range of leaf water soluble and phloem organic matter (Fig. 5). There was no significant difference among sampling positions. A diel maximum in the morning and lower values in the evening and during the night were observed for all positions collected.

Transfer of the isotopic signal from the leaf to the roots

The $\Delta^{18}O_{ws}$ of leaves explained 93% of the variation of $\Delta^{18}O$ of phloem organic matter during the whole diel course (Fig. 6). The phloem Δ equaled the leaf Δ at night and in the morning, but phloem organic matter was slightly depleted in ^{18}O from midday to evening (Fig. 6a). However, there was no significant correlation between leaf total and phloem organic matter (Fig. 6b). During the whole diel course phloem organic matter was enriched in ^{18}O compared with the bulk leaf fraction.

We assume that phloem sugar during the night represents mainly soluble sugars remobilized from starch. In order to estimate the extent to which this deviates from an integrative $\Delta^{18}O$ signature of the organic matter produced during daylight, we compared the average values from day and night (Table 2). Mean values for day $\Delta^{18}O$ were weighted for assimilation rate (Eqn 8), while for the night nonweighted averages were calculated.

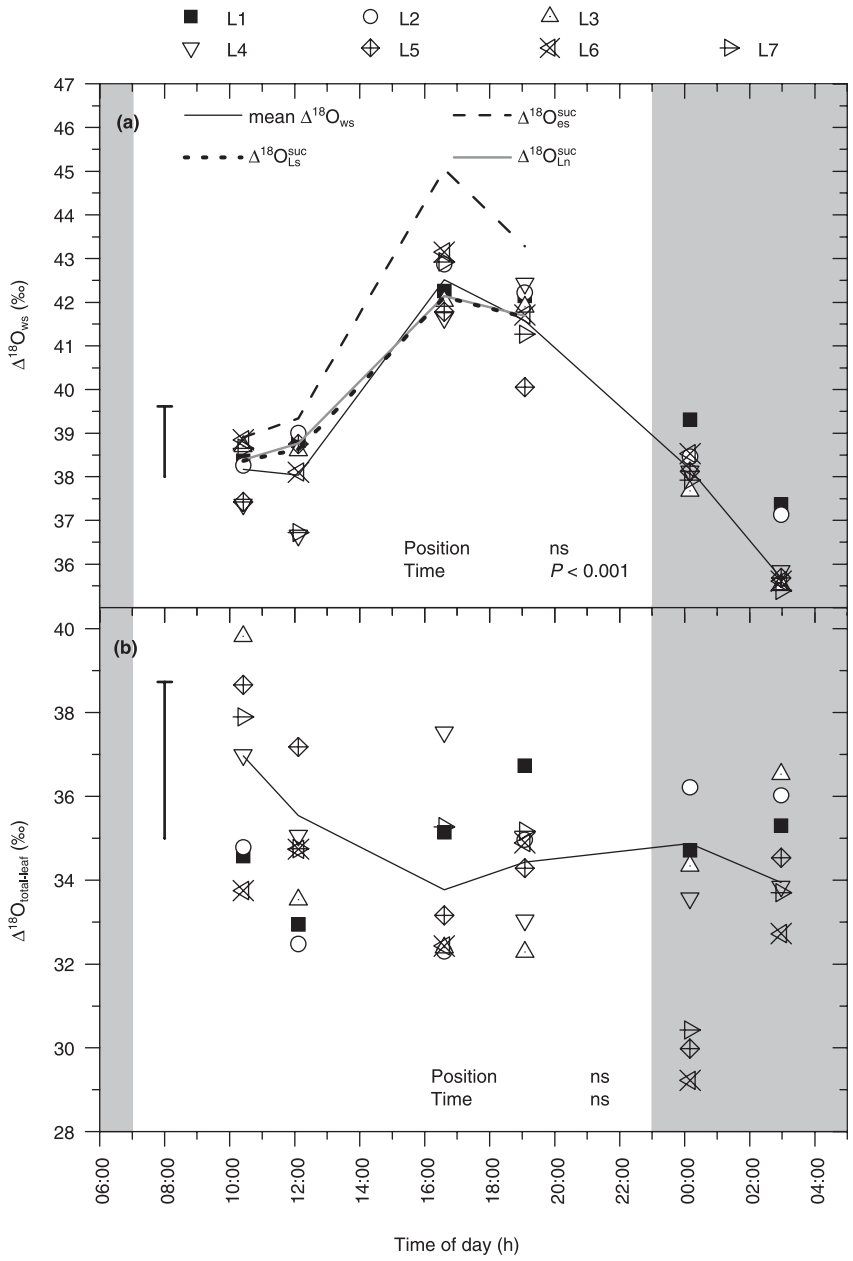

Fig. 3 (a) Predicted and observed $\Delta^{18}O$ in water-soluble organic matter and (b) observed $\Delta^{18}O$ total organic matter in leaves of *Ricinus communis* during the diel course. The symbols denote observed $\Delta^{18}O_{ws}$ (a) and $\Delta^{18}O_{total}$ (b) in the seven leaves L1 to L7. Data shown are mean values of three to four plants. In addition, the average standard error for the mean values is given. The effects of position (leaf number) and time as calculated with the GLM-ANOVA procedure are given. The thin black lines refer to canopy integrated mean values of observed $\Delta^{18}O_{ws}$ (a) and $\Delta^{18}O_{total-leaf}$ (b). Canopy integrated predicted ^{18}O enrichment of newly assimilated organic matter is indicated by the bold gray ($\Delta^{18}O^{suc}_{Ln}$), the bold black dotted ($\Delta^{18}O^{suc}_{Ls}$) and the bold black dashed line ($\Delta^{18}O^{suc}_{es}$). For calculating $\Delta^{18}O^{suc}_{Ln}$ we assumed nonsteady-state conditions and accounted for a Péclet effect; for $\Delta^{18}O^{suc}_{Ls}$ we assumed steady-state conditions and accounted for a Péclet effect and for $\Delta^{18}O^{suc}_{es}$ we assumed steady-state conditions and no Péclet effect.

