

Beyond CO₂-fixation by Rubisco – an interpretation of ¹³C/¹²C variations in tree rings from novel intra-seasonal studies on broad-leaf trees

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ABSTRACT

Evidence is presented for a very specific, seasonally recurring tri-phase carbon isotope pattern in tree rings of broad-leaf deciduous tree species. It is derived from highly resolved intra-annual measurements of ¹³C/¹²C ratios of wood and cellulose from tree rings of *Fagus sylvatica*, *Populus nigra*, *Quercus petraea* and *Morus alba*. Investigations on $\delta^{13}\text{C}$ from buds and leaves of *Fagus sylvatica* revealed a similar tri-phase $\delta^{13}\text{C}$ pattern. At the very beginning of a growing season, the $\delta^{13}\text{C}$ trend of tree rings and foliage shows a marked increase of up to 5‰. The maximum $\delta^{13}\text{C}$ -value of each vegetation period always occurs in young heterotrophic leaves shortly after bud burst and persistently in the early wood of each tree ring, when growth depends on carbon reserves. Thereafter, $\delta^{13}\text{C}$ profiles represent the autotrophic stage of the leaves, which show different patterns of variation, by and large characterized by a decline. The minimum $\delta^{13}\text{C}$ -value always shows up in the late wood of each tree ring. At the very end of each tree ring $\delta^{13}\text{C}$ -values start rising again. This increase in $\delta^{13}\text{C}$ marks the gradual switch-over to storage-dependent growth and can also be observed in senescent leaves. Seasonal changes of more than 4‰ were measured, whereas contiguous $\delta^{13}\text{C}$ values rarely differed from each other by more than 0.3‰. This tri-phase pattern cannot be explained by the common model of carbon isotope fractionation during photosynthesis. It appears to be primarily an indication of seasonal changes in down-stream processes of the carbohydrate metabolism. Environmental influences on the carbon isotope fractionation during photosynthesis are presumably of secondary importance and expressed by certain peculiarities showing up during the autotrophic phase, i.e. the mid-section of the seasonal $\delta^{13}\text{C}$ pattern.

Key-words: $\delta^{13}\text{C}$; deciduous trees; high-resolution sampling; seasonal pattern; tree rings.

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INTRODUCTION

Stable carbon isotope analysis has become an important tool in ecophysiological research. According to Farquhar and colleagues photosynthetic discrimination of ¹³CO₂ against ¹²CO₂ in C₃ plants is related to the ratio of leaf internal to leaf external CO₂ partial pressure (C_i/C_a) as controlled by stomatal conductance and driven by the rate of CO₂ assimilation (Farquhar, O'Leary & Berry 1982; Francey & Farquhar 1982; Farquhar, Ehleringer & Hubick 1989).

Since stable carbon isotope ratios (reported as $\delta^{13}\text{C}$ in per mil) of tree rings are assumed to mirror the isotope effects resulting from photosynthesis, they are frequently used for investigations of long-term temporal dynamics of plant response to environmental factors. In this respect fractionation processes beyond the leaf level were generally neglected, as being small of higher order.

Based on the model constraints of photosynthetic ¹³C discrimination against ¹²C, considerable research has for some time been focused on the significance of the isotopic composition of ¹³C/¹²C in tree rings to meteorological variables like temperature, precipitation or relative humidity (Wilson & Grinsted 1978; Mazany, Lerman & Long 1980; Stuiver & Braziunas 1987; Leavitt & Long 1991; Lipp & Trimborn 1991; Leavitt 1992; Yakir *et al.* 1994; Kitagawa & Matsumoto 1995; Panek 1995; Schleser 1995; Lipp *et al.* 1996; Liu *et al.* 1996; Switsur *et al.* 1996; Panek & Waring 1997; Robertson *et al.* 1997a, b; Zimmermann, Schleser & Bräuning 1997; Hemming *et al.* 1998; Edwards *et al.* 2000). Additionally, relationships were examined between ¹³C/¹²C ratios and soil water content (Dupouey *et al.* 1993; Walcroft *et al.* 1997; Panek & Goldstein 2001), water-use efficiency (Bert, Leavitt & Dupouey 1997; Walcroft *et al.* 1997; Duquesnay *et al.* 1998; Tang, Feng & Funkhouser 1999; Matzner, Rice & Richards 2001; Ponton *et al.* 2001), hydraulic properties of the water-conducting system of stems and branches (Panek 1996) or pollution (De Silva 1978; Peng *et al.* 1983; Martin & Sutherland 1990). Some work dealt with the $\delta^{13}\text{C}$ distribution in tree stems with height or along branches and twigs (De Silva 1978; Leavitt & Long 1986;

Schleser 1992; Panek & Waring 1995), and also with leaf anatomical properties (Schleser, Bernhardt & Hurka 1989).

Provided the $\delta^{13}\text{C}$ signature produced during primary CO_2 fixation is directly transferred into the continuously developing tissue of tree rings it should monitor the stomatal activity, as induced by environmental changes around leaves. Thus, the radial distribution of stable carbon isotopes within a ring, if adequately resolved in time should therefore reflect the different environmental conditions experienced by a tree along the vegetation period.

However, in perennial plants such as trees, the time between photosynthate production and final storage in wood is difficult to determine. Furthermore, intra-annual $\delta^{13}\text{C}$ values cannot easily be related to specific dates or weather events, because annual ring growth is not a linear process. In general, tree ring formation starts with light coloured, rapidly growing, wide and thinly walled cells [early wood (EW)] which may live only days up to a few weeks. During the summer narrow cells develop having thick walls which may live up to several months. This more dense and darker wood is called late wood (LW). This seasonal non-linear growth behaviour indicates that the ability of a tree ring to integrate environmental information depends on the longevity of its cells which is, among other factors, species dependent.

Previously published carbon isotope data of radial subsections from rings of broad-leaf and coniferous trees partly reveal rather contradictory results, since the intra-annual resolution achieved was generally poor. Nevertheless, many authors found systematic differences across annual growth rings and several studies have shown that significantly different isotope ratios can exist between early and late wood (Wilson & Grinstead 1977; Fraser, Francey & Pearman 1978; Leavitt & Long 1982, 1986, 1991; Lipp & Trimborn 1991; Leavitt 1992; Kitagawa & Wada 1993; Ogle & McCormac 1994; Hill *et al.* 1995; Switsur *et al.* 1995; Li *et al.* 1996; Livingston & Spittlehouse 1996; Ogle & McCormac 1996; Robertson *et al.* 1996; Sheu *et al.* 1996; Jordan & Mariotti 1998; Pate & Arthur 1998; Schleser *et al.* 1999; Teng *et al.* 1999; Jäggi *et al.* 2002; Leavitt 2002). In all cases

attempts were made to relate the $\delta^{13}\text{C}$ -data to the prevailing environmental, namely temperature and/or precipitation and humidity conditions along the growing season. Whether intra-annual carbon isotope data are, however, really reflecting the influence of ambient environmental factors and if so to what extent remained an open question.

The present investigation was firstly aimed at exploring whether or not a universal carbon isotope pattern exists in tree rings along the vegetation period and secondly at contributing to the assessment of seasonal isotope signature transfer from leaf level into the developing woody tissue. The study made use of a novel technique to produce highly resolved seasonal carbon isotope data down to $10\ \mu\text{m}$ resolution and was focused on broad-leaf deciduous tree species.

