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Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient

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ABSTRACT

This study investigated the influence of climate on the carbon isotopic composition ($\delta^{13}C$) and oxygen isotopic enrichment ($\Delta^{18}O$) above the source water of different organic matter pools in European beech. In July and September 2002, $\delta^{13}C$ and $\Delta^{18}O$ were determined in phloem carbohydrates and in bulk foliage of adult beech trees along a transect from central Germany to southern France, where beech reaches its southernmost distributional limit. The data were related to meteorological and physiological parameters. The climate along the transect stretches from temperate [subcontinental (SC)] to submediterranean (SM). Both $\delta^{13}C_{leaf}$ and $\Delta^{18}O_{leaf}$ were representative of site-specific long-term environmental conditions. $\delta^{13}C$ of leaves collected in September was indicative of stomatal conductance, vapour pressure deficit (VPD) and radiation availability of the current growing season. $\Delta^{18}O$ was mainly correlated to mean growing season relative humidity (RH) and VPD. In contrast to the leaves, $\delta^{13}C_{phloem}$ varied considerably between July and September and was well correlated with canopy stomatal conductance (G_s) in a 2 d integral prior to phloem sampling. The relationship between $\delta^{13}C$ and $\Delta^{18}O$ in both leaves and phloem sap points, however, to a combined influence of stomatal conductance and photosynthetic capacity on the variation of $\delta^{13}C$ along the transect. $\Delta^{18}O_{phloem}$ could be described by applying a model that included ^{18}O fractionation associated with water exchange between the leaf and the atmosphere and with the production of organic matter. Hence, isotope signatures can be used as effective tools to assess the water balance of beech, and thus, help predict the effects of climatic change on one of the ecologically and economically most important tree species in Central Europe.

Key-words: xylem sap, water balance; stomatal conductance; steady-state evaporative enrichment.

INTRODUCTION

Since the relationship between $\delta^{13}C$ in plant material and partial pressures of CO_2 in the substomatal cavity and the ambient air (c_i/c_a) was described (Farquhar, O'Leary & Berry 1982), many studies have related $\delta^{13}C$ in organic matter to different environmental parameters (e.g. Stewart *et al*. 1995; Livingston & Spittlehouse 1996; Damesin, Rambal & Joffre 1998; Korol *et al*. 1999; Cernusak *et al*. 2003a).

Various authors have also studied changes in $\delta^{13}C$ along transects of several hundred metres up to thousands of kilometres (Körner, Farquhar & Wong 1991; Sparks & Ehleringer 1997; Schulze *et al*. 1998) and successfully related $\delta^{13}C$ to environmental parameters along these gradients. However, the difficulty in interpreting the results of such studies is in the interplay of various, sometimes counteracting, effects of environmental variables (water, light, etc.) along these transects (Körner *et al*. 1991; Adams & Grierson 2001).

One major reason for these difficulties might be that, until now, ecophysiological studies on $\delta^{13}C$ in plant tissues have mainly focused on the total carbon of leaves and wood (Saurer, Aellen & Siegwolf 1997; Hultine & Marshall 2000; Fotelli *et al*. 2003). Bulk leaf and wood material, however, consist of a complex mixture of carbon with different turnover times and metabolic history. Structural carbon in deciduous trees is likely to carry an isotopic signature influenced by both the present and previous growing seasons, and the effect of carbon storage and remobilization (Kozlowski & Pallardy 2002; Helle & Schleser 2004) with

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related carbon isotope fractionation poses the main difficulty to correlating $\delta^{13}C$ signature in the total carbon fraction with meteorological or pedospheric parameters (Brendel 2001). However, because of the continuous incorporation of newly fixed carbon into structural organic matter, the total organic carbon in leaves and other tissues might – beyond all limitations – still provide a useful integrative measure of environmental influence on c_i/c_a over a longer term (Fotelli *et al*. 2003).

In contrast, the study of $\delta^{13}C$ of recently fixed carbon has been shown to give insight into the prevailing environmental conditions at the time of the sampling (Scartazza *et al*. 2004) and, as a consequence, the isotopic composition of leaf sugars (e.g. Brugnoli *et al*. 1988) and phloem-allocated carbon (e.g. Gessler *et al*. 2001; Cernusak *et al*. 2003a; Keitel *et al*. 2003) is increasingly in the centre of attention. Whereas $\delta^{13}C$ of soluble sugars of the foliage may be only representative of that part of the canopy from where the leaves were harvested, the isotope signatures of phloem-allocated sugars are thought to integrate over the whole canopy.

In a number of recent studies, $\delta^{13}C$ of the phloem sap provided a strong guide to c_i/c_a or stomatal conductance during the current growing season (Pate & Arthur 1998; Cernusak *et al*. 2003a; Keitel *et al*. 2003; Scartazza *et al*. 2004), which makes phloem isotope signatures a powerful tool for studying physiological processes related to current environmental conditions [e.g. vapour pressure deficit (VPD), soil water availability] and eliminates confounding factors of stored and reused carbon – if sampled well after the onset of the growing season.

$\delta^{13}C$ in recently fixed carbon integrates over different time scales depending on the material sampled (leaf sugars, phloem sugars), the position of sampling (twigs in the canopy, trunk phloem) and the species. Phloem sugars collected at the stem base have been shown to be indicative of canopy c_i/c_a or canopy stomatal conductance (G_s) with a time lag of 1 to 3 d depending on the species and tree height (Keitel *et al*. 2003; Gessler, Rennenberg & Keitel 2004b; Barbour *et al*. 2005; Brandes *et al*. 2006).

As both stomatal conductance and photosynthetic capacity have an influence on c_i/c_a, the main determining factor of $\delta^{13}C$ in plant organic matter cannot be easily identified.

The oxygen isotopic composition ($\delta^{18}O$) or the oxygen isotopic enrichment above the source water ($\Delta^{18}O$) of plant organic matter has been shown to provide additional information to separate the effects of stomatal conductance from the influence of changes in photosynthetic capacity on $\delta^{13}C$ (Adams & Grierson 2001), because it shares the dependence on stomatal conductance with the $\delta^{13}C$ signature but is not dependent on ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Barbour & Farquhar 2000; Scheidegger *et al*. 2000). In addition, a general synthetic model to describe $\Delta^{18}O$ of plant organic material has been recently established, which includes ^{18}O fractionation associated with phase transition, diffusion of $H_2{}^{18}O$ through the stomata and boundary layer and production of organic matter as well as the isotopic inhomogeneity of water within the leaf (Farquhar & Lloyd 1993; Barbour & Farquhar 2000; Roden, Lin & Ehleringer 2000; Farquhar & Cernusak 2005).

The combined analysis of carbon and $\delta^{18}O$ in phloem-transported organic matter has been successfully applied for studying the short-term effects of environmental parameters – as modified by aspect and silvicultural treatment – on stomatal conductance in European beech (Keitel *et al*. 2003). In contrast, $\delta^{13}C$ in the total leaf organic carbon did not reflect differences in meteorological and pedospheric parameters between a dry and wet slope of the narrow valley in Southern Germany studied (Gessler *et al*. 2001; Keitel *et al*. 2003).

In general, knowledge of the short-term (days) and longer-term (growing season) response of tree species to the variation in environmental variables like air temperature, soil water availability or radiation interception is crucial in order to estimate the effect of future climatic change on vegetation. Thus, we aimed to test if the combined analysis of carbon and oxygen isotopes in phloem organic matter and bulk leaf material could be used as a tool to characterize the water relations of European beech on a larger area scale. To exclude the influence of variation in $\delta^{18}O$ of the source water, we calculated the ^{18}O enrichment ($\Delta^{18}O$) of organic matter above the source (xylem) water. We designed a transect study with 12 field sites spanning over *c*. 875 km from Central Germany (Thüringen) to Southern France (Alpes de Haute Provence), where the area of distribution of this species is limited by water availability to validate the results obtained in a local climatic gradient (Gessler *et al*. 2001; Keitel *et al*. 2003; Gessler *et al*. 2004b).

Based on the previous observations, our main working hypotheses were:

1. Because of the complex mixture of carbon with different turnover times and metabolic history in bulk leaf organic matter, the $\delta^{13}C$ of this fraction is not representative for changing environmental, and thus, physiological conditions in beech along the selected transect. The assessment of the oxygen isotopic enrichment might, however, provide additional long-term integrating environmental information.
2. Phloem organic matter is a short-term integrating indicator for G_s for the trees from all field sites selected at different times during the growing season. If G_s is not a direct proxy for c_i/c_a under the prevailing environmental conditions (i.e. if additional factors related to Rubisco activity and carboxylation influence internal CO_2 concentration), this will be revealed by the relation between $\delta^{13}C$ and the oxygen isotopic enrichment. In addition, we hypothesized that $\Delta^{18}O$ of the phloem organic matter along the transect can be described by applying the model of Farquhar & Lloyd (1993) that takes into account the isotopic fractionation associated with $H_2{}^{18}O$ diffusion, with phase transition from liquid water to vapour and with the exchange between carbonyl oxygen and water as well as the isotopic inhomogeneity in leaf water.

