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Evaporative enrichment and time lags between $\delta^{18}O$ of leaf water and organic pools in a pine stand

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ABSTRACT

Understanding ecosystem water fluxes has gained increasing attention, as climate scenarios predict a drier environment for many parts of the world. Evaporative enrichment of ^{18}O ($\Delta^{18}O$) of leaf water and subsequent enrichment of plant organic matter can be used to characterize environmental and physiological factors that control evaporation, based on a recently established mechanistic model. In a *Pinus sylvestris* forest, we measured the dynamics of oxygen isotopic composition ($\delta^{18}O$) every 6 h for 4 d in atmospheric water vapour, xylem sap, leaf water and water-soluble organic matter in current (N) and previous year (N-1) needles, phloem sap, together with leaf gas exchange for pooled N and N-1 needles, and relevant micrometeorological variables. Leaf water $\delta^{18}O$ showed strong diel periodicity, while $\delta^{18}O$ in atmospheric water vapour and in xylem sap showed little variation. The $\Delta^{18}O$ was consistently lower for N than for N-1 needles, possibly related to phenological stage. Modelled leaf water $\Delta^{18}O$ showed good agreement with measured values when applying a non-steady state evaporative enrichment model including a Péclet effect. We determined the time lags between $\delta^{18}O$ signals from leaf water to water-soluble foliar organic matter and to phloem sap at different locations down the trunk, which clearly demonstrated the relevance of considering these time-lag effects for carbon transport, source-sink and carbon flux partitioning studies.

Key-words: *Pinus sylvestris*; oxygen isotopes; Péclet effect; phloem sap; temporal variation.

INTRODUCTION

Climate scenarios for the close future draw a picture of a warmer, drier and more variable environment for vegetation in many parts of the world (IPCC 2001). Understanding canopy, stand and ecosystem water fluxes has therefore recently gained increasing attention, well supported by the development of stable isotope tools (Yakir & Sternberg 2000; Dawson *et al.* 2002). The $^{18}O/^{16}O$ stable isotope ratio of oxygen ($\delta^{18}O$) in leaf water reflects that of source water and of leaf evaporative conditions (Dongmann *et al.* 1974; Roden & Ehleringer 1999; Barbour *et al.* 2004). During transpiration, the water isotopologues containing the lighter ^{16}O isotope diffuse faster than the heavier ones, thereby enriching the water in ^{18}O at the sites of evaporation. The extent of this evaporative enrichment is governed by the bidirectional exchange of water vapour between the leaf and its surrounding air, and is therefore affected by the vapour pressure deficit of the air (Craig & Gordon 1965).

Water in the mesophyll cells is influenced by the evaporative enrichment, because it consists of a combination of unenriched xylem water and water from the sites of evaporation (Farquhar & Lloyd 1993). The oxygen isotope signal of mesophyll cell water is imprinted on the assimilates formed inside the cell (Barbour *et al.* 2000). Because $\delta^{18}O$ in organic matter is assumed to weight the oxygen isotopic signature of leaf water by assimilation rate (Cernusak, Farquhar & Pate 2005), it provides a valuable integrative tool to study the evaporative and photosynthetic processes that drive or are associated with plant water fluxes (Yakir & Wang 1996; Riley *et al.* 2002; Bowling *et al.* 2003).

Based on the many contributions since the study of Craig & Gordon (1965; e.g. Dongmann *et al.* 1974; Farquhar & Lloyd 1993; Farquhar & Gan 2003), a general empirical model describing the evaporative enrichment of ^{18}O ($\Delta^{18}O$) of leaf water and subsequent enrichment of plant organic material relative to source water has recently been established (Farquhar & Cernusak 2005). It includes ^{18}O fractionation associated with phase transition from liquid water to vapour, diffusion of $H_2{}^{18}O$ through the boundary layer and stomata, isotopic heterogeneity of water in the leaf (due to co-occuring diffusion of ^{18}O-enriched water away from the sites of evaporation and convective transpiration stream of unenriched xylem water to the evaporative sites) and production of organic matter. It is a non-steady state model in which leaf isotopic enrichment does not adjust instantly to environmental conditions. Under controlled conditions, such a model satisfactorily described the observed

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variations in leaf water enrichment (Cernusak *et al.* 2003a; Gan *et al.* 2003). Moreover, Barbour *et al.* (2000) were able to estimate the time for sucrose exported from the leaf to reach isotopic equilibrium with leaf water.

However, the environmental and physiological controls over leaf water $\Delta^{18}O$ (Cernusak *et al.* 2005; Lai *et al.* 2006; Seibt *et al.* 2006), and the subsequent ^{18}O signal in plant organic matter (Cernusak *et al.* 2005; Keitel *et al.* 2006) have been studied in only a few *in situ* experiments. Information on ^{18}O exchange after photosynthesis is scarce, for example, little is known about the isotopic exchange during the transport of organic compounds from the leaves to the trunk. In general, no direct exchange between sucrose (which is the main carbohydrate transport form in *Pinus sylvestris*; Hansen & Beck 1994) and phloem water is expected (Cernusak, Wong & Farquhar 2003b), because the sucrose molecule contains no carbonyl bonds. Still, carbohydrate transport in the sieve tubes is highly dynamic: the unloading and retrieval of sugars can occur simultaneously in heterotrophic tissues (van Bel 2003). The sugars retrieved in the phloem may therefore modify the oxygen isotopic signal of the phloem sap, depending on their metabolic history.

Detailed mechanistic studies under field conditions are crucial to properly interpret and make use of the oxygen isotopic information in organic pools with rapid turnover (e.g. phloem sap: Keitel *et al.* 2003) or laid down in tissues that provide useful information as isotopic archives (e.g. tree rings: Saurer, Aellen & Siegwolf 1997).

During an intensive field campaign in a Scots pine forest, we measured the dynamics of $\delta^{18}O$ in atmospheric water vapour, xylem sap and leaf water, as well as in different organic matter pools (bulk leaf, leaf water-soluble organic matter and phloem sap), every 6 h during 4 d. In addition, leaf gas exchange and relevant micrometeorological variables within and above the canopy were measured. The objectives of this study are: (1) to assess the environmental and physiological controls over leaf water evaporative enrichment under field conditions; and (2) to identify a potential time lag between the ^{18}O signal of leaf water and of organic matter pools in the trees. We hypothesize that the oxygen isotope composition of leaf water will be imprinted in the newly produced organic matter with time lags related to the turnover of soluble organic matter in the leaf and to phloem transport velocity.

MATERIALS AND METHODS

Study site and experimental setup

This study was conducted at the forest meteorological research site Hartheim of the Meteorological Institute of Freiburg, a 38-year-old pine plantation in the southern upper Rhine valley, Germany (47°56′N, 7°36′E, elevation 201 m). The forest was mostly planted with Scots pine (*P. sylvestris* L.), with only a few patches of Austrian pine (*Pinus nigra* L.). All measurements were made in *P. sylvestris* plots. Most of the tree foliage was between 11 m above ground level and the top of the canopy at ca. 15 m. Plant area index (PAI) was 1.9 m^2m^{-2}, owing to recent thinning (Schindler, Türk & Mayer 2006). A detailed description of the experimental site and its management was given by Mayer *et al.* (2000) and Schindler *et al.* (2006).