MATERIALS AND METHODS

Plant material and site characteristics

Since the major aim was to assess seasonal carbon isotope behaviour, primarily driven by plant internal processes, studies were performed with the main emphasis on four broad-leaf deciduous tree species. The reason being that they show distinct seasonal interactions between reserves and growth. Investigations centered on the radial distribution of $\delta^{13}\text{C}$ in wood and/or cellulose of tree species having different wood anatomical features, namely diffuse porous species and ring porous species. Cambial activity of the chosen diffuse porous species is generally known to begin simultaneously or shortly after leaf emergence, whereas cambial activity of the ring porous trees starts several days before bud burst (e.g. Ladefoged 1952; Kozłowski 1992; Catesson & Lachaud 1993; Pilcher 1995).

The sampling sites were not primarily intended to represent climatically different stands and therefore they were only chosen from low elevation sites of central Europe (Table 1).

With the exception of the Rostrevor site all sites are characterized by poorly drained soils providing ample water to the roots. These selection criteria ensured that excep-

Table 1. Site characteristics and tree species sampled

	Hambach	Bergisch-Gladbach	Rostrevor	Neuglobsow
Species	<i>Fagus sylvatica</i> L.	<i>Populus nigra</i> L.	<i>Quercus petraea</i> (Mattuschka) Liebl.	<i>Morus alba</i> L.
Wood anatomical structure	diffuse porous	diffuse porous	ring porous	ring porous
Location	50°54' N, 6°25' E	51°4' N, 7°8' E	54°6' N, 6°12' W	53°5' N, 13°5' E
Elevation (m)	91	150	180	65
Soil	fine sand and silt loam, Pseudogley (soil with perched surface water)	silt loam, Pseudogley	stony and sandy loam, well-drained S exposed slope (5–10°)	stony silt loam, glacial till
Average annual temperature (°C)	9.7	9.5	9.4	8.7
Average annual precipitation (mm)	805	829	795	568

tional environmental extremes with regard to the available soil water content, but also climatic and topographic anomalies (e.g. altitudes) could be eliminated. Only dominant trees from stands older than 120 years, without obvious physical or chemical damage, were chosen in order to minimize the effects of competition on tree growth. At the Neuglobsow site a solitary standing mulberry tree was chosen.

High-resolution intra-annual sampling of tree rings

High-resolution intra-annual sampling was performed by using a modified sledge microtome. Wood segments approximately 8 mm × 5 mm or larger were taken from stem discs as well as cores, 5 or 12 mm in diameter, of living trees (at breast height). An important selection criteria was the selection of parallel ring groups with almost straight tree ring borders. As the curvature of growth rings varies under natural conditions, parallel ring groups were identified and selected with the aid of the gridlock of a binocular microscope. Only a limited number of consecutive rings per sample were suitable for high-resolution intra-annual isotope measurements. The chosen tree rings were always derived from adult trees aged over 50 years.

The chosen ring groups were isolated and trimmed to fit into a special sample mount attached to a fixed-blade sledge microtome (Polycut E; LEICA Microsystems, Bensheim, Germany). The sample mount of the microtome is fixed to a specially designed and constructed universal joint allowing appropriate sample adjustment without loosening the sample within the mount during the cutting process. Contiguous tangential slices of 180 μm down to 10 μm thickness were cut. Accurate sample adjustment, as well as meticulous identification of tree ring borders, and wood anatomical features such as early wood–late wood transitions were provided by visual inspection using a binocular. If needed the assignment of each micro-slicer position to wood anatomical features was documented with a digital camera (DC100; LEICA Microsystems). Details of the cutting procedure are given in Helle (1996).

Intra-annual sampling of leaves

In addition to high-resolution intra-annual investigations of carbon isotope ratios from tree rings of *Fagus sylvatica* originating from the Hambach Forest, the corresponding variations of $\delta^{13}\text{C}$ of buds and leaves (sun/shade) during 1 year were analysed for comparison.

Buds and leaves were sampled at 3 and 26 m above ground at different time-intervals: every second day immediately before and after leaf sprouting, along with bud swelling and leaf area growth during spring, namely April–June; biweekly during summer (July–October) and again every second day during leaf senescence (November). Each time 20–30 leaves or buds were harvested, oven-dried and finely ground to a powder.

Collection of atmospheric CO_2

Atmospheric CO_2 was collected every month above the upper canopy (30 m above ground) of the beech stand at Hambach Forest. Evacuated 10 L glass flasks were used for sampling. CO_2 was extracted from air by freezing water and removing nitrogen oxides by reduction in a vacuum preparation line using a copper oven (450°C).

Cellulose extraction

Cellulose was extracted from homogenized wood samples by using sodium hydroxide and sodium chlorite according to Sohn & Reiff (1942). Details of the procedure are given by Wiesberg (1974).

Stable carbon isotope analysis

Samples of leaf organic matter, bud material, total wood and/or cellulose were combusted to CO_2 either in a specifically designed vacuum combustion line or in an elemental analyser (Model NA 1500; Carlo Erba, Milan, Italy). Samples (100–200 μg) were taken for analysis of cellulose or wood. For homogeneity reasons rather large subsamples of 1.5–2 mg for bud and leaf material were used for isotope analysis.

Carbon isotope ratios of generated CO_2 were determined either on an Optima-IRMS (Micromass, Ltd. Manchester, UK) or a Finigan MAT 250 (Thermoquest GmbH, Bremen, Germany). $\delta^{13}\text{C}$ was referred to the international standard VPDB (Vienna Pee Dee Belemnite): $\delta^{13}\text{C}$ (‰) = $(R_s/R_{\text{VPDB}} - 1) \times 1000$, where R_s and R_{VPDB} are the molecular abundance ratios of carbon isotopes, $^{13}\text{C}/^{12}\text{C}$, of the sample and the standard VPDB, respectively. Sample replication, including all errors of sample preparation was generally less than $\pm 0.1\%$.

RESULTS

Seasonal $\delta^{13}\text{C}$ variations from trees with diffuse porous wood structure (beech, poplar)

Figures 1a, 2, 3a & b exhibit the seasonal fluctuations and trends of $\delta^{13}\text{C}$ -values in cellulose and/or total wood from tree rings of *Fagus sylvatica* L. (Figs 1a and 2) and *Populus nigra* L. (Fig. 3a & b). The results show that the radial distribution of carbon isotopes varied considerably across the studied intra-annual transects, namely along the growing season with time resolutions down to days in some cases. Changes of 0.5‰ to more than 4‰ were measured within a ring. However, the point-to-point variability along each isotope profile was generally low. Contiguous $\delta^{13}\text{C}$ values rarely differ from each other by more than 0.3‰.

