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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

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ABSTRACT

This study investigated the influence of meteorological, pedospheric and physiological factors on the water relations of Scots pine, as characterized by the origin of water taken up, by xylem transport as well as by carbon isotope discrimination ($\Delta^{13}C$) and oxygen isotope enrichment ($\Delta^{18}O$) of newly assimilated organic matter. For more than 1 year, we quantified δ^2H and $\delta^{18}O$ of potential water sources and xylem water as well as $\Delta^{13}C$ and $\Delta^{18}O$ in twig and trunk phloem organic matter biweekly, and related these values to continuously measured or modelled meteorological parameters, soil water content, stand transpiration (*ST*) and canopy stomatal conductance (G_s). During the growing season, $\delta^{18}O$ and δ^2H of xylem water were generally in a range comparable to soil water from a depth of 2–20 cm. Long residence time of water in the tracheids uncoupled the isotopic signals of xylem and soil water in winter. $\Delta^{18}O$ but not $\Delta^{13}C$ in phloem organic matter was directly indicative of recent environmental conditions during the whole year. $\Delta^{18}O$ could be described applying a model that included ^{18}O fractionation associated with water exchange between leaf and atmosphere, and with the production of organic matter as well as the influence of transpiration. Phloem $\Delta^{13}C$ was assumed to be concertedly influenced by G_s and photosynthetically active radiation (PAR) (as a proxy for photosynthetic capacity). We conclude that isotope signatures can be used as effective tools (1) to characterize the seasonal dynamics in source and xylem water, and (2) to assess environmental effects on transpiration and G_s of Scots pine, thus helping to understand and predict potential impacts of climate change on trees and forest ecosystems.

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INTRODUCTION

Owing to global warming as predicted by climate models (IPCC 2001), near-surface air temperature is assumed to increase in Europe within the next several decades. Model predictions are supported by trend analyses of precipitation and air temperature measurements in southwestern Germany (Mayer *et al*. 2005), and suggest an increase in mean air temperature of about 1 °C by 2050 along with a propensity for greater rainfall in winter and more frequent drought periods in summer. Such changes will have significant effects on water balance and, therefore, on forest growth and vitality of trees. A first step towards the development of climate-adapted forest management strategies is to gain a better understanding into how key parameters of the water balance of trees are affected by changes in environmental conditions.

Stand transpiration (*ST*) rates are a direct measure of water consumption by trees. Transpiration rate is a function of stomatal aperture and the water vapour gradient between the leaf internal air space and the atmosphere. Stomatal conductance is determined by numerous environmental and physiological factors such as photosynthetically active radiation (PAR), internal CO_2 pressure and water vapour pressure deficit (*VPD*) (Gao *et al*. 2002; Tuzet, Perrier & Leuning 2003). When soil water is limited, both transpiration and stomatal conductance become closely coupled with soil water potential via chemical signal transfer such as abscisic acid (Comstock 2002), thus providing information on drought reactions of trees. There is, however, a lack of information on how the interaction of different environmental factors potentially controls

stomatal conductance during the seasonal course (Matsumoto, Ohta & Tanaka 2005). It is also not clear if there are threshold values for particular factors that define if they exert control over stomatal conductance or not (Irvine *et al.* 1998).

Owing to its relationship to c_i/c_a (ratio of partial pressures of CO_2 in the substomatal cavity and the ambient air) as described by Farquhar, O'Leary & Berry (1982) and – on the other hand – the dependency of c_i on G_s (Keitel *et al.* 2003), $\delta^{13}C$ in organic matter can be used to characterize the effects of environmental parameters such as atmospheric and soil water deficit (e.g. Livingston & Spittlehouse 1996; Williams & Ehleringer 1996; Korol *et al.* 1999) on stomatal reactions.

$\delta^{13}C$ of plant organic matter is depleted compared to source carbon (CO_2) because of fractionation during diffusion through the leaves and carboxylation (Farquhar, Ehleringer & Hubick 1989a). The relationship between carbon isotope discrimination and leaf internal CO_2 concentration is explained by a two-stage model developed by Farquhar *et al.* (1982):

$$\Delta^{13}C = a + (b - a)\cdot\frac{c_i}{c_a}, \quad (1)$$

where the discrimination of ^{13}C is controlled by factors *a* (fractionation during diffusion through stomata and leaf intercellular space), *b* [discrimination during carboxylation by ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco)] and c_i/c_a. In addition to stomatal conductance, the CO_2 fixing activity of Rubisco can have an influence on c_i/c_a. As a consequence, the variation in carbon isotope fractionation cannot be directly attributed to variations in stomatal conductance. The oxygen isotope composition ($\delta^{18}O$) or the oxygen isotopic enrichment above source water ($\Delta^{18}O$) of plant organic matter has been shown to provide additional information to separate effects of stomatal conductance from the influence of changes in photosynthetic capacity (Farquhar, Barbour & Henry 1998) when stomatal conductance is assumed to be controlled by relative humidity (rH) (Scheidegger *et al.* 2000).

Analysing carbon and oxygen isotope composition or isotopic depletion/enrichment compared to a source in rapid turnover organic matter pools like phloem sugars provides information on environmental signals within the preceding hours to days (Cernusak *et al.* 2003a; Keitel *et al.* 2003; Gessler, Rennenberg & Keitel 2004; Cernusak, Farquhar & Pate 2005). Only recently, Keitel *et al.* (2006) showed that $\Delta^{18}O$ of phloem organic matter of European beech could be accurately predicted over a wide range of climatic conditions by applying a steady-state evaporative enrichment model that takes into account the isotopic gradients of leaf water caused by a Péclet effect. However, until now, an assessment of $\Delta^{18}O$ or $\Delta^{13}C$ in phloem organic matter has been done only for two different tree species (*Eucalyptus globulus* and *Fagus sylvatica*), and there have been no verified attempts at applying this model to coniferous species. In addition, evaporative enrichment models have only been applied in short-term studies, and we lack information about whether we can predict $\Delta^{18}O$ of a fast turnover organic matter pool throughout the whole growing season.

In addition to stomatal reaction, the availability of water resources in the soil affects the water balance of trees. Comparing stable isotope composition (δ^2H, $\delta^{18}O$) of xylem water with that of potential water sources has recently been accepted as a method to examine water utilization by trees (e.g. Farrington, Turner & Gailitis 1996). This is feasible as no isotope fractionation steps occur during uptake and transport of water through the xylem (Zimmermann, Ehhalt & Munnich 1967; Ehleringer & Dawson 1992; Thorburn, Walker & Brunel 1993). The main aim of previous studies assessing δ^2H and $\delta^{18}O$ of water resources (e.g. Adar *et al.* 1995; Rose, Graham & Parker 2003) was, however, the partitioning of soil water versus groundwater as main water sources. When highly different δ^2H and $\delta^{18}O$ signatures are exhibited in the water along the soil profile in combination with relatively slow variations with time, it may be possible to estimate the depth from which soil water is absorbed by trees. Still, when using such an approach, it is crucial to consider potential time lags in the isotopic signature between source water in the soil and water in the capillaries of the tree in different heights (Waring, Whitehead & Jarvis 1979).

In the present study, we aimed to asses the water balance of *Pinus sylvestris* growing in a dry environment where trees cannot reach the groundwater directly as it is located about 7 m below ground level (Reif 1996). We combined isotopic approaches with xylem flow measurements and modelling of canopy stomatal conductance (G_s) over more than 1 year. In addition to our attempt to characterize the origin of water taken up by the trees, we related $\Delta^{13}C$ and $\Delta^{18}O$ in phloem organic matter obtained from twigs and trunks to environmental and physiological parameters. We sought to assess if this combined analysis of carbon and oxygen isotopes can be used as a tool to characterize water relations of the conifer *P. sylvestris* during the growing season.

We aimed to test the following main working hypotheses: $\Delta^{13}C$ of the phloem organic matter can be used as an indicator for G_s with a time lag of a few days for the conifers species examined in a way as previously shown for a deciduous species (Keitel *et al.* 2003). If G_s is not a direct proxy for c_i/c_a, (i.e. if additional factors related to Rubisco activity and carboxylation influence c_i) this can be revealed by the relation between $\Delta^{13}C$ and the oxygen isotope enrichment (Scheidegger *et al.* 2000) – provided that G_s is mainly controlled by rH or *VPD*.

In addition, we hypothesized that $\Delta^{18}O$ of phloem organic matter can be described not only for short periods but also during the whole year by applying the model of Farquhar & Lloyd (1993) that takes into account isotopic fractionations associated with $H_2{}^{18}O$ diffusion, with phase transition from liquid water to vapour, and with exchange between carbonyl oxygen and water as well as the isotopic gradients in leaf water caused by the Péclet effect.