The measurement campaign took place from 6 June 2005, 1200 h (all times are expressed as Central European time, with a 0 to 24 h notation) to 10 June 2005, 0600 h. Samples and measurements were taken every 6 h throughout this period, at 1200, 1800, 2400 and 0600 h. Three adjacent dominant or co-dominant individuals of *P. sylvestris* were selected within reach of a truck-mounted 25 m hydraulic lift, and were used as replicates for tree-based measurements.

Micrometeorological data

Meteorological data [air temperature, relative humidity, photosynthetically active radiation, precipitation (PAR) and wind speed] were determined continuously at a measurement tower. Air temperature, humidity and vapour pressure deficit (VPD) were determined using a psychrometer according to Frankenberger (Mayer & Gietl 1976) at 2, 12 and 19 m height. Wind speed was measured with cup anemometers at 2, 6, 12, 15 and 19 m height. PAR was determined with a Li-190SZ sensor (Li-Cor, Inc., Lincoln, NE, USA) at 16 m; above-canopy precipitation was measured at 29 m. Volumetric soil moisture (TDR probes) was determined at 30 cm depth, and soil temperature (thermocouples) was measured at 3 cm depth. Data were recorded every 30 s and averaged over 30 min periods.

Collection of plant material

At each measurement time, twigs were sampled from the sunlit upper third of the canopy and were used for collection of needles, xylem sap and twig phloem. Needles were separated into current growth season (N needles) and previous growth season (N-1) needles, and were used for the extraction of needle water and needle water-soluble organic matter. In addition, trunk bark samples were taken from three heights below the live crown: high-, mid- and low-stem, at 10.0, 6.0 and 1.5 m from the ground, respectively, and were used for phloem collection. Bark samples (ca. 150 mg) were taken from the twig bark with a scalpel, and from the trunk bark with a core borer (13 mm diameter).

Brandes *et al.* (2006) observed no intracanopy gradient of $\delta^{13}C$ at the same stand, and found a strong and significant correlation between canopy-integrated stomatal conductance and leaf-level stomatal conductance that was determined in the upper part of the canopy. We conclude that the twigs sampled here adequately represent the environmental conditions of the canopy, as a prerequisite for comparing leaf level measurements, such as leaf $\delta^{18}O$, with parameters that integrate a canopy signal, such as phloem $\delta^{18}O$.

Leaf, xylem and atmospheric water

N and N-1 needles were transferred in glass tubes and immediately frozen in liquid N_2. Bulk leaf water was

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extracted from the needles by cryogenic vacuum distillation: the frozen tubes containing the needles were placed in a 80 °C water bath, connected to a vacuum system (ca. 4.10^{-2} mbar) including water traps that were cooled with liquid N_2. The water was then transferred into 2 mL vials and kept frozen until $\delta^{18}O$ analysis (see further discussion). The dried needles were ground and analysed for bulk $\delta^{13}C$ (see further discussion).

Xylem sap samples were taken according to the method presented in Keitel *et al.* (2006). One centimetre of bark was removed from the cut end of a twig. A polyethylene tube was then fitted onto the shoot at one end, and equipped with a hypodermic needle at the other. The needle was inserted in a 2 mL vial with an airtight seal, together with another needle that was connected to a hand vacuum pump. A gentle vacuum was applied, while the needles were subsequently cut off the end of the twig to facilitate xylem sap collection. The xylem sap sampled in the vials was immediately frozen in liquid nitrogen and stored at –20 °C until analysis (see further discussion).

Atmospheric water vapour was collected by cryogenic condensation. Air was pumped at 35 L h^{-1} for 2 h (centred on target measurement time) from four locations at 13 m height (mid-canopy) through a trap filled with ethanol and liquid N_2 (ca. –70 °C). The collected water was immediately transferred into 2 mL vials and kept frozen until $\delta^{18}O$ analysis (see further discussion).

Organic matter in leaf and phloem

Bark samples were washed with demineralized water immediately after collection and placed in 6 mL vials containing 2 mL of demineralized water. The samples were left to exude for 5 h as described by Rennenberg, Schneider & Weber (1996). This method was identified as the most suitable technique for assessing $\delta^{18}O$ in phloem sap (Gessler, Rennenberg & Keitel 2004). Sucrose is expected to account for more than 90% of phloem sap in trees (e.g. Pate *et al.* 1998). No direct exchange between phloem-transported sucrose and phloem water or water from the exudation solution will occur, because the sucrose molecule contains no carbonyl bounds. We therefore assume no change in the $\delta^{18}O$ of phloem sap as a consequence of the exudation procedure. Contamination of phloem exudates with cellular constituents was shown to be negligible under the experimental conditions applied (Schneider *et al.* 1996). A volume of 75 to 100 μL of phloem exudation solution was transferred in silver capsules (IVA Analysentechnik; Meerbusch, Germany), and water was evaporated at 60 °C in an oven before isotope analysis (see later discussion). We tested the issue of sealing the dried phloem samples under argon immediately after removing them from the drying oven on a separate set of samples, as addressed by Cernusak *et al.* (2003a). We indeed found that the $\delta^{18}O$ in phloem sap tended to be lower if the samples were not sealed under argon immediately, both sealed and unsealed data sets being well linearly correlated ($r = 0.980$, $P < 0.0001$, $n = 24$). We applied the following correction to our unsealed samples: $\delta^{18}O_{corrected} = 1.1 \times \delta^{18}O_{unsealed} - 2.9$.

The N and N-1 needles, frozen in liquid N_2 immediately after harvest, were microwaved to stop physiological activity and then freeze-dried. The samples were homogenized with mortar and pestle in liquid N_2. Water-soluble organic matter was extracted as follows: 50 mg of homogenized sample were incubated for 60 min at 5 °C in 1 mL demineralized water, heated at 100 °C for 1 min to precipitate proteins. The samples were cooled on ice and then centrifuged (12 000 g at 5 °C for 5 min). The 75 to 100 μL of supernatant was transferred in silver capsules, and the water was evaporated at 60 °C in an oven before isotope analysis (see later discussion).

Mass spectrometry measurements

The determination of $\delta^{18}O$ in xylem, bulk needle and atmospheric water vapour samples was established according to Gehre *et al.* (2004), using a TC/EA (high temperature conversion/elemental analyser, ThermoFinnigan, Bremen, Germany) coupled with a Delta^{Plus} XP mass spectrometer via a ConFlo III interface (Werner, Bruch & Brand 1999). The precision was < 0.15‰. In bulk needle organic matter and needle water-soluble organic matter, $\delta^{18}O$ was determined as follows: 75 to 100 μL phloem exudation solution or of water-soluble organic matter extracted from needles was transferred in silver capsules (IVA Analysentechnik) and water was evaporated at 60 °C in an oven. For bulk leaf material, 0.5 mg of homogenized dried sample was transferred in silver capsules. The samples, which contained ca. 300 μg organic O on average, were combusted in a TC/EA coupled to an isotope ratio mass spectrometer (Delta^{Plus} XL; Finnigan MAT GmbH, Bremen, Germany). The $^{18}O/^{16}O$ oxygen stable isotope ratio ($R = {}^{18}O/{}^{16}O$) is expressed using small delta notation in parts per thousand, relative to the international Vienna Standard Mean Ocean Water (VSMOW) standard, as $\delta^{18}O = \frac{R_{sample}}{R_{standard}} - 1$, where R_{sample} and $R_{standard}$ refer to the isotope ratio of the substance of interest and of the standard, respectively.