During the day, measured leaf water soluble organic matter ($\Delta^{18}O_{ws}$) matched modeled mean daytime $\Delta^{18}O^{suc}_{Ln}$, whereas measured phloem organic matter ($\Delta^{18}O_{phloem-OM}$) was significantly less enriched (by 1‰) than $\Delta^{18}O_{ws}$. At night, however, phloem and leaf soluble organic matter were equally enriched. Mean diel (day and night) $\Delta^{18}O$ did not differ significantly between leaf soluble (38.9 ± 0.7‰) and phloem organic matter (38.2 ± 1.4‰). The $\Delta^{18}O_{ws}$ pool differed by 3.1‰ between day and night, while the phloem organic matter pool differed by 2.3‰ between day and night, both being greater during daylight. The enrichment of total leaf organic matter, $\Delta^{18}O_{total-leaf}$, did not differ between light and dark period and was relatively depleted in ^{18}O compared with the other pools of organic matter during the day and not significantly different from them during the night.

Total organic matter of the stem sections (S-A to S-F) was depleted in ^{18}O compared with phloem sugars collected from the same positions (p-A to p-F). The regression line (Fig. 7a) calculated with all data from all positions is characterized by the following linear equation:

$$\Delta^{18}O_{total-stem} = 0.605\Delta^{18}O_{phloem-OM} + 8.42\ [‰]$$

At an average $\Delta^{18}O_{phloem-OM}$ of 37‰, the difference between $\Delta^{18}O_{phloem-OM}$ and $\Delta^{18}O_{total-stem}$ was 6.2‰.

