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Invited review

Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood

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The mechanistic understanding of isotope fractionation processes is increasing but we still lack detailed knowledge of the processes that determine the isotopic composition of the tree-ring archive over the long term. Especially with regard to the path from leaf photosynthate production to wood formation, post-assimilation fractionations/processes might cause at least a partial decoupling between the leaf isotope signals that record processes such as stomatal conductance, transpiration and photosynthesis, and the wood or cellulose signals that are stored in the paleophysiological record. In this review, we start from the rather well understood processes at the leaf level such as photosynthetic carbon isotope fractionation, leaf water evaporative isotope enrichment and the issue of the isotopic composition of inorganic sources (CO_2 and H_2O), though we focus on the less explored 'downstream' processes related to metabolism and transport. We further summarize the roles of cellulose and lignin as important chemical constituents of wood, and the processes that determine the transfer of photosynthate (sucrose) and associated isotopic signals to wood production. We cover the broad topics of post-carboxylation carbon isotope fractionation and of the exchange of organic oxygen with water within the tree. In two case studies, we assess the transfer of carbon and oxygen isotopic signals from leaves to tree rings. Finally we address the issue of different temporal scales and link isotope fractionation at the shorter time scale for processes in the leaf to the isotopic ratio as recorded across longer time scales of the tree-ring archive.

Keywords: cellulose, evaporative enrichment, oxygen atom exchange, phloem transport, post-photosynthetic, storage.

Introduction

Future global climate change combined with and driven by the further increase of atmospheric CO_2 concentrations is expected to markedly affect plant growth and performance and, as a consequence, the composition and spatial distribution of species in terrestrial ecosystems (IPCC 2007). One key for understanding the effect of climatic variables on plant performance over the longer term is to characterize and interpret physiological information laid down in natural archives in the past. For woody plants with secondary growth, annual rings in the xylem (generally referred to as 'tree rings') provide a datable archive that records information about the environmental and ecophysiological conditions under which atmospheric CO_2 was assimilated and partitioned to growth. Thus, tree rings allow

researchers to reconstruct climate variations (Schweingruber 1996, Briffa et al. 2002), and tree responses to that variation, far back to pre-industrial times. In one sense, tree rings are the 'fingerprints' of past climate variations and past plant performance. In recent years we have seen that tree-ring information has also been used for estimations of tree biomass and primary production (Graumlich et al. 1989, Briffa et al. 1998, Kirdyanov et al. 2003) and more recently for the validation of carbon fluxes in terrestrial ecosystems (Leblanc 1990, Biondi 1999, Babst et al. 2013, Lara et al. 2013), as well as to discern processes involved in the partitioning of photosynthate to various plant growth sinks (e.g., Gessler et al. 2009*a*).

Stable isotope ratios in tree-ring cellulose or whole wood are also increasingly used as tools to obtain retrospective insight into ecophysiological processes, especially process rates and the mitigating resistances that control plant–atmosphere exchanges of mass and energy (Marshall and Monserud 1996, 2006, Saurer et al. 1997*a*, Schleser et al. 1999, Treydte et al. 2001, 2006, 2009, Leavitt 2002, McCarroll and Loader 2004, Poussart et al. 2004, Rinne et al. 2010). Stable isotopes in tree rings combine the advantage of precise and accurate dating with the sensitivity of carbon isotope ratios (here given in delta notation as $\delta^{13}C$) and oxygen isotope ratios ($\delta^{18}O$) to biophysical mechanisms across leaf-to-tree scales (McCarroll and Loader 2004).

Due to the relationship between photosynthetic carbon isotope fractionation ($\Delta^{13}C_p$) and the ratio of leaf internal and ambient CO_2 concentration c_i/c_a or—to be more precise—of chloroplastic CO_2 concentration (c_c) and c_i (Farquhar et al. 1982), the $\delta^{13}C$ of organic matter can be used to characterize environmental effects and their influences on diffusional versus biochemical controls over photosynthesis. $\delta^{18}O$ in plant material depends on one hand on $\delta^{18}O$ of soil water, which itself is related to the isotope composition of precipitation, residence time in the soil and evaporation effects, and on the other hand on leaf water enrichment due to transpiration (Yakir and Sternberg 2000), ^{18}O fractionation between leaf water and carbonyl oxygen (Sternberg and Deniro 1983, Sternberg and Ellsworth 2011) as well as on further exchange between the oxygen of organic compounds and water in non-green tissues (e.g., Hill et al. 1995, Cernusak et al. 2005, Sternberg 2009).