Leaf gas exchange

Leaf gas exchange was measured in the upper canopy by inserting small twigs (with their needles attached) into a conifer leaf chamber connected to a portable gas exchange system (GFS3000; Heinz Walz GmbH, Effeltrich, Germany). The measurements were conducted under ambient light and temperature conditions. Twigs with both N and N-1 needles attached were placed in the chamber, with N-1 needles representing on average ca. 65% of the total needle area in the chamber. Net CO_2 and H_2O exchange rates were measured, and stomatal conductance (g_s) was subsequently calculated according to von Caemmerer & Farquhar (1981). Leaf water content was measured for both N and N-1 needles.

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Separate values of g_s were calculated for N and N-1 needles, based on (1) the overall g_s of N and N-1 needles that were inserted in the gas exchange chamber together; (2) a summertime value of 0.53 for the ratio of g_s of N-1 needles to g_s of N needles, based on the measurements of Beadle *et al.* (1985) on upper-canopy needles of *P. sylvestris*; and (3) the relative leaf area of each type of needle in the chamber. Separate transpiration rates were calculated for both needle cohorts, based on these g_s values, and assuming comparable relative humidity (RH) of ambient air and comparable leaf temperature for N and N-1 needles (g_s should not influence leaf temperature or internal water vapour pressure, because pine needles are assumed to be strongly coupled with the environment; Barbour, Walcroft & Farquhar 2002). According to Beadle *et al.* (1985), we used a summertime value of 0.61 for the ratio of assimilation rate of N-1 needles to that of N needles.

In order to test our assumptions for the ratios of transpiration rate, assimilation rate and stomatal conductance between N-1 and N needles, we performed additional experiments with approximately 4-year-old saplings collected at the field site. Saplings were collected from the site during winter and grown in a greenhouse in the natural soil from the stand. Approximately 3 months after bud break, we performed gas exchange measurements ($n = 3$ trees; 3 twigs per tree) on N and N-1 needles separately, at midday (light intensity of approximately 500 μmol m^{-2} s^{-1}, air temperature of 24 °C). We observed the following mean ratios for gas exchange parameters of N-1 to N needles: stomatal conductance, 0.51; transpiration, 0.55; and photosynthesis, 0.64. These values are comparable with the ratios given by Beadle *et al.* (1985), which we used for calculating stomatal conductance, transpiration and photosynthesis of the two different needle cohorts.

The projected area of the N and N-1 needles that were inserted into the chamber was determined with a leaf area meter (ΔT Devices, Cambridge, UK). Mean needle length was determined from a subsample of the needles. Three-dimensional leaf area was calculated assuming the needle has the shape of a half cylinder, and was used as a basis for the gas exchange values (Luoma 1997; Haberer 2002).

Leaf water $\Delta^{18}O$

The oxygen isotope ratio of oxygen in the substance of interest can be expressed as enrichement above source water, using an upper case delta notation ($\Delta^{18}O$) in parts per thousand:

$$\Delta^{18}O = \frac{R_{sample}}{R_{source}} - 1 \quad (1)$$

In the present study, xylem water was considered to be source water.

The measured enrichment of bulk needle leaf water above source water is denoted $\Delta^{18}O_B$. According to Farquhar & Gan (2003), the ^{18}O enrichment of bulk leaf water is given by

$$\Delta^{18}O_B = \varphi_x \cdot \Delta^{18}O_x + \varphi_v \cdot \Delta^{18}O_v + \varphi_L \cdot \Delta^{18}O_L \quad (2)$$

where φ_x, φ_v and φ_L are the proportions of total water associated with the longitudinal xylem, the veinlets and the lamina mesophyll, respectively, and $\Delta^{18}O_x$, $\Delta^{18}O_v$ and $\Delta^{18}O_L$ denote the evaporative enrichment of xylem, veinlet and lamina leaf water above source water, respectively. Because the models applied here (which include the Péclet effect) calculate lamina leaf water enrichment, and because lamina leaf water is also the reaction water in which assimilates are produced, it is necessary to estimate $\Delta^{18}O_L$ from measured $\Delta^{18}O_B$. According to Farquhar & Gan (2003), we assumed the water volume in the veinlets to be negligible and proposed the following procedure to estimate φ_x. In their careful and extensive study on Scots pine needle anatomy, Lin, Jach & Ceulemans (2001) showed that the contribution of xylem to the cross-sectional area of current year needles (sampled in October) was 2.2%. We prepared cross-sectional cuttings of N and N-1 needles and compared the contribution of xylem area to the cross-sectional area by visual inspection under the microscope (50-fold magnification). There was no obvious difference in the relative xylem area between the two needles classes. We therefore assumed the value of 2.2% contribution of xylem to the cross-sectional area to be valid for N and N-1 needles. For 10 needles of each cohort, we then calculated the cross-sectional area, based on needle thickness and assuming the needle to be a half cylinder. The estimated cross-sectional xylem area values were multiplied by needle length to obtain the xylem volume of a needle, which was assumed to equal the volume of vascular water. In addition, the total water content of the needles was estimated from the fresh to dry weight ratio. Xylem volume contributed 4.5 and 3.1% to total water volume of N-1 and N needles, respectively. Based on these φ_x values, we calculated $\Delta^{18}O_L$ from $\Delta^{18}O_B$, assuming needle xylem water not to be ^{18}O enriched compared with the twig xylem water that we sampled. This assumption may introduce a slight error as xylem water gets a little bit enriched as it moves along the needle (Farquhar & Gan 2003; Gan *et al.* 2003).

Steady state enrichment of water at the leaf evaporative sites ($\Delta^{18}O_{es}$) can be calculated based on a Craig–Gordon model (Craig & Gordon 1965; Dongmann *et al.* 1974; Farquhar & Lloyd 1993):

$$\Delta^{18}O_{es} = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)\frac{e_a}{e_i} \quad (3)$$

where ε^+ is the equilibrium fractionation between liquid water and vapour, ε_k accounts for the kinetic fractionation during the diffusion of water vapour from the leaf to the atmosphere, $\Delta^{18}O_v$ is the isotopic difference of atmospheric water vapour compared with source water, e_a and e_i

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represent the water vapour pressure in the atmosphere and the leaf intercellular air space, respectively.

Knowing leaf temperature (T, in K), ε^+ can be calculated following Bottinga & Craig (1969):

$$\varepsilon^+(‰) = 2.644 - 3.206\left(\frac{10^3}{T}\right) + 1.534\left(\frac{10^6}{T^2}\right) \quad (4)$$

ε_k can be estimated following Farquhar *et al.* (1989):

$$\varepsilon_k(‰) = \frac{32r_s + 21r_b}{r_s + r_b} \quad (5)$$

where r_s and r_b represent the resistance to water vapour of leaf stomata (based on measured g_s values) and boundary layer, respectively, and their respective associated fractionation factor (32 and 21‰, Cappa *et al.* 2003). Boundary layer resistance was calculated from the wind speed in the canopy measured at 12 m height and from mean needle diameter, according to Jones (1992). Calculated boundary layer resistance was generally comprised between 0.2 and 0.8 m^2 s mol^{-1} and was higher only at one time point (10 June, 0600 h: 4 m^2 s mol^{-1}).