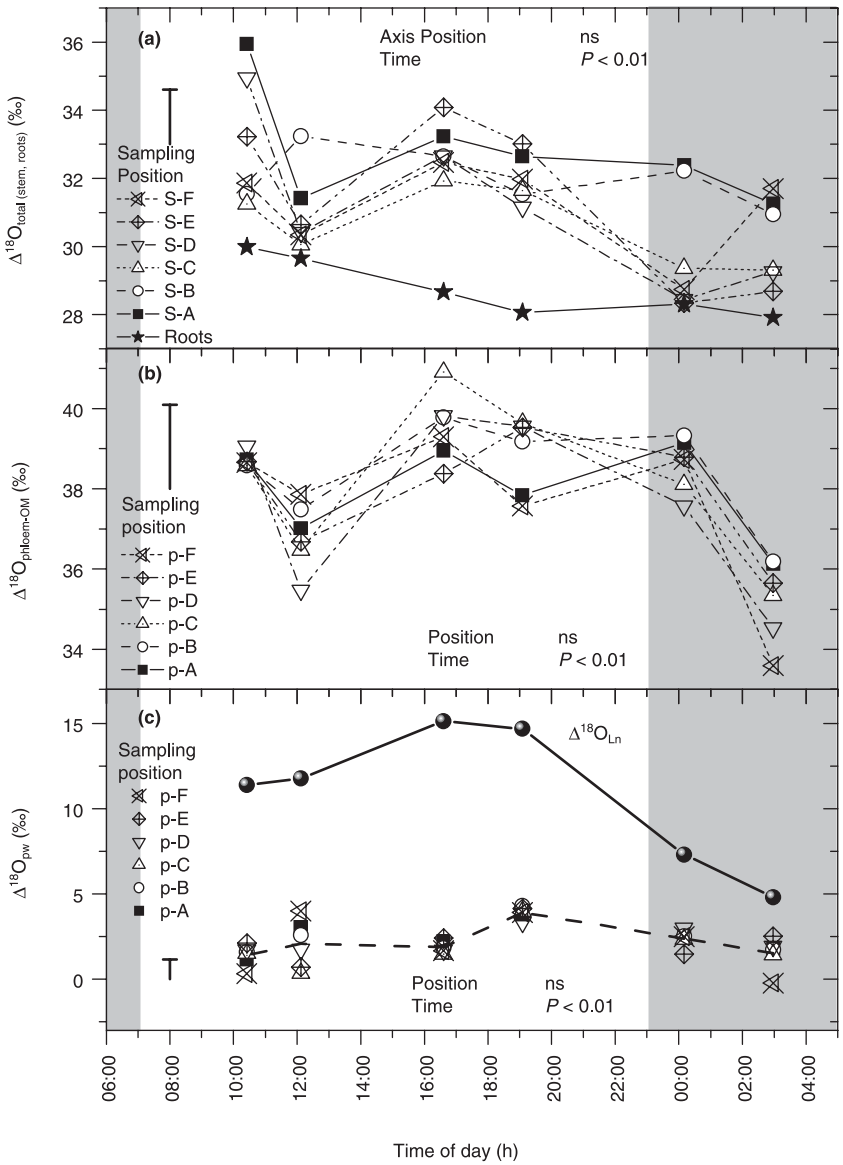

Fig. 4 $\Delta^{18}O$ in total organic matter of (a) stem sections S-A to S-F and fine roots; (b) phloem-transported organic matter and (c) $\Delta^{18}O$ in phloem sap water collected at stem positions p-A (base of the stem) to p-F (between leaves L4 and L5) (see Fig. 1) of *Ricinus communis* during the diel course. Data shown are mean values ($n = 3$–4). In addition, the average standard deviation for the mean values is given. Effects of position along the axis and of time (excluding the data for fine roots) on $\Delta^{18}O$, as calculated with the GLM-ANOVA procedure, are given. $\Delta^{18}O_{Ln}$ in (c): calculated and canopy-weighted mean lamina leaf water enrichment; the bold dashed line in (c) refers to the mean value of $\Delta^{18}O_{pw}$ from all sampling positions.

We observed that the fine roots were depleted compared with the assumed source of organic matter (phloem organic matter obtained from the lowermost sampling position (p-A)) (Fig. 7b). The depletion amounted to between 7.4 and 11.4‰. There was no significant correlation between $\Delta^{18}O_{phloem\text{-}OM}$ and $\Delta^{18}O$ of root organic matter during the diel course.

Discussion

In the present study we assessed the diel variation of water-soluble organic matter pools in the leaves and phloem of *R. communis* compared with the values calculated from nonsteady-state models of evaporative enrichment of leaf water and organic matter (Cernusak *et al.*, 2005; Farquhar & Cernusak, 2005). Previously, Barbour *et al.* (2000a) and Cernusak *et al.* (2003b) showed that $\Delta^{18}O$ of phloem organic matter collected from leaf petioles could be reasonably well explained by leaf water enrichment (either measured or calculated taking a Péclet effect into consideration). We calculated enrichment of newly produced assimilates applying a nonsteady-state evaporative enrichment model for leaf water and assuming ε_{wc} to be +27‰ ($\Delta^{18}O^{suc}_{Ln}$) and found good agreement from morning to afternoon between modeled values and measured $\Delta^{18}O$ in leaf soluble organic matter (Fig. 3a). A reasonably good agreement was also obtained when steady-state conditions were assumed modeling $\Delta^{18}O$ ($\Delta^{18}O^{suc}_{Ls}$ in Fig. 3a). We calculated $\Delta^{18}O^{suc}_{Ln}$ and $\Delta^{18}O^{suc}_{Ls}$ only for the light period when organic matter was newly assimilated. Comparable to our modeling of $\Delta^{18}O$ of leaf sugars, nonsteady-state and steady-state models for leaf water evaporative enrichment (which include the Péclet effect) both well describe measured lamina leaf water enrichment during

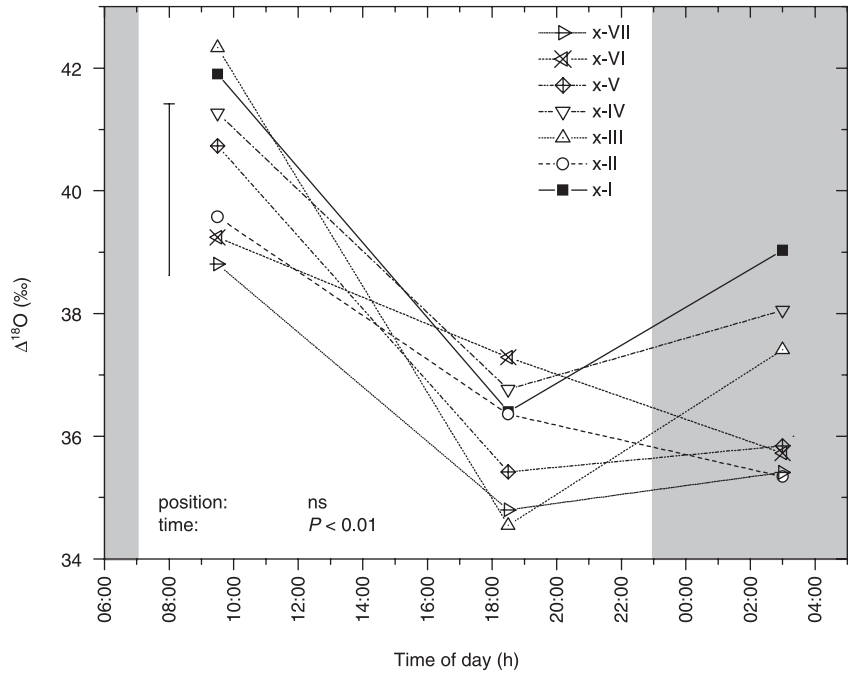

Fig. 5 $\Delta^{18}O$ in xylem organic matter of *Ricinus communis* collected at different positions (see Fig. 1) during the diel course. Data shown are mean values ($n = 3$–9). In addition, the average standard deviation for the mean values is given. Effects of position along the axis and time on $\Delta^{18}O$ as calculated with the GLM-ANOVA procedure are given.