From a physiological perspective, the combined use of the carbon and oxygen isotope ratios of tree rings can, in theory, potentially provide insight into past dynamics in stomatal conductance, crown transpiration rates, photosynthetic capacity and even the depth and seasonality of soil water uptake (Farquhar et al. 1998, Scheidegger et al. 2000, Grams et al. 2007, Gessler et al. 2009*b*). However, as recently commented on by Roden and Siegwolf (2012) several critical points must be taken into account when applying the dual-isotope approach first proposed by Scheidegger et al. (2000). Among the most important issues that need to be considered are the fact that the $\delta^{18}O$ of source and atmospheric water can vary spatially and temporarily and also that post-photosynthetic and post-evaporative oxygen atom exchange processes could affect the initial leaf-level isotope signal.

Being aware of such issues, analyses of stable isotopes in tree rings allow us to travel beyond the first-order aim of reconstructing paleoclimate, and towards the second-order aim of reconstructing responses in specific plant processes to paleoclimate.

The theory underlying stable isotope fractionation at the leaf scale, and its transference to the tree-ring archive, has been well developed, but in recent years several publications have argued that there are processes missing in the theory, especially with regard to the path from leaf photosynthate production to wood formation. These proposed post-assimilation fractionations/processes might cause at least a partial decoupling between the leaf signals that record processes such as stomatal conductance, transpiration and photosynthesis, and the wood or cellulose signals that are assumed to store the paleophysiological record.

In this review, we aim to summarize the state-of-the-art knowledge on the mechanisms that influence the carbon and oxygen isotope signals throughout the tree. In order to lay a foundation, we begin from processes at the leaf level such as photosynthetic carbon isotope fractionation and leaf water evaporative oxygen isotope enrichment but cover these topics only briefly. However, our primary aim is to extend these well-understood perspectives at the leaf scale, to the less well known 'downstream' processes related to metabolism and transport, which also have strong potential to affect isotope fractionation. We have tried to synthesize the existing knowledge on isotope fractionation at multiple scales into a framework for understanding how ecophysiological insight, which is attached to leaf processes in conventional analyses, can be obscured during the production of wood and across the longer time scales projected from tree-ring analysis. The tree-ring archive holds much insight into the coupling between forest processes and climate. However, there is still much to learn about the uncertainties that emerge when we attempt to access that insight.

^{13}C and ^{18}O stable isotope composition in trees—a short inventory of what is known and not well understood from the physiological point of view

Within the last three decades, our mechanistic understanding of the physiologically based fractionation processes of stable isotopes in plant tissues has increased considerably (Farquhar et al. 1989*a*, Yakir et al. 1994, Flanagan et al. 1997, Yakir 1998, Roden and Ehleringer 1999). Focusing on carbon isotopes, most new discoveries have improved our understanding of carbon isotope discrimination during photosynthesis

(Farquhar et al. 1982, 1989*a*, O'Leary et al. 1992, Gillon and Griffiths 1997, Warren and Adams 2006, Seibt et al. 2008; see also Table 1). By combining our understanding of photosynthetic carbon isotope discrimination with changes in the carbon isotope composition of the atmosphere, we have also been able to reconstruct historical patterns in the combustion of fossil fuel and its influence on the global carbon cycle (e.g., Keeling 1979, Gruber et al. 1999). Progress has also been made towards understanding the oxygen isotope composition and evaporative enrichment of leaf water as influences on the oxygen isotope composition of photosynthate and its conversion into biomass (Farquhar and Cernusak 2005, Barbour 2007, Barnard et al. 2007*a*, Cuntz et al. 2007, Gessler et al. 2007*b*, Ogee et al. 2007). The transference of this past knowledge, however, to interpretations of tree-ring isotope signatures is more challenging than initially considered (Gessler et al. 2009*a*, Offermann et al. 2011).

Regarding the carbon-fractionation in leaves there already exists a classic review by Farquhar et al. (1989*a*), which covers the main aspects of photosynthetic carbon isotope discrimination, and only recently Cernusak et al. (2013) reviewed the environmental drivers of variation in photosynthetic carbon isotope discrimination in terrestrial plants, and the biological processes that can either dampen or amplify the responses.

Some recent reviews have focused on specific processes mainly related to the effects of variations in mesophyll (g_m) conductance on c_c (Warren and Adams 2006, Seibt et al. 2008). g_m is more and more considered an important player in controlling photosynthesis (e.g., Flexas et al. 2012), and rapid changes due to varying environmental conditions (Douthe et al. 2012, Hommel et al. 2014) will strongly affect photosynthetic carbon isotope discrimination. Until now however, we lack information on the effects of variable mesophyll conductance on the photosynthetic isotope discrimination in adult trees under field conditions, and research in this direction is urgently needed. In addition, the effects of transpiration on the gas exchange of isotopologues of carbon dioxide (the so-called ternary effects) and thus on photosynthetic carbon isotope discrimination have been taken into account recently (Farquhar and Cernusak 2012).