The intra ring $\delta^{13}\text{C}$ variations of consecutive tree rings emerge as a seasonally recurring pattern which is rather similar in both species, beech and poplar. This seasonal carbon isotope pattern can be divided into three different sections. In the first section, at the beginning of the growing season, namely during the beginning of EW development,

$\delta^{13}\text{C}$ values rapidly rise to a maximum, which is always reached during EW development. The second section of each $\delta^{13}\text{C}$ curve can show different patterns of variation, but is generally characterized by a decline of up to 3.5‰ and reaches a minimum which is normally located in the LW region of each tree ring. Within the mid-section of every $\delta^{13}\text{C}$ -curve data can either decline gradually (e.g. Fig. 1a, 1985; Fig. 3a, 1989) or drop abruptly by up to 2‰ shortly after the EW maximum, then temporary level off, sometimes rise again slightly before the $\delta^{13}\text{C}$ -values rapidly fall again to their minimum (e.g. Fig. 1a, 1984; Fig. 3a, 1990, 1991). At the end of each LW domain, in the third section, $\delta^{13}\text{C}$ -values start rising again before crossing the tree ring border smoothly towards the EW maximum of the following tree ring.

The $\delta^{13}\text{C}$ minima and maxima of consecutive years can show very different levels, with the isotope minima of different years varying generally more than the maxima.

As illustrated in Fig. 2 the general seasonal trends of intra-annual $\delta^{13}\text{C}$ -variations are independent of whether or not total wood or cellulose is investigated. This is of importance because varying amounts of ^{13}C -depleted secondary (e.g. lignin, lipids) and ^{13}C -enriched primary (e.g. cellulose) plant products could influence the seasonal $\delta^{13}\text{C}$ pattern of growth rings (Wilson & Grinstead 1977). In any case ^{13}C depletions between 2 and 6‰ exist for aromatic compounds such as lignin relative to carbohydrates (Benner *et al.* 1987; Schmidt *et al.* 1993). In beech tree rings of different years the varying cellulose:lignin ratios might be responsible for the observed unsteady difference of 0.5–1.8‰ between

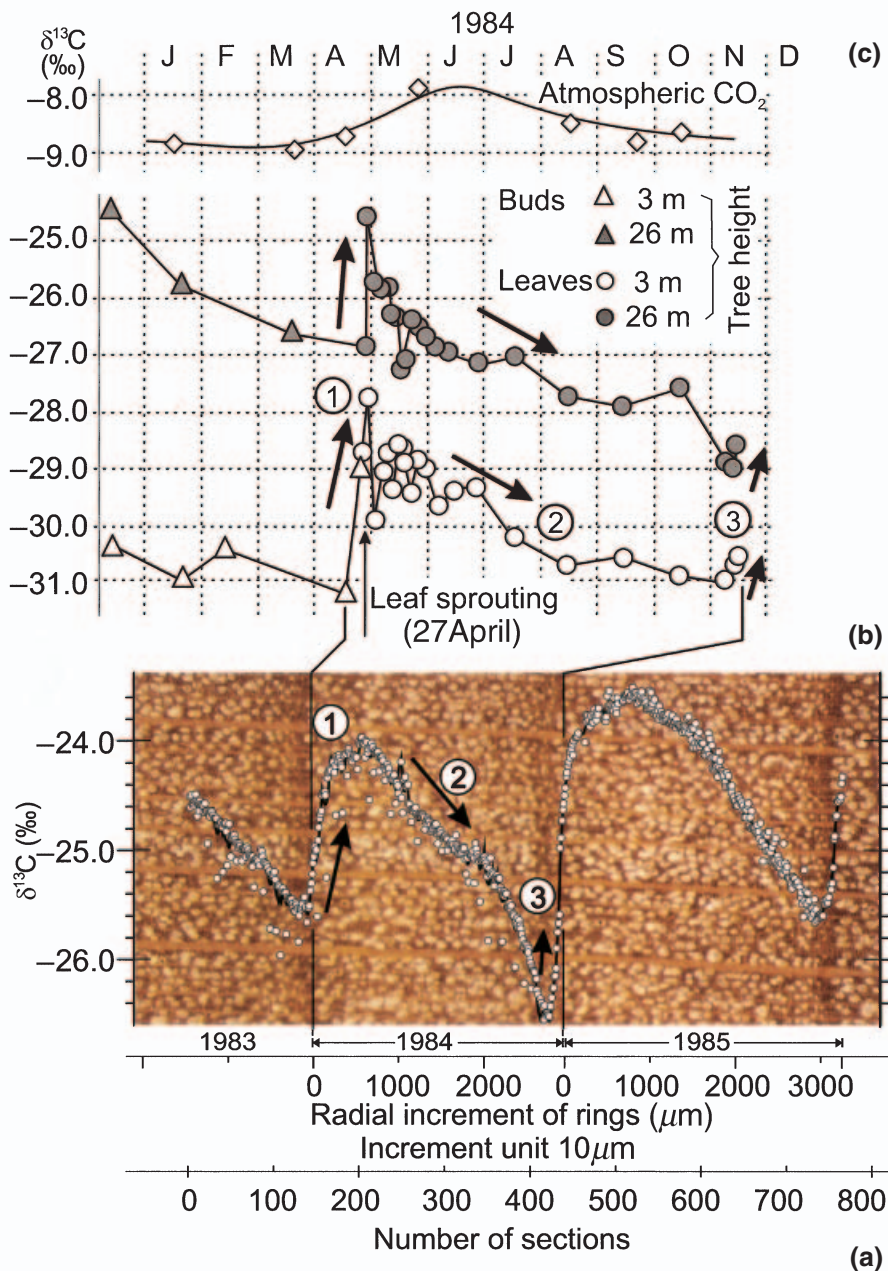


Figure 1. (a) Seasonal carbon isotope behaviour in total organic matter of tree rings (O) from *Fagus sylvatica* (beech). (b) Intra-annual carbon isotope behaviour in total organic matter of buds (▲) and sun leaves (●) from 26 m tree height and buds (△) and shade leaves (○) from 3 m height. (c) Intra-annual $\delta^{13}\text{C}$ profile of atmospheric CO_2 above the canopy at 30 m height. The three different sections of the seasonal pattern are numbered and indicated by black arrows (for details see text).

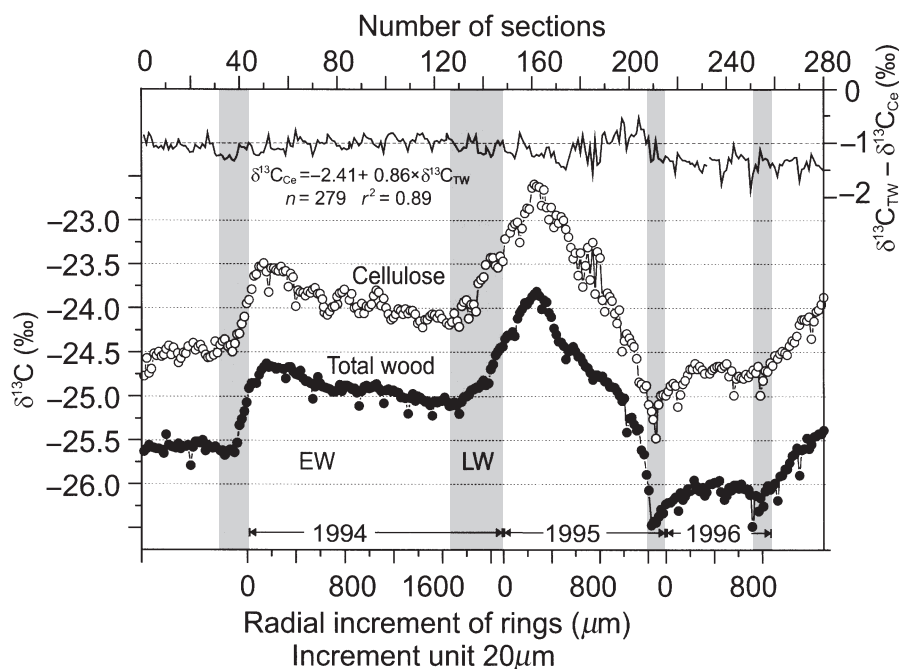


Figure 2. Comparison of seasonal carbon isotope behaviour of total wood ($\delta^{13}\text{C}_{\text{TW}}$) and the corresponding cellulose ($\delta^{13}\text{C}_{\text{Ce}}$) of tree rings of *Fagus sylvatica* (beech).