Average lamina mesophyll water is, however, expected to be less enriched than the water at the evaporative sites, resulting in an isotopic gradient between the leaf vein and the evaporative sites. The Péclet effect is the net ratio of (1) the unenriching convection of water to the leaf evaporative sites via the transpiration stream to (2) the effect of the ^{18}O-enriching diffusion of water away from the sites of evaporation. Taking into account this effect (Farquhar & Lloyd 1993), the steady state enrichment of mean lamina mesophyll water above source water ($\Delta^{18}O_{Ls}$) can be expressed as:

$$\Delta^{18}O_{Ls} = \frac{\Delta^{18}O_{es}(1 - e^{-\wp})}{\wp} \quad (6)$$

where the Péclet number is $\wp = LE/CD$, calculated from the scaled effective path length L (m), evaporation rate E (mol m^{-2} s^{-1}), molar concentration of water C (55.5 10^3 mol m^{-3}) and diffusivity of $H_2{}^{18}O$ in water D (2.66 10^{-9} m^2 s^{-1}). The scaled effective path length was estimated by fitting the non-steady state model to the measured $\Delta^{18}O_L$ under expected steady state conditions that typically occur in the end of the afternoon.

Under non-steady state conditions, the enrichment of mean lamina mesophyll water above source water ($\Delta^{18}O_{Ln}$) can be calculated following Farquhar & Cernusak (2005):

$$\Delta^{18}O_{Ln} = \Delta^{18}O_{es} - \alpha^+\alpha_k\left(\frac{(1 - e^{-\wp})}{\wp}\right)\left(\frac{\frac{d(W \cdot \Delta^{18}O_{Ln})}{dt}}{g \cdot w_i}\right) \quad (7)$$

where $\alpha^+ = 1 + \varepsilon^+$ and $\alpha_k = 1 + \varepsilon_k$; W is the lamina leaf water concentration (mol m^{-2}), t is time (s), g is the total conductance to water vapour of stomata and boundary layer ($g = \frac{1}{\gamma_s + \gamma_b}$ mol m^{-2} s^{-1}), and w_i is the mole fraction of water vapour in the leaf intercellular air spaces (mol mol^{-1}). W was estimated based on bulk leaf water content per unit area corrected for the proportion of vascular water (as described previously). The Péclet number used in the non-steady state model was estimated with the steady state model. Because the $\Delta^{18}O_{Ln}$ term occurs on both sides of Eqn 7, it is simpler to solve the equation iteratively.

The non-steady state model requires initial values for $\Delta^{18}O_{Ln}$ and W for a time point ($t_0 - 1$) preceding the first observation. We estimated this initial value for $\Delta^{18}O_{Ln}$ based on the observed diel patterns of measured $\Delta^{18}O_L$ during the entire measurement period. Our first measurement started at 1200 h (t_0) on 6 June 2005. From 3 d of measurements (7, 8 and 9 June), we calculated the fraction between $\Delta^{18}O_L$ at 1200 h and the preceding time point (0600 h). On average, the 0600 h value amounted to 67 and 53% of the 1200 h values for N and N-1 needles, respectively. Based on these fractions, we estimated the initial $\Delta^{18}O_{Ln}$ for $t_0 - 1$ from $\Delta^{18}O_L$ at t. The initial value of W was assumed to equal the one measured at 0600 h on the second day of measurement.

To calculate the mean daytime oxygen isotope enrichment above xylem water ($\Delta^{18}O$), the $\Delta^{18}O$ values of each daytime measurement time was weighted by the corresponding CO_2 assimilation rate measured (A, mol m^{-2} s^{-1}), according to Cernusak *et al.* (2005):

$$\text{weighted } \Delta^{18}O = \frac{\int A \cdot \Delta^{18}O dt}{\int A dt} \quad (8)$$

where the numerator is the daily integral of the product of A and $\Delta^{18}O$ (‰ mol m^{-2}), and the denominator is the daily integral of photosynthesis (mol m^{-2}).

STATISTICAL ANALYSIS

Data were analysed using R 2.2.1 (R Development Core Team 2005). Differences between variables or with the null value were determined with a Student's t-test. Time series analysis was carried out using cross-correlations: a Pearson correlation coefficient between two time series variables was calculated for a number of lags comprised between –10 and 10 times the 6 h interval between two time points. The mean value and associated error that are given for a variable over a time period represent that of the average over the three replicate trees ($n = 3$) of the variable considered.

In order to assess if the data set for $\delta^{18}O$ in leaf water and leaf organic matter contained a periodic component, a periodogram was calculated using the spectral analysis function of Number Cruncher Statistical Software 2004 (NCSS, Kaysville, UT, USA), according to Brandes *et al.* (2006). Equation 9, which is written in the form of a multiple regression, describes the model for the periodic component X_t of a data set with a sum of k frequencies:

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$$X_t = \sum_{j=1}^{k} a_j W_{tj} + \sum_{j=1}^{k} b_j Z_{tj} + e_t \quad (9)$$

where $a_j = R_j \cos(d_j)$, $b_j = -R_j \sin(d_j)$ and $W_{tj} = \cos(f_j t)$, $Z_{tj} = \sin(f_j t)$, in which R is the amplitude of the variation, f is the frequency of the periodic variation measured in numbers of radians per unit time, d is the phase, e_t is the random error (noise) of the time series, t is the time period number.

For the spectral analysis, the total sum of squares as a measure of the variation is separated into amounts associated with each frequency (or wavelength λ, $\lambda = 2\pi/f$). The sum of squares of a particular frequency as a proportion of the total sum of squares at an appropriate frequency range ($n = 20$) is $I(f_k)$:

$$I(f_k) = \frac{N}{4\pi}(a^2{}_k + b^2{}_k) \quad (10)$$

The time series used for the spectral analysis were corrected for series average and trend. Since samples were taken every 6 h, a wavelength of four equals 24 h.

RESULTS

Micrometeorological conditions

Across the sampling period, mean day and night temperatures at 12 m height were 14.4 and 9.3 °C, respectively, with maximum and minimum hourly averages of 18.5 and 14.7 °C during daytime and 14.7 and 3.6 °C during nighttime, respectively. Mean soil temperature at 3 cm depth and volumetric water content at 30 cm depth were 13.5 °C (hourly average maximum and minimum of 15.4 and 10.8 °C, respectively) and 19.9% (hourly average maximum and minimum of 21.0 and 19.9%, respectively). Light rainfall occurred during the night between 6 and 7 June (total of 1.88 mm rainfall above canopy). This resulted in a lower VPD during the night of 6 June and a smaller morning increase of VPD on 7 June compared with the other days (Fig. 1).

Stomatal conductance values that were calculated for both leaf cohorts and used in the calculation of leaf water enrichment are given in Fig. 1. Peak g_s values occurred at midday, with a maximum on 8 June (189 and 100 mmol m^{-2} s^{-1} for N and N-1 needles, respectively).