In addition, we aimed to test if we could decipher the soil depth from which water is absorbed throughout the year by comparing the oxygen and hydrogen isotopic composition in precipitation, soil water at different depths and xylem water.

MATERIAL AND METHODS

Site description

Field experiments were carried out in an approximately 40-year-old Scots pine plantation (*Pinus sylvestris* L.) at the Hartheim forest meteorological experimental site (201 m a.s.l.; 47°56′N and 7°37′E) operated by the Meteorological Institute of the University of Freiburg. The pine stand is located in the southern upper Rhine plain near the village of Hartheim in Germany close to the river Rhine and the French border. Annual precipitation amounts to 667 mm and mean annual temperature to 9.8 °C according to the nearby weather station Bremgarten for the period 1951–1980 (Mayer *et al*. 2002). During the growing season (April–September), potential evaporation frequently exceeds precipitation (Tajchman 1972). Mean temperature during the growing season is 15.4 °C. Soil profiles are characterized as a calcaric Fluvisol (Hädrich & Stahr 1997) according to the Food and Agriculture Organization (FAO) classification, with a mean depth of the topsoil of 40 cm. Soil textural class is sandy silt, and soil density amounts to 1.0–1.4 g cm^{-3}. The pH of the mineral soil varies between 7.2 and 8.1 (Hädrich & Stahr 1997).

Because of different measures of river regulation (between 1817 and 1959), the groundwater table has receded and is at present 7 m below the surface in this former flood plain forest area.

The historical vegetation was an alluvial forest that was replaced by drought-resistant Scots pine forests in the middle of the last century (Mayer *et al*. 2002).

Mean height of the trees was 14.3 m; stand density was 800 trees ha^{-1}. Basal sap wood area amounted to 22.12 cm^2 m^{-2}, and projected *LAI* was 2.07 after a thinning in autumn 1993 (Mayer *et al*. 2002). Further thinning in 2003 decreased basal sap wood area and *LAI* to 17.69 cm^2 m^{-2} and 1.47, respectively, at the time of the studies. Understorey vegetation consists mainly of *Brachypodium pinnatum*, *Carex alba* and *Carex flacca* (Wedler *et al*. 1996).

Meteorological parameters

Dry and wet bulb temperatures were measured at a height of c. 15 m on a meteorological tower. Down- and upwelling short-wave radiation (pyranometers, type CM21; Kipp & Zonen, Delft, the Netherlands) as well as down- and upwelling long-wave radiation (pyrgeometers, type CG1, Kipp & Zonen) were measured immediately above the Scots pine forest canopy at 16 m above-ground level. Photosynthetic photon flux density (LI-190SA; Li-Cor, Lincoln, NE, USA) was recorded above the canopy. Precipitation was monitored at the top of the 30 m tower. All sensors were sampled every 30 s. A data acquisition system (CR23X; Campbell, Logan, UT, USA), which calculates 10 min average values and for precipitation, 10 min totals, was stationed at the experimental site. *VPD* was calculated from dry and wet bulb temperature.

Potential evapotranspiration rates (E_{pot}) were obtained using the hydrological model BROOK90 (Federer, Vorosmarty & Fekete 1996), which is based on the approach by Shuttleworth & Wallace (1985).

Transpiration and canopy stomatal conductance

Prevailing conditions of water availability were analysed by measuring xylem water flow as a measure for tree water use applying Granier-style probes and by scaling up flow densities from a single tree to the stand level (Granier *et al*. 1996). Xylem sap flow was measured on 12 trees with thermocouple probes as described by Granier (1985, 1987).

Sap flow densities (*SFD*s) (L m^{-2} sapwood area s^{-1}) were determined every 5 min and were calculated as means of 30 min. Daily sums of *ST* based on the stand ground area were upscaled using the following equation:

$$ST = SA \cdot \sum SFD \quad (\mathrm{mm\,d^{-1}}) \text{ equals } (\mathrm{L\,m^{-2}\,d^{-1}}), \tag{2}$$

where *SA* is the stand sapwood area (m^2 m^{-2}) and Σ*SFD* is the *SFD* summed up over 24 h (L m^{-2} d^{-1}). Studying radial sap flow patterns of *P. sylvestris*, Nadezhdina, Cermak & Ceulemans (2002) found that active sapwood is restricted to the outer 60% of the stem radius. By analysing the sapwood area using the displacement method with a basic fuchsin/periodic acid dye for trees of different radii, a correlation was found between stem disc area at 1.3 m height and sapwood area (data not shown). From this relation, we calculated a mean sapwood area of 17.69 cm^2 m^{-2}, which was used for the *ST* calculation. Values of single trees were multiplied with a correction factor of 0.86 to incorporate the reduction of *SFD* at a depth of 20 to 40 mm (relative to the cambium) (Köstner *et al*. 1996).

Based on sap flux densities, mean daily canopy stomatal conductance (G_s, mmol m^{-2} s^{-1}) was calculated using a simplified Penman–Monteith equation according to Pataki, Oren & Phillips (1998) & Keitel *et al*. (2003):

$$G_s = \frac{G_c}{LAI} = \frac{\gamma \cdot \lambda \cdot P}{\rho \cdot c_p \cdot VPD \cdot R \cdot T_a} \cdot \frac{J_s \cdot SA}{LAI} \cdot 10^3, \tag{3}$$

where G_c is canopy conductance; γ is the psychrometric constant (kPa K^{-1}); λ is the latent heat of vapourization (J kg^{-1}); ρ is the density of moist air (kg m^{-3}); c_p is the heat capacity of moist air (J kg^{-1} K^{-1}); P is the atmospheric pressure (Pa); R is the ideal gas constant (8.31 J mol^{-1} K^{-1}]; T_a is air temperature (K); J_s is mean daily sap flux density given in kg m^{-2} s^{-1}, and *LAI* is the leaf area per ground area given in m^2 m^{-2}. VPD is given in kPa.

These calculations assume that (1) stem capacitance can be neglected, and that (2) canopy aerodynamic conductance is much larger than G_s as reported by Whitehead & Jarvis (1981), which means that aerodynamic resistance can be neglected in comparison with stomatal resistance. In short-term experiments lasting 9 d, Brandes *et al*. (2006) showed that canopy stomatal conductance (G_s) was comparable to mean leaf level stomatal conductance (g_s) as determined with a portable gas exchange measurement device.

Plant material

Samples were collected biweekly at approximately 1100 h Central European Time (CET). Twigs of the lower, yet fully sun-exposed crown were collected from eight dominant or co-dominant trees. Trees were chosen randomly, but alternately, to avoid repetitive sampling that could produce artefacts because of wounding responses.

Phloem exudate collection was performed according to the method described in detail by Gessler *et al*. (2004). Active phloem tissue was cut from the twigs using a scalpel and from the trunks at 1.3 m height using a punch, and was subsequently separated from older bark tissue. From 24 February 2004 to 6 October 2004, additional phloem samples were collected from eight trees at each sampling date. Tissues were rinsed with water in order to remove cellular constituents that might have been released from destroyed cells at the site of cutting, and resin from resin reservoirs destroyed during sampling. Bark pieces were incubated in 2 mL H_2O for 5 h at room temperature. Thereafter, the exudates were centrifuged. Previous studies (Schneider *et al*. 1996) with different species showed that contamination of phloem exudates with cellular constituents using the exudation technique are negligible under the experimental conditions applied.

Xylem sap was collected from sampled twigs and small branches. At the cut basal (or proximal when related to the main trunk) end of the twig, bark tissue was removed to avoid contamination with phloem sap. We applied a gentle vacuum with a hand pump to the cut end of the sampled branch that was sealed with silicon putty in 2 mL glass vials. Vacuum was maintained, and xylem sap was collected while small segments of branch wood were repeatedly cut from the apical end of the shoot.

Xylem water from twigs of trees is often extracted applying the cryogenic distillation technique (Ehleringer, Roden & Dawson 2000). In order to test if the mild vacuum extraction method we used was comparable to distillation, we harvested larger branches from the crown of seven pine trees and then sampled two twigs of a comparable order of ramification (with a diameter of approximately 1 cm) from each larger branch. A section of one twig (after removing the bark) was subjected to cryogenic distillation as described by Ehleringer *et al*. (2000). The other twig was used for mild vacuum extraction. Our assessment revealed that $\delta^{18}O$ of xylem water did not differ significantly between the two methods (vacuum extraction, –4.13 ± 0.19‰; cryogenic distillation, –4.32 ± 0.42‰; $P < 0.05$).