Similarly, evaporative isotope enrichment in leaf water has been broadly discussed in recent review articles and mixing of different water pools in the leaf as well as non-steady-state effects due to changes in leaf water content have been taken into account (Farquhar et al. 1998, 2007, Farquhar and Cernusak 2005). Given this past coverage, we will address the topics of fractionation during CO_2 assimilation and H_2O transpiration only briefly and refer the reader to the above-mentioned reviews for in-depth information. Instead, we will focus more on the more recently discovered, and less broadly discussed, processes that occur between the leaf and wood cambium (Figure 1). This includes fractionation processes that we refer to as 'post-photosynthetic' (Jäggi et al. 2002, Badeck et al. 2005) or 'post-carboxylation' fractionation (Gessler et al. 2008), and oxygen atom exchange between organic compounds and surrounding water (DeNiro and Epstein 1981, Sternberg et al. 1986). Given their importance for the long-time isotope records archived in the tree rings, we will, however, start with shedding some light on the effects of variations of the carbon and water sources.

The primary sources: CO_2 and H_2O

Variability in the isotopic composition of CO_2 and H_2O

Atmospheric CO_2 is the principal carbon source for terrestrial plants. Its carbon isotope variability is important for plant physiologists as the isotopic composition of newly assimilated plant organic matter ($\delta^{13}C_p$ in ‰) depends on the one hand on isotope fractionation ($\Delta^{13}C$ in ‰) during diffusion and biochemical assimilation (Farquhar et al. 1982), and on the other hand on the isotopic composition of the inorganic source CO_2 ($\delta^{13}C_{CO_2}$).

$$\delta^{13}C_p = \frac{\delta^{13}C_{CO_2} - \Delta^{13}C}{1 + \Delta^{13}C} \quad (1)$$

Currently the mean $\delta^{13}C$ of atmospheric CO_2 is ~– 8‰. This value, however, changes seasonally with relative ^{13}C depletion during winter and enrichment during summer, particularly in the northern hemisphere (e.g., Levin et al. 1995), and is becoming more negative over time (~0.02–0.03‰ year^{-1}, according to the data available from the CU-INSTAAR/NOAA-CMDL network for atmospheric CO_2; http://www.esrl.noaa.gov/gmd/). This progressive decline is mainly caused by progressive increases in the emission of ^{13}C-depleted CO_2 during fossil fuel combustion (cf. Table 1). As a reaction to this atmospheric decline, $\delta^{13}C$ values in tree-ring records worldwide have shown a downward trend of 1–2‰ starting around 1800–1850 AD (e.g., Leavitt 1993, Marshall and Monserud 1996, McCarroll and Loader 2004, Treydte et al. 2007). Since this trend biases the climatic signal in the tree-ring record, it is commonly removed in paleoclimatic analyses. Within closed canopies, also vertical gradients in the $\delta^{13}C$ of source CO_2 are observed due to the near-soil accumulation of CO_2 (depleted in ^{13}C compared with tropospheric CO_2) from respiration (Werner and Gessler 2011). Atmospheric $\delta^{13}C$ variations and potentially within canopy gradients should thus be considered when comparing species differing in their growth cycles and stand structures, in distantly separated samples (either in time or in space) and certainly when assessing the low-frequency domain in tree-ring time series.

Within the global hydrologic cycle, the $\delta^{18}O$ of water is subject to several fractionations. On one hand, light isotopes evaporate more rapidly than their heavier counterparts, and thus water vapour is isotopically depleted in ^{18}O with respect to source water (Craig and Gordon 1965). On the other hand, the opposite