total wood ($\delta^{13}\text{C}_{\text{TW}}$) and cellulose ($\delta^{13}\text{C}_{\text{Ce}}$) (Fig. 2). It should be noted, however, that the difference between $\delta^{13}\text{C}_{\text{TW}}$ and $\delta^{13}\text{C}_{\text{Ce}}$ in a tree ring is almost always more or less constant, but may show differences in different years.

Figure 3b shows the $\delta^{13}\text{C}$ -pattern of cellulose from two different cross-sections of a poplar tree ring formed in AD 1991. The ring width of cross-section A (8.8 mm) is almost twice as wide as that of cross-section B (4.6 mm). Microscopic examination of stained wood micro-sections revealed that cross-section A is less lignified, characterizing it as tension wood, namely reaction wood of the leaning stem. For comparison of isotope results the ring widths were normalized. Each intra-annual $\delta^{13}\text{C}$ -value was given a relative position based on 100% for total ring width, assuming that the increment in growth in each direction is similar on a relative basis along the vegetation period. After normalization, the two $\delta^{13}\text{C}$ patterns were quite similar in both the tri-phase seasonal trend and the absolute values, except for two possible outliers in each series. The minima appeared at slightly different relative positions which indicates that relative ring growth may not be equal in both directions. However, this rather good match has to be confirmed by further investigations, as studies on the circumferential $\delta^{13}\text{C}$ variability of cellulose have shown high variability between different radii of up to 4‰ (e.g. Tans & Mook 1980; Nguyen-Queyrens *et al.* 1998).

Seasonal $\delta^{13}\text{C}$ -variations of beech foliage

In order to investigate and evaluate seasonal changes in photosynthetic ^{13}C -discrimination against ^{12}C relative to the $\delta^{13}\text{C}$ changes found in tree rings, $\delta^{13}\text{C}$ -values of buds and leaves of *Fagus sylvatica* L. sampled at 3 m (shade leaves) and 26 m (sun leaves) height during a year (Fig. 1b) were analysed.

In the foliage, a sharp increase of 2–3‰ was observed at the very beginning of the vegetation period (end of April). The highest $\delta^{13}\text{C}$ values were measured in leaves sampled 2 d after bud burst. Shortly thereafter $\delta^{13}\text{C}$ -values of leaves drop by approximately 1.5‰. During the vegetation period (end of May to beginning of November) $\delta^{13}\text{C}$ -values varied slightly and gradually fell by 2‰. Along with leaf senescence $\delta^{13}\text{C}$ -values started to rise again at the very end of the vegetation period, which can also be seen in the final LW section.

A similar pattern of $\delta^{13}\text{C}$ along with leaf aging was also found in other studies on *Fagus sylvatica* and *Acer grandidentatum* (Leavitt & Long 1985; Damesin & Lelarge 2003) and even in tropical tree species (Terwilliger *et al.* 2001). These studies, as well as that by Schleser (1990) demonstrated that the observed pattern cannot be attributed to changes in the content of differently labelled metabolic fractions within whole leaf organic matter, since they revealed a constant offset in $\delta^{13}\text{C}$ between whole leaf organic matter and cellulose. Although at different absolute levels, identical patterns in whole leaf material, cellulose and soluble sugars were found. However, the rapid change of $\delta^{13}\text{C}$ from low values at bud stage to the highest values shortly after leaf emergence has rarely been recognized mostly because of coarse temporal resolution of sampling.

In order to assess the possible effect of changing atmospheric source values on the photosynthetic $^{13}\text{C}/^{12}\text{C}$ ratio of organic matter we measured the annual variation of $\delta^{13}\text{C}$ in atmospheric CO_2 directly above the canopy. Although only a few measurements were made the results corroborate the well-known trend of more negative $\delta^{13}\text{C}$ -values during winter and less negative values during the vegetation period due to strong photosynthetic ^{13}C discrimination. In our case $\delta^{13}\text{C}$ -values of atmospheric CO_2 increased by approximately 1‰ during summer. From Fig. 1b it is evi-