Collection of precipitation and soil water

Precipitation and soil water samples were collected biweekly at the same time when plant material was collected. Precipitation was sampled above the canopy, using collection vessels designed after specification of the IAEA (2002) to avoid isotopic fractionation by evaporation of rainwater stored in the rain gauge. The special construction of these vessels prevents moisture exchange with the atmosphere. Soil samples were taken at depths of 0–2, 2–20 and 20–40 cm, packed in air-tight plastic bags and stored in a refrigerator until further treatment.

Soil samples were analysed gravimetrically for water content, and soil water was extracted using the toluene distillation method (Revesz & Woods 1990).

Carbon, oxygen and hydrogen isotope composition

From phloem exudates, 200 μL aliquots were transferred into silver ($\delta^{18}O$ analysis) or tin ($\delta^{13}C$ analysis) capsules and oven dried at 70 °C. Chromosorb (10 mg) was added into the tin capsules to improve combustion for $\delta^{13}C$ analysis.

Samples were combusted in an elemental analyser (NA 2500; CE Instruments, Milan, Italy) for $\delta^{13}C$ analysis and in a high temperature conversion/elemental analyser (TC/EA; Finnigan MAT GmbH, Bremen, Germany) for $\delta^{18}O$ analysis, both coupled to an isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT GmbH) by a Conflo II interface (Finningan MAT GmbH). SDs were ±0.1 and ±0.4‰ for measurements of $\delta^{13}C$ and $\delta^{18}O$, respectively.

Cernusak *et al*. (2003a) reported that $\delta^{18}O$ of phloem organic matter from *E. globulus* varies depending on whether the sample cups were sealed under argon immediately upon removal from the drying oven, or whether they were folded so as to not form a gas-tight seal. The authors presume that the difference is caused by adsorption of water vapour from the atmosphere onto the surface of the dried sugars when the sample is not enclosed in a gas-tight cup. We tested this presumption on a separate set of 24 samples by sealing the dried phloem samples under argon immediately after removing them from the drying oven. We indeed found that the $\delta^{18}O$ in phloem organic matter was lower if the samples were not sealed under argon, 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) phloem samples: $\delta^{18}O_{corrected} = 1.14 \times \delta^{18}O_{unsealed} - 2.88$.

For the $\delta^{18}O$ measurement of xylem sap samples, 0.3 μL of sap was injected by a GCPal autosampler (CTC, Zwingen, Switzerland) into a high TC/EA modified according to Gehre *et al*. (2004), coupled online to an isotope ratio mass spectrometer (Delta ^{plus} XP) via a ConFlo III interface (both Finnigan MAT GmbH). SDs for measurements were ±0.2‰.

Analyses of δ^2H and $\delta^{18}O$ of precipitation and soil water, as well as δ^2H of xylem water were conducted using a Finnigan MAT Delta S dual inlet isotope ratio mass

spectrometer (IRMS). For δ^2H analysis, 1 μL of the sample was injected into an H-device using a chrome reduction method at 900 °C that subsequently produces hydrogen gas. $\delta^{18}O$ measurements were performed by equilibrating 5 mL of the sample with CO_2 for 11 h at 20 °C within an equilibration unit. Analytical precision was better than ±0.2 and ±1‰ for $\delta^{18}O$ and δ^2H, respectively.

The isotopic values are expressed in delta notation (in ‰ units), relative to Vienna Pee Dee Belemnite (VPDB) for carbon and Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen.

Isotopic calculations for organic matter and oxygen isotope theory

To eliminate the influence of source water on $\delta^{18}O$ and of atmospheric CO_2 on $\delta^{13}C$, respectively, in phloem organic compounds, $\Delta^{18}O$ and $\Delta^{13}C$ were calculated. $\Delta^{18}O$ defines the enrichment of phloem organic compounds compared to xylem sap signature and was calculated using the following equation:

$$\Delta^{18}O_{org} = \frac{\delta^{18}O_{org} - \delta^{18}O_s}{1 + \delta^{18}O_s}, \quad (4)$$

where $\Delta^{18}O_{org}$ is the enrichment of phloem organic compounds in ^{18}O above source (i.e. xylem) water; $\delta^{18}O_{org}$ is the oxygen isotope composition of phloem organic compounds, and $\delta^{18}O_s$ is the oxygen isotope composition of xylem water. The discrimination of ^{13}C during assimilation ($\Delta^{13}C$) was calculated using the equation:

$$\Delta^{13}C_{org} = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{org}}{1 + \delta^{13}C_{org}}, \quad (5)$$

where $\Delta^{13}C_{org}$ is the depletion of ^{13}C in phloem organic compounds compared to the source C isotope composition, which is that of atmospheric CO_2($\delta^{13}C_{CO_2}$). $\delta^{13}C_{org}$ is the carbon isotope composition of phloem organic compounds. $\delta^{13}C$ values of atmospheric CO_2 were derived from measurements recorded at the nearby Schauinsland measurement station of the Umweltbundesamt between 1977 and 1992 (Levin, Graul & Trivett 1995). This station is approximately 20 km from the field site examined here. Mean annual peak-to-peak amplitude was 0.8‰ during that period. A slight annual decrease of 0.02‰ was recorded in the first 10 years, but no long-term trend was observed thereafter. We thus used averaged monthly values (range: –7.7 to –8.5‰) of the last 5 years for calculating $\Delta^{13}C$.

The enrichment of water at the sites of evaporation can be calculated with the following model (Craig & Gordon 1965; Farquhar & Lloyd 1993):

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)\frac{e_a}{e_i}, \quad (6)$$

where ε^+ is the equilibrium fractionation between liquid water and vapour at the air–water interfaces; ε_k is the kinetic fractionation during diffusion of water vapour from the leaf intercellular gaseous space to the atmosphere (calculated according to Farquhar *et al*. 1989b), taking into account new determinations of the isotopic effect for diffusion of $H_2{}^{18}O$ in air (Cappa *et al*. 2003); $\Delta^{18}O_v$ is the difference in $\delta^{18}O$ between source water and atmospheric water, and e_a/e_i is the ratio of external to internal water vapour pressure. Average lamina mesophyll water is, however, supposed to be less enriched than the water at the evaporative sites because of the influx of unenriched xylem water into the leaf. Steady-state enrichment of mean lamina leaf water ($\Delta^{18}O_L$) depends on the steady-state enrichment at the evaporative site of the leaf ($\Delta^{18}O_e$) and on the lamina radial Péclet number $\wp$ (Farquhar *et al*. 1993; Farquhar & Gan 2003):

$$\Delta^{18}O_L = \frac{\Delta^{18}O_e(1 - e^{-\wp})}{\wp}, \quad (7)$$

where the Péclet number is defined as EL/CD, where E and L are transpiration rates (mol m^{-2} s^{-1}) and scaled effective path length (m), respectively; 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}). Oxygen isotope signatures in organic plant material record the ^{18}O signature of the water in which they were formed (Epstein, Thompson & Yapp 1977; Yakir & Deniro 1990). Enrichment of ^{18}O above source water in the newly produced organic matter ($\Delta^{18}O_p$) depends on $\Delta^{18}O$ of average lamina mesophyll water ($\Delta^{18}O_L$, Eqn 7) and on the equilibrium fractionation between organic carbonyl oxygen and water (ε_{wc}; +27‰; Cernusak, Wong & Farquhar 2003b). We, thus, applied Eqn 7, taking into account ε_{wc} for predicting $\Delta^{18}O$ of phloem organic matter ($\Delta^{18}O_{calc}$).

For this calculation, we made the following assumptions according to Keitel *et al*. (2006): water vapour was in isotopic equilibrium with source water, as often assumed to be valid during the growing season in Europe (Förstel & Hützen 1983; Saurer, Borella & Leuenberger 1997). In that case, $\Delta^{18}O_v$ in Eqn 6 equals $-\varepsilon^+$. Keitel *et al*. (2006) revealed that making this assumption is justified when calculating ^{18}O enrichment of phloem organic matter in Central Europe. Different from Keitel *et al*. (2006) working with a broadleaf species, we assumed here that leaf temperature equals air temperature according to Barbour, Walcroft & Farquhar (2002). These authors showed that needles of *Pinus radiata* were strongly coupled to the environment, making leaf temperature, e_i and ε^+ scale directly to air temperature.