Figure 1. Field sites (open circles) along the transect from Central Germany to south-eastern France. Germany: 1, Mühlhausen; 2, Gasseldorf; 3, Höglwald; 4 and 5, Tuttlingen; 6, Renquishausen; 7, Dietenbach. Switzerland: 8, Hofstetten, Basel. France: 9, Grenoble; 10–12, Montagne de Lure (MdL), Sisteron. Leaves, phloem sap/exudates and xylem sap were harvested in 2002 along the transect. (Map source: European Commission: http://europa.eu.int).

MATERIALS AND METHODS

Site descriptions and characteristics

Twelve field sites were chosen along a transect from mid-Germany (Mühlhausen, State of Thüringen) to Southern France [Montagne de Lure (MdL), Department des Alpes de Haute Provence] in order to achieve a climate gradient from temperate–semi-(or sub-) continental to submediterranean (SM) (Fig. 1). The distance between the outermost two sites is 875 km. To increase the variability of environmental parameters, we included in this transect (1) the two sites from the local (topo)climatic gradient caused by different exposure [south-west (SW) versus north-east (NE)] as described by Keitel *et al*. (2003); and (2) a height gradient at the southernmost site from 1030 to 1650 m above sea level (a.s.l.) Table 1 depicts the main characteristics of the field sites along the transect.

Mühlhausen (Thüringen, Germany)

The field site east of Mühlhausen is located in the community forest of Mühlhausen, 'Mühlhäuser Hardt'. The forest

Table 1. Field sites along the transect from north-eastern to south-western Europe

Site number	Location	Latitude (°N)	Longitude (°E)	Altitude (m a.s.l)	Climate	T_a^{mean} (°C)	P_{mean} (L m^{-2})	Aspect	Slope (°)	Soil textural class	Soil type	BA (m^2 ha^{-1})
1	Mühlhausen	51.22	10.45	*c.* 400	Temperate (SC)	7.0	700	Plane	–	Loamy clay	Leptosol	35.6
2	Gasseldorf	49.80	11.20	*c.* 300	Temperate (SC)	7.4	903	W to SW	22	Silt	Leptosol	35.2
3	Höglwald	48.30	11.10	540	Temperate (SC)	7.3	800	Plane	–	Loam	Cambisol	34.7
4	Tuttlingen NE	48.00	8.40	750	Temperate (SO)	6.6	856	NE	37	Loam	Leptosol	20
5	Tuttlingen SW	48.00	8.40	750	Temperate (SO)	6.6	856	SW	25	Loam	Leptosol	27
6	Renquishausen	48.08	8.90	890	Temperate (SO)	6.3	910	Plane	–	Loam	Leptosol	28.8
7	Dietenbach	48.00	7.85	280	Temperate (SO)	10.5	950	Plane	–	Sandy silt	Cambisol	32.4
8	Hofstetten	47.47	7.52	500	Temperate (SO)	10.0	885	Plane	–	Silty loam	Leptosol	46.3
9	Grenoble	45.05	5.67	*c.* 450	Temperate (SO)	6.3	1450	W to NW	30	Clay loam	Cambisol	34.5
10	MdL I	44.07	5.83	1030	SM	12.4	736	S	5	Loam	Leptosol	27.4
11	MdL II	44.07	5.83	1250	SM	11.3	736	E	13	Loam	Leptosol	27.4
12	MdL III	44.07	5.83	1650	SM	10.0	736	SW	22	Loam	Leptosol	27.4

Leaves, phloem and xylem sap were harvested twice in 2002. The main site characteristics are listed: latitude and longitude, altitude, climate, long-time average mean annual air temperature (T_a^{mean}) and annual sum of precipitation (P_{mean}), slope angle and aspect, soil textural class, soil type and BA of the stand.
a.s.l., above sea level; SC, subcontinental; SW, south-west; NE, north-east; SO, suboceanic; NW, north-west; SM, submediterranean; S, south; MdL, Montagne de Lure; E, east; BA, basal area.

mainly consists of 100-year-old beech trees (90%) with an average height of 29 m and some admixed species, mainly *Fraxinus excelsior* and *Acer pseudoplatanus*.

Gasseldorf (Bavaria, Germany)

The field site in the Fränkische Schweiz is located on a slope near Gasseldorf close to Forchheim. The forest is between 80 and 100 years old and contains mainly European beech (95%) with some *Picea abies* (5%).

Höglwald (Bavaria, Germany)

The field site Höglwald is situated 50 km west–north-west (NW) of Munich. The soil is very acidic on the surface (pH 2.7–3.6) with the pH increasing to 4.5 at 2 m depth. The whole forest consists of *c*. 50% *Fagus sylvatica* and 50% *P. abies*. We selected a beech-dominated part of the forest (Gessler *et al*. 1998). The average height of beech is 32 m.

Tuttlingen (Baden-Württemberg, Germany)

Tuttlingen-Möhringen is located in southern Germany, about 100 km south–SW from Stuttgart in a low mountain range (Schwäbische Alb) and is the site of a previous study (Keitel *et al*. 2003).

The two sites examined are located on the opposing slopes (not more than 1000 m apart) of a single, narrow valley. One experimental site faces to the NE and the other to the SW. On both sites, European beech is the dominant species making up >90% of the total basal area (BA) of adult trees. The average age of the adult beech trees is 70–80 years with a mean height of between 25 and 27 m (for a detailed site description, see Gessler *et al*. 2004a).

Renquishausen (Baden-Württemberg, Germany)

The forest at this site is dominated by 90- to 115-year-old beech trees (85%) with scattered *F. excelsior*, *Acer campestre*, *Prunus avium* and *Pinus sylvestris* (15%) and has an average height of 28–30 m.

Dietenbach (Baden-Württemberg, Germany)

The field site Dietenbach is located close to Freiburg with a forest consisting of *F. sylvatica* (*c*. 45%), *F. excelsior* (*c*. 12%), *A. pseudoplatanus* (*c*. 12%), *Quercus rubra* (*c*. 11%), *Carpinus betulus* (*c*. 8%) and *Quercus robur* (*c*. 7%).

Hofstetten (Solothurn, Switzerland)

The field site in Hofstetten (close to Basel, Switzerland) is part of the Swiss Canopy Crane Project, University of Basel. The forest is about 120 years old with tree heights between 32 and 38 m. The stand is dominated by *F. sylvatica* L., *Quercus petraea* and *Q. robur* with subdominant representatives of other deciduous species and conifers (Pepin & Körner 2002).

Grenoble (Isère, France)

This field site is located on a slope *c*. 12 km south of Grenoble. The forest is mainly dominated by beech (70%) with scattered *Quercus pubescens*, *A. campestre* and *P. avium*. In the past, the forest was partly used as coppice, so that the trees are of varying age (40–80 years). Hence, only the oldest trees with a height of *c*. 25 m were sampled.

MdL (Alpes de Haute Provence, France)

The field sites are located on a mountain range (MdL) in the Provence close to St. Etienne-les-Orgues near Sisteron. Plant material was harvested from three field sites on the lower, middle and upper slopes of this mountain [referred to as MdL I (low), MdL II (middle) and MdL III (high)]. The 12-m-high, 90-year-old forest was used as coppice until 70 years ago and, then, transferred to high forest. Today, *F. sylvatica* is dominant with some *Q. pubescens* and *Sorbus torminalis*.

Plant material

Phloem, leaves and xylem sap were sampled in 16–21 July and 10–15 September 2002.

Phloem sap collection (bleeding and exudation techniques)

The phloem sap was collected at about 0900 h Central European Time (CET) at breast height of six adult beech trees per site according to the method described by Pate & Arthur (1998) for *Eucalyptus globulus* and by Gessler *et al*. (2001) for European beech. The bark was cut on a length of *c*. 5 cm to the depth of the wood at about 15% to the horizontal, using a single-sided razor blade. The 'bleeding' phloem sap was either collected immediately with a Pasteur pipette after the incision was made or not at all. Because no bled phloem sap could be obtained at the sites at the MdL, a modified phloem exudation technique as described by Gessler *et al*. (2004b) was applied. Small pieces of bark [(*c*. 150 mg fresh weight (FW)] with a diameter of about 1.0 cm were collected from the stem at breast height using a corer, and washed with double-demineralized water. Subsequently, the bark pieces were placed in 6 mL vials containing 2 mL double-demineralized water and left for 5 h. Previous studies (Schneider *et al*. 1996) with beech and spruce showed that contamination of phloem exudates with cellular constituents using the exudation technique can be neglected under the experimental conditions applied. Additionally, Gessler *et al*. (2004b) showed that the isotopic composition of the phloem samples is not affected by the sampling technique, which is a prerequisite for the comparison of the different phloem samples.

Leaf and xylem sap collection

Sun branches were collected from the ground using extendable loppers. Six to eight leaves were harvested evenly

distributed over the branch and frozen in liquid nitrogen until further analysis.

The $\delta^{18}O$ of the source water is one determining factor of the $\delta^{18}O$ in organic material. To rule out the effects of differing $\delta^{18}O$ of the source water, the $\delta^{18}O$ of xylem sap of the trees at the respective sites, which is assumed to carry the same isotopic signature as the source water (Yakir 1998), was determined. For the extraction of xylem sap samples, 1 cm of the bark was removed at the cut end of the branch. A PE-tube was then fitted onto the shoot, which was inserted via a needle into a vial with an airtight seal. The connection to a vacuum pump was made using the same setup (PE-tube and needle inserted into the same vial). A gentle vacuum was applied to the branch, and the leaves were subsequently cut off the end of the branch to facilitate the xylem sap collection. The xylem sap was frozen in liquid nitrogen and stored at –80 °C until analysis.