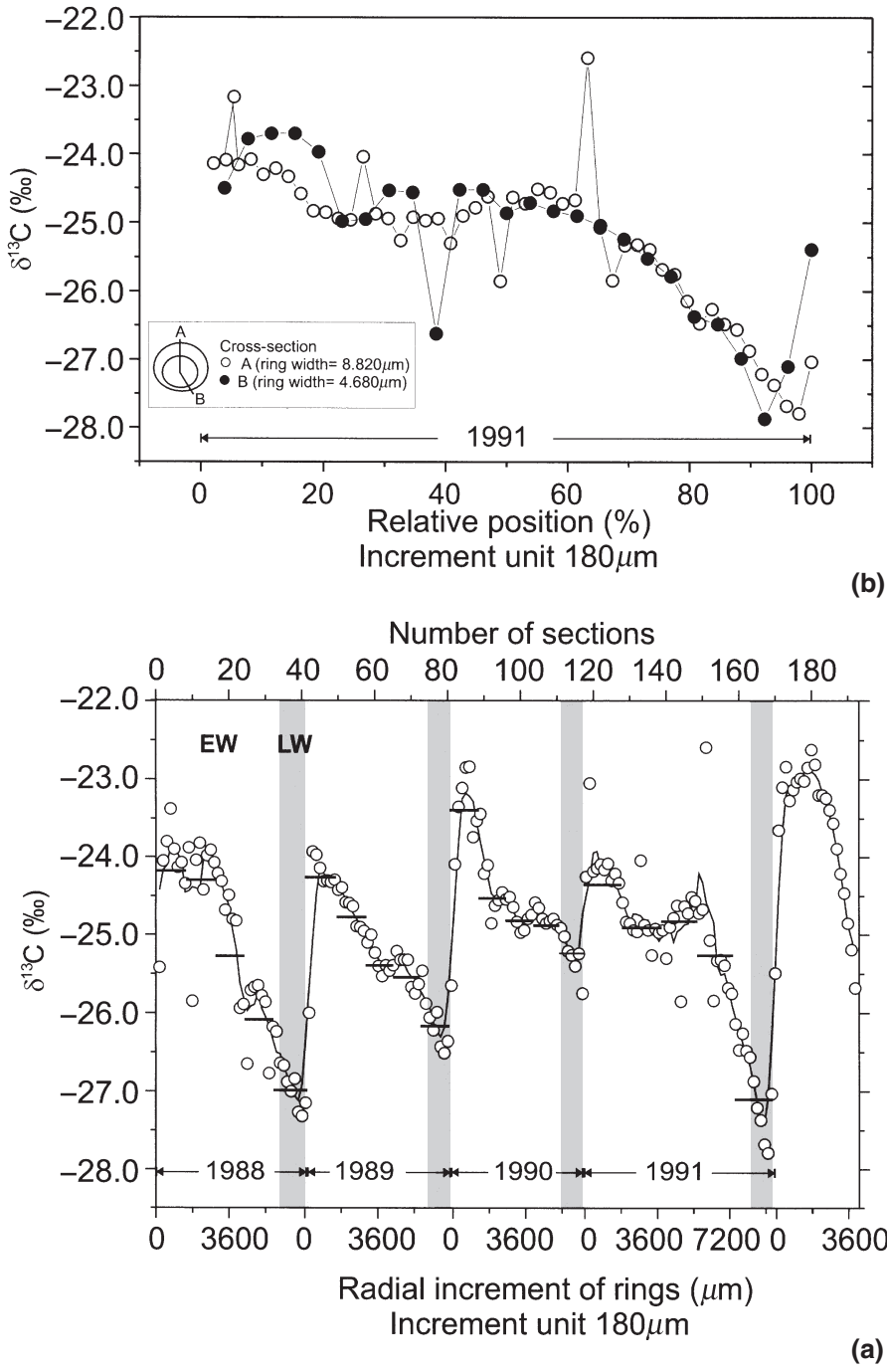


Figure 3. (a) Seasonal carbon isotope variations in cellulose of tree rings from *Populus nigra* (poplar). Horizontal lines indicate the mean $\delta^{13}\text{C}$ values of high-resolution data for five equal ring subdivisions. (b) Seasonal carbon isotope variations in cellulose of two different cross-sections from one annual ring of *Populus nigra* (poplar).

dent that the increase in $\delta^{13}\text{C}$ of organic matter mainly occurs during the time of leaf emergence in spring. Therefore, the general change in $\delta^{13}\text{C}$ of atmospheric CO_2 , representing the source value for any isotope composition of plant organic matter does not account for the observed 2–3‰ increase in $\delta^{13}\text{C}$ of young foliage. Moreover, during May/June the $\delta^{13}\text{C}$ trends of atmospheric CO_2 and organic matter were antipodal.

However, Fig. 1b illustrates that the $\delta^{13}\text{C}$ -values of organic matter from 3 m height are depleted by 2–4‰ in comparison with organic matter from 26 m height. This agrees with previous observations of a strong linear corre-

lation between leaf $\delta^{13}\text{C}$ and height in the tree (Vogel 1978; Schleser & Jayasekera 1985; Schleser 1992; Buchmann & Ehleringer 1998; Buchmann, Hinckley & Ehleringer 1998). The relative depletion of leaf $\delta^{13}\text{C}$ at 3 m height reflects the uptake of soil-respired, ^{13}C -depleted CO_2 , in conjunction with a higher ^{13}C fractionation in the shaded leaves of the lower canopy caused by wider stomatal apertures, namely by higher stomatal conductance, relative to upper leaves. Nevertheless, the main characteristics of the seasonal $\delta^{13}\text{C}$ patterns are all the same at both heights. Hence, the changing $\delta^{13}\text{C}$ -source value cannot explain the large $\delta^{13}\text{C}$ variations of leaf tissue along the vegetation period.

It is interesting to note that the ^{13}C -content of buds varies notably during the winter months. From December to March $\delta^{13}\text{C}$ -values fall by more than 1‰, before their $\delta^{13}\text{C}$ -values increase rapidly alongside bud swelling and bud burst.

Radial distribution of $\delta^{13}\text{C}$ in ring-porous wood (oak, mulberry)

Oak and mulberry belong to the group of deciduous tree species having ring-porous wood. Most interestingly, the EW formation of oak and mulberry, similar to that of most other species with ring-porous wood, generally starts before bud burst. Its development is completed before the leaves have fully expanded, namely before they are capable of positive net photosynthesis (Ladefoged 1952; Kozłowski 1992; Catesson & Lachaud 1993; Pilcher 1995). Consequently, these trees can not use carbon from current assimilates for EW formation but have to use carbon from previous years, accumulated mainly as starch during the preceding summer months, namely during the time of their LW formation.

Figures 4a and 5 show the typical seasonal $\delta^{13}\text{C}$ patterns of cellulose from two tree rings of oak (*Quercus petraea* (Matt.) Liebl.) and mulberry (*Morus alba* L.), respectively. In view of their ring widths, resolutions of 40 μm for oak and 180 μm for mulberry were chosen for intra-annual $\delta^{13}\text{C}$ measurements.

In agreement with the observations on the diffuse porous deciduous tree species, a distinct recurring $\delta^{13}\text{C}$ -pattern occurs. Again, the tri-phase development can be observed.

An enrichment in $\delta^{13}\text{C}$ of EW is followed by a subsequent decline during LW formation and increasing $\delta^{13}\text{C}$ -values at the very end of each vegetation period, that is, the last part of the LW domain. Minima and maxima of the years analysed are at the same level for oak, but for mulberry the minimum in 1959 is approximately 1.5‰ higher than in 1960, and the maxima differ by approximately 1‰.

Some authors reported a visual correlation between the mean $\delta^{13}\text{C}$ -value of the LW for a given year and that of the EW of the following year (e.g. Switsur *et al.* 1995). However, our findings illustrate that it is very unlikely that $\delta^{13}\text{C}$ of the LW-section of a ring correlates with the EW-section of the following year by means of absolute values, because EW of broad-leaf deciduous trees generally exhibits an enrichment in ^{13}C relative to the preceding or following LW.

DISCUSSION

Our high-resolution measurements revealed a rather similar picture for $\delta^{13}\text{C}$ changes in both tree rings and foliage of *Fagus sylvatica*, although $\delta^{13}\text{C}$ -values in wood were less variable than in leaf $\delta^{13}\text{C}$. During the growing season $\delta^{13}\text{C}$ of foliage declines by 4.5‰, whereas $\delta^{13}\text{C}$ of the corresponding tree ring shows a difference of 2.6‰ between maximum and minimum $\delta^{13}\text{C}$ (Fig. 1a & b). The similarity of both curves may indicate that they result from the same physiological and biochemical processes.