ε^+ was calculated from air temperature according to Bottinga & Craig (1969). ε_k was calculated according to Farquhar *et al*. (1989b), taking into account new determinations of the isotopic effect for diffusion of $H_2{}^{18}O$ in air (Cappa *et al*. 2003). The range for ε_k was 31.2–32.0‰. We assumed aerodynamic resistance << stomatal resistance when calculating ε_k. Values for the effective length L generally vary between 0.004 and 0.17 m (Wang, Yakir & Avishai 1998; Barbour & Farquhar 2004). Thus, we tested values for L of 0.005, 0.015, 0.025, 0.04, 0.055 and 0.07 m. In addition, we calculated $\Delta^{18}O_{calc}$ ignoring a potential Péclet effect.

$\Delta^{18}O_{calc}$ was modelled with the mean day-time values of G_s, air temperature, e_a, e_i and transpiration rate from different times (1–5 d) preceding phloem sampling.

Statistical analyses

Statistical analyses were conducted using SPSS 10.05 (SPSS Inc., Chicago, IL, USA) Correlations were calculated using the bivariate correlation procedure. Significance of correlation was calculated according to Sachs (1984). Regression lines between $\delta^{13}C$ and $\delta^{18}O$ were determined by linear regression analysis. Differences between mean values were estimated applying Student's *t*-test.

RESULTS

Meteorological and hydrological conditions

Total precipitation (P) during the study period amounted to 1008 mm (Fig. 1a). Almost 400 mm of precipitation fell during the growing season (April–September). The highest monthly precipitation value was recorded in October 2004 (170 mm), the lowest in April 2004 (12 mm). Daily values of P were variable with a maximum of 50.4 mm on 26 October 2004.

Maximum air temperature (T_a) was 27.5 °C in August 2003, and minimum values of –4.5 °C were detected in January 2004 (Fig. 1b). E_{pot} followed the same trend as air temperature with a total sum of 1239 mm during the investigation period and 697 mm during the growing season.

Relative humidity showed minimum values at the beginning of August 2003 and July 2004, and there was a longer period of low rH < 65% between mid April and early May 2004.

Soil water contents in the upper soil horizon (0–40 cm; Fig. 1d) were close to the permanent wilting point (PWP = 46.8 mm) in summer and autumn 2003. In October 2003, soil water content increased to values above 100 mm and remained close to saturation (field capacity = 125.6 mm) until April 2004. Soil water content varied between 20 and 105 mm in summer 2004, and reached field capacity again in November 2004.

Transpiration and canopy stomatal conductance

Highest daily *ST* values in 2003 were between 1.2 and 1.3 mm d^{-1} during several days in September and October. *ST* decreased in the middle of October and remained at approximately 0.24 mm d^{-1} between November 2003 and mid-March 2004 (Fig. 2). Highest *ST* during the growing season was slightly above 2 mm d^{-1}, and average transpiration was 1.29 mm d^{-1}. Transpiration was coupled to atmospheric evaporative demand because daily sums of transpiration showed dependency ($R^2 = 0.51$, $P < 0.0001$) on mean daily *VPD* with a regression line characterized

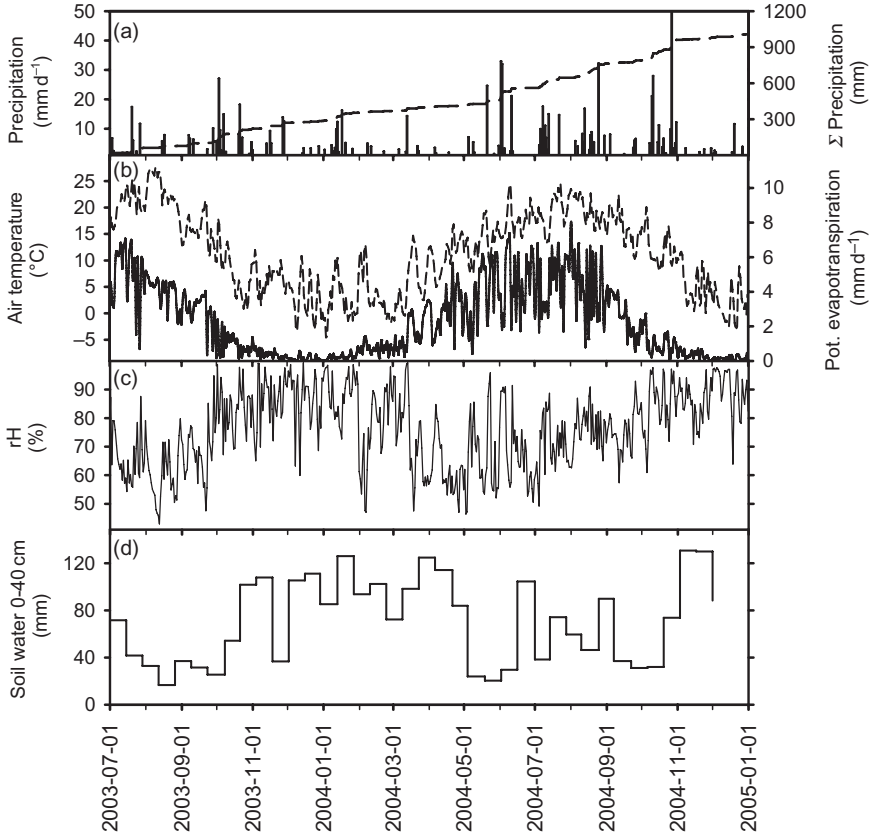

Figure 1. Meteorological and hydrological parameters at the experimental site of Hartheim from July 2003 to December 2004. (a) Daily sum (mm d^{-1}) and cumulative precipitation, (b) daily mean air temperature (°C) (dashed line) and potential evapotranspiration (E_{pot}) (mm d^{-1}) (solid line), (c) relative humidity (rH) (%) and (d) soil water content (mm) in the soil layer 0–40 cm.

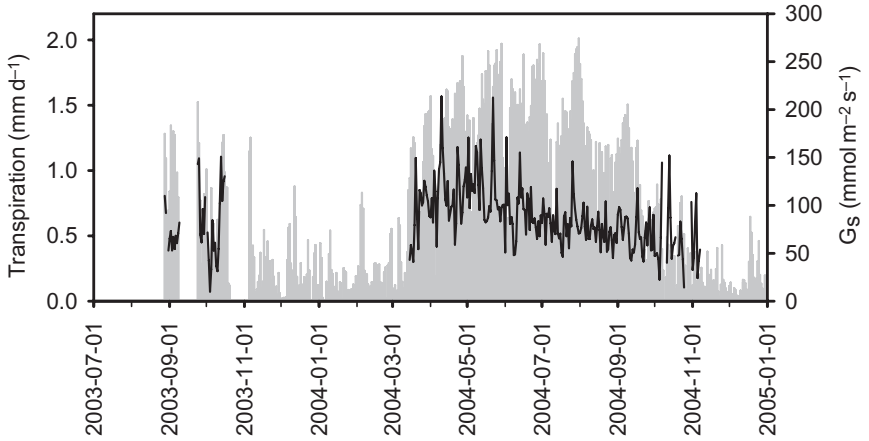

Figure 2. Daily sums of stand transpiration (*ST*) (grey bars) and mean daily canopy stomatal conductance (G_s) (black line) from August 2003 to December 2004. Data gaps for ST in September and October 2003 are due to a data logger malfunction. Using the Penman–Monteith equation canopy stomatal conductance could not be calculated during winter.

by the following linear equation: $ST(\text{mm d}^{-1}) = 0.0012(\text{mm d}^{-1}\ \text{Pa}^{-1}) \times VPD(\text{Pa}) + 0.2338(\text{mm d}^{-1})$. G_s calculated from the simplified Penman–Monteith equation was not realistic between 17 October 2003 and 14 March 2004, and from 8 November to 31 December 2004 because of T_a approaching 0 and rH close to 100% (Keitel *et al.* 2003). Throughout the measurement period, G_s values were eliminated at times when rH exceeded 90% and T_a was below 0 °C. During the periods when calculations were possible, mean daily G_s values were on average 87 mmol m^{-2} s^{-1} and varied between 10 and 214 mmol m^{-2} s^{-1}. Whereas soil water content was not related significantly to G_s ($P = 0.272$), an increase in *VPD* decreased maximum G_s (Fig. 3). Value pairs (G_s/*VPD*) were grouped by the *VPD* value in a way that each step of 100 Pa was categorized as one *VPD* range group. The maximum G_s value from each group was used to complete a regression analysis. The linear equation for the regression line was $G_s(\text{mmol m}^{-2}\ \text{s}^{-1}) = -0.102(\text{mmol m}^{-2}\ \text{s}^{-1}\ \text{Pa}^{-1}) \times VPD(\text{Pa}) + 206(\text{mmol m}^{-2}\ \text{s}^{-1})$ with $R^2 = 0.91$ and $P < 0.0001$.