Transpiration and evaporation

The daily sums of transpiration of beech trees on a per-square-metre-ground-floor basis and ground evaporation were calculated using the water balance model WBS3, a forest-hydrological model that requires daily mean values of air temperature and daily total precipitation as meteorological inputs (Matzarakis *et al*. 2000). The time-independent input variables of the WBS3 simulations are as follows: BA of the stand, the percentage of deciduous and coniferous trees, type of soil, maximum useable water storage capacity of the soil, slope angle, slope direction and geographical latitude. For evaporation, transpiration and interception of forests, validations of WBS3 showed a good agreement between the results from model calculations and measurements for different areas and slopes (Fritsch 1998; Matzarakis *et al*. 2000). Recently, a comparative assessment of the WBS3 and the more complex BROOK90 yielded satisfactory results. Transpiration rates determined with the WBS3 model were also compared with the transpiration rates calculated from sap flow measurements with Granier style sensors. For beech forests located on the two aspects of the experimental site Tuttlingen, we found a reasonably high correlation between daily sums of transpiration calculated from the WBS3 model and those determined from sap flow measurements (Nahm *et al*. 2006).

G_s

Because stomatal conductance is one of the factors determining $\delta^{13}C$ of organic carbon, the mean daily G_s (in millimoles per square metre per second) (Table 2) was calculated using a simplified Penman–Monteith equation as recently applied by Keitel *et al*. (2003) according to Pataki, Oren & Phillips (1998):

$$G_s = \frac{G_c}{\mathrm{LAI}} = \left[\frac{\gamma \lambda P}{\rho(c_p)(\mathrm{VPD})(R)(T)}\right]\left[\frac{\mathrm{TR}}{\mathrm{LAI}}\right]10^3 \quad (1)$$

where G_c is the canopy conductance, γ is the psychrometric constant (kPa K^{-1}), λ is the latent heat of vaporization (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}), VPD is the water vapour pressure deficit (kPa), P is the atmospheric pressure (Pa), R is the gas constant (8.31 m^3 Pa mol^{-1} K^{-1}), T is the air temperature (K), TR is transpiration on a ground area basis as obtained from the WBS3 model output (L m^{-2} d^{-1}) and converted to the unit kg m^{-2} s^{-1} and LAI is the leaf area index of beech (m^2 m^{-2}). This calculation assumes that canopy aerodynamic conductance is much larger than G_s as reported by Whitehead & Jarvis (1981) (i.e. aerodynamic resistance can be neglected compared to stomatal resistance).

Table 2. Means and sums of meteorological and physiological parameters characterizing the field sites during the growing season 2002 (May to September)

Site number	Location	T_a (°C)	RH (%)	R (L m^{-2})	VPD (kPa)	Sun (h)	TR (L m^{-2})	EV (L m^{-2})	G_S (mmol m^{-2} s^{-1})
1	Mühlhausen	15.7	72.6	312	0.51		208.7	40.23	38
2	Gasseldorf	15.5	75.7	449	0.46		212.05	77.82	35
3	Höglwald	16.0	77.1	631	0.45		301.18	31.49	55
4	Tuttlingen NE	13.6	80.2	551	0.40		235.33	57.86	33
5	Tuttlingen SW	13.9	80.1	551	0.41		206.42	69.41	40
6	Renquishausen	14.3	81.1	551	0.43		176.34	43.31	64
7	Dietenbach	18.2	67.5	563	0.76		239.36	56.14	34
8	Hofstetten	17.0	76.1	526	0.49	892	265.47	44.92	60
9	Grenoble	18.7	69.7	478	0.69	1028	182.64	88.59	20
10	MdL I	19.4	60.9	453	0.93	1410	175.27	74.96	19
11	MdL II	18.7	63.2	453	0.85	1410	149.83	99.33	16
12	MdL III	17.8	64.9	453	0.68	1410	138.54	109.28	15

T_a, mean air temperature; RH, mean relative humidity; R, sum of rainfall; VPD, mean vapour pressure deficit; Sun, sum of sunshine hours; TR, sum of transpiration; EV, sum of evaporation; G_s, mean canopy stomatal conductance; NE, north-east; SW, south-west; MdL, Montagne de Lure.

Meteorological parameters

The meteorological parameters [daily sum of precipitation, mean daily temperature, mean daily relative humidity (RH), sunshine hours] were provided by the 'Deutsche Wetterdienst' (DWD), the Meteorological Institute of the University of Freiburg, Meteo Suisse and Meteo France from stations at or nearby the mentioned field sites.

Determination of sugars in the phloem sap

For the determination of soluble carbohydrates, 5–10 μL of phloem sap was diluted 1:1000 and phloem exudates 1:50 with demineralized water. Aliquots of 100 μL were injected into a high-performance liquid chromatography (HPLC) system (DX 500; Dionex, Idstein, Germany) as described by Gessler *et al*. (2002). Separation of sugars was achieved on a CarboPac 1 separation column (250×4.1 mm; Dionex) with 36 mM NaOH as an eluent at a flow rate of 1 mL min^{-1}. Carbohydrates were measured by means of a pulsed amperometric detector equipped with an Au working electrode (DX 500; Dionex). Individual carbohydrates that eluted 8–16 min after injection were identified and quantified by internal and external standards.

Isotope analysis and definitions

The leaf material was oven-dried for 3 d at 65 °C, ground and homogenized with a ball mill to a fine powder, and aliquots were transferred into tin ($\delta^{13}C$ analysis) or silver ($\delta^{18}O$ analysis) capsules. The phloem sap was pipetted into the tin ($\delta^{13}C$ analysis) or silver ($\delta^{18}O$ analysis) capsules and oven-dried for 60 min at 65 °C. Dried samples of 'phloem-bleeding sap' (5–10 μL), 'phloem exudates' (30–100 μL) and leaves (1–2 mg) were combusted in an elemental analyser (NA 2500; CE Instruments, Milan, Italy) for $\delta^{13}C$ and in a high-temperature conversion/elemental analyser (TC/EA Finnigan 165 MAT GmbH, Bremen, Germany) for $\delta^{18}O$ analyses, both coupled to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT GmbH) by a Conflo II interface (Finnigan MAT GmbH) as described in detail by Gessler *et al*. (2004b). The tin capsules contained 0.5 mg of chromosorb (Chromosorb 102, 60–80 mesh; Sigma-Aldrich Chemie GmbH, Munich, Germany) to allow the proper combustion of phloem organic matter in the elemental analyser. For the analysis of $\delta^{18}O$ in the xylem sap, an aliquot (1 μL) was pipetted into silver capsules and sealed. As evaporation enriches the water with the heavier oxygen isotope, the samples were prepared and dropped into the autosampler only a short time before pyrolysis of the sample. 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. Isotopic compositions ($\delta^{13}C$, $\delta^{18}O$) are expressed as deviations from these standards:

$$\delta = \frac{R_{\text{plant material}}}{R_{\text{standard}}} - 1 \tag{2}$$

where R is the isotope ratio ($^{13}C/^{12}C$ or $^{18}O/^{16}O$) of the plant material and the standard, respectively.

The $\delta^{18}O$ of organic matter in leaves and phloem was expressed as an enrichment above the source water ($\Delta^{18}O$):

$$\Delta^{18}O = \frac{\delta^{18}O_{\text{plant material}} - \delta^{18}O_{\text{source water}}}{1 + \delta^{18}O_{\text{source water}}} \tag{3}$$

The $\delta^{18}O$ of xylem water was regarded as a representative of the source water $\delta^{18}O$. For the calculation of $\Delta^{18}O$ of phloem organic matter at both time points and of leaf organic matter in July, the xylem $\delta^{18}O$ at the respective time points was chosen. For the calculation of the leaf $\Delta^{18}O$ in September, a mean value of $\delta^{18}O$ of the xylem water harvested in July and September was calculated for each site.

Oxygen isotopic theory and calculations

Steady-state enrichment of water at the evaporative site of the leaf ($\Delta^{18}O_e$) can be described according to Dongmann *et al*. (1974), where ε^+ is the equilibrium fractionation (‰) between liquid water and water vapour, ε_k is the kinetic fractionation (‰) during water vapour diffusion (Farquhar *et al*. 1989), $\Delta^{18}O_v$ is the isotopic difference (according to Eqn 3) of water vapour in the atmosphere compared to the source water and e_a/e_i is the ratio of ambient to intercellular water vapour concentration.

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)\frac{e_a}{e_i} \tag{4}$$

ε^+ can be calculated according to Bottinga & Craig (1969) from a regression equation relating it to leaf temperature, T (in kelvin):

$$\varepsilon^+ = 2.644 - 3.206\left(\frac{10^3}{T}\right) + 1.534\left(\frac{10^6}{T^2}\right) \tag{5}$$

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

$$\varepsilon_k = \frac{32r_s + 21r_b}{r_s + r_b} \tag{6}$$

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

The average lamina mesophyll water is, however, supposed to be less enriched than the water at the evaporative sites. In general, this difference is mainly supposed to be dependent on: (1) the diffusion of ^{18}O-enriched water away from the sites of evaporation; and (2) the convection of unenriched xylem water via the transpiration stream in the opposite direction.