Table 2 Comparison of modeled and measured $\Delta^{18}O$ in leaf and phloem organic matter during day and night

	Mean $\Delta^{18}O$ day	Mean $\Delta^{18}O$ night	
$\Delta^{18}O^{suc}_{Ln}$	40.6 ± 0.6 a		
$\Delta^{18}O_{ws}$	40.0 ± 0.5 a	36.9 ± 1.0 a	different at $P < 0.01$
$\Delta^{18}O_{total\text{-}leaf}$	35.0 ± 0.5 c	34.4 ± 2.5 a	not different
$\Delta^{18}O_{phloem\text{-}OM}$	39.0 ± 1.3 b	36.7 ± 1.1 a	different at $P < 0.05$

Mean $\Delta^{18}O$ of newly produced organic matter as calculated from Eqn 5 taking an enrichment of organic matter of 27‰ compared with water into account ($\Delta^{18}O^{suc}_{Ln}$) and of leaf water soluble ($\Delta^{18}O_{ws}$), leaf bulk ($\Delta^{18}O_{total\text{-}leaf}$) and phloem organic matter ($\Delta^{18}O_{phloem\text{-}OM}$) during day and night are shown. Day values were weighted for assimilation rate. Letters a–c indicate homogeneous groups when mean $\Delta^{18}O$ of different fraction during day or during night where compared.

the light period. Mainly during night the prediction of leaf water enrichment is improved by the application of nonsteady-state models (Cernusak *et al.*, 2005). The ^{18}O enrichment of leaf water-soluble organic matter was overestimated when no Péclet effect was taken into account ($\Delta^{18}O^{suc}_{es}$). The difference between $\Delta^{18}O^{suc}_{es}$ on the one hand and $\Delta^{18}O^{suc}_{Ln}$ and $\Delta^{18}O^{suc}_{Ls}$ on the other strongly exceeds the errors calculated for the models.

The soluble pool of organic matter appears to be in, or close to, isotopic equilibrium with lamina leaf water even though environmental and physiological conditions change during the day. However, we do not have data for the period between 12 : 00 h and 17 : 00 h, and it is likely that the peak in leaf water enrichment occurred within this period (i.e. before 17 : 00 h). With this in mind the data might suggest a slight lag, as the measured sugar enrichments are less than the modeled leaf water early in the day, and greater at 17 : 00 h. Assuming a sugar pool size of *c.* 60 mmol C m^{-2} (A. Gessler, unpublished), and an assimilation rate of *c.* 10 μmol m^{-2} s^{-1}, the time lag would be approx. 2 h. From midday to evening, when the most intensive change of environmental conditions occurred, $\Delta^{18}O$ of leaf soluble organic matter differed slightly from phloem sugars collected directly below the canopy whereas both values were comparable during night and in the morning (Fig. 6). Barbour *et al.* (2000a) showed that it took approx. 3.5 h for phloem sugars harvested at the petiole to reach an isotopic equilibrium. However the phloem equilibration time will exceed the equilibration time of the leaf pool, as it includes the time of transport from the leaf lamina to the petiole.

Thus, even though phloem transport velocity is *c.* 0.75 m h^{-1} in *R. communis* (Peuke *et al.* 2001), it is likely that phloem sugars isotopically lag the current environmental conditions, as previously observed for trees (Gessler *et al.*, 2004).

However, no obvious time-lag was observed among $\Delta^{18}O$ of phloem organic matter collected from different positions along the axis. In addition, there was no significant basipetal gradient in $\Delta^{18}O$ of phloem organic matter. There was, however, a diminution of the diel amplitude between leaves and phloem: the leaf soluble sugar enrichments increased by *c.* 4.5‰ from morning until afternoon and then diminished by *c.* 8‰ until 03 : 00 h, whereas the corresponding changes in the phloem organic matter were approx 3 and 4‰. Taking