Isotopic signatures of precipitation, soil water and xylem water

Figure 4a reveals the hydrogen and oxygen isotopic composition of precipitation above the canopy and the monthly mean values weighted for the amount of precipitation. The mean value of the entire measurement period was c. –53‰ (range: –7 to –144‰) for δ^2H and –7.8‰ (range: –1.6 to –19.2‰) for δ^{18}O. A clear annual pattern with isotopically enriched precipitation from April to October and relatively depleted precipitation during winter (November–March) was evident. The weighted monthly means of δ^{18}O and δ^2H were significantly correlated with mean monthly air temperatures [$\delta^{18}\text{O}(‰) = 0.373(‰\ °\text{C}^{-1}) \times \text{T}(°\text{C}) - 11.895(‰)$, $R^2 = 0.6$, $P < 0.01$; $\delta^2\text{H}(‰) = 2.9278(‰\ °\text{C}^{-1}) \times \text{T}(°\text{C}) - 84.505(‰)$, $R^2 = 0.58$, $P < 0.01$].

The annual patterns of δ^{18}O and δ^2H in precipitation could be found again in the isotopic composition of soil water (Fig. 4b,c). However, soil water δ^{18}O and δ^2H were damped in their annual variation compared to precipitation. The scattering of the values around the annual mean value, described by the variance (σ^2), was reduced, for example, for δ^{18}O from 13.5‰ for precipitation to 11.6‰ (soil water 0–2 cm), 8.0‰ (soil water 2–20 cm) and finally to 2.7‰ for soil water in 20–40 cm depth. Xylem δ^{18}O and δ^2H were generally comparable to signatures of middle and lower parts of the soil profile (2–20 cm and 20–40 cm) from March to October. Differing from soil and rain water, there was only a slight decline in δ^{18}O and δ^2H of xylem water during winter. As a consequence, xylem water δ^{18}O and δ^2H signatures were greater when compared with potential source water from November to February.

Δ^{13}C of organic matter

Δ^{13}C of organic matter in trunk and twig phloem varied between 16.5 and 19.3‰, and between 17.3 and 20.2‰, respectively (Fig. 5a). Δ^{13}C of twig phloem was positively

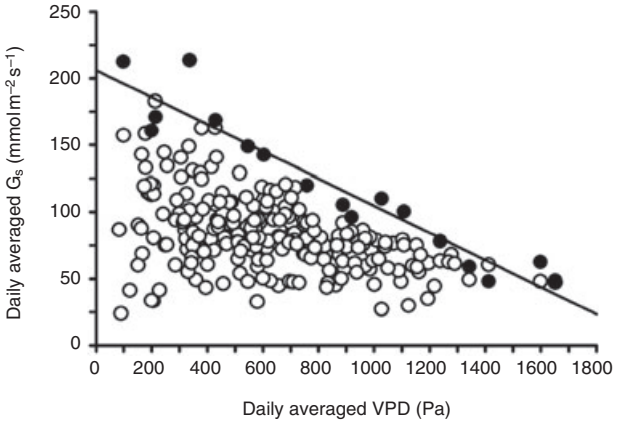

Figure 3. Daily averaged G_s versus daily averaged *VPD* of all days on which G_s could be calculated. The whole range of *VPD* values during the investigation period was divided into *VPD* groups with a range of 100 Pa each. Closed symbols show maximum G_s values for each of the 17 *VPD* groups. Open symbols show all other measured data points.

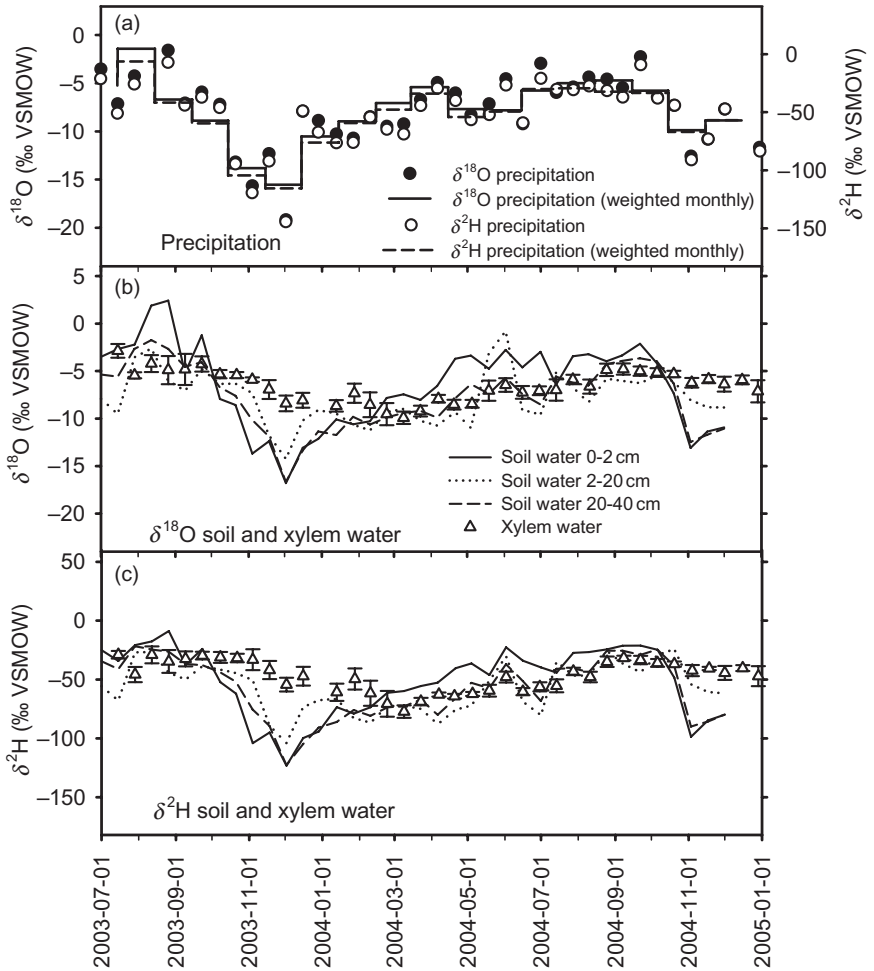

Figure 4. Hydrogen and oxygen isotope composition of precipitation, soil water and xylem water at the experimental site of Hartheim from July 2003 to December 2004. (a) δ^{18}O and δ^{2}H of precipitation, (b) δ^{18}O and (c) δ^{2}H values of soil water at different depths and xylem water. Xylem water values are means ± SD of eight trees.

related to Δ^{13}C of trunk phloem ($R^2 = 0.41$, $P < 0.0001$), and the regression line was below the 1 : 1 line [$\Delta^{13}C_{trunk}(‰) = 0.5859 \times \Delta^{13}C_{twig}(‰) + 6.8137(‰)$]. Applying Student's *t*-test, a significant ($P < 0.001$) difference between average values of trunk phloem Δ^{13}C and twig phloem Δ^{13}C was revealed. Twig phloem organic carbon was depleted in ^{13}C on average by 1.1‰ compared to trunk phloem.

Correlation analysis between Δ^{13}C and meteorological and physiological parameters did not reveal significant relations. Not even moderate correlations could be found between Δ^{13}C and P, *ST*, T_a, rH, *VPD*, global radiation (GR), PAR and G_s, respectively. Even if time lags of 1–7 d between environmental/physiological traits and a potentially detectable influence on phloem Δ^{13}C were assumed (Keitel *et al*. 2003), correlation coefficients were always < 0.5 (data not shown).

Δ^{18}O of organic matter

Δ^{18}O in trunk and twig phloem varied throughout the year with amplitudes of 13.5 and 13.3‰ (Fig. 5b), respectively, which were about 4.5 times as high as those of Δ^{13}C. Trunk phloem Δ^{18}O was strongly related to twig phloem Δ^{18}O [$\Delta^{18}O_{trunk}(‰) = 0.8447 \times \Delta^{18}O_{twig}(‰) + 5.8442(‰)$ $R^2 = 0.81$, $P < 0.0001$). Student's *t*-test showed a significant ($P < 0.001$) difference between average values of trunk phloem Δ^{18}O and twig phloem Δ^{18}O. Trunk phloem signatures were on average 0.8‰ higher throughout the measurement period. From September 2003 to March 2004, Δ^{18}O values exhibited a steady increase of c. 4‰. In April 2004, Δ^{18}O of twig and trunk phloem increased dramatically until they reached values of up to 42‰ in mid May, then they decreased until July 2004. In November and December 2004, values were c. 2‰ lower than in the year before.