Leaf, xylem and atmospheric $\delta^{18}O$

Leaf water $\delta^{18}O$ showed strong diel variation (Fig. 1), and was significantly correlated with VPD: $R^2 = 0.50$ ($P = 0.047$) and 0.68 ($P = 0.004$) for N and N-1 needles, respectively. The amplitude of diel variation of $\delta^{18}O$ was significantly larger for N-1 needles than for N needles ($P = 0.002$), with mean values during the precipitation-free period of 11.5 ± 0.4‰ and 4.8 ± 0.6‰, respectively. At most sampling times, N-1 needle water was significantly more enriched in ^{18}O than N needle water. The lowest $\delta^{18}O$ values in both needle cohorts were measured during the night between 6 and 7 June, during the precipitation event. Xylem water $\delta^{18}O$ showed little variation throughout the measurement campaign, with an overall mean and SE of –6.1 ± 0.2‰ (Fig. 1). The diel variation of $\delta^{18}O$ of atmospheric water vapour was more apparent (Fig. 1), with more negative values (reaching –20.1 ± 0.5‰) during daytime than during nighttime (reaching –12.1 ± 0.4‰).

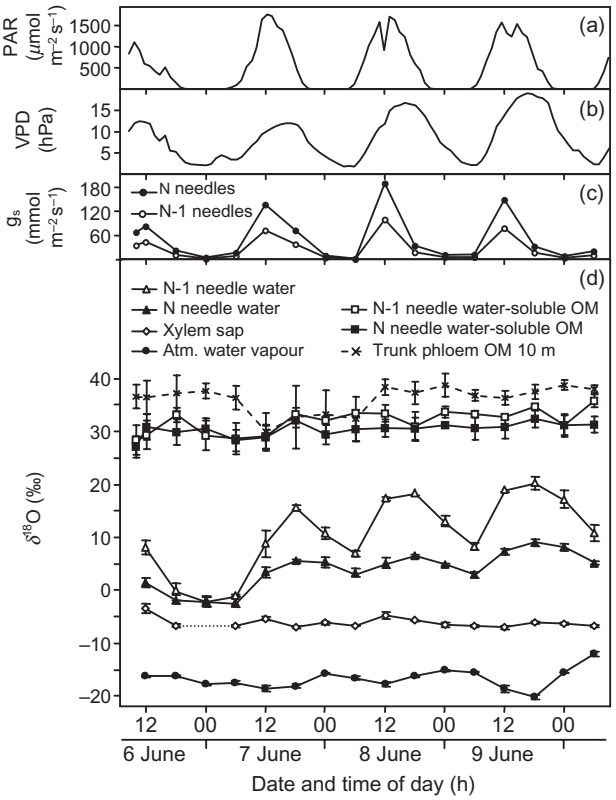

Figure 1. Diel variation of photosynthetically active radiation (PAR; panel a), vapour pressure deficit (VPD; panel b), stomatal conductance (g_s, panel c) and $\delta^{18}O$ in different ecosystem compartments in June 2005 (panel d), including atmospheric water vapour (circles), xylem sap (diamonds), needle water (triangles) and water-soluble organic matter (OM, squares) in current year needles (N, closed symbols) and previous year needles (N-1, open symbols), and trunk phloem sap sampled at 10 m height (crosses). Bars represent ± SE, $n = 3$.

Leaf water $\Delta^{18}O$

Observed $\Delta^{18}O_L$ ranged between 4.1 and 14.5‰ in N needles, and between 5.2 and 24.9‰ in N-1 needles (Fig. 2). In both needle cohorts, daytime values of $\Delta^{18}O_L$ were consistently lower than calculated $\Delta^{18}O_{es}$. The calculated $\Delta^{18}O_{es}$ was similar for both needle cohorts. The difference between $\Delta^{18}O_{es}$ and $\Delta^{18}O_L$ during the day was generally larger for N needles than for N-1 needles. At night, $\Delta^{18}O_L$ was higher than $\Delta^{18}O_{es}$ for N-1 needles.

The scaled effective path length (L) that was used to calculate the Péclet number in Eqns 6 and 7 was estimated

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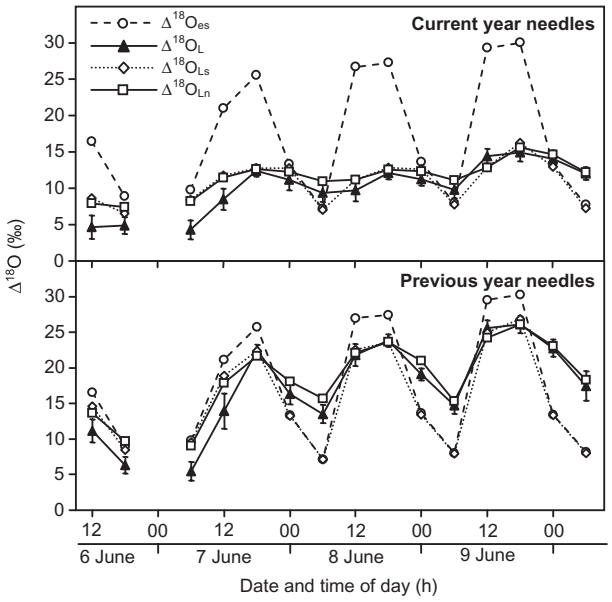

Figure 2. Diel variation of lamina mesophyll water enrichment above source water $\delta^{18}O$ in current year needles (top panel) and previous year needles (bottom panel), as measured ($\Delta^{18}O_L$, triangles) and predicted with steady state ($\Delta^{18}O_{Ls}$, diamonds) and non-steady state ($\Delta^{18}O_{Ln}$, squares) approaches. Predicted steady state enrichment of water at the needle evaporative sites ($\Delta^{18}O_{es}$, circles) is also shown. Bars represent ± SE, $n = 3$.

based on the discrepancy between predicted $\Delta^{18}O_{es}$ and observed $\Delta^{18}O_L$ at 1800 h, when $\Delta^{18}O_L$ was assumed to be at steady state. Over all measurement days, the best fit between observed $\Delta^{18}O_L$ at 1800 h and $\Delta^{18}O_{Ls}$ was obtained for L values of 0.05 m for N-1 needles and 0.15 m for N needles, which resulted in an average Péclet number at 1200 h of 0.42 and 1.7 for N-1 and N needles, respectively. Although high, these values are well within the range observed for other species ($0 < \wp < 1.8$, Wang, Yakir & Avishai 1998; $0.009 < L < 0.2$, Barbour & Farquhar 2003). While the predictions of mean lamina leaf water enrichment that assumed isotopic steady state ($\Delta^{18}O_{Ls}$) were in good agreement with the observed enrichment during the day, they underestimated $\Delta^{18}O_L$ of N-1 needles at night. From 7 June onwards, non-steady state modelling described $\Delta^{18}O_L$ quite adequately in N-1 needles over the complete diel cycle, whereas in N needles, this approach slightly overestimated $\Delta^{18}O_L$ at night (Fig. 2).

Organic matter $\delta^{18}O$ in leaf and phloem

Compared with that of leaf water, the amplitude of variation of $\delta^{18}O$ was smaller for leaf water-soluble organic matter and phloem sap. Water-soluble organic matter $\delta^{18}O$ values ranged from 28.5 to 35.8‰ in N-1 needles and 27.2 to 32.4‰ in N needles. Phloem sap $\delta^{18}O$ values ranged from 28.9 to 36.7‰ for twig phloem (Fig. 1). Bulk needle organic matter $\delta^{18}O$ showed no clear pattern (data not shown), with an overall mean and SE of 24.9 ± 1.0‰ and 25.8 ± 1.3‰ for N-1 and N needles, respectively.