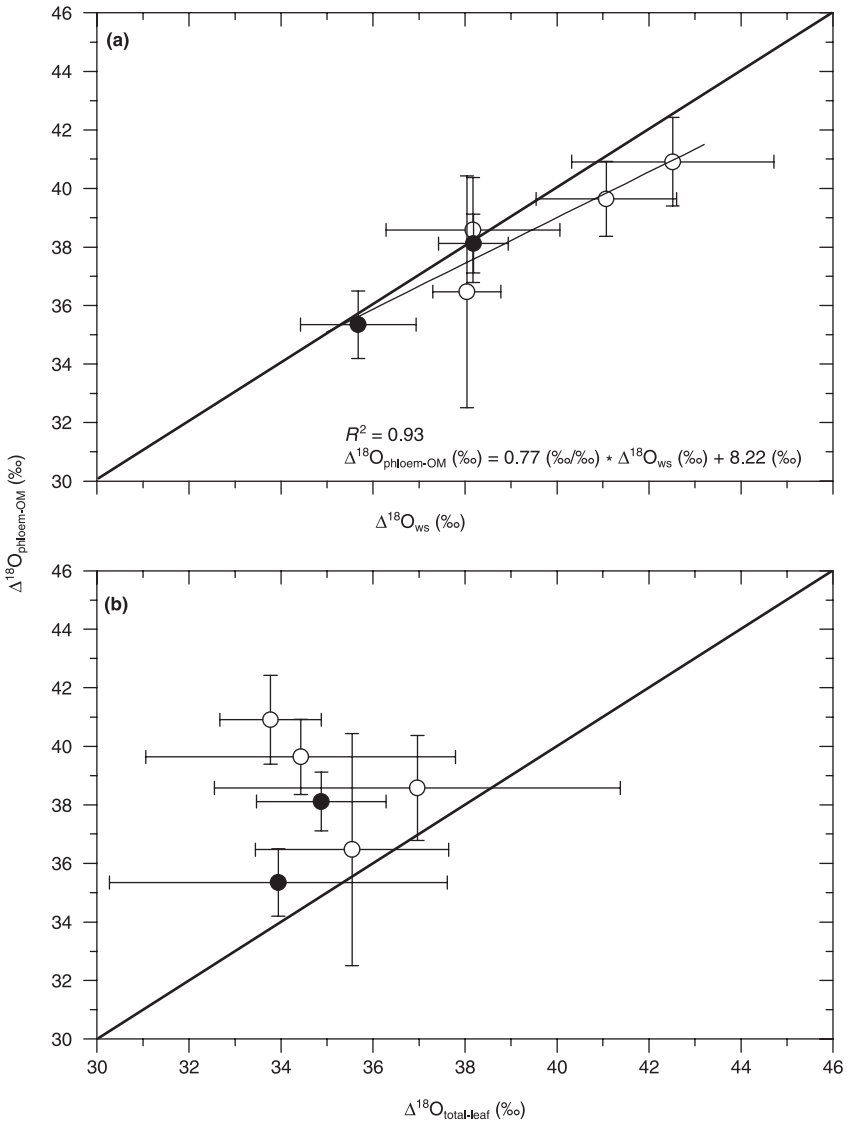

Fig. 6 Relation in *Ricinus communis* plants between $\Delta^{18}O$ of phloem transported organic matter sample (*y*-axis) directly below the canopy (Position p-C see. Fig. 1) and (a) leaf area weighted $\Delta^{18}O$ of water-soluble matter (*x*-axis) and of (b) total organic matter of leaves (*x*-axis). Data shown are means ± SD (n = 3–4). The closed circles are night values. Bold lines denote 1 : 1 lines. The thin black line in (a) is the linear regression line defined by the linear equation given.

into account that the average diel $\Delta^{18}O$ did not differ significantly between leaf soluble and phloem organic matter we can conclude that the isotopic signal imprinted on newly assimilated carbohydrates in leaves as integrated over the whole day is conserved in the carbohydrates during phloem transport. However, the diel dynamics are altered during phloem loading. The small but consistent reduction in mean daytime $\Delta^{18}O$ in phloem organic matter compared with the soluble organic matter fraction in the leaves (Table 2) might be caused by the different isotopic equilibrium times of the leaf and phloem pools. However, we cannot rule out the possibility that organic compounds newly assimilated in the green tissues in the stems of *R. communis* contribute partly to phloem organic matter. In the stems, reaction water is not or only slightly enriched and sugars fixed there should have a $\Delta^{18}O$ of approx. 27‰ (Cernusak *et al.*, 2005), which is well below the enrichment for carbohydrates assimilated in leaves.

The dynamic Münch mass flow model recently reviewed by van Bel (2003) proposes that while a proportion of the sucrose from sieve tubes is released during phloem transport, carbohydrates are also transported back into the sieve tubes in stem tissues (Minchin & Thorpe, 1987). According to our results the retrieved organic matter is either isotopically similar to the released sugars, or the amount of carbohydrates reloaded is too low to alter the $\Delta^{18}O$ of phloem organic matter significantly.

Mean daily assimilation-weighted $\Delta^{18}O^{suc}_{Ln}$ or foliar $\Delta^{18}O_{ws}$ should be representative of the oxygen isotope enrichment of transitory starch laid down during the day. Whereas during daylight triose phosphates are the main export forms leaving the chloroplast, starch breakdown during the night releases maltose as the major export compound (Weise *et al.*, 2004). Assuming that phosphatases break the O–P bond, the number of exchangeable oxygen atoms can be estimated (Sternberg