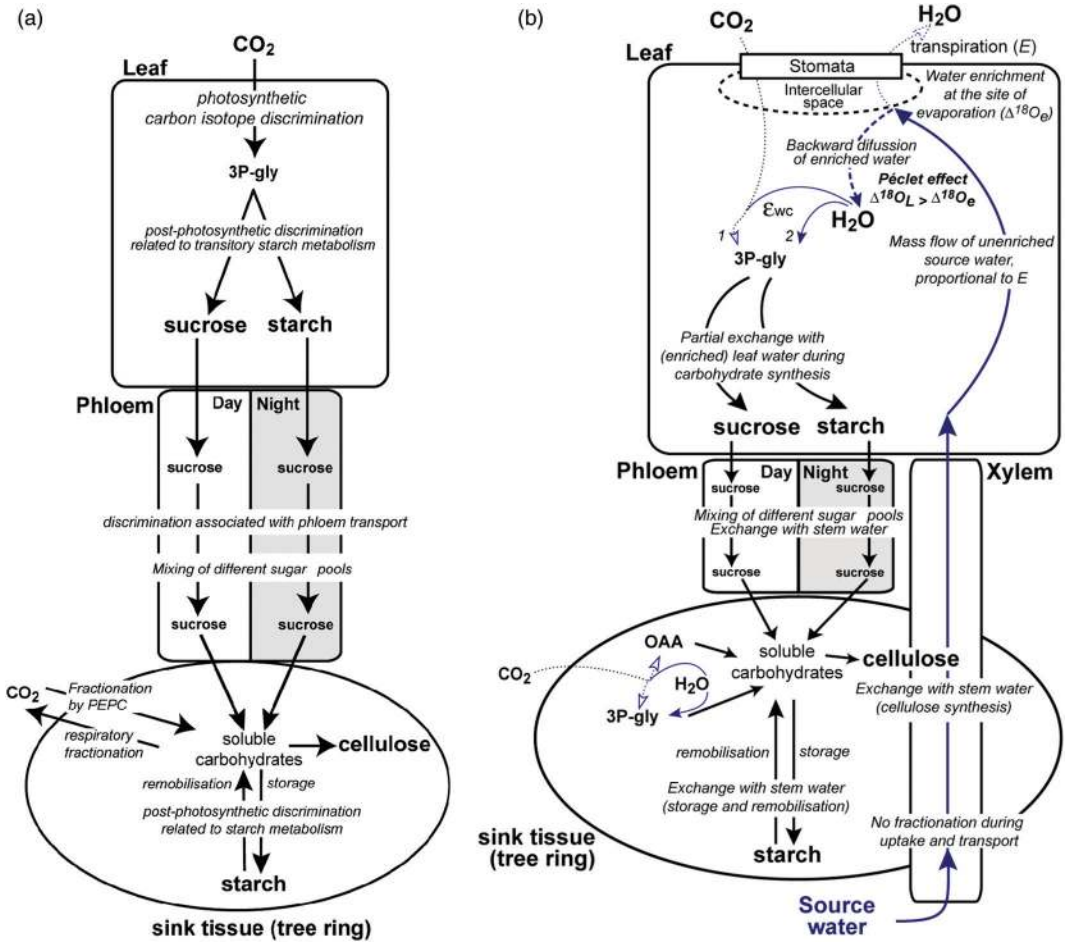

Figure 1. Overview of the different processes influencing the carbon (a) and oxygen (b) isotope signature, from primary sources (CO_2 and H_2O, respectively) to tree-ring cellulose, going through different organic and inorganic pools. PEPC, phosphoenolpyruvate carboxylase; $\Delta^{18}O_e$ and $\Delta^{18}O_L$, isotopic enrichment of water at the site of evaporation and of mean lamina leaf water, respectively; ε_{wc}, equilibrium fractionation factor between oxygen in water and carbonyl groups; OAA, oxaloacetate; 3P-gly, 3-phosphoglycerate.

occurs during precipitation, when the heavier isotopologues condense more readily due to the slightly stronger intermolecular bond strength (Gat 1996). The amount of this fractionation varies according to temperature and altitude, resulting in a composition of precipitation that favours the heavier isotopes in positive relation with temperature, and in negative relation with altitude, distance from moisture sources and local precipitation amount (Dansgaard 1964, but see also Aggarwal et al. 2012). Therefore, analyses of precipitation $\delta^{18}O$ can reveal intra-annual to multi-decadal information about regional weather patterns, and even large-scale atmospheric circulation dynamics, depending on geographical region and season (Baldini et al. 2008, Field 2010). Only recently, it has been shown that canopy processes can alter the isotopic composition of precipitation reaching the ground. Koeniger et al. (2008) observed that sublimation of snow intercepted by the canopy causes ^{18}O isotopic enrichment of the snowpack below the canopy and the stream flow from forested areas. After precipitation has reached the ground, there are several potential fractionation processes that can occur before the water oxygen isotope composition becomes imprinted in the organic matter of plant tissues. The most important one takes place within the soil, as evaporation affects the original isotopic signal, causing depth gradients with the highest ^{18}O enrichment in the upper soil layers. So the residence time and depth/location of soil water taken up by plants are important determinants of the oxygen isotope ratio of xylem water (see, e.g., Saurer et al. 1997*b*, Moreno-Gutiérrez et al. 2012).