However, the difference in $\delta^{13}\text{C}$ between foliage and tree ring is not constant. It increases from approximately 1‰ at the stage of maximum enrichment during bud burst to approximately 2.4‰ at the time of minimum $\delta^{13}\text{C}$ -values in

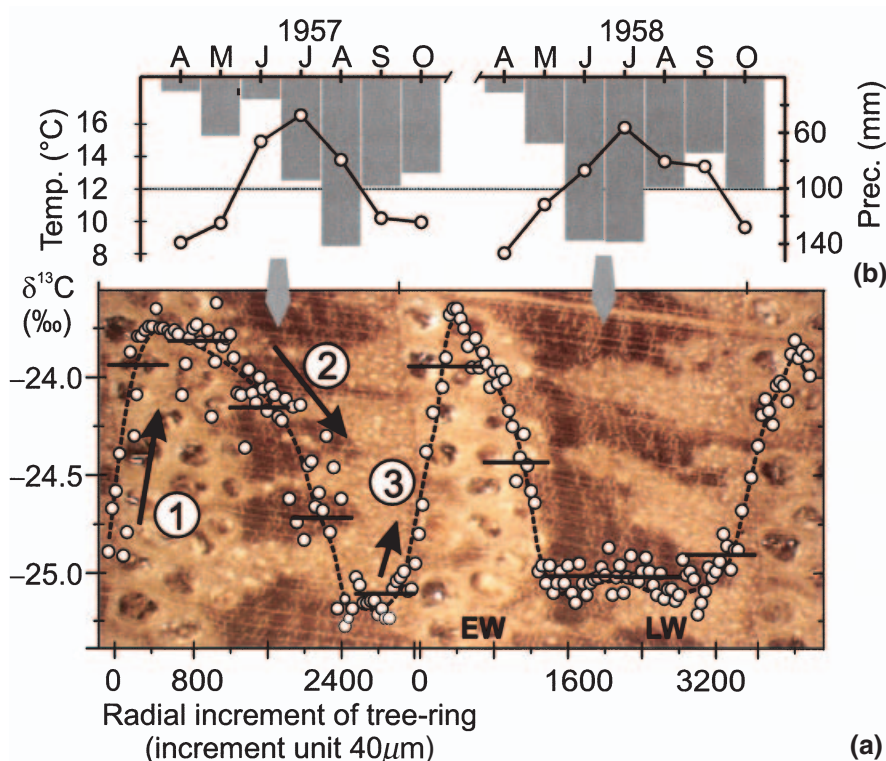


Figure 4. (a) Seasonal carbon isotope variations in cellulose of tree rings from *Quercus petraea* (oak). Horizontal lines indicate the mean $\delta^{13}\text{C}$ values of high-resolution data for five equal ring subdivisions. (b) Seasonal variations of monthly mean air temperature and precipitation (April–October).

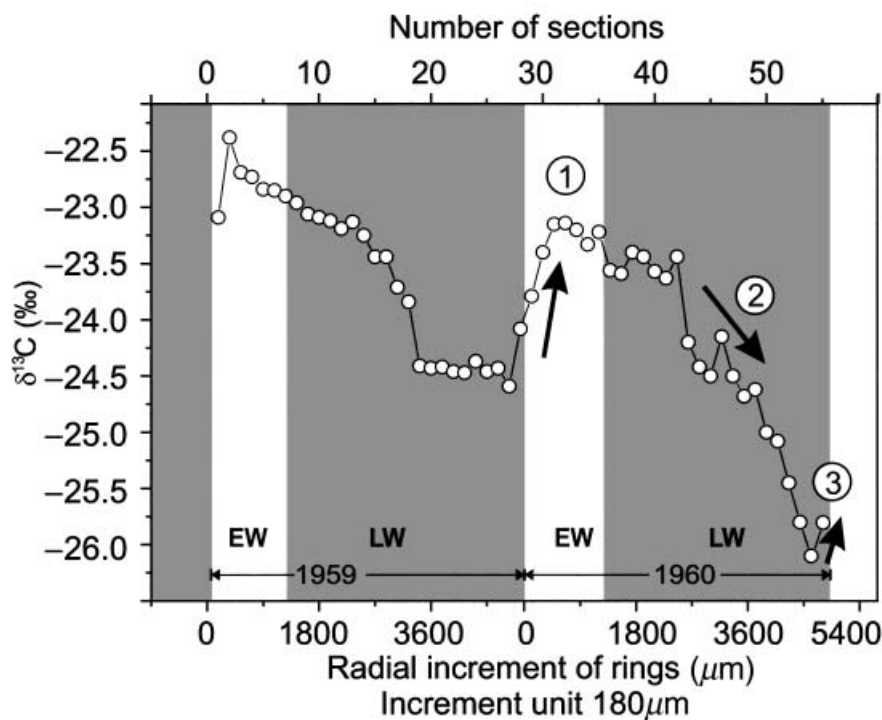


Figure 5. Seasonal carbon isotope variations in cellulose of tree rings from *Morus alba* (mulberry). Grey shaded area marks the late wood section of each ring.

autumn. Note, that the leaves provide an integrated record of $\delta^{13}\text{C}$ which will be progressively dampened by prior $\delta^{13}\text{C}$ signals as the leaves enlarge. Therefore, new leaf material formed later in the growing season is even more depleted than the whole leaf $\delta^{13}\text{C}$ -values depicted in Fig. 1b.

Nonetheless, the highest $\delta^{13}\text{C}$ -values observed in the foliage occur at highest growth rates, namely at bud burst stage and the highest $\delta^{13}\text{C}$ -values of tree rings probably occur with the highest cambial activity of the season. The $\delta^{13}\text{C}$ -peaks observed in young foliage and EW probably occur simultaneously, since bud swelling and cambial activity of beech start at about the same time.

Our results are in agreement with recent findings of seasonal carbon isotope patterns of soluble sugars in phloem sap of a trunk, as well as soluble sugars, starch and respiratory CO_2 of leaves and current year shoots of *Fagus sylvatica* (Keitel *et al.* 2003; Damesin & Lelarge 2003).

$\delta^{13}\text{C}$ of wood versus $\delta^{13}\text{C}$ of foliage

We ascertained a relative enrichment of ^{13}C in wood tissue in comparison with the bulk leaf organic matter, which is in good agreement with findings by other authors (Leavitt & Long 1982, 1986; Schleser 1992, 1999; Miller, Williams & Farquhar 2001; Damesin & Lelarge 2003). This general offset in $\delta^{13}\text{C}$ between foliage and woody parts cannot be ascribed to varying contents of differently labelled chemical compounds like ^{13}C -enriched amino acids and cellulose or ^{13}C -depleted lipids and lignin. Several studies have shown that the enrichment of wood relative to foliage apparently remains, even if only single chemical components, such as cellulose (Francey *et al.* 1985; Leavitt & Long 1986;

Schleser 1990, 1992) or fructose, glucose, sucrose and starch extracted from leaves and stem material (Damesin & Lelarge 2003) are compared.

The cause for the observed isotope shift between foliage and stem is probably not due to phloem transport itself, since only small $\delta^{13}\text{C}$ gradients with tree height exist (e.g. Leavitt & Long 1986; Schleser 1992). However, some authors assume the $\delta^{13}\text{C}$ shift as being related to phloem loading and unloading processes (e.g. Damesin & Lelarge 2003).

Several studies have shown that leaf starch exhibits an enrichment in ^{13}C of up to 4‰ in comparison with far less polymerized sugars or total leaf organic matter (Brugnoli *et al.* 1988; Gleixner *et al.* 1993; Borland *et al.* 1994; Le Roux *et al.* 2001). Therefore, another or additional reason for the observed $\delta^{13}\text{C}$ shift between leaf and stem carbon may exist. One cause might be the large dependence of stem growth on ^{13}C -enriched sugars proceeding at night, originating from mobilized ^{13}C -enriched transitory starch stored in the chloroplasts during the day.