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 & Lloyd 1993).

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

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

According to Barbour & Farquhar (2000), the enrichment of ^{18}O in organic matter above the source water in a particular tissue ($\Delta^{18}O_p$) depends on the $\Delta^{18}O$ of the average lamina mesophyll water ($\Delta^{18}O_L$) on the proportion of oxygen atoms exchanging with water during the synthesis of cellulose (p_{ex}), the portion of unenriched source water in the tissue (p_x) and on the equilibrium fractionation between organic oxygen and water (ε_{wc}; +27‰) (Yakir & Deniro 1990):

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

p_{ex} can be calculated according to Barbour & Farquhar (2000) where y (Eqn 9) is the proportion of hexose phosphates that cycle through to triose phosphates before being incorporated into cellulose,

$$p_{ex} = 0.2 + y\left(0.6 + \frac{0.2}{2-y}\right) \quad (9)$$

A detailed derivation of the equation is given by Barbour & Farquhar (2000).

p_x is calculated according to Cernusak, Wong & Farquhar (2003b) where ρ is the proportion of ^{18}O-enriched water coming into the cell either by diffusion or phloem transport as opposed to not enriched xylem-transported water (equals source water). $\Delta^{18}O_{pw}$ is the enrichment of phloem or diffusive water above the source water.

$$p_x = 1 - \rho\left(\frac{\Delta^{18}O_{pw}}{\Delta^{18}O_L}\right) \quad (10)$$

We applied Eqn 8 for predicting the $\Delta^{18}O$ of phloem organic matter for the 12 field sites from the mean daytime values of environmental and physiological parameters determined or modelled for the 2 d period preceding the phloem sap sampling in July and September.

For this calculation, we made the following assumptions: water vapour was in isotopic equilibrium with the source water, as often assumed for European summer conditions (Förstel & Hützen 1983; Saurer *et al*. 1997). In that case, $\Delta^{18}O_v$ in Eqn 4 equals $-\varepsilon^+$. The difference between air temperature and leaf temperature was calculated according to a leaf energy balance model described in detail by Barbour *et al*. (2000a) and Cernusak *et al*. (2003a) assuming an average surface area of a single leaf of 10 cm^2. The isothermal net radiation as an input parameter for the energy balance model was estimated as described by Barbour *et al*. (2000a) assuming canopy-averaged photosynthetically active radiation (PAR) to amount to 600 μmol m^{-2} s^{-1}. The internal water vapour concentration (e_i) was assumed to equal the leaf temperature-dependent saturation concentration.

For an effective length, we chose $L = 0.015$ m according to Farquhar & Cernusak (2005). As already mentioned, we assumed aerodynamic resistance << stomatal resistance as reported by Whitehead & Jarvis (1981) when calculating ε_k from Eqn 6. For phloem organic matter that originates directly from sugars produced in the leaves, we assumed p_x (Eqn 10) to be 0 as the oxygen isotopic enrichment of the reaction water (leaf water) equals Δ_L.

We used Eqn 8 to relate $\Delta^{18}O$ of total leaf ($\Delta^{18}O_p$ in Eqn 8) to phloem organic matter by substituting $\Delta^{18}O_L$ by $\Delta^{18}O$ of phloem organic matter minus ε_{wc} as previously done by Cernusak, Farquhar & Pate (2005). This is justified as phloem organic matter reflects $\Delta^{18}O$ of the water in which it was formed plus ε_{wc}. We assumed here p_{ex} to be 0.4 as previously observed for the leaves of *E. globulus* and considered to be close to the mean value of p_{ex} from all literature data (Cernusak *et al*. 2005). p_x was assumed to be 1, as the major part of the structural organic matter was produced during leaf formation with non-enriched water.

Statistics

Statistical analyses were conducted using SPSS 10.05 (SPSS, Chicago, IL, USA) and NCSS 2004 (NCSS, Kaysville, UT, USA). Correlations between $\delta^{13}C$ and environmental parameters were calculated using the bivariate correlation procedure. Regression lines (e.g. between $\delta^{13}C$ and G_s and $\delta^{13}C$ and $\Delta^{18}O$) were determined by linear or non-linear regression analysis. Differences in isotopic signatures between sites were calculated, applying one-way analysis of variance (ANOVA) procedures, differences between two times for a particular site were assessed with Student's *t*-test.

RESULTS

Climate, transpiration, evaporation and stomatal conductance

The mean air temperature (T_a) during the growing season 2002 differed by 5.8° between the warmest and the coldest sites (Table 2). RH showed a comparable but inverse pattern along the transect with the lowest mean values at the MdL and the highest values at the Renquishausen and the two Tuttlingen sites. As a consequence, VPD calculated from T_a and RH showed maxima and minima comparable to T_a. The lowest rainfall during the 2002 growing season was observed at the northernmost site (Mühlhausen), whereas at the Höglwald site (at a distance of *c*. 320 km) more than 300 mm of additional precipitation was detected. Sunshine hours, which were only available for the sites in France and Switzerland, increased in the southern direction.

The modelled sums of transpiration were highest at the Höglwald site and below 200 mm at the Renquishausen, Grenoble and MdL sites. Evaporation was calculated to amount between *c*. 40 mm (Mühlhausen) and 109 mm (MdL III). The mean stomatal conductance during the growing season ranged from 15 to 64 mmol m^{-2} s^{-1}. The

lowest values were detected at the French sites, whereas the highest conductance occurred at the Höglwald, Hofstetten and Renquishausen sites.

Carbon isotope signatures in total leaf and in phloem organic matter and phloem sugar concentrations

Significant differences in $\delta^{13}C$ of the total foliar organic matter ($\delta^{13}C_{leaf}$) were observed between the sites of the transect at both sampling times (Fig. 2a), and values varied from –26.5 to –32‰. For a particular site, no significant intra-annual differences could be observed. Hence, the $\delta^{13}C_{leaf}$ values from both dates were highly correlated ($R^2 = 0.90$, $P < 0.001$). In both months, the foliage was most depleted in ^{13}C at the Hofstetten field site amounting to –31.73‰ in July and –32.06‰ in September. The most positive $\delta^{13}C_{leaf}$ values were found in leaves from the southernmost location at the MdL in France (*c*. –26.25 to –28.75‰).

In contrast to leaves, $\delta^{13}C$ in phloem organic matter ($\delta^{13}C_{phloem}$) not only varied among sites, but also showed strong and significant differences between the two different sampling times (Fig. 2b). In the northern part of the transect, phloem organic matter was enriched in ^{13}C in September as compared to July, whereas in the southern part either no significant change or depletion (Hofstetten) was detected. In July, phloem organic matter was most ^{13}C enriched at the Grenoble, Hofstetten and MdL sites at the lowest elevation. In September, the highest $\delta^{13}C_{phloem}$ values were observed at the two northernmost sites of the transect, and the strongest ^{13}C depletion occurred in the phloem of beech from Höglwald and the medium elevation at the MdL. In contrast to the $\delta^{13}C_{leaf}$ signatures, the Hofstetten site was not found to have significantly more negative values in phloem organic matter than the other sites. Carbohydrate concentrations in the phloem sap were analysed for the nine sites where bleeding sap could be obtained (Fig. 2c). Sucrose was found to be the dominant sugar in the collected phloem sap, and made up between 91.6 and 99.9% of total sugar in June and September 2002. Sugar concentrations mainly varied between *c*. 430 and 630 mM. Site-dependent patterns of $\delta^{13}C_{phloem}$ and sugar concentrations were not synchronous, and hence, no significant regression relation was obtained between the two parameters ($R^2 = 0.06$, $P > 0.05$).

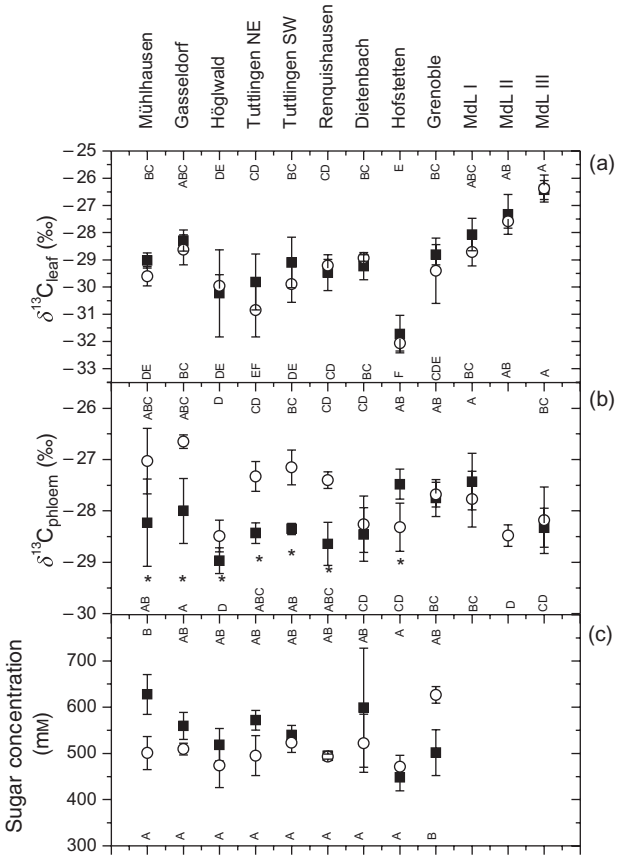

Figure 2. Carbon isotope signatures of total organic matter in the leaves (a) of phloem-transported carbon (b) and phloem sugar concentrations (c) from beech trees of 12 sites along the transect in July (black squares) and September (white circles) 2002. Data shown are mean values ± SD from measurements of six trees per site. Asterisks indicate significant differences between July and September measurements for a particular site. Letters A to F indicate significant differences between sites as determined by one-way analysis of variance (July at the top, September at the bottom of a layer). NE, north-east; SW, south-west; MdL, Montagne de Lure.

