

# Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.)

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## ABSTRACT

At eight different dates during the 2000 growing season,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were determined in the phloem of adult beech trees growing in natural beech stands in south-west Germany differing in stand density and local climate. In addition, stand transpiration, precipitation, photosynthetic active radiation, relative air humidity, water pressure deficit of the air, air and soil temperature, soil water potential, and sugar concentration of the phloem sap were determined directly and evaporation and canopy stomatal conductance were modelled. All parameters were related to  $\delta^{13}\text{C}$ . The study aimed to identify the time integral within which the  $\delta^{13}\text{C}$  of organic compounds transported in the phloem is an indicative measure of these environmental influences.  $\delta^{13}\text{C}$  of soluble carbon transported in the phloem was well correlated with mean stomatal conductance in a two-day integral prior to phloem sampling but did not depend on either light intensity or soil water availability. A strong positive relationship between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  pointed to observed variation in  $\delta^{13}\text{C}$  of phloem sap being a result of variation in stomatal conductance. Bulk leaf  $\delta^{13}\text{C}$  was a poor indicator of changes in environmental conditions during the growing season. From these results we conclude that the analysis of  $\delta^{13}\text{C}$  in soluble carbon transported in the phloem is a reliable indicator of short-term changes in  $C_i/C_a$ . In contrast, the  $\delta^{13}\text{C}$  of structural carbon in beech foliage represents an integration of a range of factors that mask short-term influences responsible for  $C_i/C_a$ .

**Key-words:**  $\delta^{13}\text{C}$ ;  $\delta^{18}\text{O}$ ; carbohydrates; stomatal conductance; water availability.

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## INTRODUCTION

The determination of carbon isotope composition in plant tissues is now widely used by plant eco-physiologists to integrate the influence of a range of environmental factors on plant performance. Due to their effects on internal concentration of  $\text{CO}_2$  ( $C_i$ ), intercepted radiation as well as atmospheric and soil water deficits modify the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  (expressed as deviation from PDB standard ( $\delta^{13}\text{C}$ )) in plant carbon (e.g. Leavitt & Long 1986; Livingston & Spittlehouse 1996; Korol *et al.* 1999). Stomatal closure due to water deficits reduces  $C_i$ , leading to an increase in  $\delta^{13}\text{C}$  (e.g. Guehl, Fort & Ferhi 1995; Lauteri *et al.* 1997). Plant water potential has hence been related to  $\delta^{13}\text{C}$  in leaves and wood and in turn to the availability of water (Damesin, Rambal & Joffre 1998; Warren, McGrath & Adams 2001). Alternatively, under light-limiting (but not water-limiting) conditions, photosynthesis and, consequently,  $C_i$  depends on radiation. As a consequence, organic carbon in leaves from the shaded part of the crown of trees is less depleted in  $^{13}\text{C}$  as compared to the sun-exposed crown (Leavitt & Long 1986).

The range of other influences on  $\delta^{13}\text{C}$  in plant organic matter includes external factors such as altitude and nutrition and plant internal factors such as hydraulic architecture of the water transport system (e.g. Högberg, Johannisson & Hällgren 1993; Walcroft *et al.* 1996; Korol *et al.* 1999; Hultine & Marshall 2000; Warren & Adams 2000).

The main difficulty in correlating  $\delta^{13}\text{C}$  signature in leaves of trees or tree rings with meteorological or edaphic parameters is the effect of carbon storage and re-translocation and related fractionation of carbon isotopes. The general consequences of re-use of carbon assimilated during previous growing seasons for the  $\delta^{13}\text{C}$  signatures of current leaves (and wood), remain poorly understood and of obvious significance, especially for deciduous trees. Although different authors (e.g. Dupouey *et al.* 1993; Livingston & Spittlehouse 1996; Macfarlane & Adams 1998; Barbour,

Walcroft & Farquhar 2002) observed moderate to strong relationship between  $\delta^{13}\text{C}$  in the wood of annual growth rings and different parameters of water balance or water availability, Geßler *et al.* (2001) showed that both, radial growth and  $\delta^{13}\text{C}$  in tree rings of beech exhibited a variable time-lag with environmental conditions (rainfall, radiation) that precluded a significant correlation. In deciduous trees such as European beech, foliage that develops in spring is formed mainly from stored carbon and nutrients (Millard 1996; Kozłowski & Pallardy 1997) – newly assimilated carbon is mixed with the previously stored carbon to form the new leaves. Clearly, efforts to interpret  $\delta^{13}\text{C}$  signatures of current leaves in relation to prevailing conditions of light and moisture *without* understanding the influence of stored and re-used carbon are likely to fail (Brendel 2001).

A number of recent studies suggest that the  $\delta^{13}\text{C}$  of the phloem sap provides a strong guide to  $C_i/C_a$  during the actual growing season (Adams & Grierson 2001). Yoneyama *et al.* (1997) first reported carbon isotope ratios in phloem sap of wheat and Pate & Arthur (1998) and Geßler *et al.* (2001) developed and applied suitable methodologies for studying carbon isotopes in phloem sap in trees including eucalypts and European beech.

Development of the theoretical basis for the discrimination of stable isotopes of oxygen within plants, coupled with empirical studies demonstrating its practical application, has been another recent and significant development in ecophysiology. The  $\delta^{18}\text{O}$  signature shares dependence on stomatal conductance with  $\delta^{13}\text{C}$  signature but is not dependent on RubisCo activity (Farquhar, Barbour & Henry 1998; Barbour *et al.* 2000a). Hence, combined analysis of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  in plant organic matter may help separate the effects of stomatal conductance and carbon fixation on  $\delta^{13}\text{C}$  (Scheidegger *et al.* 2000; Xu *et al.* 2000; Adams & Grierson 2001).

In the present study we assessed the effects of water availability [soil water potential ( $\Psi_s$ ); precipitation ( $P$ )], radiation [photosynthetic active radiation (PAR)] and related environmental [air ( $T_a$ ) and soil ( $T_s$ ) temperature; relative air humidity ( $RH$ ); water pressure deficit of the air ( $VPD$ ); evaporation ( $E$ )] and physiological factors [stand transpiration ( $ST$ ); canopy stomatal conductance ( $G_s$ )] on the  $\delta^{13}\text{C}$  signature in the phloem. The study aimed to identify the time integral within which  $\delta^{13}\text{C}$  is an indicative measure for these environmental factors. A field experiment in a beech stand in southern Germany (Geßler *et al.* 2001; Fotelli *et al.* 2002) includes paired sites that differ mainly in aspect (south-west versus north-east) on either side of a small valley and, within each site, replicated plots of differing stand density. A previous study at these sites (Geßler *et al.* 2001) showed that water availability was least in the warm-dry south-west facing site as compared to the north-east facing site. However, analysis of phloem sap  $\delta^{13}\text{C}$  suggested this interpretation may have been confounded by differences in radiation. In the present study, both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  signatures of phloem sap carbon were used to attempt to overcome this problem.

## MATERIALS AND METHODS

### Site description

#### General description

The experimental sites used for the present study are located in southern Germany (longitude: 8°40' E; latitude: 48°00' N), about 100 km south-south-west from Stuttgart in a low mountain range (Schwäbische Alb, 740–760 m a.s.l.). Mean annual regional air temperature measured at a climate station of the DWD (Deutscher Wetterdienst, Offenbach, Germany), about 4 km from the experimental sites, is about 6.6 °C, and mean temperature during the growing season (May to October) about 11.5 °C. Mean annual precipitation is 856 mm with monthly maxima in June and July. The sum of precipitation during the growing season (May to October) amounts to 410 mm.