We did not measure a significant difference in $\delta^{18}O$ between phloem sap sampled at different locations or between needle water-soluble organic matter in N and N-1 needles. However, calculation of the mean difference in phloem sap $\delta^{18}O$ between sampling locations over the measurement campaign revealed a consistent but small ^{18}O enrichment of 10-m-trunk phloem sap compared with twig phloem sap, although highly variable and therefore not significant ($P = 0.057$, Fig. 3). Moreover, phloem sap was generally more depleted in ^{18}O, the lower the sampling locations were situated down the trunk (Fig. 3).

Spectral analysis was carried out to quantitatively assess the periodicity of the $\delta^{18}O$ signal in leaf water and leaf water-soluble organic matter. A clear periodicity was identified for leaf water $\delta^{18}O$, with a wavelength that was close to 4, corresponding to 24 h (Fig. 4). At the same wavelength, we observed an absolute maximum for $I(f)$ of $\delta^{18}O$ in the water-soluble organic matter of N-1 needles, and a local maximum of that of N needles. However, these peaks were much less prominent than those of leaf water, and we could observe other peaks at different wavelengths, both of which indicated a less strong periodic component. In both leaf water-soluble organic matter and leaf water, the periodic component of $\delta^{18}O$ was greater for N-1 than for N needles.

Mean oxygen isotopic enrichment above xylem water weighted by daily photosynthesis rate was calculated for both leaf water and leaf water-soluble organic matter, according to Cernusak *et al.* (2005). The 4 d average of the difference between photosynthesis-weighted daily ^{18}O enrichment of leaf water-soluble organic matter and leaf water amounted to 25.9 ± 1.9‰ (mean ± SE, N-1 needles) and 28.5 ± 1.9‰ (N needles).

Time-lagged correlations

We found significant time-lagged correlations ($P \leq 0.05$) between $\delta^{18}O$ in needle water and in various organic matter pools in the trees (Fig. 5). The ^{18}O signal in needle

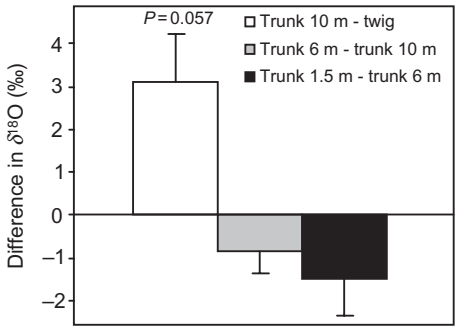

Figure 3. Averaged difference over the measurement campaign between the $\delta^{18}O$ of phloem sap sampled from the twig and the 10-m-trunk position (open bar), from the 10-m-trunk and the 6-m-trunk position (grey bar), and from the 6-m-trunk and the 1.5-m-trunk position (closed bar), averaged over the measurement campaign. Bars represent ± SE, $n = 3$. P represents the significance level for a difference from zero.

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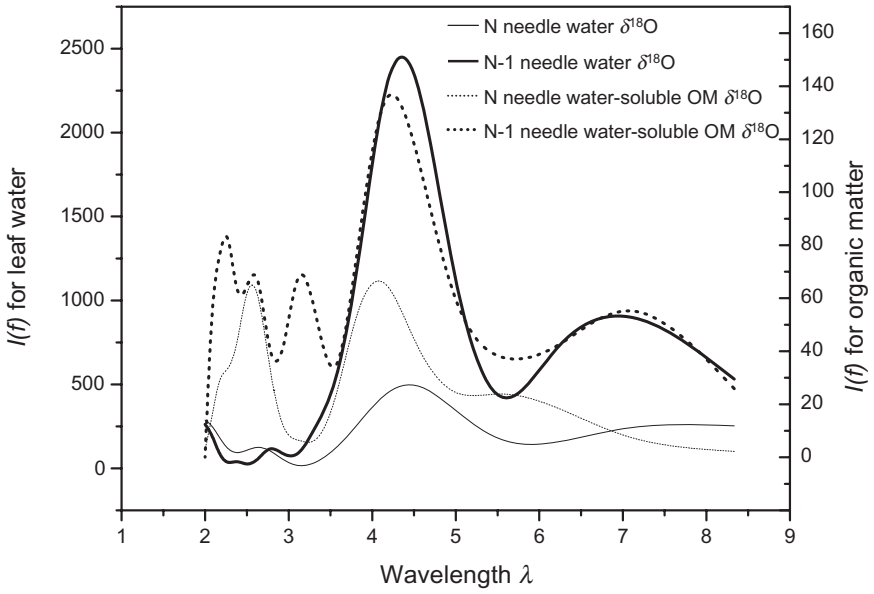

Figure 4. Periodogram in the wavelength scale for the time series of $\delta^{18}O$ in leaf water of current year needles (N, thin solid line) and previous year needles (N-1, bold solid line), as well as in water-soluble organic matter (OM) of N needles (thin dotted line) and N-1 needles (bold dotted line). A wavelength of 4 corresponds to a 24 h periodicity. Note that $I(f)$ is dimensionless.

water-soluble organic matter was significantly correlated with $\delta^{18}O$ in needle water, with a time lag of 0 to 12 h depending on the needle type: the highest correlation coefficient was 0.66 and 0.60 for a 6 h lag in N and N-1 needles, respectively. Trunk phloem sap $\delta^{18}O$ at 10 m significantly lagged $\delta^{18}O$ in needle water-soluble organic matter by 6 h for N needles and 12 h for N-1 needles (correlation coefficient of 0.62 and 0.50), and also lagged $\delta^{18}O$ in leaf water by 12 to 24 h (the highest correlation coefficient was for an 18 h lag: 0.61 for N needles and 0.60 for N-1 needles, respectively). The $\delta^{18}O$ in trunk phloem sap at 6 m lagged $\delta^{18}O$ at 10 m by 0 to 6 h (highest correlation coefficient of 0.74 for a 6 h lag), and $\delta^{18}O$ in trunk phloem sap at 1.5 m lagged $\delta^{18}O$ at 6 m by 0 to 6 h (highest correlation coefficient of 0.71 for a 0 h lag).

We found no significant correlation (at any time lag) between micrometeorological data and $\delta^{18}O$ measurements in any organic matter pools.

DISCUSSION

Leaf water $\Delta^{18}O$

We aim to assess the factors that control evaporative ^{18}O enrichment in lamina leaf water by comparing observed $\Delta^{18}O_L$ with values obtained from a model of increasing complexity. The model defined by Eqn 3 mainly considers fractionation associated with water diffusion, phase transition and water vapour pressure differences of the gas phase inside and outside the leaf, under the assumption of isotopic steady state ($\Delta^{18}O_{es}$). Equation 6 adds a Péclet effect ($\Delta^{18}O_{Ls}$) and Eqn 7 furthermore supposes leaf isotopic non-steady state ($\Delta^{18}O_{Ln}$).