Possible causes for the tri-phase seasonal $\delta^{13}\text{C}$ pattern in broad-leaf deciduous trees

The Farquhar model of carbon isotope discrimination during photosynthesis explains the general depletion in ^{13}C of tree organic matter relative to atmospheric CO_2 . However, it is obvious that this model does not explain the observed seasonal $\delta^{13}\text{C}$ pattern in tree rings and foliage of deciduous broad-leaf trees. Highest $\delta^{13}\text{C}$ -values appear at times of no or negative net photosynthesis when tree growth largely depends on reserves. The isotope values tend to decline

during the period from spring to summer in which air temperatures at the European sites studied generally increase along with decreasing humidity. The model clearly predicts highest $\delta^{13}\text{C}$ -values in organic matter formed during summer, that is, during the time of high temperature and low humidity resulting in decreased stomatal aperture and low C_i/C_a . As demonstrated in Fig. 1a–c the seasonal changes in $\delta^{13}\text{CO}_2$ of the atmosphere are too small and different in timing or trend to be considered as a cause. Therefore, none of the factors (changing atmospheric $\delta^{13}\text{CO}_2$ or C_i/C_a) that influence stable carbon isotope ratios between atmospheric $\delta^{13}\text{CO}_2$ and $\delta^{13}\text{C}$ of leaf assimilates can be considered responsible for the specific tri-phase isotopic pattern found in structural carbohydrates of buds, leaves and rings of broad-leaf deciduous trees.

We therefore suggest that the observed tri-phase $\delta^{13}\text{C}$ -pattern is primarily an indication of certain seasonal changes in specific biochemical and plant physiological changes of the carbohydrate metabolism beyond photosynthesis. The importance of photosynthate storage was strengthened earlier by results from modelling efforts on pine (Hemming *et al.* 2001). However, in contrast to deciduous tree species, tree growth of pine is not dependent on stored carbon during spring. Early wood production is known to depend almost entirely on current photosynthate (Dickmann & Kozlowski 1970; Glerum 1980; Barbour, Walcroft & Farquhar 2002).

Entering into biochemical reasoning, the kinetic isotope effects on reactions connected to a branching of metabolic pathways have to be considered, which lead to carbon pools of differently ^{13}C -labelled metabolic fractions. The extent of discrimination is dependent on the pool-sizes of intermediate carbohydrates and on metabolic flux rates in the different directions, leading to depletion in one direction and enrichment in another, simply on the basis of what is called 'isotopic balance' (e.g. Schmidt & Gleixner 1998).

Section 1: $\delta^{13}\text{C}$ enrichment during the early vegetation period

At the beginning of the vegetation period tree growth depends on reserves, mainly stored as starch in the previous year(s). Starch is accumulated in parenchymatic tissues of the sapwood (woody rays) or the phloem cells of the inner bark of branches, stem and roots during summer and autumn. Most of it is dissolved during winter for cold resistance and again resynthesized from sugar at the end of dormancy. In spring, when the period of fast growth begins, starch is mobilized again and transported from storage to meristematic tissue (e.g. Essiamah & Eschrich 1985; Lacoite *et al.* 1993; Sauter & Van Cleve 1994; Kozlowski & Pallardy 1997; Damesin & LeLarge 2003).

Assuming that the biochemical processes and kinetic isotope effects involved in starch formation in the amyloplast are similar to those in the chloroplast, the starch reserves stored in woody tissue during winter should be enriched in ^{13}C in comparison with sugars such as sucrose and hexoses. Consequently, the incorporation of starch-derived, ^{13}C -

enriched carbon can result in the observed isotopically heavier EW, buds and young leaves. In fact starch $\delta^{13}\text{C}$ -values are highest at bud burst as demonstrated by Damesin & Lelarge (2003).

The observed successive ^{13}C enrichment of EW and emerging leaves requires, however, an additional isotope effect. The fast formation of structural organic matter removes carbon from the pool of carbon reserves and diminishes it. Assuming that isotopically 'light' molecules are turned over faster than 'heavy' molecules the initial $\delta^{13}\text{C}$ -value of the reserve pool becomes enriched with time. As a consequence all organic carbon (structural and non-structural) becomes enriched during spring growth. Since starch is the starting material for all the metabolic turnover processes in this period of heterotrophic growth this compound should reach the highest spring enrichment in conjunction with lowest concentrations in comparison with intermediates such as sucrose, hexoses and trioses. Structural organic matter such as cellulose shows less spring enrichment in $\delta^{13}\text{C}$. This reasoning is supported by findings that the starch content is indeed lowest after budburst while simultaneously showing its highest $\delta^{13}\text{C}$ -values (Damesin & Lelarge 2003).

The enrichment of organic matter might also be promoted by carbon isotope partitioning between anabolic and catabolic metabolism. Gleixner *et al.* (1993) found that catabolic reactions preferentially use 'lighter' sugar molecules (triose-phosphate) to make ATP available, whereas 'heavier' ones are involved in polymerization, for example, cell wall formation. Bud burst and EW formation are accompanied by very high respiration rates in order to meet the energy requirements of the tree in this period of fast growth (e.g. Müller 1954; Teskey & McGuire 2002). In early spring, branch and stem respiration rise independently of xylem temperature, which is caused by an increase in metabolic activity of branch parenchymatic tissues associated with the phenological development of buds.

Maximum respiration rates probably occur at about the time in which the maximum $\delta^{13}\text{C}$ enrichment of emerging leaves and/or highest cambial activity in the stem is reached. Müller (1954) has undoubtedly demonstrated that during this time period the respiration rate far exceeds the normal rate. In terms of the observed enrichment in $\delta^{13}\text{C}$ of organic matter, the maintenance of isotopic balance during carbon isotope partitioning demands ^{13}C depletion in the corresponding respired CO_2 . Unfortunately, no measurements of $\delta^{13}\text{C}$ on the corresponding stem- or bud-respired CO_2 were pursued in this study. Measurements by Damesin & Lelarge (2003) revealed a decrease in $\delta^{13}\text{C}$ of respired CO_2 at the beginning of the vegetation period. Lowest $\delta^{13}\text{C}$ values were recognized at the bud burst stage corresponding to highest ^{13}C enrichment of leaf and stem organic matter of the investigated beech tree.

A fourth reason for the observed spring enrichment may be due to the fact that carbon once exported from the leaf to the woody tissue is generally enriched in ^{13}C by up to 2‰ (e.g. Keitel *et al.* 2003). After storage during winter the use

of this carbon for spring growth should lead to an enrichment of newly formed organic matter.

Section 2: $\delta^{13}\text{C}$ decline during the main vegetation period

As soon as the young leaves become net exporters of photosynthates, and cambial activity slows down growth switches from storage material to current photosynthates. The direction of phloem sap flow is reversed from up- to down-stream and the sugar pools in the tree are replenished by assimilates which carry the $\delta^{13}\text{C}$ signal determined by isotope fractionation processes as governed by stomatal conductance or photosynthetic capacity. Assuming that currently produced assimilates are more or less depleted in ^{13}C in comparison with the remaining sugar reserves the carbon pool used for growth is gradually being diluted in ^{13}C , leading to progressively declining $\delta^{13}\text{C}$ -values of sugars. Their gradual incorporation into newly formed cell wall material leads to the observed subsequent decline of $\delta^{13}\text{C}$ in organic matter.