The experimental sites are located on the two opposing sides (not more than 1000 m apart) of a single, narrow valley. One experimental site faces to the north-east (NE) and the other to the south-west (SW). Rainfall does not vary significantly across the valley (Geßler *et al.* 2001). The slope at both sites is moderately steep (NE: 58–100%; SW: 36–58%). Soil profiles are characterized as Terra fusca – Rendzina derived from limestone (Weissjura beta and gamma series) and are shallow on both sites, averaging less than 0.20 m depth of topsoil before becoming dominated by parent rock interspersed with pockets of organic matter and mineral soil. The soil profile at the SW site is especially rocky, containing more than 40% (volumetric basis) rocks and stones (> 63 mm diameter) in the top 0.20 m of the soil and increasing to 80% below 0.50 m. The soil at the NE site contains 15% rocks and stones in the uppermost 0.20 m of the soil and approximately 30% below 0.50 m. Soil pH ( $\text{H}_2\text{O}$ ) is 5.7 in the surface organic layer and 7.5 at 0.60 m depth.

On both sites, European beech (*Fagus sylvatica* L.) is the dominant species making up more than 90% of the total basal area 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. The difference in aspects (NE, SW) produces a difference in radiation interception per  $\text{m}^2$  of inclined surface area with higher energy available on the SW site (Geßler *et al.* 2001). According to retrospective analyses of meteorological data, as well as the growth and water status of adult beech trees (Geßler *et al.* 2001) and beech seedlings (Fotelli *et al.* 2002), the SW-exposed site has permanently lower availability of water and higher air temperatures, than the NE-exposed site. Thus, the understorey vegetation differs between the two sites and the classification of the stand on the NE site is a *Hordelymo-Fagetum* and on the SW site, a *Carici-Fagetum* (Oberdorfer 1992).

On each site, two silvicultural (thinning) treatments plus controls (unthinned) were established in March 1999. The experimental design consisted of two blocks, each containing a single plot (each approx. 0.53 ha in size) of the two silvicultural treatments plus a control plot. The total basal area (BA) of trees on the untreated control plots varied

between sites – on the NE site the mean BA in control plots was close to 27 m<sup>2</sup> ha<sup>-1</sup>, while on the SW site the control plot BA was about 20 m<sup>2</sup> ha<sup>-1</sup>. The two thinning treatments on both sites were BA = 15 and 10 m<sup>2</sup> ha<sup>-1</sup>. Thinning decreased the leaf area index (*LAI*) from 5.16 (control) to 3.15 (BA = 15 m<sup>2</sup> ha<sup>-1</sup>) and 1.68 (BA = 10 m<sup>2</sup> ha<sup>-1</sup>) on the NE site, and from 5.12 to 3.24 and 2.12 on the SW site. One year after thinning, the density of understorey vegetation, other than natural regeneration of beech, increased in the thinned stands, in comparison with the controls (by approximately 25% on the NE site, and approximately 8% on the SW site; T. Paul & A. Reif, personal comm.).

### Radiation, humidity and air temperature

Data from all microclimatic sensors were recorded every 30 s by CR23X-dataloggers (Campbell Scientific, Logan, UT, USA) and means calculated and stored every 30 min. *T<sub>a</sub>* and *RH* were measured by HMP45D-Sensors (Vaisala, Helsinki, Finland) 1.5 m above ground (Holst *et al.* 2001). PAR was determined by Li-190SZ-Sensors (LiCor Inc., Lincoln, NE, USA). From the beginning of the growing season 2000 until October 2000 PAR measurements were only performed at 1.5 m above ground. Since October 2000, PAR has been available from measurements taken 6 m above the canopy for the NE and SW aspects. For the time before October, PAR above canopy level was calculated from PAR measurements below the canopy.

For a homogeneous stand the extinction of radiation in a canopy can be described by the Bouguer–Lambert law (Eqn 1):

$$PAR_{bc} = PAR_{ac} \exp(-LAI \cdot k) \quad (1)$$

where *PAR<sub>ac</sub>* is the PAR measured above the canopy, *k* is the extinction coefficient, and *PAR<sub>bc</sub>* is the PAR reaching the ground (Baldocchi *et al.* 1984; Yang, Miller & Montgomery 1993; Kull *et al.* 1999).

The value of *k* was determined from measurements of PAR below and above canopy level during the 2001 growing season. Assuming comparable daily and seasonal courses of *k* between the two years, PAR was calculated by inverting Eqn 1.

PAR was measured via horizontally mounted radiation sensors that were influenced by the different aspect of the plots. Whereas in the morning the SW plot was shaded by the slope, in the afternoon the NE plot was influenced by a limiting horizon. On a monthly basis, the NE slope has only about 80–85% of PAR of the SW slope in winter when the elevation of the sun is less than in summer and the limited horizon has a strong influence. Throughout the growing season, and when the sun is close to its maximum elevation at this latitude, there is almost no difference in monthly PAR for both sites. The major difference between the sides of the valley that brings about the drier and warmer conditions on the SW slope is the differences in aspect: the SW slope is exposed to longer periods of radiation whereas the NE slope is more sheltered in the afternoon.

### Soil water potential ( $\Psi_s$ ) and soil temperature ( $T_s$ )

The value of  $\Psi_s$  was determined in four replicates at soil depths of 20, 40 and 60 cm using pressure transducer tensiometers (T4; UMS, Munich, Germany). Soil water potential was measured on the SW and NE control plots in large canopy gaps and under closed canopy. The  $\Psi_s$  measured in the large canopy gaps was regarded as representative of the water availability in the thinning treatments with a BA of 10 m<sup>2</sup> ha<sup>-1</sup>, whereas the  $\Psi_s$  under the canopy was used for the control plots (S. Augustin, personal comm.). The  $\Psi_s$  data were collected every 30 min and calculated as daily means. The value of *T<sub>s</sub>* was measured with Pt100 sensors at a soil depth of 5 cm at the control and the 10 m<sup>2</sup> ha<sup>-1</sup> treatment and were also calculated as daily means.

### Transpiration, evaporation and canopy stomatal conductance

Prevailing conditions of water availability and climate were analysed by measuring xylem (water) flow densities using Granier-style probes and by scaling up flow densities from a single tree to the stand level (e.g. Granier *et al.* 1996). Flow densities in the water-conducting sapwood of beech were determined using the constant-heating method according to Granier (1985) and Köstner *et al.* (1996). Flux densities (*FD*; ml cm<sup>-2</sup> sapwood area s<sup>-1</sup>) were determined every 5 min in seven to nine beech trees per plot and calculated as means of 30 min. Flux densities were converted to estimates of water use/stand transpiration (*ST*) using the stand sapwood area (*SA*; cm<sup>2</sup> m<sup>-2</sup>) assuming that sap flux densities are uniform through the cross-sectional sap wood area according to the following equation (Granier *et al.* 1996):

$$ST = SA \cdot FD \quad (2)$$

Half hourly values of stand transpiration were summed for 24 h (1 m<sup>2</sup> d<sup>-1</sup> or mm d<sup>-1</sup>). The *SA* value of 25 representative trees of different breast height diameters (BHD) on each site was determined according to Glavac *et al.* (1989) and extrapolated to the stand level according to Granier *et al.* (1996).

Evaporation (*E*) was calculated using the water balance model WBS3, a forest-hydrological model that requires daily mean value of air temperature and daily total precipitation as meteorological inputs (Matzarakis *et al.* 2000). Time-independent input variables of the WBS3-simulations are: basal area of the stand, mixing ratio of deciduous trees, mixing ratio of coniferous trees, type of soil, maximum useable storage capacity of the soil, slope angle, slope direction and geographical latitude. For evapotranspiration, *E*, transpiration and interception of forests, validations of WBS3 showed a good agreement between results from model calculations and measurements for different areas and slopes (Fritsch 1998; Matzarakis *et al.* 2000).