Oxygen isotope signatures in xylem water and in total leaf and phloem organic matter

$\delta^{18}O$ in xylem water ($\delta^{18}O_{xylem}$) varied significantly between the different sites and, for a particular site, between the two measurement dates (Fig. 3a). In both months, the strongest ^{18}O depletion was observed at the northernmost site, whereas the sites in Switzerland and France generally displayed the highest $\delta^{18}O_{xylem}$. At both dates, there was a significant latitudinal and longitudinal influence on $\delta^{18}O_{xylem}$ with signatures decreasing by between 0.7 and 1.1‰ with increasing eastern longitude and by 0.7–0.9‰ with increasing northern latitude. Because of these differences in source water, the $\delta^{18}O$ of leaf and phloem organic matter was calculated as enrichment above the xylem water ($\Delta^{18}O_{phloem}$, $\Delta^{18}O_{leaf}$). In contrast to carbon isotopes in leaves, there were not only significant differences in $\Delta^{18}O_{leaf}$ between sites, but also intra-annual differences for a particular site (Fig. 3b). On all sites, $\Delta^{18}O_{leaf}$ increased from July to September by between 2.8 and 7.2‰. The ranking of sites according to their $\Delta^{18}O$ values did, however, not change: in both months, the highest ^{18}O enrichment was observed with beech from the French sites and from Mühlhausen and Höglwald. Thus, foliar $\delta^{18}O$ showed strong correlation between the values from July and September ($R^2 = 0.76$, $P < 0.001$) despite the intra-annual increase.

$\Delta^{18}O_{phloem}$ varied between sites and sampling time (Fig. 3c). In both months, the highest ^{18}O enrichment was observed at the northernmost site, whereas $\Delta^{18}O_{phloem}$ was

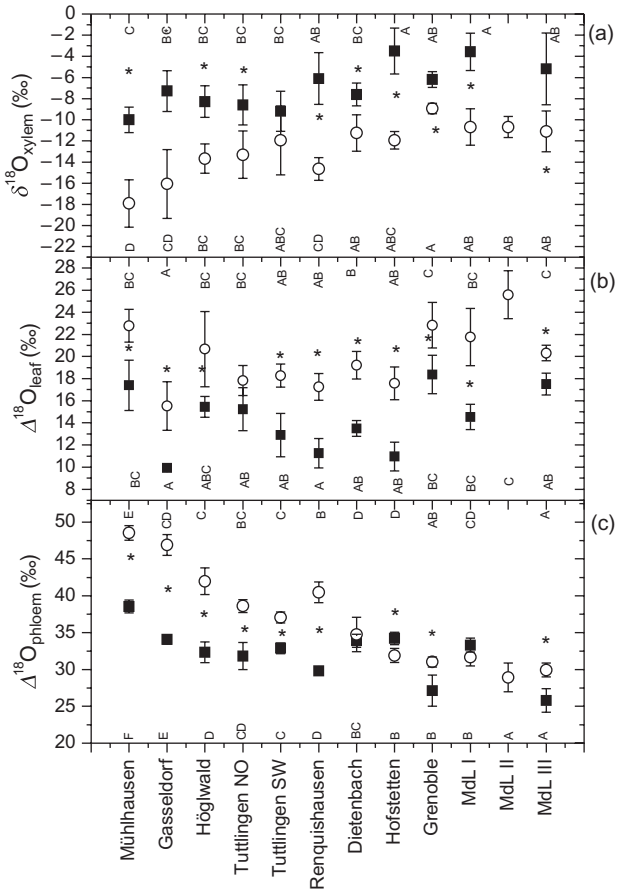

Figure 3. Oxygen isotope signatures of xylem water (a) given in oxygen isotopic composition ($\delta^{18}O$) and in total organic matter in the leaves (b) and of phloem-transported carbon (c) both given as enrichment above the source water ($\Delta^{18}O$) from beech trees of 12 sites along the transect in July (black squares) and September (white circles) 2002. Data shown are mean values ± SD from measurements of six trees per site. Asterisks indicate significant differences between July and September measurements for a particular site. Letters A to F indicate significant differences between sites as determined by one-way analysis of variance (July at the top, September at the bottom of a layer). NE, north-east; SW, south-west; MdL, Montagne de Lure.

always lowest at one of the French sites. In contrast to foliage, where a general increase in $\Delta^{18}O_{leaf}$ between July and September was observed for all sites, the intra-annual variation in phloem $\delta^{18}O$ was greater. Whereas there was a significant increase in $\delta^{18}O_{phloem}$ at most of the sites from July to September, no change was observed at Dietenbach and the lowest site at the MdL (MdL I) and enrichment even decreased at Hofstetten.

Correlation between isotope signatures and climatic and physiological parameters

Both $\delta^{13}C_{leaf}$ and $\Delta^{18}O_{leaf}$ showed site-typical patterns along the transect, even though $\Delta^{18}O_{leaf}$ increased during the growing season. Thus, we assumed site-specific factors being responsible for this observation and related the isotopic signatures in total organic matter from leaves collected in September to altitude, latitude, longitude and to mean T_a, RH, sunshine hours, transpiration, evaporation and stomatal conductance of the current (2002) growing season (Table 3). Neither $\delta^{13}C_{leaf}$ nor $\Delta^{18}O_{leaf}$ was significantly related to latitude or longitude. $\delta^{13}C_{leaf}$ was positively related to altitude with an increase in $\delta^{13}C$ of 0.22‰ per 100 m. Altitude strongly influenced sunshine hours ($R = 0.87$, $P = 0.05$) and transpiration ($R = -0.68$, $P = 0.01$) all of which correlated significantly with $\delta^{13}C_{leaf}$. In addition, $\delta^{13}C_{leaf}$ showed significant negative relation to RH and G_s. $\Delta^{18}O_{leaf}$ correlated significantly with T_a, RH and VPD, whereas no correlation was observed between $\Delta^{18}O_{leaf}$ and transpiration. The linear regression line between RH and $\Delta^{18}O_{leaf}$ had a slope of –0.19‰/%.

In contrast to foliar isotopic composition, $\delta^{13}C_{phloem}$ and $\Delta^{18}O_{phloem}$ values were highly variable among sites and with time, suggesting short-term responses to environmental/physiological parameters.

Correlation analyses were performed for the means or sums of T_a, RH, sunshine hours, transpiration, evaporation and stomatal conductance 1–22 d prior to the time of phloem sap collection (from July and September) according to Keitel *et al.* (2003). G_s was found to be the main influence on $\delta^{13}C_{phloem}$ along the transect (Table 4a). The highest correlation coefficient was determined for a time

Table 3. Correlation between $\delta^{13}C$ and $\delta^{18}O$ of total leaf organic matter (September values) and geographical, meteorological and physiological parameters

	Altitude	Latitude	Longitude	T_a	RH	VPD	Sun	TR	EV	G_s	R
$\delta^{13}C$	**0.63**	–0.46	–0.34	0.43	**–0.64**	0.55	**0.88**	**–0.75**	**0.72**	**–0.65**	–0.34
	0.03	*0.13*	*0.28*	*0.16*	*0.02*	*0.06*	*0.04*	*< 0.01*	*< 0.01*	*0.02*	*0.26*
$\Delta^{18}O$	0.32	–0.47	–0.47	**0.63**	**–0.72**	**0.70**	0.54	0.07	0.33	**–0.57**	–0.41
	0.31	*0.12*	*0.12*	*0.03*	*0.01*	*0.02*	*0.34*	*0.23*	*0.28*	*0.05*	*0.18*

For correlation analysis, the mean values or sums of T_a, RH, VPD, Sun, TR, EV, G_s and R as displayed in Table 2 were used. In the first row, Pearson's correlation coefficients are displayed; in the second row (in italics), significance levels are given. Bold figures indicate Pearson's correlation to be significant.

T_a, mean air temperature; RH, mean relative humidity; VPD, mean vapour pressure deficit; Sun, sum of sunshine hours; TR, sum of transpiration; EV, sum of evaporation; G_s, mean canopy stomatal conductance; R, sum of rainfall.