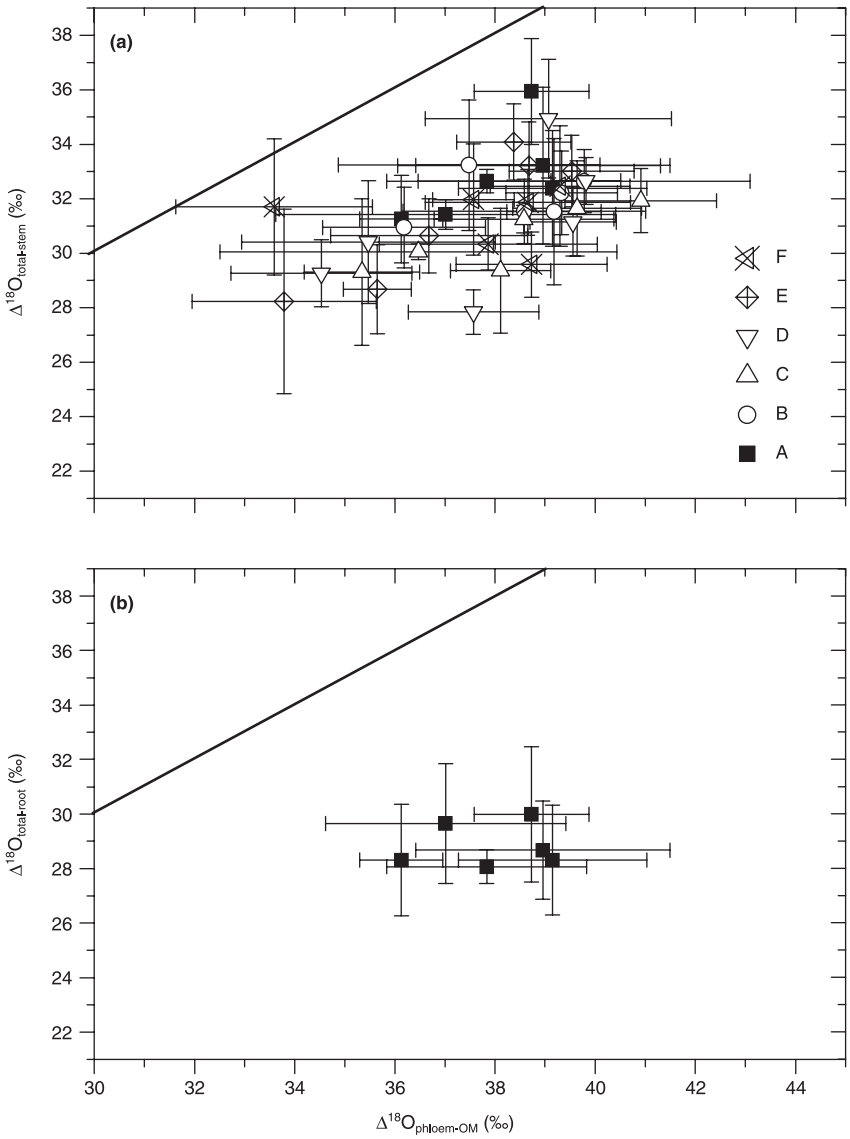

Fig. 7 Relation in *Ricinus communis* plants between $\Delta^{18}O$ of phloem organic matter and (a) total organic matter of stem sections along the axis (positions A–F) and (b) fine roots. Data shown are means ± SD (n = 3–4). The bold black lines denote the 1 : 1 line. In (b) phloem organic matter obtained from the lowermost sampling position (p-A in Fig. 1) was used for correlation.

et al., 1986). Thus, during starch hydrolysis one oxygen atom per hexose molecule produced can be exchanged with medium water (when we ignore the α1–6 glycosidic bonds). Assuming a rapid equilibrium between glucose–phosphate and fructose–phosphate (Farquhar *et al.*, 1998), which are the precursors for sucrose synthesis, two further oxygen atoms can be exchanged per hexose molecule. During the formation of the α1–2β glycosidic bond one equilibrated oxygen atom per molecule of sucrose is, however, lost. As a consequence, five out of 11 oxygen atoms in sucrose generated from starch are exchanged. Since calculated mean leaf water $\Delta^{18}O$ during the night was 6.1‰ (cf. Fig. 4) and mean daytime $\Delta^{18}O_{ws}$ was 40‰ (Table 2), $\Delta^{18}O$ of sucrose produced from hydrolysed starch is calculated to amount to *c.* 36.9‰ (i.e. $6/11 \times 40 + 5/11 \times (27 + 6.1)$), a value comparable to the measured ^{18}O enrichment in leaf water soluble (36.9‰) and phloem organic matter (36.7‰) (Table 2). In addition to sucrose, the phloem sap of *R. communis* also contains amino acids, organic acids and inorganic ions (Hall & Baker, 1972; Jeschke & Pate, 1991). However, sucrose makes up approx. 80–89% of phloem organic matter and Cernusak *et al.* (2003b) found no strong difference in the oxygen isotope enrichment between bulk phloem material and purified sucrose.

The ^{18}O enrichment of total stem organic matter was considerably lower than that of phloem organic matter. When cellulose and other structural compounds are produced from sugars unloaded from the phloem, exchange with unenriched reaction water of the stem occurs (Barbour & Farquhar, 2000). Cernusak *et al.* (2005) reviewed the literature for information on the proportion of oxygen atoms in sugar that exchange with water during cellulose synthesis (p_{ex}). They observed a high consistency for p_{ex} among a wide range of

species with a mean value of 0.42 when substrates for tissue synthesis were carbohydrates. Since the bulk organic material of the stem sections analysed here consists (apart from cellulose) of a mixture of various compounds, the portion of oxygen atoms exchanged may differ from 0.42.

Compared with phloem organic matter, but also with bulk stem material, total organic matter in roots is depleted in ^{18}O (Figs 4A and 7B). The mean daily value of enrichment is *c.* 28.7‰ and thus, not much greater than ε_{wc}. This might point to the fact that, on a daily average basis, part of the evaporative ^{18}O enrichment imprinted from the leaf water on the newly assimilated organic matter is lost in the roots which is also indicated by the small diel variation in $\Delta^{18}O$ of root total organic matter compared with the stem (Fig. 4a). One possible explanation for that observation is associated with CO_2 refixation in the roots by phosphoenolpyruvate carboxylase (PEPC). Badeck *et al.* 2005 observed CO_2 fixation by PEPC in roots of *Phaseolus vulgaris* to exceed net respiration rates. From these findings the authors estimated that PEPC can potentially refix CO_2 at rates of several 10% of the gross respiration rate in roots. As a consequence, we suggest that PEPC refixation may influence $\Delta^{18}O$ of organic matter in the below ground tissues. As assimilation of organic matter in the roots takes place within unenriched reaction water, and as the carboxyl oxygens of malate – the product of PEPC refixation – are less ^{18}O enriched than carbonyl oxygen (Schmidt *et al.*, 2001), we can explain the relatively low $\Delta^{18}O$ values of root organic matter.