Effects of changing CO_2 concentration on $\delta^{13}C$

Direct measurements of CO_2 trapped in Antarctic and Greenland ice caps indicate that recent changes (i.e., during the last 150 years) in atmospheric CO_2 composition exceed those attained in the last half a million years, showing a dramatic increase from ~270 μmol mol^{-1} during most of the Holocene to present values over 350 μmol mol^{-1} (Indermuhle et al. 1999). As mentioned above, variations in atmospheric CO_2 concentration come with a depletion in $\delta^{13}C_{CO_2}$, but changes in the CO_2 concentration itself might also affect the $\delta^{13}C$ of plant material and thus indicates

Table 1. Processes and their environmental drivers influencing the carbon isotope signature of organic compounds from CO_2 fixation to cellulose synthesis. VPD, vapour pressure deficit; T_{air}, air temperature; T_{leaf}, leaf temperature; T_{soil}, soil temperature; PAR, photosynthetic active radiation; g_s and g_m, stomatal and mesophyll conductance, respectively; c_a, atmospheric CO_2 concentration; c_i, leaf internal CO_2 concentration; PEPC, phosphoenolpyruvate carboxylase; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid.

Tissue	Isotope fractionation or isotopologue mixing process	Underlying physical, physiological or biochemical mechanism	Environmental drivers	Effect on $\delta^{13}C$ of organic matter
Photosynthesis				
Atmospheric CO_2	Long-term and intra-annual variations in $\delta^{13}C$ of CO_2 (Keeling et al. 1995)	*Long term*: mixing of CO_2 from fossil fuel combustion into the atmosphere *Intra-annual*: seasonal variation in biogenic source and sink strength and in anthropogenic CO_2 source strength	*Long term*: anthropogenic with modifications due to inter-annual variations in global air temperature (Keeling et al. 1995) *Intra-annual*: temperature, PAR, soil moisture on the hemisphere scale	*Temporal*: continuous decrease in $\delta^{13}C$ of newly fixed organic matter over years. Intra-annual variation in $\delta^{13}C$ of organic matter without change in photosynthetic discrimination Increase in c_a: *Temporal*: if c_a–c_i remains constant and thus c_i/c_a increases $\delta^{13}C$ of newly fixed organic matter decreases
	Potential effects of an increase in c_a on c_i/c_a and thus photosynthetic carbon isotope fractionation			
Leaves/chloroplast	Photosynthetic carbon isotope discrimination (Farquhar et al. 1982)	Fractionation associated with CO_2 diffusion, carboxylation dark-/photorespiration	VPD and soil moisture via effects on g_s and g_m PAR, T_{air}, T_{leaf} T_{air}, T_{leaf}	*Temporal*: short-term variation of $\delta^{13}C$ in new assimilates during the day, day-to-day, seasonal, inter-annual and long-term (decadal/centennial/millennial) variations *Spatial*: decrease in $\delta^{13}C$ in the lower canopy with less PAR availability
Post-photosynthetic processes				
Leaves/chloroplast	Post-carboxylation fractionation during transitory starch metabolism (Rossmann et al. 1991, Tcherkez et al. 2004)	Kinetic and equilibrium isotope effects of the aldolase reaction during C–C bond breaking and making	Day length and PAR (regulating transitory starch accumulation and daytime partitioning between C allocated to starch and to sucrose)	*Temporal*: day sucrose becomes ^{13}C depleted compared with 3-phospho-D-glycerate and starch and night sucrose becomes ^{13}C enriched; day-night differences in $\delta^{13}C$ of leaf-exported sugars
Leaves/symplast	Interaction of post-carboxylation isotope fractionation in leaf metabolic pathways and phloem loading (Hobbie and Werner 2004)	Various kinetic and equilibrium isotope effects causing lipids and lignins remaining in the leaves to be depleted and sugars and cellulose to be enriched in ^{13}C	Not known	*Spatial*: leaf-exported sugars become ^{13}C enriched as compared with primary assimilates
Phloem (twig/trunk)	C isotope discrimination in interaction with phloem transport (Gessler et al. 2009*a*)	Continuous unloading of sucrose from the phloem, metabolic conversion of part of the sucrose and reloading of the rest. Lignin and other substances produced become ^{13}C depleted (kinetic and equilibrium isotope effects) and the retrieved sugars thus ^{13}C enriched. Potential involvement of a kinetic isotope effect of the invertase reaction	Not known Potentially associated with phloem turnover, and thus to assimilation and transport rates.	*Spatial*: increase in ^{13}C enrichment of phloem sugars along the transport pathway
Phloem (stem/trunk)	Mixing of sugar pools with different metabolic history during phloem transport (Brandes et al. 2006)	The continuous unloading and re-loading drive the pool mixing	Not known	*Temporal*: day–night differences become less pronounced along the phloem transport path
Stem/trunk	Bark photosynthesis	Re-fixation of ^{13}C-depleted respired CO_2 and fractionation proportional to respiration rate/photosynthesis rate	Light, leaf phenology	*Spatial and temporal*: branch and stem section with stronger contribution of bark photosynthesis will have more negative $\delta^{13}C$ values. Contribution of bark photosynthesis might vary with foliation in deciduous stands over the year