During daytime, the observed $\Delta^{18}O_L$ for N and N-1 needles was adequately described only when a Péclet effect was taken into account in the steady state leaf water model. This is consistent with previous studies, in which steady state predictions of evaporative site water enrichment ($\Delta^{18}O_{es}$) overestimate actual values of $\Delta^{18}O_L$ during the day, when transpiration rates are high (Flanagan, Marshall & Ehleringer 1993; Wang *et al.* 1998). During nighttime, the steady state predictions for leaf water enrichment ($\Delta^{18}O_{Ls}$) were lower than the observed $\Delta^{18}O_L$, especially in N-1 needles. Similar results have been reported in previous studies for several other species (Flanagan & Ehleringer 1991; Cernusak, Pate & Farquhar 2002; Cernusak *et al.* 2005). To further evaluate the relevance for including the Péclet effect to the model, we calculated the difference between predicted $\Delta^{18}O_{es}$ and observed $\Delta^{18}O_L$, normalized against $\Delta^{18}O_{es}$ (Fig. 6), as suggested by Gan *et al.* (2002). The term, $1\text{-}\Delta^{18}O_L/\Delta^{18}O_{es}$, represents the proportion of unenriched water in leaf water (after correction for vascular water). The average and SE during daytime of the precipitation-free period was 0.5 ± 0.03 and 0.2 ± 0.03 for

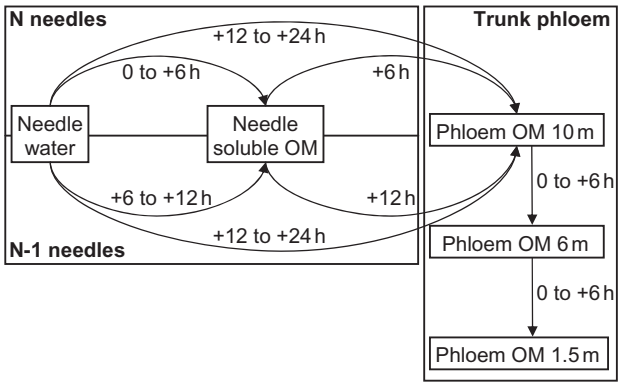

Figure 5. Significant time-lagged correlations ($P \leq 0.05$) between $\delta^{18}O$ in different tree compartments: needle water and water-soluble organic matter (OM) for current year (N) and previous year (N-1) needles, phloem sap in the trunk bark at three sampling heights (10, 6 and 1.5 m height).

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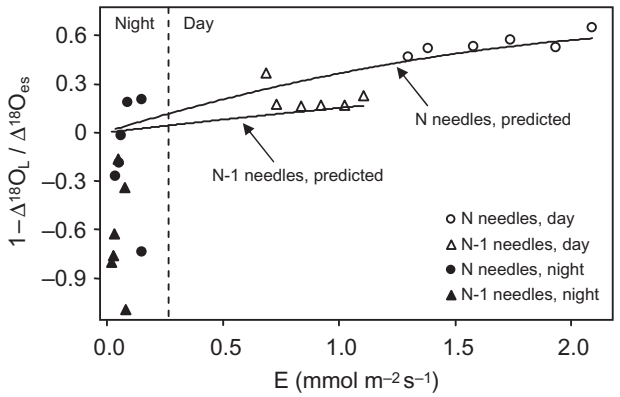

Figure 6. Relationship between the transpiration rate of current year (N, circles) and previous year (N-1, triangles) needles and the discrepancy between the observed enrichment of mean lamina leaf water ($\Delta^{18}O_L$) and the steady state prediction of water enrichment at evaporative sites ($\Delta^{18}O_{es}$), during daytime (open symbols, 1200 and 1800 h) and nighttime (closed symbols, 2400 and 0600 h) of the precipitation-free period. The predicted values are based on scaled effective path length of 0.15 and 0.05 m for N and N-1 needles, respectively.

N and N-1 needles, respectively. As expected during nighttime and early morning, when transpiration was minimal, the steady state model without the Péclet effect performed poorly. Under daytime conditions that were close to steady state, we observed an overall increase of $1\text{-}\Delta^{18}O_L/\Delta^{18}O_{es}$ as transpiration increased, and values from both N and N-1 needles were in good agreement agreed with the predicted values calculated according to Barbour *et al.* (2000). This is consistent with the findings of Barbour *et al.* (2000) and constitutes the prerequisite for including the Péclet effect in the general model (Eqn 6).

Moreover, nighttime predictions were much improved by applying a non-steady state enrichment model that took into account the changes in leaf water concentrations. Daytime predictions with the non-steady state model did not improve considerably those with the steady state model. However, the less accurate predictions of the non-steady state model during the first four sampling times during the day may be related to the rainfall events (e.g. VPD; Fig. 1) compared with the otherwise dry measurement period, and associated impacts on *L*. Keitel *et al.* (2006) assumed *L* to be strongly dependent on atmospheric and pedospheric water conditions. Because plants can quickly react with an increase in hydraulic conductance to a decrease in VPD (probably mediated by the enhanced expression and activity of aquaporins, for a recent review see Tyerman, Niemietz & Bramley 2002), and potentially a shorter water pathway and thus a decrease in *L*, our estimate of *L* (which was constant over the measurement period) under wet conditions might have been too high.

The non-steady state model does not fully explain the diel amplitude of $\Delta^{18}O_L$. In particular, it overestimates observed ^{18}O enrichment in the leaf water during night (e.g. 7 to 8 July in N-1 needles and during most of the measurement period in N needles; Fig. 2), possibly because of an underestimation of the amount of unenriched (xylem) water in the needles. Our calculated lamina leaf water enrichment ($\Delta^{18}O_L$) is based on measured bulk leaf water ^{18}O enrichment ($\Delta^{18}O_B$), corrected for the volume of water in the xylem vessels. Gan *et al.* (2003) assumed that the water pools of the ground tissue associated with the veins, denoted ground tissue capacitance by Farquhar & Gan (2003), is enriched to the same extent as vein xylem water. In pine needles, the veins are located in a central cylinder enclosed in an endodermis with suberinized cell walls (Braune, Leman & Taubert 1983). Even though this casparian strip has been shown to be more permeable for water and solutes than endodermal transport barriers in the roots (Wu *et al.* 2005), the water in the cells in the needle central cylinder could be less enriched, and thus more xylem-like than mesophyll water.

In general, observed $\Delta^{18}O_L$ was consistently lower for N than for N-1 needles throughout the precipitation-free period. This is in agreement with the assumed higher transpiration rates in developing current year needles at that time of the year (Beadle *et al.* 1985). Higher transpiration rates result in a larger contribution of unenriched xylem water to the mean lamina leaf water pool (cf. Eqn 6). Cernusak *et al.* (2005) also observed lower $\Delta^{18}O_L$ values associated with dampened diurnal variations in developing as compared with fully expanded leaves of *Eucalyptus globulus*. The difference in $\Delta^{18}O_L$ that we measured between N and N-1 needles is likely related to foliage phenological stage, with the younger needles transpiring more than the fully grown ones. This pattern might not be found later in the growing season, when the current year needles are fully developed. Indeed, no significant difference was found among $\Delta^{18}O$ of needles of different cohorts in a *Pinus pinaster* (Ait.) stand after summer (J. Ogée *et al.*, pers. comm.).