Table 4. Correlation between (a) $\delta^{13}C$ and (b) $\Delta^{18}O$ in the phloem and physiological and meteorological parameters along the transect in July and September 2002, averaged over different time spans prior to phloem sap collection

(a)

	Days							
$\delta^{13}C$ versus	1	2	3	4	5	6	7	22
G_s	–0.40	**–0.55****	–0.52*	–0.52*	–0.46*	–0.35	–0.30	–0.23
TR	–0.46*	–0.46*	–0.48*	–0.49*	–0.50*	–0.51*	–0.50*	–0.45*
EV	0.24	0.34	0.41	0.40	0.36	0.34	0.33	0.37
T_a	–0.11	–0.11	–0.10	–0.11	–0.13	–0.17	–0.20	–0.06
RH	–0.12	–0.28	–0.25	–0.22	–0.16	–0.08	–0.02	0.23
VPD	0.08	0.16	0.20	0.20	0.14	0.07	0.01	–0.20
R	0.38	0.34	0.23	0.11	0.17	0.15	0.17	0.05
Sun	0.10	0.51	0.54	0.51	0.39	0.36	0.31	0.18

(b)

	Days							
$\Delta^{18}O$ versus	1	2	3	4	5	6	7	22
G_s	0.22	0.12	0.08	0.06	0.04	0.02	0.02	0.03
T_a	0.01	0.10	0.11	0.12	0.13	0.15	0.14	0.22
RH	0.06	0.02	0.01	–0.05	–0.06	–0.09	–0.06	–0.04
R	–0.33	–0.24	–0.24	–0.23	–0.25	–0.26	–0.24	0.08
TR	–0.05	0.00	0.01	0.02	0.02	0.02	0.01	0.03
VPD	0.01	0.05	0.04	0.07	0.07	0.10	0.07	0.10
EV	0.03	–0.01	0.01	0.04	0.08	0.1	0.08	0.04
Sun	0.33	0.02	–0.06	0.07	0.18	0.17	0.11	–0.03

*$P < 0.05$, **$P < 0.01$.

The table shows Pearson's correlation coefficients for bivariate correlation analysis between $\delta^{13}C$/ $\delta^{18}O$ of all phloem samples collected at 11 sampling locations in July plus 12 sampling locations in September and the respective environmental parameters as mean values of 1–22 d prior to the phloem sap collection; $n = 23$ (exception of sunshine hours only for sites in Switzerland and France, $n = 5$).

G_s, mean canopy stomatal conductance; TR, sum of transpiration; EV, sum of evaporation; T_a, mean air temperature; RH, mean relative humidity; VPD, mean vapour pressure deficit; R, sum of rainfall; Sun, sum of sunshine hours.

integral of 2 d prior to phloem sap collection. For longer time integrals, the correlation coefficient decreased steadily and was no longer significant for time integrals longer than 5 d. The only other parameter significantly correlated with $\delta^{13}C_{phloem}$ was found to be transpiration. However, correlation coefficients were weaker and more or less constant over the observed time period.

Figure 4 shows the regression line between G_s with a time integral of 2 d and $\delta^{13}C_{phloem}$ with a slope of –0.01‰ $mmol^{-1}\ m^2\ s$. If July and September were analysed separately, a significant dependency between $\delta^{13}C_{phloem}$ and G_s was found in July ($R^2 = 0.39$, $P < 0.05$) but not in September ($R^2 = 0.11$, $P = 0.28$).

$\Delta^{18}O_{phloem}$ was not significantly related to any of the meteorological or physiological parameters tested (Table 4b).

Correlation between modelled and measured phloem $\Delta^{18}O$

According to Eqn 8, we calculated the $\Delta^{18}O$ of phloem organic matter as expected from the mean daytime climatic conditions and physiological parameters during the 2 d before the July and September phloem sampling. The squares and circles in Fig. 5 show the calculated values assuming a scaled effective path length, L, of 15 mm plotted against the measured phloem $\Delta^{18}O$.

For four points in July – highlighted as black/white squares – the calculated values differ strongly from the measured ones. These four points represent the four southernmost sites assessed in July (Hofstetten, Grenoble and MdL I and III), which were then characterized by RH values <70%, whereas for all other sites in July and all sites examined in September, the RH ranged between 71 and 93%. When these four data points were excluded from the regression analysis, a line with a slope close to the 1:1 line (1.28‰/‰; $R = 0.62$; $P > 0.01$) was produced. Values for L in literature range broadly between 4 and 54 mm (Cernusak *et al.* 2005). Thus, we subjected $\Delta^{18}O$ calculated (1) with L values of 4 and 54 mm; and (2) disregarding leaf water isotopic inhomogeneities because of the Peclét effect (C–G in Fig. 5) to a regression analysis with measured values. The latter model (2) explained best the relation between calculated and measured $\Delta^{18}O$ in phloem organic matter yielding an R of 0.65 ($P < 0.01$).

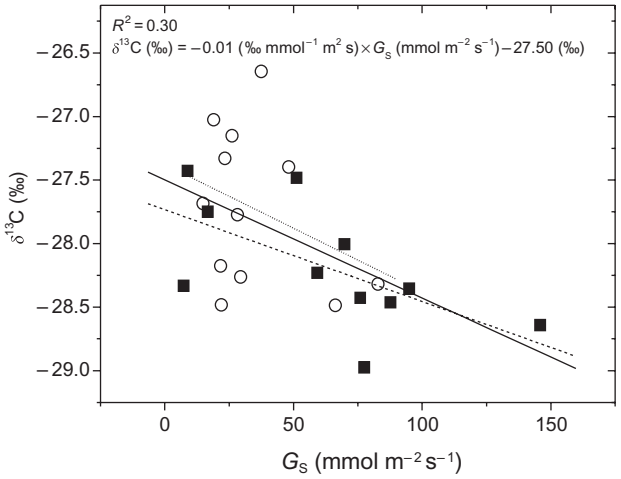

Figure 4. Regression between carbon isotopic composition (δ^{13}C) in phloem sap and the average daily mean stomatal conductance (G_s) over a period of 2 d prior to sampling along the transect from mid-Germany to southern France. The regression analysis (solid line; $R^2 = 0.30$, $P < 0.01$) includes measurements from 11 sampling locations in July 2002 (black squares) and 12 sampling locations in September 2002 (white circles). The dashed line shows the result of a separate regression analysis for July ($R^2 = 0.39$, $P < 0.05$) and the dotted line for September ($R^2 = 0.11$, $P = 0.28$). All data points shown are mean values from six trees per site.

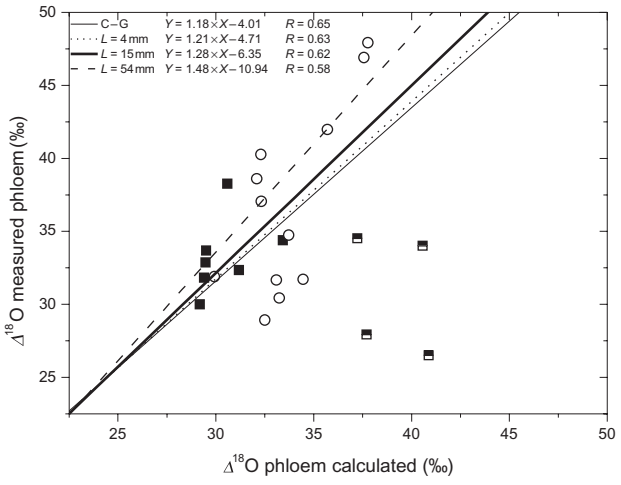

Figure 5. Regression between modelled and measured phloem organic matter oxygen isotopic enrichment (Δ^{18}O). According to Eqns 7 and 8, Δ^{18}O was predicted using a scaled effective path length (L) of 15 mm and plotted against measured values (bold solid line). The figure shows values from 11 sampling locations in July 2002 (squares) and 12 sampling locations in September 2002 (white circles). Four July values from sites where mean relative humidity 2 d before the phloem sampling was <70% are given as black/white squares. These points were omitted for the calculation of the linear regressions. Regressions were calculated for the data shown and additionally for Δ^{18}O modelled assuming L to be 4 mm (dotted line) and 54 mm (dashed line) and ignoring the Péclet effect (C–G, thin solid line).

Correlation between δ^{13}C and Δ^{18}O

The relationship between δ^{13}C and Δ^{18}O of foliage and phloem sap was quantitatively examined in addition to the correlation analyses between δ^{13}C and physiological and meteorological parameters, to separate the carboxylation-related effects on δ^{13}C from diffusional ones. Figure 6a shows that the variation in $\Delta^{18}O_{leaf}$ harvested in September was higher among sites as compared to $\delta^{13}C_{leaf}$ resulting in a slope of the regression line of 0.19‰ δ^{13}C per 1‰ Δ^{18}O. A significant positive linear relationship was observed between $\delta^{13}C_{phloem}$ and $\Delta^{18}O_{phloem}$ (Fig. 6b). When the September value for the Höglwald site was regarded as an outlier value (displayed in brackets), R^2 amounted to 0.44. The slope was 0.08‰ δ^{13}C per 1‰ Δ^{18}O, and thus, lower as for the regression line obtained for leaves. If the results for July and September were analysed separately, a significant