As stomatal conductance is one of the factors determining  $\delta^{13}\text{C}$  of organic carbon, mean daily canopy stomatal conductance (*G<sub>s</sub>*, mmol m<sup>-2</sup> s<sup>-1</sup>; Fig. 2) was calculated using

a simplified Penman–Monteith equation (according to Pataki, Oren & Phillips 1998):

$$G_s = \frac{G_c}{LAI} = \frac{\gamma \cdot \lambda \cdot P}{\rho \cdot c_p \cdot VPD \cdot R \cdot T} \cdot \frac{J_s \cdot SA}{LAI} \quad (3)$$

where  $G_c$  is canopy conductance,  $\gamma$  is the psychrometric constant ( $\text{kPa K}^{-1}$ ),  $\lambda$  is the latent heat of vaporization ( $\text{J kg}^{-1}$ ),  $\rho$  is the density of moist air ( $\text{kg m}^{-3}$ ),  $J_s$  is mean daily sap flux density ( $\text{g m}^{-2} \text{s}^{-1}$ ),  $c_p$  is the heat capacity of moist air ( $\text{J kg}^{-1} \text{K}^{-1}$ ),  $VPD$  is the water vapour pressure deficit ( $\text{kPa}$ ),  $P$  is the atmospheric pressure ( $\text{Pa}$ ),  $R$  is the gas constant ( $8.31 \text{ m}^3 \text{ Pa mol}^{-1} \text{ K}^{-1}$ ),  $T$  is air temperature ( $\text{K}$ ),  $J_s$  is mean daily sap flux density ( $\text{g m}^{-2} \text{s}^{-1}$ ) and  $LAI$  is the leaf area ( $\text{cm}^2 \text{ m}^{-2}$ ). This calculation assumes, that: (1) sap flux densities are uniform throughout the cross-sectional sapwood area; (2) that stem capacitance can be neglected; and (3) that 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.

### Plant material

Phloem sap was collected at about 0900 h MEZ at breast height of four to six adult beech trees per aspect and treatment according to the method described by Pate *et al.* (1998) for *Eucalyptus globulus* and Geßler *et al.* (2001) for European beech. The bark was cut with a scalpel to the depths of the wood at about 15% to horizontal. The 'bleeding' phloem sap was collected immediately with a Pasteur pipette. Since no phloem sap could be obtained in early spring (Geßler *et al.* 2001) the first samples were collected at the end of May. In total, phloem sap was collected on eight sampling days during the growing season 2000 (29 May, 28 June, 18 July, 1 August, 15 August, 29 August, 12 September, 25 September). Different trees were used at each sampling date in order to avoid the cumulative effects of bark damage.

On selected sampling dates [28 April (bud burst), 18 July, 25 September] four trees were climbed in each plot and foliage (expanding leaves on the first date, fully expanded leaves on the other dates) was sampled from two branches excised from the sun-exposed part of the tree crowns. Since  $\delta^{13}\text{C}$  signatures of leaves increased with increasing branch length (data not shown), samples were taken at a fixed position (approximately 3 m branch length) in the sun-crown.

Different trees were used for phloem sap and foliage sampling in order to avoid artefacts in the composition of phloem sap as a consequence of the harvest of foliage. Material from neighbouring trees of the same social class (co-dominant or dominant) was collected where required to prevent cumulative damage to sample trees.

### Determination of sugars in the phloem sap

For the determination of soluble carbohydrates, 5–10  $\mu\text{L}$  of phloem sap were diluted to 500  $\mu\text{L}$  with demineralized

water. Aliquots of 100  $\mu\text{L}$  were injected into a high-performance liquid chromatography system (Dionex DX 500; Dionex, Idstein, Germany). Separation of sugars was achieved on a CarboPac 1 separation column ( $250 \times 4.1 \text{ mm}$ ; Dionex) with 36 mM NaOH as an eluent at a flow rate of  $1 \text{ mL min}^{-1}$ . Carbohydrates were measured by means of a pulsed amperometric detector equipped with an Au working electrode (Dionex DX 500; Dionex). Individual carbohydrates that eluted 8–16 min after injection were identified and quantified by internal and external standards.

### Carbon and oxygen isotope composition

Leaf material was oven-dried for 3 d at  $65^\circ\text{C}$  and, subsequently, ground and homogenized with a ball mill into a fine powder. Samples of 1–2 mg were transferred into tin capsules (Thermo Quest, Milan, Italy). An aliquot of undiluted phloem (10  $\mu\text{L}$ ) was pipetted into tin ( $\delta^{13}\text{C}$  analysis) or silver ( $\delta^{18}\text{O}$  analysis) capsules and oven-dried for 30 min at  $65^\circ\text{C}$ .

Samples were injected into an elemental analyser (NA 2500; CE Instruments, Milan, Italy) for  $\delta^{13}\text{C}$  analysis and into a high temperature conversion/elemental analyser (TC/EA; Finnigan MAT GmbH, Bremen, Germany) for  $\delta^{18}\text{O}$  analysis, both coupled to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT GmbH) by a ConFlo II interface (Finnigan MAT GmbH). The isotopic values are expressed in delta notation (in ‰ units), relative to VPDB (Vienna Pee Dee Belemnite) for carbon and VSMOW (Vienna Standard Mean Ocean Water) for oxygen.

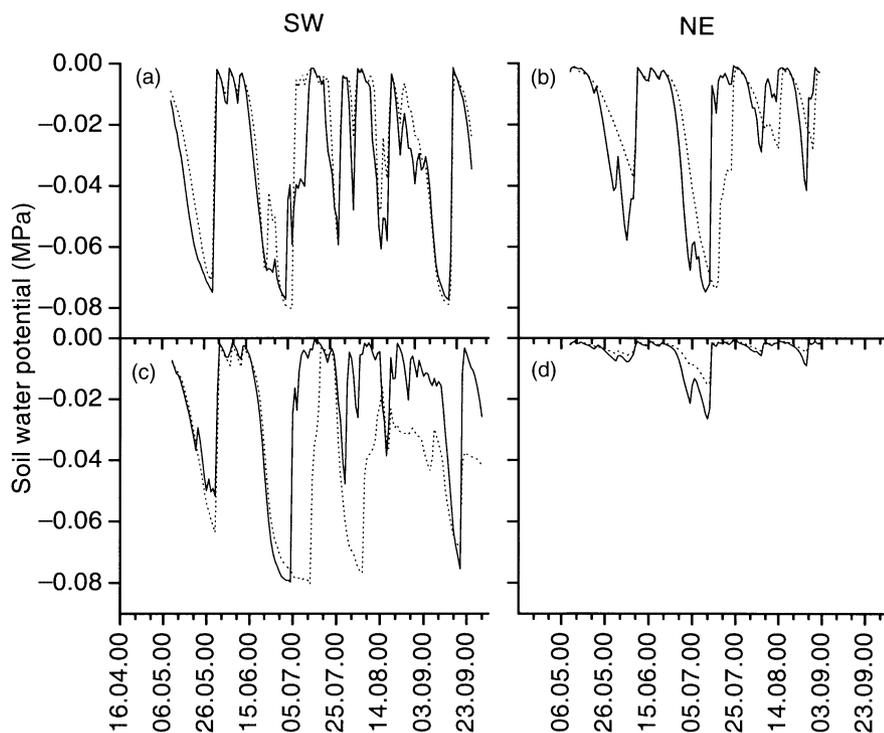
### Statistical analysis

Statistical analyses were conducted using SPSS 10.05 (SPSS, Inc., Chicago, IL, USA). The effects of site (NE, SW) and treatment (thinning) on the measured parameters were assessed using univariate GLM-ANOVA procedures. Correlations between  $\delta^{13}\text{C}$  and environmental parameters were calculated using the bivariate correlation procedure. Regression lines between  $\delta^{13}\text{C}$  and  $G_s$  and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were determined by linear regression analysis.