The observed successive changes in $\delta^{13}\text{C}$ of whole leaf organic matter and leaf cellulose may indicate that even after the leaves are fully expanded, they change structure along the vegetation period and/or continue to thicken. The subsequent addition of newly formed material to already existing old organic matter could well explain the observed declining trend. It is interesting to note that investigations on the starch content of beech trees indeed, revealed a steady increase during the summer with a peak in late September (Damesin & Lelarge 2003).

However, this explanation can not hold for the similar pattern as observed in the tree rings. In woody parts of the tree the cambium continuously produces new cell rows. Each thin section of wood contains a certain number of cell rows being formed within a certain time step. Whereas, old leaves always contain cell wall material being produced right at the beginning of the vegetation period, thin sections of LW do not. The similarity of the $\delta^{13}\text{C}$ pattern in foliage and wood rather points to a slow turnover of the sugar pool in the phloem sap which is providing carbon to the meristematic cambium.

Another reason for the ^{13}C decline in the mid-section may be a carbon isotope partitioning between growth, namely the formation of new cell walls, and storage, i.e. the formation of starch. Based on this hypothesis the increasing accumulation of ^{13}C -enriched starch during summer probably leads to correspondingly ^{13}C -depleted wood.

An uptake of ^{13}C -depleted stem respiratory CO_2 might additionally have an impact. The CO_2 concentration within the xylem can be very much higher than in the atmosphere (Hari, Nygren & Korpilahti 1991; Teskey & McGuire 2002). A large fraction of stem-respired CO_2 dissolved in xylem water is transported towards and into leaves where it is re-fixed by photosynthesis. Increasing stem respiration along with increasing summer temperatures could therefore, at least partly, be responsible for the declining $\delta^{13}\text{C}$ trend in organic matter, even though the solubility of CO_2 in water

abates with increasing temperature. Detailed investigations will be necessary to validate this hypothesis.

Section 3: $\delta^{13}\text{C}$ increase at the very end of the vegetation period

The increase in $\delta^{13}\text{C}$ of late LW can be assigned to changes in the carbohydrate metabolism accompanying leaf senescence during autumn. Export of carbon from the leaves is decreasing. At a certain stage they are not even self-supporting. It is most likely that the same processes that are responsible for the ^{13}C enrichment in EW of the early vegetation period come into play again.

Tree ring formation subsequently becomes dependent on reserves. As shown for beech and poplar the amount of soluble sugars and starch in stem and leaves is slightly decreasing at this time of the season (Sauter & van Cleve 1994; Damesin & Lelarge 2003).

Since respiratory CO_2 from stem or leaves can no longer be re-fixed in photosynthesis, carbon isotope partitioning between anabolic and catabolic metabolism might show effects with respiration of ^{13}C -depleted CO_2 leaving the ^{13}C -enriched organic matter behind.

Climatic significance of seasonal $\delta^{13}\text{C}$ -variations

The mid-section of the tri-phase ^{13}C pattern in tree rings exhibits different peculiarities that are most probably due to varying environmental influences on leaf carbon isotope discrimination. This will be demonstrated by comparing the radial carbon isotope distribution of oak tree rings (Fig. 4a) with corresponding monthly means of temperature and precipitation of the vegetation periods for the years 1957 and 1958 from the climatically sensitive site near Rostrevor (Fig. 4b).

The months of June and July of 1957 were particularly dry and warm. Following the model constraints the tree responded with narrow stomatal apertures and the produced assimilates had a relatively high ^{13}C content, causing a fairly slow decline of $\delta^{13}\text{C}$ across the LW. In 1958 $\delta^{13}\text{C}$ -values fall to a minimum at the end of EW, showing no significant ^{13}C variations further on. Indeed, the weather conditions during the summer months of the year 1958 varied little, June, July and August were well balanced with regard to precipitation, being within a range that causes no isotopic variations. Presumably water supply and temperature conditions just varied within the optimum range of tree growth inducing no stress on the tree. Note, that the tree ring from 1958 is 12% wider than the ring formed in 1957 due to better LW growth. Merely based on the intra-seasonal $\delta^{13}\text{C}$ pattern this can be traced back to better growth conditions during the early phase of LW growth.

Nevertheless, some modifications can, in general, be expected by material temporarily falling back upon ^{13}C -enriched reserves. Trees use both stored and currently produced carbohydrates, often concurrently, for growth (Kozłowski & Pallardy 1997). Intermittent flushes of growth involving recurrent formation and opening of buds,

fruiting as well as short-term enhancement of cambial activity, at least in some parts of the tree, will probably have an influence on the seasonal progression of $\delta^{13}\text{C}$ in tree rings. Especially, during the early part of the mid-section within the tri-phase $\delta^{13}\text{C}$ pattern a short-term increase may occur also when growth becomes dependent on reserves again, such as after defoliation caused by insect outbreaks (own unpublished results).

In Figs 3a and 4a horizontal lines were plotted indicating the mean $\delta^{13}\text{C}$ -values of high-resolution data for five equal subdivisions of a tree ring. It becomes clear that the tri-phase nature of the intra-ring $\delta^{13}\text{C}$ pattern can not be detected from investigations at such low resolution. In addition, results from low-resolution studies probably underestimate the maximum $\delta^{13}\text{C}$ enrichment of early wood and the minimum $\delta^{13}\text{C}$ values by 1‰ or more. Therefore, low-resolution studies on intra-ring $\delta^{13}\text{C}$ do not fully assess seasonal variability. However, the $\delta^{13}\text{C}$ development of the mid-section of the seasonal pattern, which is probably most affected by environmental factors, may frequently be represented by investigations at low resolution providing suitable information for reconstruction of seasonal environmental changes.

CONCLUSIONS

The seasonal tri-phase carbon isotope pattern in rings and foliage of broad-leaf trees is mainly due to post-photosynthesis fractionation processes. Seasonal variations of carbon isotope fractionation during photosynthesis are of much lower magnitude compared with the total intra-annual isotope fluctuations. The reasons for this pattern appear to be quite complex, because tree ring material might completely or partly originate from previous years, varying pool sizes of transitory carbohydrates which in addition undergo a number of chemical modifications involving isotope fractionations. Principally, the tri-phase intra-annual $\delta^{13}\text{C}$ pattern must be caused by the carbon isotope partitioning within the seasonal interplay of accumulation and remobilization of storage material, varying respiration rates and tree growth.

Unfortunately, the present knowledge about carbohydrate partitioning during the transfer of photosynthates into tree rings is poor. A quantitative estimate of the metabolic and isotopic carbon balance in a complicated system like a tree is presently impossible.

In future, high-resolution studies on radial stable isotope distributions in tree rings in conjunction with ecophysiological studies on carbohydrate transfer within trees will provide a better knowledge on the biochemical and physiological processes underlying tree ring formation.

Based on this improved knowledge on carbohydrate metabolism in trees, future high-resolution intra-annual investigations on tree rings using the microtome technique or more sophisticated laser ablation technique will probably allow a retrospective view on plant physiological processes underlying perennial/woody plant response to environmental or climate change.

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