Leaf organic matter $\delta^{18}O$

Even though the diel patterns of $\delta^{18}O$ were strongly attenuated in soluble organic matter, we observed a periodicity that was close to 24 h. The $\delta^{18}O$ of soluble carbohydrates generally ranges between +28 and +36‰, and correlates with the isotopic signal of water within which they were formed (see review by Schmidt, Werner & Rossmann 2001). The $\delta^{18}O$ of water-soluble organic matter in N and N-1 leaves was well within the range of +28 to +36‰ mentioned previously. The ^{18}O enrichment of water-soluble organic matter above leaf water was also as expected: over the 4 d period assessed, the difference between mean daily photosynthesis-weighted $\Delta^{18}O$ of leaf water and leaf water-soluble organic matter (25.9 ± 1.9‰ for N-1 needles and 28.5 ± 1.9‰ for N needles) supported the observed average difference of 27.8‰ in *E. globulus* (Cernusak *et al.* 2005). The slightly different enrichment observed here might be attributed to the presence of organic compounds other than carbohydrates in the needles with differences in ^{18}O enrichment.

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Time-lagged correlations

We were able to follow the $\delta^{18}O$ signal from leaf water to leaf organic matter, with significant time lags that could reach 12 h. In addition, we observed a dampened diel periodicity of $\delta^{18}O$ in needle water-soluble organic matter compared with that of $\delta^{18}O$ in needle water. These findings can be explained by turnover times of the leaf soluble organic matter pool in the range of 6 to 12 h, even when we assume that the newly produced organic compounds entering the pool are isotopically equilibrated with lamina leaf water. Leaf water has been shown to reach within 1‰ of the steady state value within ca. 35 min, depending on the transpiration rate (Wang & Yakir 1995). However, especially at night when stomatal aperture is small, isotopic equilibration may take much longer, because leaf water residence time may increase to several hours (Farquhar & Cernusak 2005). Soluble leaf organic matter is expected to take much longer to equilibrate isotopically compared with water, if it does equilibrate at all, because the isotope exchange may take longer than the turnover time of needle water-soluble organic matter (Farquhar, Barbour & Henry 1998; Schmidt *et al.* 2001; Werner 2003; Sternberg *et al.* 2006). Barbour *et al.* (2000) measured a delay of ca. 3.5 h before sucrose exported by a leaf of *Ricinus communis* (L.) reached a new steady state. Because we consider here not only sucrose but a complex assortment of leaf water-soluble organic compounds, and because we assess variable day and night conditions, we could assume even longer mean turnover times than these 3.5 h. In that case, however, at least part of the $\delta^{18}O$ of organic matter produced during the day might also be influenced by carbohydrates released from transitory starch during the night. Thus, the oxygen isotope composition of these compounds may be determined not only by the photosynthesis-weighted $\delta^{18}O$ of leaf water of the preceding day, but also by the exchange with less-enriched nighttime leaf water during starch hydrolysis.

We followed the isotopic signal of leaf water ^{18}O not only as it was incorporated into leaf organic matter but also in the trunk bark phloem sap. The significant time-lagged correlations between trunk bark phloem sap at different sampling heights resulted in a phloem transport velocity ranging from 0.5 to 1 m h^{-1}, as reported in other studies (Zimmermann & Braun 1971; Keitel *et al.* 2003; Gessler *et al.* 2004). We found no significant correlation between phloem sap sampled at twig level and any of the other compartments in which we measured $\delta^{18}O$. This indicates that either our measurement frequency was not high enough to pick up the twig phloem signal, or that $\delta^{18}O$ of twig phloem sap was neither correlated to leaf-level $\delta^{18}O$ nor to trunk bark phloem $\delta^{18}O$. Moreover, the consistent $\delta^{18}O$ depletion of twig phloem sap compared with 10-m-high trunk phloem sap (also observed by Brandes *et al.* 2006 during the seasonal course at the same site) points to additional carbon assimilation in the photosynthetic bark of *P. sylvestris* in twigs. Organic matter assimilated in the bark (an environment where the reaction water is not or only slightly ^{18}O enriched) should have a $\Delta^{18}O$ of 27‰ (Cernusak *et al.* 2005), well below the enrichment for sugars fixed in leaves. If the twig phloem sap included considerable amounts of sugars formed in the twig bark, whereas that at the trunk level did not, this would explain the relatively depleted $\delta^{18}O$ signal of twig phloem as compared with 10-m-trunk phloem sap. Another possible hypothesis is the differential signature of sugars depending on their allocation in the plant, the more depleted $\delta^{18}O$ signal corresponding to leaf-allocated sugars, and the less depleted $\delta^{18}O$ signal corresponding to sugars allocated to reserve organs such as trunk or roots.

In contrast to water-soluble leaf organic matter, phloem-allocated carbon in *P. sylvestris* mainly consists of sucrose (Hansen & Beck 1994) and, thus, should be enriched by approximately 27‰ above the $\delta^{18}O$ of leaf water in which it was formed (Schmidt *et al.* 2001). However, based on the Münch hypotheses of phloem transport and subsequent studies (see review by van Bel 2003), the $\delta^{18}O$ of sugars is expected to be altered by the continuous loading and unloading of the phloem. Sugars unloaded from transport phloem might undergo metabolic conversion in the non-enriched reaction water of stems before they are reloaded into the sieve tubes. This might result in a decrease in $\delta^{18}O$ of phloem organic matter as it is transported. In order to compare the $\delta^{18}O$ of phloem sap with the $\delta^{18}O$ of leaf water, we assumed that (1) the leaf area ratio of N and N-1 needles in the gas exchange chambers was representative for the whole canopy; and (2) phloem-exported sugars were imprinted only by leaf water enrichment during assimilation by RuBisCo (i.e. we did not take into account the possible post-photosynthetic fractionation of sugars that can be incorporated into transitory starch during the day and remobilized at night). Based on our time-lag analysis, we considered that the ^{18}O signal in leaf water would appear 18 h later in the 10-m-trunk phloem sap, and could then be used to calculate canopy-integrated leaf water enrichment at the time phloem sugars were produced. Applying this procedure, 10-m-trunk phloem sap was found to be enriched by 25.3 ± 4.0‰ above leaf water, on average over the 4 day period, which is consistent with a depletion of the theoretical 27‰ enrichment above leaf water as phloem is transferred away from the leaf.

Trunk bark phloem tended to become more depleted in ^{18}O as it was sampled at a lower position, supporting the expected pattern. Because part of the sucrose from the sieve tubes is released during phloem transport [two-thirds of which is transported back into the sieve tubes, Minchin & Thorpe (1987)], sucrose transformations outside the sieve tubes and related O exchange with unenriched xylem water is possibly responsible for the increased depletion of phloem sap in ^{18}O as it is transported.

CONCLUSION

Our study showed clearly that the Péclet effect has to be included in the evaporative enrichment model to adequately describe daytime ^{18}O enrichment of leaf water and that non-steady state conditions should be taken into account during the night. The assessment of $\delta^{18}O$ in

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different organic matter pools revealed consistent time-lagged correlations that have direct implications for carbon transport and source-sink studies in trees. These time lags reflect an explicit temporal factor between environment and the $\delta^{18}O$ of carbon compounds that are likely to be respired by roots and soil heterotrophic microorganisms. Thus, understanding the magnitude and controls of such time lags will have important implications for partitioning net ecosystem carbon fluxes.

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