## RESULTS

### Soil water potential and soil temperature

Figure 1 shows the seasonal patterns of  $\Psi_s$  at depths of 20 and 40 cm (60 cm not shown for clarity) during the growing season 2000. Data shown are for the closed canopy (Fig. 1a & b) and large canopy gaps (Fig. 1c & d) – supposed to be representative for the thinning treatments with a BA of  $10 \text{ m}^2 \text{ ha}^{-1}$  – on the SW and NE site. In general,  $\Psi_s$  was more negative on the SW site. This pattern was most pronounced at both soil depths in May and August/September. Reduced canopy cover increased water availability on the NE site as indicated by the increase in  $\Psi_s$ . On the SW site, there was only a slight increase in  $\Psi_s$  as a consequence of reduced canopy cover.  $T_s$  showed comparable seasonal pat-



**Figure 1.** Soil water potential on the SW and NE aspect under the closed canopy of the control sites (a, b) and in large canopy gaps (c, d). Soil water potential was measured using four replicate tensiometers placed at depths of 20 cm (—) and 40 cm (- - -).

terns among aspects and control and thinning (BA of  $10 \text{ m}^2 \text{ ha}^{-1}$ ) treatments (data not shown). Mean  $T_s$  during the growing season amounted to  $13.1^\circ\text{C}$  on the NE and  $13.9^\circ\text{C}$  on the SW control site. Thinning to a BA of  $10 \text{ m}^2 \text{ ha}^{-1}$  increased soil temperatures on both sites: mean  $T_s$  during the growing season amounted to  $14.4^\circ\text{C}$  on the NE site and to  $15.1^\circ\text{C}$  on the SW site.

### Transpiration, evaporation and canopy stomatal conductance

The sum of stand transpiration during the growing season 2000 ( $\Sigma_{ST}$ ) was comparable between the NE and SW sites whereas the seasonal distribution of  $ST$  varied between the two aspects, with higher  $ST$  at the beginning of the growing season (until mid June) on the SW site (Fig. 2). Thinning reduced  $ST$  on both sites to c. 70% ( $15 \text{ m}^2 \text{ ha}^{-1}$ ) and approximately 50% ( $10 \text{ m}^2 \text{ ha}^{-1}$ ) of that of the control stand.

Evaporation from the soil was comparable between sites and increased with progressive thinning to approximately 130% ( $15 \text{ m}^2 \text{ ha}^{-1}$ ) and 180% ( $10 \text{ m}^2 \text{ ha}^{-1}$ ) of that from the control plots.

Mean  $G_s$  during the entire growing season amounted to approximately  $85 \text{ mmol m}^{-2} \text{ s}^{-1}$  in the control treatments on both sites (Fig. 2). At the beginning of the growing season,  $G_s$  was higher on the SW control site compared to the respective treatment on the NE site (in May, SW:  $55 \text{ mmol m}^{-2} \text{ s}^{-1}$ ; NE:  $37 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). This pattern changed in midsummer (in July: SW:  $103 \text{ mmol m}^{-2} \text{ s}^{-1}$ ; NE:  $170 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and at the end of the growing season (in September: SW:  $50 \text{ mmol m}^{-2} \text{ s}^{-1}$ ; NE:  $71 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). Thinning reduced mean  $G_s$  slightly on the NE site (mean

$G_s$  during the growing season:  $80 \text{ mmol m}^{-2} \text{ s}^{-1}$  at a BA of  $15 \text{ m}^2 \text{ ha}^{-1}$  and  $67 \text{ mmol m}^{-2} \text{ s}^{-1}$  at a BA of  $10 \text{ m}^2 \text{ ha}^{-1}$ ) and more intensively on the SW site ( $61 \text{ mmol m}^{-2} \text{ s}^{-1}$  at a BA of  $15 \text{ m}^2 \text{ ha}^{-1}$  and  $48 \text{ mmol m}^{-2} \text{ s}^{-1}$  at a BA of  $10 \text{ m}^2 \text{ ha}^{-1}$ ).

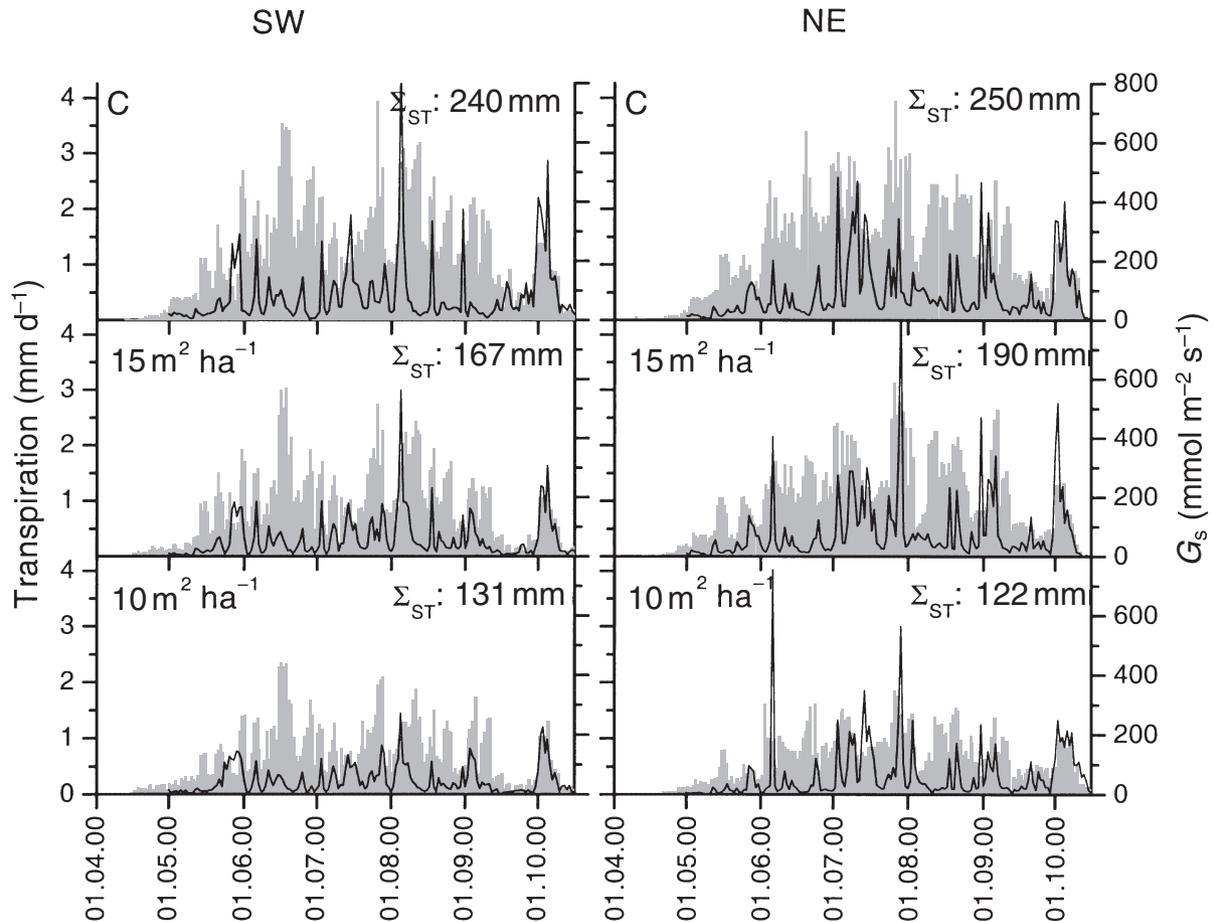
### $\delta^{13}\text{C}$ in the foliage and phloem

During the whole growing season,  $\delta^{13}\text{C}$  in foliage of beech was not significantly different between sites or among treatments (Table 1). In April, immediately after bud break, when young leaves were expanding,  $\delta^{13}\text{C}$  showed a seasonal maximum. The mean  $\delta^{13}\text{C}$  signature of leaves from both sites and all treatments amounted to  $-24.5\%$  in April and decreased to  $-26.7\%$  in July and further to  $-27.3\%$  in September.

In contrast, significant effects of site and treatment on  $\delta^{13}\text{C}$  on phloem sap were observed, especially at the beginning of the growing season with a tendency towards decreased  $^{13}\text{C}$  depletion on the SW site and in the thinning treatments (Table 1). During the seasonal course  $\delta^{13}\text{C}$  showed a maximum on all sites and treatments in June.