Stem/trunk	Carbon isotope fractionation of PEPC for anaplerotic processes (Cernusak et al. 2009, Gessler et al. 2009c)	Net fractionation against ^{12}C associated with CO_2 hydration equilibrium and PEP carboxylation	N-supply, salt- and drought stress (increasing PEPC activity)	*Spatial and temporal*: depending on the PEPC activity in a given tissue or during a given period heterotrophic tissues will become ^{13}C enriched, but effects on cellulose or lignin are not clear
Stem/trunk	Respiratory isotope fractionation (Ghashghaie et al. 2003, Priault et al. 2009)	Fragmentation of the respiratory substrate molecule with heterogeneous intramolecular isotope distribution (fragmentation fractionation) and kinetic isotope effects in glycolysis and TCA cycle in combination with the commitment of pathways	T_{air}, T_{soil}, water logging (affect the ratio between PDH- and TCA cycle produced CO_2); various stresses (affect the commitment of secondary metabolite pathways)	*Temporal*: secondary metabolites derived from PEP, pyruvate and acetyl CoA become more or less depleted in ^{13}C compared with glucose. Effects on cellulose or lignin are not clear
Stem/trunk	Fractionation during heterotrophic starch synthesis	Aldolase, pentosephosphate pathway	Seasonal variations of T_{air}, PAR and soil moisture determine together with internal regulation the rate and timing of starch accumulation and breakdown	*Temporal*: seasonal variation of $\delta^{13}C$ with relative ^{13}C enrichment of sugars and structural compounds during periods of storage reserve remobilization

a physiological response to environmental change. Indeed, opposed to the trends in $\delta^{13}C_{CO_2}$, several studies on tree rings (Duquesnay et al. 1998, Saurer et al. 2003, 2004, Battipaglia et al. 2013, Hereş et al. 2013, Voltas et al. 2013, Granda et al. 2014), herbarium material (Beerling 1996, Köhler et al. 2010) and sub-fossil leaves (Beerling 1996) have shown an increase in plant $\delta^{13}C$ from pre-industrial to present times, although this trend varied considerably among species. In general, these trends have been interpreted as evidence of increasing water-use efficiency in response to atmospheric CO_2 changes. However, since the recent CO_2 increase is associated with global climate change (in particular temperature, vapour pressure deficit and the hydrological regimes) it is difficult to disentangle CO_2 and climate effects on $\delta^{13}C$ in retrospective studies. Furthermore, although an increase in water-use efficiency in response to increasing CO_2 concentrations is generally expected in C_3 plants (see Franks et al. (2013) for an extensive review), higher internal CO_2 concentrations could lead to unaffected or even higher $\Delta^{13}C$ (Picon et al. 1997). Unfortunately, whereas the effect of elevated CO_2 (>350 ppm) on plant metabolism has been the subject of extensive research, only a few experimental studies have been performed to understand plant responsiveness under low CO_2 environments, such as those prevailing at the Last Glacial Maximum and during most of the Holocene (Policy et al. 1993, Sage 1995). Changes in anatomical features, such as stomatal density, in response to increasing CO_2 (Woodward and Kelly 1995, Beerling and Royer 2002, Gagen et al. 2011) might further complicate the interpretation of $\delta^{13}C$ in tree-ring records.

Whole wood or cellulose, that is the question

Wood is a complex mixture of different organic compounds. The major components of wood are α-cellulose, hemicelluloses, lignin and solvent-extractable substances (mainly sugars and resins) (Kollmann 1955). Due to their different biosynthetic pathways, these components show differences in their carbon and oxygen isotope composition (see, e.g., Epstein et al. 1976, Gray and Thompson 1977, Leavitt and Danzer 1993, Borella et al. 1998, Barbour et al. 2001). Although pioneering studies on the stable isotope composition of tree rings were performed using whole wood (e.g., Craig 1954, Libby et al. 1976), the analysis of a single chemical component (usually cellulose) was ultimately recommended by Epstein et al. (1976). The following reasons have been offered for focusing on cellulose. First, mobile compounds such as resins or sugars might move along the parenchyma cells between several tree rings, potentially adding 'noise' to high-resolution climate reconstruction (Leavitt and Danzer 1993, McCarroll and Loader 2004, Harlow et al. 2006). Second, the relative contribution of the different compounds in wood might vary from one year to another, as well as within a single tree ring, causing additional uncertainties (Epstein et al. 1976, Borella et al. 1998). Third, whereas