Xylem organic matter is more strongly enriched in ^{18}O than is organic matter in the roots. Ranging between 42‰ and 35‰ during the diel course $\Delta^{18}O$ is much closer to phloem organic matter along the stem. We consequently conclude that the major part of xylem organic matter is not coming from the roots but originating from phloem-to-xylem exchange, thus representing current evaporative enrichment conditions in leaves. When comparing the diel patterns of $\Delta^{18}O$ in phloem and xylem organic matter a substantial time-lag becomes obvious. Whereas maximum $\Delta^{18}O$ values were observed in the afternoon for phloem organic matter, ^{18}O enrichment in xylem organic matter was highest during night and in the early morning. The time-lags might be related to the timing of xylem loading during the diel course. Future research on whole-plant cycling of organic matter will have to address the underlying causes in detail.

In conclusion, the ^{18}O isotopic enrichment of the water soluble organic matter pool in leaves during the course of a day can be reasonably well explained by current evaporative enrichment models (working hypothesis (1)). We also showed (working hypothesis (2)) that $\Delta^{18}O_{phloem\text{-}OM}$ during the night is influenced by leaf water enrichment during both day and night (i.e. during starch synthesis in the light and remobilization in the dark).

We also showed that ^{18}O enrichment of phloem organic matter reflected that of water-soluble leaf organic matter when averaged over the whole day; during the diel course, however, there was a diminution of the diel amplitude of the oxygen isotope enrichment in phloem as compared to leaf soluble organic matter. Once loaded into the phloem, the diel $\Delta^{18}O$ pattern of organic matter did not change during basipetal transport in the sieve tubes. We therefore conclude that the isotopic signal imprinted on newly assimilated organic matter in the leaf can be found in phloem organic matter when averaged over a diel cycle and (working hypothesis (3)) that phloem sugars generally conserve the oxygen isotope signal during transport to the stem base. These findings are of central importance for studies which assess $\Delta^{18}O$ in phloem organic matter in the field for characterizing the water balance of plants as affected by environmental conditions (e.g. Cernusak *et al.*, 2003a; Keitel *et al.*, 2006). When calculating or modeling transpiration (Cernusak *et al.*, 2003a) and stomatal conductance (Brandes *et al.*, 2007) from $\Delta^{18}O$ in phloem organic matter it is normally assumed that no postphotosynthetic exchange of organic oxygen during phloem loading or transport occurs. We showed here that this assumption is justified when averaging $\Delta^{18}O$ over the whole day. However, diel variations influenced by night-time starch remobilization may have to be taken more strongly into account when applying $\Delta^{18}O$ approaches in the field. There is a strong need to verify our observation in other species, especially trees, under field conditions.

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References

Badeck FW, Tcherkez G, Nogues S, Piel C, Ghashghaie J. 2005. Post-photo synthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon. *Rapid Communications in Mass Spectrometry* **19**: 1381–1391.

Barbour MM, Farquhar GD. 2000. Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant Cell and Environment* **23**: 473–485.

Barbour MM, Fischer RA, Sayre KD, Farquhar GD. 2000a. Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Australian Journal of Plant Physiology* **27**: 625–637.

Barbour MM, Schurr U, Henry BK, Wong SC, Farquhar GD. 2000b. Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean. Evidence in support of the Peclet effect. *Plant Physiology* **123**: 671–679.

Barbour MM, Andrews TJ, Farquhar GD. 2001. Correlations between oxygen isotope ratios of wood constituents of *Quercus* and *Pinus* samples from around the world. *Australian Journal of Plant Physiology* **28**: 335–348.

Barbour MM, Walcroft AS, Farquhar GD. 2002. Seasonal variation in delta C-13 and delta O-18 of cellulose from growth rings of *Pinus radiata*. *Plant, Cell & Environment* **25**: 1483–1499.

Barbour MM, Roden JS, Farquhar GD, Ehleringer JR. 2004. Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Peclet effect. *Oecologia* **138**: 426–435.

van Bel AJE. 2003. The phloem, a miracle of ingenuity. *Plant, Cell & Environment* **26**: 125–149.

Bottinga Y, Craig H. 1969. Oxygen isotope fractionation between CO_2 and water, and the isotopic composition of marine atmospheric CO_2. *Earth and Planetary Science Letters* **5**: 285–295.

Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams MA, Gessler A. 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris* – effects of photosynthetic and post-photosynthetic carbon isotope fractionation. *Global Change Biology* **12**: 1922–1939.

Brandes E, Wenninger J, Koeniger P, Schindler D, Rennenberg H, Leibundgut C, Mayer H, Gessler A. 2007. Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. *Plant, Cell & Environment* **30**: 113–127.

Cappa CD, Hendricks MB, DePaolo DJ, Cohen RC. 2003. Isotopic fractionation of water during evaporation. *Journal of Geophysical Research-Atmospheres* **108**: 4525. doi: 10.1029/2003JD003597.

Cernusak LA, Pate JS, Farquhar GD. 2002. Diurnal variation in the stable isotope composition of water and dry matter in fruiting *Lupinus angustifolius* under field conditions. *Plant Cell and Environment* **25**: 893–907.