### Correlation of phloem $\delta^{13}\text{C}$ with physiological, climatic and pedospheric parameters

In contrast to  $\delta^{13}\text{C}$  in leaves, carbon isotope signature in phloem sap varied between sampling dates during the growing season and also between sites and treatments. Therefore we tested: (1) which environmental parameter(s) was (were) responsible for the  $\delta^{13}\text{C}$  patterns of phloem allocated carbon; and (2) quantified the time integral within which  $\delta^{13}\text{C}$  was influenced by this (these) parameter(s).



**Figure 2.** Mean daily stand transpiration ( $ST$ ; grey bars) and canopy stomatal conductance ( $G_s$ ; black line) of adult beech trees on the SW and NE aspect in the control (C) and the thinning treatments ( $15 \text{ m}^2 \text{ ha}^{-1}$ ;  $10 \text{ m}^2 \text{ ha}^{-1}$ ).  $\Sigma_{ST}$  refers to the sum of transpiration during the growing season (15 April 2000–30 October 2000).

Correlation analyses were performed between  $\delta^{13}\text{C}$  of phloem-transported carbon and different physiological ( $ST$ ,  $G_s$ ) meteorological ( $E$ ,  $T_a$ ,  $RH$ ,  $VPD$ ,  $PAR$ ) and pedospheric parameters ( $\Psi_s$  in 20, 40 and 60 cm;  $T_s$ ). These different parameters were calculated as mean values of 1–22 d prior to the time of phloem sap collection (Table 2).

Canopy stomatal conductance ( $G_s$ ) was clearly and closely related to  $\delta^{13}\text{C}$  (greatest  $R$ ) within a time integral of 2 d prior to phloem sap collection. Figure 3 shows the relationship between these two parameters. For longer time integrals,  $R$  decreased steadily. Although  $\delta^{13}\text{C}$  was not correlated significantly with  $ST$ ,  $E$  and  $\Psi_s$ , significant influences of  $T$ ,  $RH$  and  $VPD$  were observed. The correlation coefficients, however, were distinctly smaller than observed for  $G_s$ . For  $RH$  and  $VPD$ , the correlation coefficient was greatest when the values were averaged for a period of 3 d prior to phloem sap sampling. The correlation between  $\delta^{13}\text{C}$  and temperature was more or less constant for the observed period of time.  $\delta^{13}\text{C}$  was not correlated significantly with the amount of  $PAR$  (as measured above the canopy, line  $PAR$  in Table 2). The value of  $C_i$  and hence  $\delta^{13}\text{C}$ , depends on radiation only under light-limiting conditions. As it may be supposed that these conditions prevailed only in the

unthinned control treatments, the correlation between  $\delta^{13}\text{C}$  and  $PAR$  was calculated exclusively for control stands on both sites (line  $PAR_C$  in Table 2), additionally. However, when the canopy was closed and not influenced by selective felling,  $\delta^{13}\text{C}$  did also not depend significantly on  $PAR$ .

### $\delta^{18}\text{O}$ in the phloem sap

In addition to correlation analysis between  $\delta^{13}\text{C}$  and physiological, meteorological and pedospheric parameters, we examined the relationship between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of phloem sap (Fig. 4). A strong and positive linear relationship ( $R^2 = 0.63$ ;  $P < 0.001$ ) was observed between the two parameters with a slope of  $0.90\text{‰ } \delta^{13}\text{C}$  per  $1\text{‰ } \delta^{18}\text{O}$  (i.e.  $1.11\text{‰ } \delta^{18}\text{O}$  per  $1\text{‰ } \delta^{13}\text{C}$ ).

Correlation analyses between  $\delta^{18}\text{O}$  in the phloem sap and environmental parameters produced – as observed for  $\delta^{13}\text{C}$  – highest correlation coefficients for a 2–3 d integral prior to sampling (Table 3). The value of  $\delta^{18}\text{O}$  was influenced less intensively by  $G_s$  ( $R = -0.69$ ) as compared to  $\delta^{13}\text{C}$ , whereas correlation to the atmospheric parameters  $RH$  ( $R = 0.68$ ) and  $VPD$  ( $R = 0.58$ ) produced higher correlation coefficients. No correlation to  $\Psi_s$  and  $T_s$  was observed.

**Table 1.** Effects of site (S; north-east, NE; south-west, SW) and treatment (T; control, 15 m<sup>2</sup> ha<sup>-1</sup>, 10 m<sup>2</sup> ha<sup>-1</sup>) on carbon isotope composition  $\delta^{13}\text{C}$  of phloem and leaves of beech

Site/treatment	Date								
	28.04.00	29.05.00	28.06.00	18.07.00	01.08.00	15.08.00	29.08.00	12.09.00	25.09.00
$\delta^{13}\text{C}$ in phloem									
NE-C		-29.9 ± 0.2	-27.1 ± 0.7	-29.8 ± 0.4	-27.6 ± 0.1	-27.8 ± 0.4	-27.7 ± 0.1	-27.3 ± 0.3	-27.2 ± 0.4
NE-15		-28.9 ± 0.5	-25.7 ± 0.1	-29.3 ± 0.1	-27.9 ± 0.3	-27.7 ± 0.3	-26.9 ± 0.7	-27.1 ± 0.3	-27.7 ± 0.3
NE-10		-29.4 ± 0.7	-25.9 ± 0.4	-29.9 ± 0.3	-27.8 ± 0.1	-27.7 ± 0.2	-27.0 ± 0.3	-26.9 ± 0.2	-27.4 ± 0.2
SW-C		-30.3 ± 0.1	-25.7 ± 0.7	-29.4 ± 0.5	-27.7 ± 0.2	-27.8 ± 0.2	-26.8 ± 0.3	-26.8 ± 0.2	-27.1 ± 0.2
SW-15		-30.1 ± 0.3	-25.7 ± 0.7	-28.5 ± 0.3	-27.6 ± 0.5	-27.6 ± 0.2	-26.8 ± 0.4	-26.6 ± 0.3	-27.2 ± 0.4
SW-10		-29.8 ± 0.5	-25.4 ± 0.6	-28.3 ± 0.2	-27.6 ± 0.1	-27.2 ± 0.2	-27.2 ± 0.2	-26.3 ± 0.3	-27.2 ± 0.3
Main effects		S: ** T: *	S: *** T: NS	S: *** T: ***	S: NS T: NS	S: NS T: NS	S: NS T: NS	S: * T: *	S: NS T: NS
$\delta^{13}\text{C}$ in leaves									
NE-C	-24.6 ± 0.3			-26.9 ± 1.0					-27.3 ± 0.7
NE-15	-24.1 ± 0.2			-26.4 ± 0.4					-27.4 ± 1.0
NE-10	-24.9 ± 0.9			-26.7 ± 0.6					-27.3 ± 0.8
SW-C	-25.0 ± 0.9			-27.3 ± 0.5					-28.0 ± 0.6
SW-15	-24.1 ± 0.4			-26.2 ± 0.1					-26.7 ± 0.4
SW-10	-24.6 ± 0.7			-26.7 ± 0.7					-27.3 ± 0.9
Main effects	S: NS T: NS			S: NS T: NS					S: NS T: NS

The significance of the main effects from analysis of variance and the standard deviation ( $n = 4$ ) for each treatment are shown (NS, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ).