Table 1 shows Pearson's correlation coefficients between Δ^{18}O in the twig phloem (Table 1a) or trunk phloem (Table 1b) and various climatic and physiological parameters. Time lags between recorded parameters and corresponding Δ^{18}O were between 0 (parameters recorded on the day of phloem sampling) and 5 d. At a significance level of $P < 0.01$, weak or moderate correlations were found between Δ^{18}O in the twig phloem and *ST*, rH, *VPD*, PAR and GR; the highest correlations with rH, *VPD*, PAR and GR arose with a time lag of 1 d. Correlations of Δ^{18}O from trunk phloem organic matter with the same parameters (Table 1b) depicted similar results: for rH and *VPD*, the highest correlations were obtained with a time lag of 1 d, and there were no clear differences when different

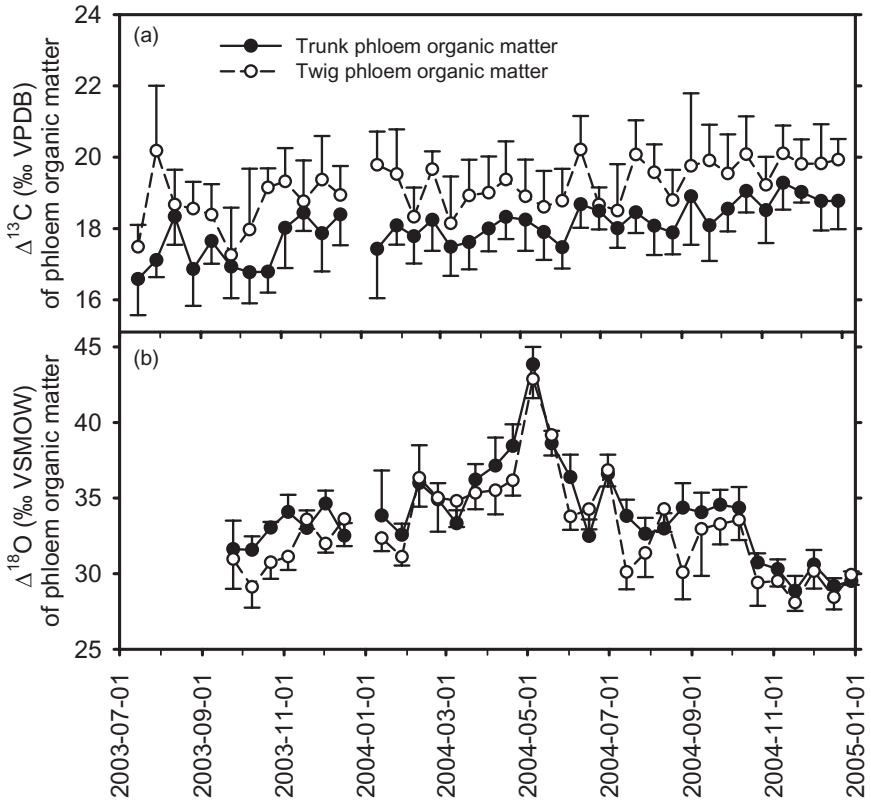

Figure 5. $\Delta^{13}C$ (a) and $\Delta^{18}O$ (b) of organic matter from trunk phloem (closed circles) and twig phloem (open circles). Data shown are mean values + or – SD from eight trees sampled for twig and trunk phloem except for the period from 10 February 2004 to 6 October 2004 where 16 trees were sampled for trunk phloem.

time lags were applied for PAR and GR. With both, $\Delta^{18}O$ of twig and trunk phloem, T_a and P were only weakly correlated. G_s and *ST* showed moderate correlation at time lags > 2 d.

In addition, $\Delta^{18}O_{calc}$ modelled with a scaled effective path length of 0.055 m was related to measured $\Delta^{18}O$ in the twig and trunk phloem. The highest correlation coefficients (0.80, twig phloem; 0.81, stem phloem) were obtained when a time lag of 2 d was taken into account.

Figure 6 shows the quantification of the relationship between measured $\Delta^{18}O$ in the twig and trunk phloem organic matter and $\Delta^{18}O_{calc}$ by means of regression analysis. $\Delta^{18}O_{calc}$ was computed for different L or by not taking into account the Péclet effect for a time lag of 2 d. The highest R^2 values were obtained for twig ($R^2 = 0.64$) and trunk phloem ($R^2 = 0.66$) organic matter, assuming L to be 0.055 m, whereas higher and lower values for L decreased R^2. The linear equations for the regression ($L = 0.055$ m) lines were $\Delta^{18}O_{calc}(‰) = 0.993 \times \Delta^{18}O$ twig phloem (‰) + 1.506(‰) and $\Delta^{18}O_{calc}(‰) = 0.973 \times \Delta^{18}O$ trunk phloem (‰) + 1.326(‰).

The slope of the regression line between $\Delta^{18}O$ and $\Delta^{13}C$ is required for analysis of the conceptual model proposed by Scheidegger *et al*. (2000). Plotting $\Delta^{13}C$ of phloem organic matter versus $\Delta^{18}O$ produced a horizontal scatter cloud with a regression slope not significantly differing from 0 [$\Delta^{13}C(‰) = -0.0814 \times \Delta^{18}O(‰) + 21.333(‰)$; $R^2 = 0.08$, $P = 0.25$].

DISCUSSION

The aim of the present study was to assess the influence of meteorological, pedospheric and physiological factors on the water relations of Scots pine, as characterized by the carbon isotope discrimination ($\Delta^{13}C$) and oxygen isotope enrichment ($\Delta^{18}O$) of newly assimilated organic matter during the entire year. In addition, we aimed to describe the origin of water absorbed by these conifers via a comparison between $\delta^{18}O$ and $\delta^{2}H$ of xylem water with potential source water.

The meteorological conditions at the experimental site during the investigation period were characterized by a very warm and dry summer 2003 and a dry spring period in 2004 followed by a relatively wet summer and autumn in 2004. We were thus able to characterize water relations of Scots pine within a wide range of meteorological conditions.

Transpiration and canopy stomatal conductance

Daily sums of *ST* in Hartheim were comparable with those of 70-year-old *P. sylvestris* trees in a mixed forest stand with similar stand density (Meiresonne *et al*. 2003). Irvine *et al*. (1998) reported a decline in transpiration in Scots pines when volumetric soil water content decreased below 12%. However, the trees investigated in our study did not show

Table 1. Correlation between $\Delta^{18}O$ in organic compounds of the twig (a) or trunk phloem sap (b) and daily means/sums of physiological and meteorological parameters and $\Delta^{18}O_{calc}$ of the day of sampling and 1 to 5 d prior to sampling

(a)	Time lag (d)					
$\Delta^{18}O$ twig versus	0	1	2	3	4	5
G_s	0.32	0.27	0.31	**0.60**	**0.45**	**0.71**
ST	**0.37**	**0.49**	**0.56**	**0.56**	**0.53**	**0.51**
T_a	0.12	0.28	*0.33*	*0.34*	0.27	0.21
rH	–0.33	**–0.63**	**–0.50**	–0.28	**–0.44**	**–0.43**
VPD	0.28	**0.52**	*0.38*	0.23	*0.37*	0.28
P	0.12	–0.29	–0.09	–0.23	–0.08	–0.25
PAR	0.32	**0.53**	**0.46**	**0.42**	**0.52**	*0.36*
GR	0.31	**0.52**	**0.45**	**0.40**	**0.51**	*0.35*
$\Delta^{18}O_{calc}$	**0.62**	**0.72**	**0.80**	**0.49**	0.32	0.25
(b)	Time lag (d)					
$\Delta^{18}O$ trunk versus	0	1	2	3	4	5
*G*s	0.32	0.21	0.29	**0.61**	0.33	**0.69**
ST	**0.40**	**0.42**	**0.55**	**0.59**	**0.57**	**0.67**
T_a	0.19	*0.32*	*0.37*	*0.35*	0.30	0.23
rH	**–0.36**	**–0.58**	**–0.52**	–0.28	*–0.40*	**–0.49**
VPD	0.29	**0.46**	*0.38*	0.21	*0.34*	*0.38*
P	0.22	–0.06	–0.06	–0.15	0.14	–0.25
PAR	0.33	**0.50**	**0.48**	**0.48**	**0.54**	**0.50**
GR	0.33	**0.50**	**0.46**	**0.46**	**0.54**	**0.50**
$\Delta^{18}O_{calc}$	**0.71**	**0.76**	**0.81**	**0.54**	*0.42*	0.33

Values in italics and bold are significant at the 0.05 and 0.01 level, respectively.
G_s, canopy stomatal conductance; *ST*, stand transpiration; T_a, air temperature; rH, relative humidity; *VPD*, water vapour pressure deficit; P, precipitation; PAR, photosynthetic active radiation; GR, global radiation; $\Delta^{18}O_{calc}$, calculated enrichment of phloem organic matter.

reactions of G_s towards decreased soil water contents with values approaching the PWP (PWP = 46.8 mm equals 11.7 volume%). This difference might be explained by the fact that the Hartheim trees were experiencing 'normal' conditions within the period of our measurements because they are adapted to comparable soil water depletion during their individual development. As G_s appears not to be influenced by soil/root-borne signals, transpiration rates should change according to the atmospheric water demand. This is in agreement with the correlation found between *VPD* and *ST*, and the connection between *VPD* and G_s. Whereas transpiration is closely coupled to atmospheric water demand, stomatal conductance is more likely to be controlled physiologically and probably triggered on different control levels. Figure 3 shows that only the maximum of G_s for a given *VPD* range is strongly controlled and limited by atmospheric water demand, whereas no general correlation between G_s and *VPD* can be established. We therefore have to assume that additional factors (which might be, e.g. connected to photosynthetic activity and CO_2 demand) influence G_s.