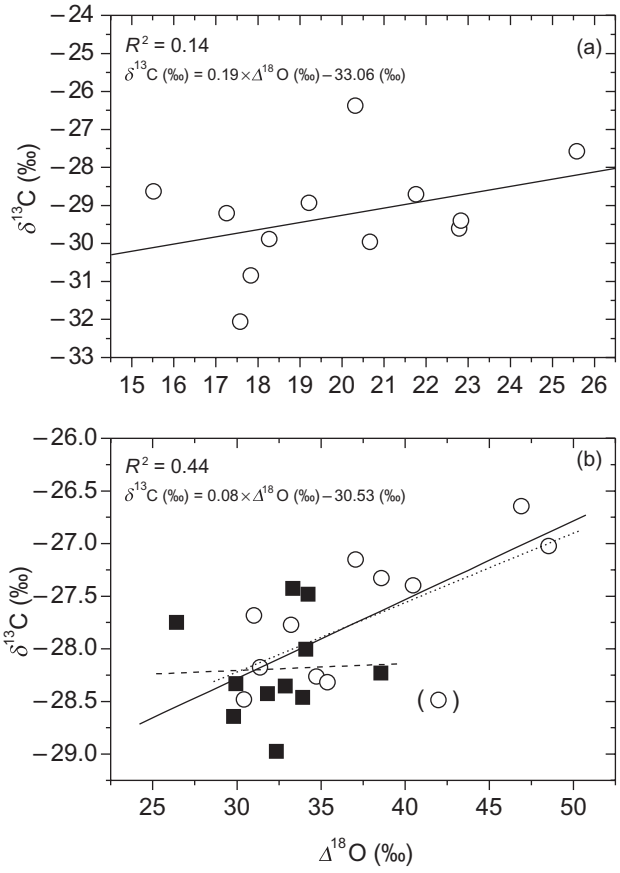

Figure 6. Regression between carbon isotopic composition (δ^{13}C) and oxygen isotopic enrichment (Δ^{18}O) of the organic matter of leaves (a) and phloem sap (b) along the transect from mid-Germany to southern France. (a) Leaf samples from 11 sampling locations in September (white circles). (b) Phloem sap was harvested from 11 sampling locations in July 2002 (black squares) and 12 sampling locations in September 2002 (white circles). The value of the Höglwald site was regarded an outlier value and is displayed in brackets. The regression analysis was performed using the July plus the September values without the outlier (bold line). In addition, the results of separate regression analyses for July (dotted line) and September (dashed line) data are shown.

relationship was found in September ($R^2 = 0.72$, $P < 0.001$), but not in July.

Relation between isotopic signatures of leaf and phloem organic matter

In Fig. 7a, the $\delta^{13}C_{leaf}$ is plotted against the $\delta^{13}C_{phloem}$. In general, with the exception of beech trees from the two highest elevated sites from the MdL (I: September; II: July and September), phloem carbon was isotopically heavier than the total leaf carbon. The enrichment in ^{13}C in phloem organic matter relative to the total leaf carbon amounted to between 0.3 and 4.25‰. However, no significant linear relation between the two parameters was observed.

For oxygen, strong isotopic enrichment was also observed in $\Delta^{18}O_{phloem}$ as compared to $\Delta^{18}O_{leaf}$ (Fig. 7b). In July, the relative enrichment was higher than in September and amounted to *c*. 17‰ as the average of all sites.

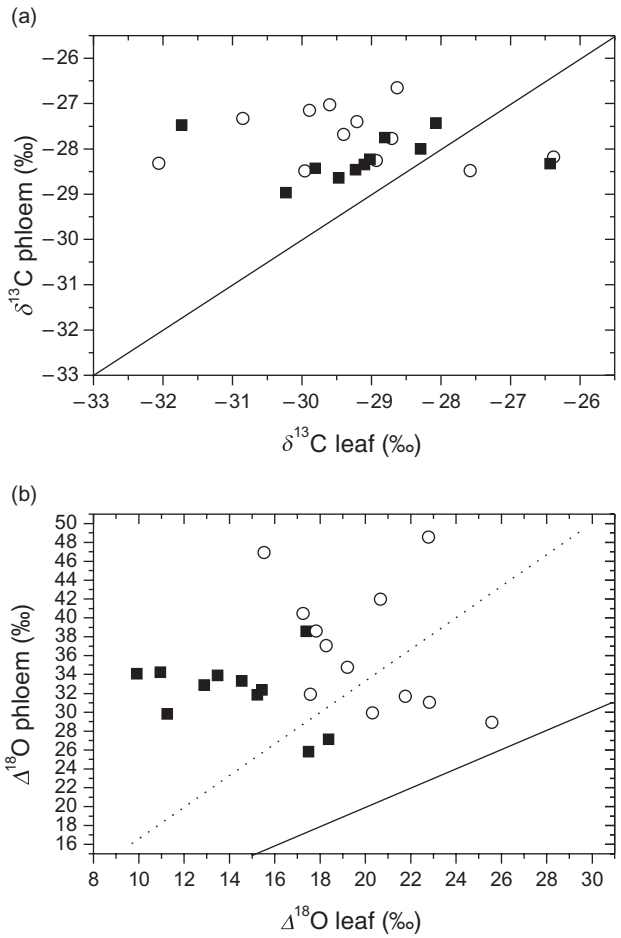

Figure 7. Carbon (a) and oxygen (b) isotope composition of total leaf organic matter plotted against phloem carbon isotopic composition ($\delta^{13}C$) and oxygen isotopic enrichment ($\Delta^{18}O$). Data shown are mean values for each site ($n = 6$) from July (black squares) and September (white circles). The black lines represent the 1:1 lines; the dotted line in (b) refers to $\Delta^{18}O$ of total leaf organic matter calculated from the phloem organic matter according to a modified Eqn 8 assuming p_{ex} to be 0.4 and p_x to be 1.

When $\Delta^{18}O_{leaf}$ is assumed to be related to $\Delta^{18}O_{phloem}$ according to $\Delta^{18}O_{leaf} = \Delta^{18}O_{phloem}\ (1 - p_{ex}\ p_x)$ with p_{ex} amounting to 0.4 and p_x to 1, the dotted line shown in Fig. 7b is obtained. This line is located much closer to the scattered points as compared to the 1:1 line. However, there was no significant linear correlation between $\Delta^{18}O_{phloem}$ and $\Delta^{18}O_{leaf}$.

DISCUSSION

In the present study, we aimed to test if the combined analysis of carbon and $\delta^{18}O$ in the heterogeneous organic matter of leaves and in the short-term turnover phloem carbohydrate pool could be used to assess water relations in beech on a time scale of a few days to the growing season.

The $\delta^{13}C_{leaf}$ varied significantly along the transect, was specific for a particular field site without changing from summer to autumn and was correlated with physiological/environmental parameters which might influence c_i/c_a via diffusional restriction or Rubisco activity/carboxylation efficiency. Only the strong depletion in ^{13}C observed in the leaves from Hofstetten may point to the fact that leaves collected from that site may have not been from the fully sun-exposed part of the crown.

The altitudinal gradient observed was in the same range as described for other tree and herbaceous species (e.g. Körner *et al*. 1991; Hultine & Marshall 2000; Warren, McGrath & Adams 2001). One main reason for decreasing isotope discrimination with increasing elevation is assumed to be the reduction of O_2 partial pressure and an associated increase in assimilation rates (Körner *et al*. 1991). If stomatal conductance is unaffected by altitudinal gradients or has even a tendency to decrease with increasing elevation as observed in the present study ($R = -0.43$), decreased internal CO_2 concentrations can account for the observed change in $\delta^{13}C$.

In addition, altitudinal changes in leaf morphology which influence CO_2 diffusion and N content, which is assumed to be a proxy for carboxylation efficiency, have been implicated for altitudinal variation in carbon isotope composition. Because there was no significant altitude-related variation of leaf N content ($R = -0.30$, $P = 0.31$ for September), we have to assume that carbon isotope discrimination was not affected by Rubisco concentration.

There was, however, a strong correlation between foliar $\delta^{13}C$ and sunshine hours – a proxy for intercepted radiation. Thus, increasing light availability is likely to add to increasing carboxylation efficiency with increasing altitude.

On the other hand, $\delta^{13}C_{leaf}$ was also significantly related to parameters associated with atmospheric and tree water balance (especially RH, transpiration and G_s).

These results indicate a combined influence of stomatal diffusion (G_s) and carboxylation (radiation and potentially altitude) on internal CO_2 concentration, and subsequently, photosynthetic carbon isotope discrimination, and thus, should also be reflected by the relation between $\Delta^{18}O$ and $\delta^{13}C$ (Scheidegger *et al*. 2000).

The proposed conceptual model assumes that the $\Delta^{18}O$ signatures of organic matter share dependence on stomatal conductance with $\delta^{13}C$ signatures, but are not dependent on Rubisco activity (Scheidegger *et al*. 2000; Barbour *et al*. 2004). In general, the $\Delta^{18}O$ of the mean laminar leaf water is imprinted on newly synthesized organic matter (with an offset of *c*. 27‰; see Eqn 8) (Sternberg & Deniro 1983; Barbour *et al*. 2000b; Cernusak *et al*. 2003b).

Table 3 shows that the $\Delta^{18}O_{leaf}$ from September is highly related to mean RH, T_a and VPD of the current growing season, and thus, is in good agreement with the theory. The slope for the regression line (–0.19‰/%) between the RH and the $\Delta^{18}O$ is largely in agreement with the ones observed for cotton plants (Farquhar, Barbour & Henry 1998) and oak tree rings (Switsur & Waterhouse 1998; both –0.12‰/%). Over the longer term, a strong coupling between atmospheric water demand and G_s is assumed for beech (Granier, Loustau & Breda 2000; Gessler *et al*. 2004a), which is nicely reproduced by the water balance model WBS3 (G_s versus VPD: $R = –0.71$, $P = 0.009$; G_s versus RH: $R = 0.77$, $P = 0.003$). Thus, the $\Delta^{18}O_{leaf}$ in beech could be shown to be a measure of G_s and can be reasonably applied for regression analysis with $\delta^{13}C$ according to the conceptual model of Scheidegger *et al*. (2000).