### $\delta^{13}\text{C}$ and carbohydrates in the phloem sap

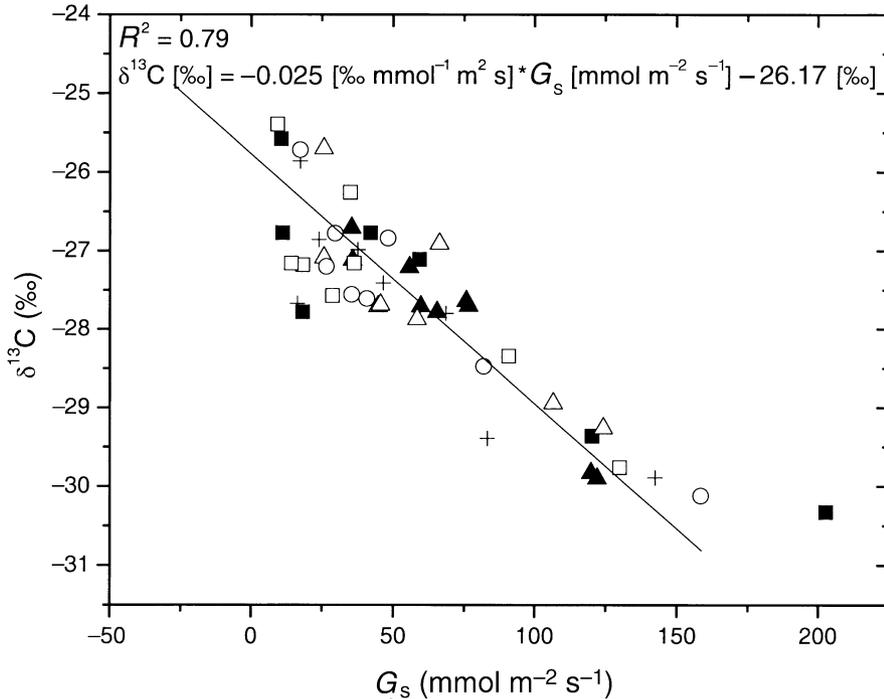
Sucrose, the dominant sugar in the collected phloem sap, made up between 95 and 99.8% of total sugar between June and September. In May however, when sugar concentra-

tions were at a minimum (170–240 mM), fructose and glucose together made up approximately 40% of total sugar. In the different treatments on the SW site, seasonal patterns of both  $\delta^{13}\text{C}$  and sugars were comparable and maxima and minima of both variables were observed on the same

**Table 2.** Correlation between  $\delta^{13}\text{C}$  in the phloem and physiological, meteorological and pedospheric parameters, averaged over different time spans prior to phloem sap collection

$\delta^{13}\text{C}$ versus	Days										
	1	2	3	4	5	6	7	10	14	18	22
$G_s$	-0.82**	-0.89**	-0.65**	-0.59**	-0.59**	-0.57**	-0.55**	-0.51**	-0.43**	-0.36*	-0.27
$ST$	0.24	0.26	0.26	0.20	0.19	0.18	0.19	0.20	0.23	0.22	0.23
$E$	0.03	0.04	0.05	0.07	0.06	0.09	0.07	0.08	0.07	0.06	0.06
$T_a$	0.45**	0.42**	0.42**	0.31*	0.27	0.27	0.34*	0.49**	0.50**	0.47**	0.41**
$RH$	-0.47**	-0.51**	-0.62**	-0.57**	-0.52**	-0.45**	-0.42**	-0.48**	-0.38*	-0.32*	-0.09
$VPD$	0.40**	0.43**	0.46**	0.40**	0.41**	0.41**	0.45**	0.40**	0.32*	0.43**	0.28*
$P$	-0.01	0.10	-0.43**	-0.32*	-0.22	-0.23	-0.22	-0.14	-0.11	-0.24	-0.09
$PAR$	-0.18	-0.13	0.09	-0.07	-0.01	-0.02	-0.01	0.04	0.12	0.12	0.14
$PAR_c$	-0.29	-0.24	-0.24	-0.21	-0.13	-0.15	-0.23	-0.09	-0.01	0.05	0.05
$\Psi_s$ 20	0.08	0.09	0.08	0.07	0.08	0.04	0.01	-0.09	-0.16	-0.24	-0.29
$\Psi_s$ 40	0.01	-0.03	-0.06	-0.05	-0.04	-0.04	-0.04	-0.08	-0.13	-0.15	-0.15
$\Psi_s$ 60	0.14	0.12	0.10	0.09	0.10	0.10	0.09	0.04	0.00	-0.01	0.02
$T_s$	0.03	0.02	0.10	0.08	0.07	0.11	0.09	0.06	0.06	0.05	0.05

The table shows Pearson's correlation coefficients for bivariate correlation analysis between  $\delta^{13}\text{C}$  of all phloem samples collected at eight points of time during the growing season from each site and treatment (except for the correlation with (1)  $T_s$  and  $\Psi_s$ : only control/closed canopy and 10 m<sup>2</sup> ha<sup>-1</sup> treatments/canopy gaps and (2)  $PAR_c$ : only control treatments) and the respective stomatal conductance ( $G_s$ ), transpiration ( $ST$ ), evaporation ( $E$ ), air temperature ( $T_a$ ), relative humidity ( $RH$ ), precipitation ( $P$ ), water vapour pressure deficit of the air ( $VPD$ ),  $PAR$ , soil water potential in 20, 40 and 60 cm ( $\Psi_s$  20, 40 and 60) and temperature of the soil ( $T_s$ ) as mean values of 1–22 d prior to the phloem sap collection.  $n = 16$ –48; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

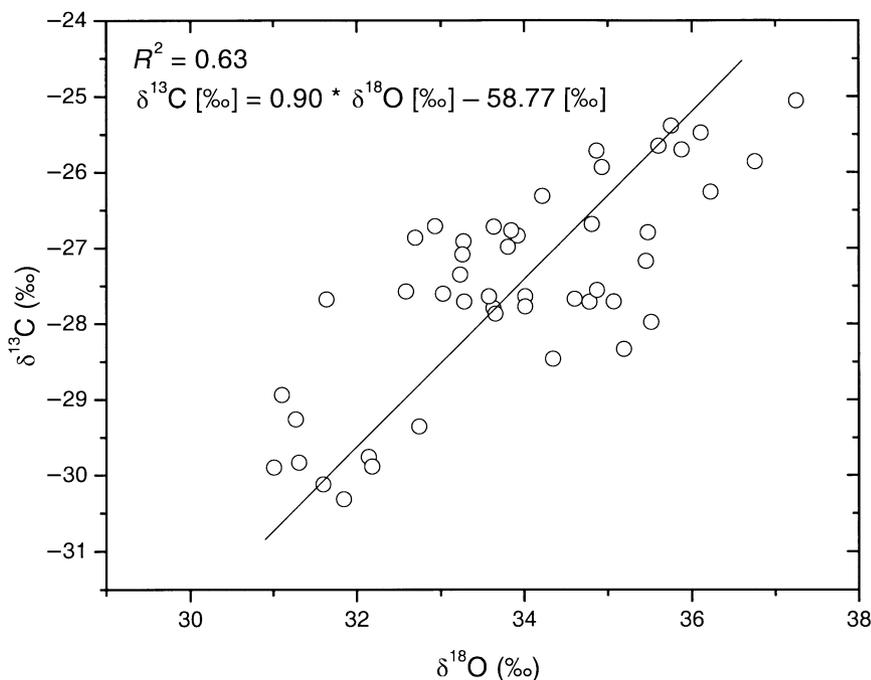


**Figure 3.** Regression between  $\delta^{13}\text{C}$  in phloem sap and the average daily mean stomatal conductance ( $G_s$ ) over a period of 2 d prior to sampling. The regression analysis includes measurements from eight sampling times during the growing season 2000 (29 May, 28 June, 18 July, 1 August, 15 August, 29 August, 12 September, 25 September) made in all three treatments (control and the two thinning treatments BA 15  $\text{m}^2 \text{ha}^{-1}$  and BA 10  $\text{m}^2 \text{ha}^{-1}$ ) on both aspects (NE and SW). SWC, ■; SW 15  $\text{m}^2 \text{ha}^{-1}$ , □; SW 10  $\text{m}^2 \text{ha}^{-1}$ , ○; NEC, ▲; NE 15  $\text{m}^2 \text{ha}^{-1}$ , △ NE, 10  $\text{m}^2 \text{ha}^{-1}$ , +.

sampling date (Fig. 5). Hence, a strong and significant regression relation was obtained between the two parameters (for all treatments on the SW site:  $R^2 = 0.59$ ;  $P < 0.001$ ). Comparable and synchronous seasonal patterns of phloem sugars and  $\delta^{13}\text{C}$  were not observed for the different treatments on the NE site. Thus, the regression relation between the two parameters was weak and not significant ( $R^2 = 0.13$ ,  $P > 0.05$ ).