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

Cernusak LA, Wong SC, Farquhar GD. 2003b. Oxygen isotope composition of phloem sap in relation to leaf water in Ricinus communis. *Functional Plant Biology* **30**: 1059–1070.

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

Dongmann G, Nürnberg HW, Förstel H, Wagener K. 1974. On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. *Radiation and Environmental Biophysics* **11**: 41–52.

Farquhar GD, Cernusak LA. 2005. On the isotopic composition of leaf water in the non-steady state. *Functional Plant Biology* **32**: 293–303.

Farquhar GD, Gan KS. 2003. On the progressive enrichment of the oxygen isotopic composition of water along a leaf (Reprinted from *Plant, Cell & Environment*, **26**: 801–819, 2003). *Plant Cell & Environment* **26**: 1579–1597.

Farquhar GD, Lloyd J. 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer JR, Hall AE, Farquhar GD, eds. *Stable isotopes and plant carbon-water relations*. San Diego, CA, USA: Academic Press, 47–70.

Farquhar GD, Hubick KT, Condon AG, Richards RA. 1989. Carbon isotope discrimination and water-use efficiency. In: Rundel PW, Ehleringer JR, Nagy KA, eds. *Stable isotopes in ecological research*. New York, NY, USA: Springer, 21–46.

Farquhar GD, Barbour MM, Henry BK. 1998. Interpretation of oxygen isotope composition of leaf material. In: Griffiths H, ed. *Stable isotopes – integration of biology, ecological and geochemical processes*. Oxford, UK: Bios Scientific Publishers Ltd, 27–74.

Fehri A, Letolle R. 1977. Transpiration and evaporation as the principal factors in oxygen isotope variations of organic matter in land plants. *Physiologie Vegetale* **15**: 363–370.

Gan KS, Wong SC, Yong JWH, Farquhar GD. 2002. O-18 spatial patterns of vein xylem water, leaf water, and dry matter in cotton leaves. *Plant Physiology* **130**: 1008–1021.

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 Biology* **6**: 721–729.

Hall SM, Baker DA. 1972. chemical composition of *Ricinus* phloem exudate. *Planta* **106**: 131–&;.

Jäggi M, Saurer M, Fuhrer J, Siegwolf R. 2003. Seasonality of delta^{18}O in needles and wood of *Picea abies*. *New Phytologist* **158**: 51–59.

Jeschke WD, Pate JS. 1991. Modeling of the partitioning, assimilation and storage of nitrate within root and shoot organs of castor bean (R*icinus communis* L.). *Journal of Experimental Botany* **42**: 1091–1103.

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 & Environment* **26**: 1157–1168.

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

Minchin PEH, Thorpe MR. 1987. Measurement of unloading and reloading of photo-assimilate within the stem of bean. *Journal of Experimental Botany* **38**: 211–220.

Peuke AD. 2000. The chemical composition of xylem sap in *Vitis vinifera* L. cv. riesling during vegetative growth on three different franconian vineyard soils and as influenced by nitrogen fertilizer. *American Journal of Enology and Viticulture* **51**: 329–339.

Peuke AD, Rokitta M, Zimmermann U, Schreiber L, Haase A. 2001. Simultaneous measurement of water flow velocity and solute transport in xylem and phloem of adult plants of *Ricinus communis* over a daily time course by nuclear magnetic resonance spectrometry. *Plant, Cell & Environment* **24**: 491–503.

Roden JS, Ehleringer JR. 1999. Hydrogen and oxygen isotope ratios of tree-ring cellulose for riparian trees grown long-term under hydroponically controlled environments. *Oecologia* **121**: 467–477.

Roden JS, Lin GG, Ehleringer JR. 2000. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochimica et Cosmochimica Acta* **64**: 21–35.

Rogers LA, Dubos C, Cullis IF, Surman C, Poole M, Willment J, Mansfield SD, Campbell MM. 2005. Light, the circadian clock and sugar perception in the control of lignin biosynthesis. *Journal of Experimental Botany* **56**: 1651–1663.

Saurer M, Aellen K, Siegwolf R. 1997. Correlating delta C-13 and delta O-18 in cellulose of trees. *Plant, Cell & Environment* **20**: 1543–1550.

Scheidegger Y, Saurer M, Bahn M, Siegwolf R. 2000. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* **125**: 350–357.

Schmidt HL, Werner RA, Rossmann A. 2001. O-18 pattern and biosynthesis of natural plant products. *Phytochemistry* **58**: 9–32.

Smith AM, Zeeman SC, Thorneycroft D, Smith SM. 2003. Starch mobilization in leaves. *Journal of Experimental Botany* **54**: 577–583.

Sternberg L, Deniro MJ. 1983. Bio-geochemical implications of the isotopic equilibrium fractionation factor between oxygen atoms of acetone and water. *Geochimica et Cosmochimica Acta* **47**: 2271–2274.

Sternberg LDL, Deniro MJ, Savidge RA. 1986. Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. *Plant Physiology* **82**: 423–427.

Wang XF, Yakir D. 1995. Temporal and spatial variations in the oxygen-18 content of leaf water in different plant species. *Plant Cell & Environment* **18**: 1377–1385.

Weise SE, Weber APM, Sharkey TD. 2004. Maltose is the major form of carbon exported from the chloroplast at night. *Planta* **218**: 474–482.

Yakir D, Deniro MJ. 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. *Plant Physiology* **93**: 325–332.