The combination of the two isotopes C and O (Fig. 6a) supports the hypothesis drawn from the correlation analysis between $\delta^{13}C$ and environmental parameters that both factors, stomatal conductance and carboxylation, govern $\delta^{13}C$.

In contrast to previous studies (Gessler *et al*. 2001; Keitel *et al*. 2003), the combined analysis of $\delta^{13}C$ and $\Delta^{18}O$ of the total leaf organic matter of beech here proved to be indicative of environmental differences between sites. In the previous studies, the variation of environmental parameters was achieved by differences in local (topo-) climate (SW- versus NE-exposed aspect) and by applying different degrees of thinning to beech stands. Under these conditions, differences in climatic conditions, and thus, physiological reactions were much smaller as compared to the present study. As an example, maximum differences in mean growing season temperature between sites amounted to 2 °C in the study described by Keitel *et al*. (2003), whereas the variation along the climatic gradient assessed here was 5.8 °C (Table 2). Thus, we have to revise hypothesis 1 at least partially: $\delta^{13}C_{leaf}$ can be considered a measure for changing environmental conditions and related physiological responses. It is, however, likely that because of the complex composition of this carbon pool, smaller differences in (local) climate are not resolved.

The carbon and oxygen isotopic signatures of the total leaf organic matter were not related to short-term variations of environmental or physiological parameters during the growing season. This is in agreement with the findings of Hemming *et al*. (2005) who did not find a relation between prevailing environmental conditions averaged over different time periods prior to harvest and $\delta^{13}C_{leaf}$ for different species (including *F. sylvatica*) at sites distributed all over Europe.

In contrast to $\delta^{13}C_{leaf}$, $\delta^{13}C_{phloem}$ differed strongly between sampling times and sites, and ranking of the sites according to their $\delta^{13}C_{phloem}$ values changed between July and September, suggesting a strong influence of short-term variation of meteorological and/or physiological parameters.

However, no significant correlation was found between $\delta^{13}C_{phloem}$ and the environmental parameters T_a, RH, VPD and R of varying time integrals. Yet, comparable to the previous studies of Keitel *et al*. (2003) and Gessler *et al*. (2004b), we here also observed a significant but weaker relation (R^2: –0.79 in Keitel *et al*. (2003) versus –0.30 in this study) between $\delta^{13}C_{phloem}$ and mean G_s of the previous 2 d, and the regression analysis (Fig. 4) produced a line with a slope comparable to the assessment of $\delta^{13}C_{phloem}$ in the local climatic gradient (Keitel *et al*. 2003; Gessler *et al*. 2004b). The relatively high variation in $\delta^{13}C$ of phloem organic matter in September within a small range of G_s might point either to a stronger influence of carboxylation or already indicate the onset of remobilization of carbon from senescing leaves. However, because the variation of the data points from both month can be described significantly by one regression line (Fig. 4), we have to assume that comparable physiological mechanisms govern phloem $\delta^{13}C$ in July and September. In contrast to the long-term trend over the whole growing season, we did not observe a significant coupling between VPD or RH and G_s over the short term, which again supports the assumption of Keitel *et al*. (2003) and Gessler *et al*. (2004a,b) that under particular conditions, G_s may not only be controlled by atmospheric factors but also by different and potentially varying influences like soil water availability. This assumption is supported by the results of Scartazza *et al*. (2004) who observed a strong correlation between current soil water content and both $\delta^{13}C_{phloem}$ of beech and $\delta^{13}C$ of ecosystem respiration.

When the regression analyses between phloem oxygen and carbon isotope composition are compared between the present study and the local climatic gradient assessed by Keitel *et al*. (2003), one main difference emerges: whereas in the previous study, a slope of approximately 1 indicated a sole influence of G_s on $\delta^{13}C_{phloem}$, we here observe patterns indicative for the combined effects of carboxylation and stomatal limitation in both months as already observed for leaves. This finding is supported by the moderate correlation between phloem $\delta^{13}C_{phloem}$ and sunshine hours 2–4 d prior to sampling which was, however, not significant because of the lack of this information for the major part of sites assessed.

The $\Delta^{18}O$ in phloem organic matter was not directly related to any of the meteorological or physiological parameters examined (Table 4b). However, the model applied here, which takes into account the ^{18}O fractionation associated with the exchange of water between leaves and the atmosphere and with the production of organic matter, is able to explain at least part of the variation of phloem organic matter along the transect (Fig. 5). For our model application, we assumed the water vapour of the atmosphere to be in isotopic equilibrium with the source water. However, it is known that stronger changes in RH during

the diurnal cycle combined with slow leaf water turnover can result in deviations from this steady state (Wang & Yakir 1995). The stomatal conductances were lowest for the southernmost sites (between 17 and 39 mmol m^{-2} s^{-1}) in July, which can contribute to a long leaf water turnover time. The low daytime RH values of the four southernmost sites examined in July might, thus, point to such a temporal disequilibrium. It is also worth mentioning that in September, the MdL values were again to the right of the regression line. In addition, it is likely that the term L is not constant with environmental conditions and time. Plants can quickly react with reduced tissue hydraulic conductance to drought stress, a reaction which is supposed to be mainly mediated by reduced expression and/or activity of aquaporins (for a recent review, see Tyerman, Niemietz & Bramley 2002). It can be assumed that the high atmospheric water demand at the four respective sites in July might also have resulted in reduced hydraulic conductance in the mesophyll cells, and thus, increased L.

When the four deviating values were disregarded, there was a moderate correlation with a regression line close to the 1:1 relationship between calculated and measured $\Delta^{18}O$ of phloem organic matter. R increased with decreasing L. However, it should again be considered that L is likely not to be constant, but might vary between sites and time points.

We can accept hypothesis 2 because we found, indeed, a correlation between G_s and $\delta^{13}C_{phloem}$ when a transport time for the assimilates from the leaves to the stem base of approximately 2 d is taken into account. However, the connection between these two parameters is likely to be weakened by the additional influence of Rubisco carboxylation activity. Whereas Keitel *et al*. (2003) observed a variation of $\delta^{13}C_{phloem}$ between –25.5 and –30.5‰ in a comparable range of G_s as determined here, the (putatively levelling) combined effects of light availability/carboxylation efficiency and stomatal conductance on c_i reduced the $\delta^{13}C_{phloem}$ variation (Fig. 4). Thus, the additional assessment of oxygen isotope signatures is crucial for a proper interpretation of the information obtained with $\delta^{13}C$.

Even though the $\Delta^{18}O$ of the phloem organic matter was not directly related to environmental parameters, it could at least be partially predicted by applying the model described by Farquhar & Lloyd (1993). The four deviating values point to the fact that it might be necessary to apply non-steady state models as proposed by Cernusak *et al*. (2005) to describe the $\Delta^{18}O$ of organic matter and to define L more carefully when applying such models in field studies.

In contrast to our observations (Fig. 7b), Cernusak *et al*. (2005) found a close linear relation between $\Delta^{18}O_{leaf}$ and $\Delta^{18}O_{phloem}$ in *E. globulus* along a climatic gradient. Jäggi *et al*. (2003) also showed that the $\delta^{18}O$ of the total organic matter of current year needles of *P. abies* corresponded well with $\delta^{18}O$ of fast-turnover soluble organic matter. Thus, we may assume that the isotopic composition of beech leaves is, to a lesser extent, influenced by the labile and, to a higher extent, by the structural pool of organic matter produced during leaf development as compared to the two evergreen species. This assumption is also supported (1) by the fact that the model assuming the total leaf organic matter produced with non-^{18}O-enriched water describes the relation between $\Delta^{18}O_{phloem}$ and $\Delta^{18}O_{leaf}$ better than a 1:1 line; (2) by the lack of correlation between $\delta^{13}C_{leaf}$ and $\delta^{13}C_{phloem}$; and (3) the little variation observed in $\delta^{13}C_{leaf}$ between July and September.

In addition to carbon and oxygen isotopic composition, the phloem sugar concentration has been shown to be a guide to the water balance of eucalyptuses. Pate & Arthur (1998) and Cernusak *et al*. (2003a) observed a strong relationship between sugar concentration and $\delta^{13}C$ and $\Delta^{18}O$ in *E. globulus*. Comparable patterns were also observed in beech under dry conditions, but not when water availability was higher (Keitel *et al*. 2003). Because we did not observe any correlation between $\delta^{13}C_{phloem}$ and sugar concentration in our transect study, we have to conclude that such interrelation was confounded by the great climatic differences between sites (i.e. by the fact that not only water-limited sites were included in this study).

CONCLUSIONS

Combining the analyses of $\delta^{13}C$ and $\Delta^{18}O$ in organic matter pools with long (leaves) and short (phloem) turnover times, we could show that isotope signatures can be used as effective tools to assess the water balance of beech along a climatic gradient. Together with the application of models for evaporative ^{18}O enrichment in organic matter, this approach can help to characterize environmental and physiological factors controlling water relations of trees.

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