## DISCUSSION

With the present approach, we combined the analysis of carbon isotopes in phloem sap (Yoneyama *et al.* 1997; Pate & Arthur 1998; Geßler *et al.* 2001), that putatively allows the analysis of short-term changes in  $C_i/C_a$ , with the model of variation in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  proposed by Scheidegger *et al.* (2000). This model can, theoretically, differentiate between



**Figure 4.** Regression between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of the organic matter of phloem sap. The figure includes measurements from eight different sampling times during the growing season 2000 (29 May, 28 June, 18 July, 01 August, 15 August, 29 August, 12 September, 25 September) made in all three treatments (control and the two thinning treatments BA 15  $\text{m}^2 \text{ha}^{-1}$  and BA 10  $\text{m}^2 \text{ha}^{-1}$ ) on both aspects (NE and SW).

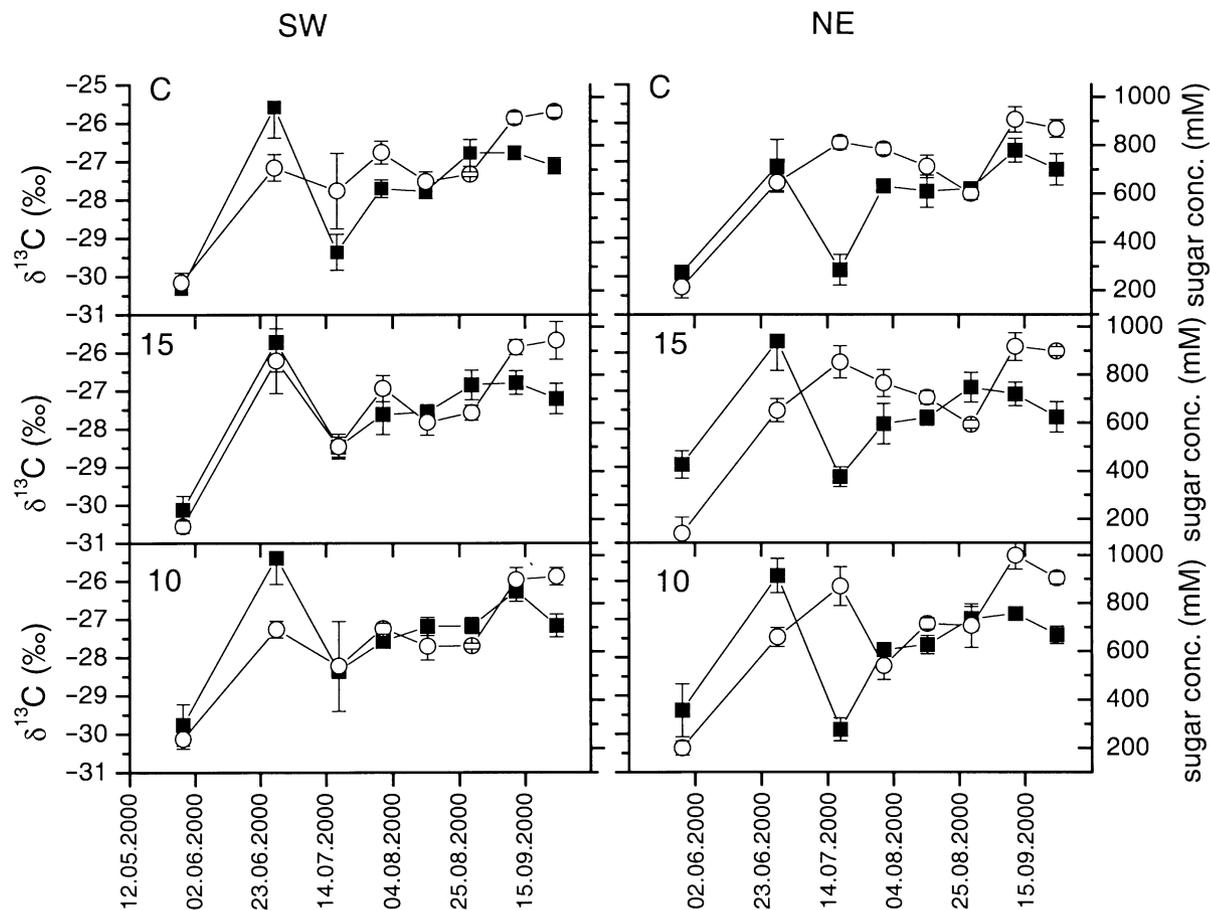
**Table 3.** Correlation between  $\delta^{18}\text{O}$  in the phloem and physiological, meteorological and pedospheric parameters, averaged over different time spans prior to phloem sap collection

$\delta^{18}\text{O}$ versus	Days			
	1	2	3	4
$G_s$	-0.60**	-0.69**	-0.66**	-0.61**
$ST$	0.36*	0.40**	0.39*	0.35*
$E$	0.21	0.34*	0.29	0.24
$T_a$	0.48**	0.51**	0.48**	0.47**
$RH$	-0.58**	-0.61**	-0.68**	-0.63**
$VPD$	0.56**	0.56**	0.58**	0.49**

The table shows Pearson's correlation coefficients for bivariate correlation analysis between  $\delta^{13}\text{C}$  of all phloem samples collected at eight points of time during the growing season from each site and treatment. All environmental variables shown in Table 2 were tested, but only the ones producing significant correlation are displayed. Highest correlation coefficients were obtained for integrals 2–4 d prior to the phloem sap collection, hence only these integrals are shown.  $n = 48$ ; \*,  $P < 0.05$ , \*\*,  $P < 0.01$ .

the effects on  $C_i$  of stomatal conductance and those of carbon fixation. In addition, we related  $\delta^{13}\text{C}$  signatures in the phloem to different environmental parameters averaged over different periods of time in order to characterize the time integral within which  $\delta^{13}\text{C}$  of phloem carbon is an indicator for particular environmental conditions.

Our study was conducted within replicated sites that differed in aspect (NE versus SW) and, hence, in radiation intercept and air temperature (Geßler *et al.* 2001), and in silvicultural treatment, that increased variation in water availability, temperature and light availability. Thinning resulted in a decrease in stand transpiration as previously observed for beech stands (Breda, Granier & Aussenac 1995) and a slight increase in evaporation from the soil on both sites. However, soil water availability for the remaining trees, as indicated by soil water potential (Fig. 1), increased only on the NE site under the open canopy – it remained constant or even decreased on the SW site, even though it never decreased under a value  $< -0.08$  MPa. However, even a small depletion of soil water availability within the observed range may influence water balance of drought-sensitive beech, as indicated by decreasing plant



**Figure 5.** Seasonal pattern of carbon isotope composition (■) and sugar concentrations (○) in the phloem sap of beech from different sites (SW, NE) and different treatments (control,  $15 \text{ m}^2 \text{ ha}^{-1}$  and  $10 \text{ m}^2 \text{ ha}^{-1}$ ) during the growing season 2000. Data shown are means ( $\pm$  SD) from four to six trees per site and treatment.

water potential and xylem flow rates (Geßler *et al.* 2001) and changes in N metabolism (Fotelli *et al.* 2002).

Our initial hypothesis was that the differences in water availability (and radiation) between sites and treatments were reflected by  $\delta^{13}\text{C}$  in the phloem. In spite of this, no significant correlation was observed between  $\delta^{13}\text{C}$  in the phloem sap and water potential at soil depths of 20, 40 or 60 cm when the whole growing season was considered (Table 2). In contrast, Geßler *et al.* (2001) could attribute site-specific differences (NE versus SW) in phloem  $\delta^{13}\text{C}$  to differences in soil water availability in 1999. However, this effect was only observed during a one-month drought period with low amounts of rainfall, whereas in 2000 precipitation was evenly distributed during the growing season. Monthly rainfalls were not less than 90 mm and extreme shortages of water were unlikely.