Isotopic signatures of precipitation, soil water and xylem water

The isotopic composition of precipitation is influenced by different fractionation effects (Moser & Rauert 1980; Kendall & McDonnell 1998). The most important effect at the plot scale is the seasonal effect due to temperature differences with isotopically lighter precipitation in winter months and heavier in summer as shown for the present study in Fig. 4a.

The calculated relationship between weighted monthly mean values of $\delta^{18}O$ in precipitation and air temperature are in line with the worldwide relationship reported by Clark & Fritz (1997) [$\delta^{18}O(‰) = 0.338(‰\ °C^{-1}) \times T(°C) - 11.99(‰)$; $R^2 = 0.815$]. It must, however, be mentioned here that on a larger (or even worldwide) scale, elevation effects also influence $\delta^{18}O$ of precipitation (Barbour, Andrews & Farquhar 2001).

In spring, summer and autumn 2004, both $\delta^{18}O$ and $\delta^{2}H$ of xylem water were in a range comparable to soil water of

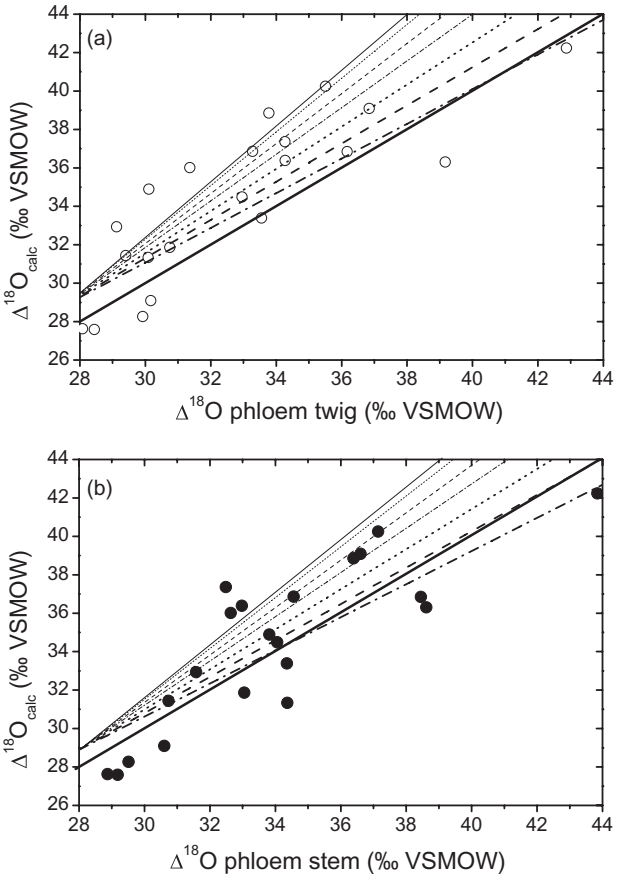

Figure 6. Modelled $\Delta^{18}O$ of phloem organic matter ($\Delta^{18}O_{calc}$) plotted against measured $\Delta^{18}O$ in the twig (a) in trunk phloem (b) organic matter assuming a time lag of 2 d. Regression relations were calculated with $\Delta^{18}O_{calc}$ computed for different values of *L* (thin dotted line, 0.005 m; thin dashed line, 0.015 m; thin dash-dotted line, 0.025 m; bold dotted line, 0.04; bold dashed line, 0.055; bold dash-dotted line, 0.07; thin solid line, Péclet effect not taken into account). The bold solid line denotes the 1:1 line. The circles displayed are the data points for $L = 0.055$.

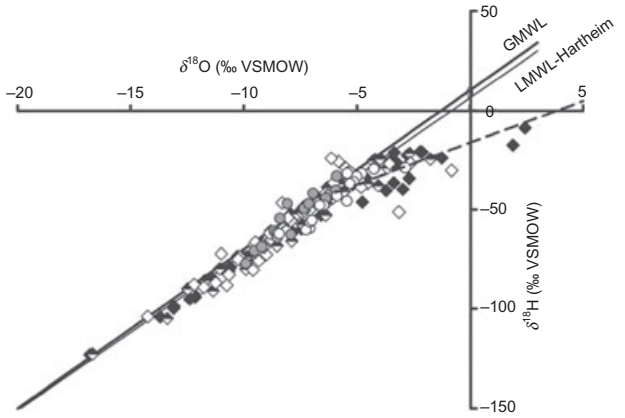

Figure 7. $\delta^{18}O$ versus $\delta^{2}H$ values of soil water at different depths (filled diamonds, 0–2 cm; semi-filled diamonds, 2–20 cm; and open diamonds, 20–40 cm) and xylem water (open circles, summer period July–October 2003 and May–October 2004; grey filled circles, winter period November 2003–April 2004 and November and December 2004). The black dashed line is the regression line between summer values (July–October) of $\delta^{18}O$ and $\delta^{2}H$ in soil water (0–2 cm); GMWL (global meteoric water line): $\delta^{2}H(‰) = 8(‰/‰) \times {}^{18}O(‰) + 10(‰)$; LMWL (local meteoric water line): $\delta^{2}H(‰) = 7.85(‰/‰) \times {}^{18}O(‰) + 6.2(‰)$.

2–20 cm depth. In August 2003 when soil water availability was very low and superficial desiccation of soil has to be assumed, xylem water isotopic composition equalled that of soil water from deeper soil layers, thus confirming our working hypothesis. In contrast to the main growing season, oxygen and deuterium signatures in twig xylem water were higher compared with soil water at all three depths during late autumn and winter of both 2003 and 2004 (Fig. 4b,c).

Two main possible factors can explain this observation. Firstly, if isotopically enriched phloem water (Cernusak *et al.* 2005) is transferred to the xylem, where it mixes with xylem water, this could result in ^{18}O and ^{2}H enrichment during periods of low xylem-flow rates. Evaporative influences would be indicated by a change in the slope of the regression line between $\delta^{18}O$ and $\delta^{2}H$ of xylem samples compared to the local meteoric water line (LMWL) (Gonfiantini 1986).

Figure 7 shows the $\delta^{2}H$ and $\delta^{18}O$ values of the soil water samples at different depths, and the xylem samples from winter and summer. Soil water samples collected in summer from the uppermost soil layer exhibited a lower slope (4.2‰/‰) for the relationship between $\delta^{2}H$ and $\delta^{18}O$ compared with the LMWL as a result of evaporative enrichment. In contrast, xylem samples are well represented by the LMWL. We can therefore exclude any influence of evaporation on the isotopic composition of xylem water.

The second explanation can be related to the time it takes for water to be taken up from the soil and transported to the canopy in winter, when temperature and transpiration rates are low (Figs 1 & 2). In an experiment initiated in June 2004 in which trees of the same stand were irrigated with deuterium-labelled water, a transport time of 5 d from the soil to the twigs was detected when average daily sums of transpiration were 1.18 mm (Wenninger & Brandes, unpublished results). This result is well within the range of water transit times during the growing season for different conifers (Meinzer *et al.* 2006). As daily transpiration sums were 0.2 mm from mid-October 2003 to February 2004 and 0.17 mm from November to December 2004, transport times of 30 and 33 d, respectively, can be assumed. In addition, tree stems can function as water-storage pools with residence times for water of up to 44 d in some conifers (Meinzer *et al.* 2006). During winter, recharge of sapwood water storage over a period of several months has been observed (Waring *et al.* 1979). Thus, it is most likely that the deviation of the isotopic composition of xylem water from soil water in winter is due to slow water transport within the xylem and a mixing of newly absorbed water with isotopically heavier stored water. Water storage in stems may also attenuate the variations in $\delta^{18}O$ and $\delta^{2}H$ in xylem water as compared to soil water during the growing season.