cellulose and hemicelluloses are deposited into secondary cell walls during cellular expansion, lignin is deposited after its cessation, resulting in a time lag between the incorporation of both compounds into whole wood (Boerjan et al. 2003). Finally, unlike cellulose, the lignin chemical composition of wood is not constant, and its complex biosynthetic pathway and associated isotope fractionations include several unresolved aspects (Boerjan et al. 2003). Consequently, cellulose has been the material of choice in most tree-ring studies (see references in Switsur and Waterhouse 1998, McCarroll and Loader 2004); this is despite the fact that cellulose purification is time consuming, and has no clear endpoint (Leavitt and Danzer 1993, Loader et al. 1997, Borella et al. 1998).

Although the use of a single constituent, such as cellulose, is justified on theoretical grounds, many studies have shown similar results when whole wood and purified cellulose are compared; in many cases a constant offset is observed, but the strength of the correlation to climate variation is conserved (Borella et al. 1998, Barbour et al. 2001, Loader et al. 2003, Harlow et al. 2006). Moreover, the potential effect of time lags between cellulose and lignin deposition seems to be small even for ultra-high-resolution studies (i.e., intra-annually within tree rings) (Helle and Schleser 2004, Kagawa et al. 2006*a*). Most of the comparative studies reporting similar strength of the climate signal among wood, cellulose and lignin have focused on $\delta^{13}C$ (e.g., Loader et al. 2003, Robertson et al. 2004, Ferrio and Voltas 2005, Harlow et al. 2006). Whether these conclusions are applicable to the case for $\delta^{18}O$ is still unclear. In some studies, strong positive correlations between the $\delta^{18}O$ of whole wood and cellulose have been reported (Borella et al. 1999, Barbour et al. 2001, Sidorova et al. 2008, Gori et al. 2012) and both cellulose and lignin $\delta^{18}O$ have been shown to be related to the $\delta^{18}O$ of precipitation. In contrast, other studies have shown poor correlations between whole wood and cellulose $\delta^{18}O$ and weaker climate signals in whole wood (Gray and Thompson 1977, Ferrio and Voltas 2005, Sass-Klaassen et al. 2005, Battipaglia et al. 2008), and even slightly negative relationships between cellulose and lignin $\delta^{18}O$ (Gray and Thompson 1977, Ferrio and Voltas 2005). Roden and Farquhar (2012) observed a good correlation between whole wood and α-cellulose for $\delta^{13}C$, whereas for $\delta^{18}O$ the correlation was weaker and highly variable between the two species studied (*Eucalyptus globulus* Labill. and *Pinus radiata* D.Don). The reasons for such contrasting results might include the complexity of lignin biosynthesis, starting from the fact that part of the oxygen in lignin precursors is originally derived from molecular oxygen (Boerjan et al. 2003, Shi et al. 2010), and not exclusively from water, as it is in the case of sugars and cellulose. Furthermore, the oxygen in lignin may be subject to partial exchange with xylem water (Schmidt et al. 2001). The amount of this exchange probably varies among species, and is likely influenced by several (unknown) environmental and physiological variables. Thus, correlations between cellulose and lignin might be strong when the amount of exchange between oxygen in lignin precursors and water is high, but weak when such exchange is low (Barbour et al. 2001). More work is needed to understand the way in which the oxygen isotope signal is transferred into lignin, and its variability with climate and among species. We see potentially that after more intensive research it can be finally concluded that past climate signals are best recorded in $\delta^{18}O$ of whole wood rather than with cellulose or lignin (Gori et al. 2012). In the meantime, the most sensible working procedure is to test cellulose extraction at least with a subset of samples, and use whole wood if the values are well correlated with those of cellulose.