Moreover, the amounts of water transpired by the trees (Fig. 2) also did not reflect the soil water availability ( $R^2 = 0.09$ ,  $P = 0.05$  for  $\Psi_{s20}$ ;  $R^2 = -0.07$ ,  $P = 0.11$  for  $\Psi_{s40}$ ;  $R^2 = -0.04$ ,  $P = 0.40$  for  $\Psi_{s60}$ ). On the contrary, transpiration was significantly, albeit weakly correlated with vapour pressure deficit ( $R^2 = 0.3$ ,  $P < 0.001$ ). This weak correlation is an indicator of the number of influences on transpiration and their variation in influence throughout the growing season (Jones 1998). The weak correlations between stand transpiration and vapour pressure deficit on the one hand and between  $\delta^{13}\text{C}$  in the phloem and vapour pressure deficit on the other hand, additionally illustrated the lack of interdependence between  $\delta^{13}\text{C}$  in the phloem and transpiration (Table 2).

In contrast to the factors discussed above, carbon isotope composition was closely related to calculated stomatal conductance (Fig. 3), especially when conductance was integrated over a two-day period prior to phloem sampling. If phloem transport rates between  $0.5 \text{ m h}^{-1}$  and  $1.0 \text{ m h}^{-1}$  are assumed (Zimmermann & Braun 1971), transport of newly assimilated carbohydrates, from the foliage of the canopy to the base of the trunk, should take between 25 and 54 h in the trees examined. Hence, it is plausible that carbohydrates collected at the base of the trunk of beech carry an isotopic signal that is representative for physiological or environmental conditions of the previous two days.

There was also a significant – albeit less strong – correlation of phloem  $\delta^{13}\text{C}$  to  $VPD$ ,  $RH$  and  $T_a$ . This finding indicates – as observed for transpiration – that  $G_s$  may not only be controlled by atmospheric factors but by different and potentially varying influences during the growing season. Since  $\delta^{13}\text{C}$  in the phloem did not correlate with PAR, it is concluded that  $C_i$  was only affected by stomatal constraints and not by limitations in carbon fixation under the environmental conditions prevailing at the sites studied in 2000. This finding is in contrast to results of a previous study carried out in 1999 at the same sites (Geßler *et al.* 2001), which showed evidence that the increase in light availability due to thinning influenced phloem  $\delta^{13}\text{C}$  when water supply was limited. Again, this observation was made in a drought period in 1999, whereas there were no comparable meteorological conditions during the 2000 growing season.

In order to test the hypothesis that  $\delta^{13}\text{C}$  was only affected by  $G_s$  and not by the activity of the RubisCo, we correlated  $\delta^{13}\text{C}$  of organic compounds in the phloem with  $\delta^{18}\text{O}$ . This procedure proposed by Scheidegger *et al.* (2000) and Barbour *et al.* (2002) based on suggestions from Farquhar, Ehleringer & Hubick (1989), and Farquhar *et al.* (1998) provides a qualitative distinction between the effects on  $\delta^{13}\text{C}$  of stomatal conductance from those of a change in photosynthetic capacity. Enrichment of  $^{18}\text{O}$  in leaf water depends on the ratio of the water pressures in the atmosphere and in the gaseous space within the leaf and is a function of back-diffusion of water from the sites of evaporation being opposed by convection of source water to these sites via transpiration (Dongman *et al.* 1974; Farquhar *et al.* 1998; Barbour *et al.* 2002). A range of studies show that this enrichment in leaf water is reflected in the oxygen isotope signature of organic matter synthesized in the leaves and, putatively, transported in the phloem (DeNiro & Epstein 1979, Yakir 1992; Farquhar *et al.* 1998; Roden & Ehleringer 1999a, b; Barbour *et al.* 2000a). Since greater stomatal conductance cools the leaf and reduces internal water vapour pressure,  $\delta^{18}\text{O}$  in organic matter is a potentially useful tool to characterize stomatal conductance, independent from effects of carbon fixation (Adams & Grierson 2001). The significant correlation between  $\delta^{13}\text{C}$  of the organic compounds in the phloem and  $G_s$  (Table 3;  $R = -0.69$ ;  $P < 0.01$ ) supports this hypothesis.

If the source of variation of  $\delta^{13}\text{C}$  in organic matter is changing photosynthetic capacity,  $\delta^{18}\text{O}$  should be unaffected. We have shown that this was not the case – there was instead a strong positive correlation between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  (Fig. 4), a pattern related to stomatal and not photosynthetic control of  $\delta^{13}\text{C}$  (Farquhar *et al.* 1998; Scheidegger *et al.* 2000). The slope of the relationship between the two parameters (1.11‰ increase in  $\delta^{18}\text{O}$  per 1‰ increase in  $\delta^{13}\text{C}$ ) was within the range described in literature (between 0.32‰  $\delta^{18}\text{O}$  per 1‰  $\delta^{13}\text{C}$  (Sternberg, Mulkey & Wright 1989) and 2.9‰  $\delta^{18}\text{O}$  per 1‰  $\delta^{13}\text{C}$  (Barbour *et al.* 2000b)).

The observed differences between the coefficients obtained for correlation between  $\delta^{13}\text{C}$  or  $\delta^{18}\text{O}$  and environmental parameters (Tables 2 & 3) again confirm theoretical assumptions, that  $\delta^{13}\text{C}$  is mainly controlled by  $G_s$  under non-limiting light conditions (Farquhar, O'Leary & Berry 1982), whereas the ratio of water pressure of the air outside and inside the leaf, the latter influenced by  $G_s$ , is the parameter controlling evaporative enrichment of  $^{18}\text{O}$  (Farquhar *et al.* 1998; Adams & Grierson 2001).

A strong relationship has previously been demonstrated between sugar concentrations and  $\delta^{13}\text{C}$  in the phloem sap of *Eucalyptus globulus*. Both increased when water availability decreased (Pate *et al.* 1998; Pate & Arthur 1998). Geßler *et al.* (2001), however, were unable to observe any significant correlation between  $\delta^{13}\text{C}$  and sugar concentration in the phloem of European beech at the same stands examined here. Nevertheless, the previous study was of relatively short duration (sampling in August and September 1999) and used combined data from SW and NE sites. Figure 5 shows that a close correlation between sugar con-

centration and carbon isotope composition does exist for beech, but only on the SW site. This finding is again in agreement with the results of Pate & Arthur (1998), who described an almost exactly synchronous seasonal pattern of sugar concentrations and  $\delta^{13}\text{C}$  but only for a site with low water availability – correlations were less strong on a well-watered site. It is concluded that determination of sugar contents in the phloem of beech is not a reliable indicator for  $\delta^{13}\text{C}$  as proposed by Pate & Arthur (1998) for *E. globulus*.

Leaf  $\delta^{13}\text{C}$  did not vary between sites and treatments, hence reflecting poorly the temporary differences in environmental conditions between the different aspects and stand densities. The strong enrichment of bursting buds with  $^{13}\text{C}$  in April is likely to be a result of remobilization of carbon originating from stored starch that carries a  $\delta^{13}\text{C}$  signature that is about 4‰ greater than triose-P originating from the pentose phosphate cycle (Schmidt & Gleixner 1998). The reasons for this greater  $\delta^{13}\text{C}$  signature of stored and remobilized carbon remain a subject for further research.

We conclude that the analysis of  $\delta^{13}\text{C}$  in soluble carbon transported in the phloem is a reliable indicator of short-term changes of  $C_i/C_a$ . In leaves,  $\delta^{13}\text{C}$  is masked by the long-term integrating  $\delta^{13}\text{C}$  of structural carbon. If additional analysis of  $\delta^{18}\text{O}$  is performed, this approach can be used to differentiate between the effects of stomatal conductance and carbon fixation within a time integral of a few days.

## ACKNOWLEDGMENTS

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