$\Delta^{13}C$ and $\Delta^{18}O$ of organic matter

In general, it is assumed that $\Delta^{13}C$ and $\Delta^{18}O$ of short-turn over carbon pools in leaves or phloem are strongly dependent on c_i/c_a (or on proxies for this value) (for $\Delta^{13}C$) and on G_s (for $\Delta^{18}O$), respectively (Brugnoli *et al.* 1988; Barbour & Farquhar 2000; Barbour *et al.* 2000; Cernusak *et al.* 2003a; Keitel *et al.* 2003; Scartazza *et al.* 2004). Keitel *et al.* (2003) and Gessler *et al.* (2004) showed that G_s could be used as a proxy for c_i/c_a in beech and, consequently, they found a relation between carbon isotope signature of trunk phloem organic matter and G_s, taking into account that transport of newly produced assimilates from the canopy to the stem base took approximately 2 d. In contrast to previous observations, no such correlation was observed for Scots pine trees in this study.

Because of the lack of a direct linear dependency between $\Delta^{13}C$ and environmental or physiological parameters, we may conclude that there is a combined influence resulting from several parameters. In short-term assessments during 9 d within the same stand, Brandes *et al.* (2006) showed that the combined influence of stomatal diffusion and carboxylation determined internal CO_2 concentration and, consequently, photosynthetic carbon isotope discrimination of pine trees. The authors applied a multiple regression model including G_s and assimilation rate as determinants that explained 55% of the variation in c_i/c_a.

According to the conceptual model of Scheidegger *et al.* (2000), this interaction should also be reflected by the relation between $\Delta^{18}O$ and $\Delta^{13}C$. $\Delta^{18}O$ of recently synthesized organic matter should share – according to that model – the dependence on stomatal conductance with $\Delta^{13}C$ but should not be dependent on Rubisco activity (Barbour *et al.* 2004). When a combined influence of stomatal conductance and carboxylation efficiency on carbon isotope discrimination is assumed, plotting $\Delta^{13}C$ (y-axis) versus $\Delta^{18}O$ (x-axis) should result in a regression line with a slope close to 0 as also observed for our data. However, a prerequisite for Scheidegger's model is that stomatal conductance is

strongly guided by rH or *VPD* because e_a/e_i is in fact mainly controlling $\Delta^{18}O$ (Eqn 6). As shown in Fig. 3, only maximum G_s but not G_s in general is controlled by *VPD* in this study. Consequently, we have to assume that the Scheidegger model is not applicable in our case and have to reject the working hypothesis that the relation between $\Delta^{18}O$ and $\Delta^{13}C$ can be used as a tool to differentiate between influences of stomatal diffusion and carboxylation efficiency on $\Delta^{13}C$.

To overcome this constraint, we applied a multiple regression model with G_s and PAR (as a proxy for assimilation rate) as independent variables to explain the variability of $\Delta^{13}C$ during the entire year in a manner comparable to the regression analysis Brandes *et al*. (2006) used for determining c_i/c_a in their short-term experiment. However, such an approach applied on the whole year data set did not reveal any significant results ($P = 0.56$). Keitel *et al*. (2006) hypothesized that the relative influence of G_s and carboxylation on the carbon isotope composition of phloem organic matter from beech varied during the growing season, resulting in stronger dependency of $\delta^{13}C$ on G_s in July compared with September. We can assume that a comparable variable influence of the two determinants on $\Delta^{13}C$ during the year prevents single or multiple regression models from being significant in our study.

$\Delta^{18}O$ in both trunk and twig phloem organic matter was significantly correlated with rH with a time lag of 1 or 2 d, whereas no significant relation was found with G_s. After 3–5 d, a significant correlation with G_s was found, however, (1) without a concomitant correlation with rH and (2) with a positive correlation coefficient. Therefore, and because time lags between atmospheric or physiological conditions during assimilation and the isotopic signal in phloem organic matter collected at different positions including the stem base from beech and pine are reported to lie between 1 and 2 d (Keitel *et al*. 2003; Gessler *et al*. 2004; Brandes *et al*. 2006) we have to assume these relations not to be causal.

On the other hand, the model we applied to predict evaporative ^{18}O enrichment, which takes into account the ^{18}O fractionation associated with the exchange of water between leaves and the atmosphere and with production of organic matter, is able to explain up to 66% of the variation of phloem organic matter during the year with a realistic time lag. In addition, the model shows that besides e_a/e_i, a Péclet effect, mainly driven by transpiration, has to be taken into account when estimating $\Delta^{18}O$ of organic matter. The highest regression coefficient was obtained when a scaled effective path length of 0.055 m was assumed. This value is well within the range observed for other tree species including conifers (Wang *et al*. 1998; Cernusak *et al*. 2005; Farquhar & Cernusak 2005; Pendall, Williams & Leavitt 2005). When comparing modelled $\Delta^{18}O$ to ^{18}O enrichment in both twig and trunk phloem organic matter, a slight offset from the 1:1 line was observed.

This offset and the fact that 34–36% of the variation of $\Delta^{18}O$ cannot be explained by the regression line shown in Fig. 6 may be due to the simplified assumptions made when applying the steady-state model for calculating evaporative enrichment.

For our model application, we assumed atmospheric water vapour to be in isotopic equilibrium with source (xylem) water. In regions not located close to the coast, air moisture is in fact often near equilibrium with local precipitation water (e.g. Dansgaard 1964; Gat 1998). However, at the local scale, significant short-term variation can occur in $\delta^{18}O$ of water vapour mainly associated with changes in wind direction and not related to evapotranspiration (Yakir 1998) and may, thus, temporarily prevent equilibrium conditions. In addition, for our model, we assume isotopic steady-state conditions for leaves. However, stronger changes in rH during the diurnal cycle combined with slow leaf water turnover are known to result in deviations from this steady state (Wang & Yakir 1995) and can potentially lead to an overestimation of $\Delta^{18}O$. Furthermore, it is likely that the term L does not remain constant with environmental conditions, leaf age and time (Pendall *et al*. 2005). Plants can quickly react with reduced tissue hydraulic conductance to drought stress, a reaction that is supposed to be mainly mediated by reduced expression and/or activity of aquaporins (for a recent review, see Tyerman, Niemietz & Bramley 2002).

Moreover, we have to assume that – especially during spring – remobilization of starch from the previous year needles might affect $\Delta^{18}O$ in phloem organic matter. The ^{18}O enrichment of sucrose remobilized from starch is partially uncoupled from current leaf water enrichment. When we assume that part of the oxygen atoms in sucrose generated from starch to be exchangeable with the reaction water via carbonyl hydration (Farquhar *et al*. 1998), we must consider a mixed influence of leaf water conditions during the time of starch storage and current conditions.

Organic matter from twig phloem was consistently depleted in ^{13}C and in ^{18}O compared to that from stem phloem throughout the year. Other studies conducted on Scots pine have hypothesized that the smooth bark surfaces of twigs are capable of photosynthetic CO_2 refixation (Linder & Troeng 1980), whereas this does not occur in trunks that have thick, rough barks. Carbon refixed from respired CO_2 by photosynthetic bark is strongly depleted in ^{13}C compared with the carbon fixed by leaves (Cernusak *et al*. 2001). Additionally, organic matter refixed in the bark where reaction water is not or only slightly enriched and should have a $\Delta^{18}O$ of approximately 27‰ (Cernusak *et al*. 2005), well below the enrichment for sugars fixed in leaves. If the bark phloem exudates from twigs included some sugars formed by refixation, whereas those from the trunks did not (or – when we assume that part of the refixed sugars is transported in basipetal direction – contained a lower amount), this would explain the variation between the two in isotopic composition.

CONCLUSIONS

Comparing the natural isotopic composition of soil and xylem water gives insight into the potential for trees to acquire water from different soil layers depending on water

availability during summer, when transpiration rates are high.

Whereas $\Delta^{13}C$ in phloem was not a direct predictor for recent stomatal reaction, the measurement and modelling of $\Delta^{18}O$ in organic matter combined with the assessment of *ST* and canopy stomatal conductance proved to be effective tools in assessing the water balance of pine trees at that dry site, thus helping to understand and predict potential impacts of climate change on trees and forest ecosystems.

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