Post-photosynthetic carbon isotope fractionation and its effect on the $\delta^{13}C$ of organic matter

Day and night—diel variations in $\delta^{13}C$ as a consequence of transitory starch storage and remobilization

For downstream photosynthetic carbon isotope discrimination (cf. Table 1; Figure 1a), there are possible post-carboxylation fractionations at the leaf level that could cause the $\delta^{13}C$ of extracted tree-ring cellulose to deviate from that predicted using leaf-scale models. During the light period, starch is produced and accumulated in the chloroplasts of leaves, and the production of sucrose in the cytoplasm involves ^{13}C-depleted triose phosphates exported from the chloroplast. This ^{13}C depletion is a direct consequence of transitory starch synthesis, which favours ^{13}C during production of fructose-1,6-bisphosphate by aldolase (EC 4.1.2.13) (Rossmann et al. 1991, Gleixner and Schmidt 1997). During the night, sucrose synthesis involves degradation of the ^{13}C-enriched starch via maltose (Weise et al. 2004) and, as a consequence, leaf-exported sucrose during the night should also be enriched in ^{13}C. And indeed, in adult *Pinus sylvestris* L. and eucalypt trees the $\delta^{13}C$ of sugars exported from leaves to twig phloem varies diurnally with amplitudes of up to 1.7‰ (Brandes et al. 2006, Gessler et al. 2007*a*, Kodama et al. 2008). Since these phloem-transported sugars are the main carbon source for cellulose during wood production, diel variations in $\delta^{13}C$ may affect the carbon isotope composition of cellulose extracted from tree rings (Tcherkez et al. 2007). We know that there are diel variations in the expression of key enzymes involved in lignin (Rogers et al. 2005) and cellulose (Harmer et al. 2000, Solomon et al. 2010) biosynthesis with highest gene expression and thus cambial activity during night. If both prerequisites (diel pattern in $\delta^{13}C$ in the trunk and mainly night-time production of tree-ring tissue) were true, the $\delta^{13}C$ in tree-ring cellulose would not necessarily reflect photosynthesis-weighted c_i/c_a, as is derived in leaf-scale models, and might lead to an overestimation of intrinsic photosynthetic water-use efficiency (A/g_s; Farquhar et al. 1989*b*). According to Tcherkez et al. (2007), the $\delta^{13}C$ of tree-ring cellulose could at least in theory deviate by −6‰ (enrichment) to +2‰ (depletion) from the $\delta^{13}C$ of newly assimilated carbon

depending on the proportion of carbon derived from the two sucrose pools (i.e., day versus night). In the following section, however, we indicate that the day–night difference in the $\delta^{13}C$ of leaf-exported sugars might be of minor or even no importance for the tree-ring isotope composition.

Mixing of sugar pools during phloem transport

There are strong indications that although the circadian dynamics as discussed above might play a role in determining the $\delta^{13}C$ of leaf and twig cellulose, the mixing of sugar pools during transport between the leaf and trunk cambium could ameliorate or totally prevent the diel effect. Brandes et al. (2006) and Kodama et al. (2008) observed dampening of short-term variations in $\delta^{13}C$ as caused by transitory starch accumulation and breakdown with increasing transport distance (twig phloem to the trunk base phloem in *P. sylvestris*). The authors assumed that this was due to mixing of sugar pools of different age and metabolic history along the transport path. Gessler et al. (2007*a*) also reported that the amplitude of diel variation in $\delta^{13}C$ decreased from 2.5‰ in twig phloem sugars to <0.5‰ at the trunk base in *Eucalyptus delegatensis* R.T.Baker. A possible explanation for mixing of sugar pools during basipetal transport might be provided by the dynamic Münch mass flow model (see the review by Van Bel 2003). The model proposes that while a proportion of the sucrose from sieve tubes is released during phloem transport, approximately two-thirds of it is recovered and transported back into the sieve tubes (Minchin and Thorpe 1987). In other words, the sieve tubes represent a system of 'leaky pipes' (Van Bel 2003) that lose and partially retrieve sugars during transport. This loss and retrieval of sugars may allow the mixing of different pools and thus dampen short-term (diel) variations in $\delta^{13}C$. It is, however, unclear whether this mixing effect occurs in all tree species and under all environmental conditions.

Carbohydrate dynamics in heterotrophic tissues: storage, remobilization, respiration and re-fixation

Non-structural carbohydrates (NSCs) sustain the metabolic processes of the cambium, including the production of wood. A number of studies report a significant relationship between seasonal variations in NSC concentrations in the cambium and the annual dynamics of wood formation, and this relationship is conserved among different species, habitats and major taxonomic groups (e.g., angiosperms and gymnosperms) (Stewart et al. 1973, Sundberg et al. 1993, Uggla et al. 2001, Deslauriers et al. 2009, Giovannelli et al. 2011, Simard et al. 2013). At the beginning of the growing season, NSC concentrations increase and peak when resource demand is highest. This is also when the greatest number of xylem cells are in the enlargement and cell wall thickening phases (Simard et al. 2013). The high seasonal demand for NSCs in spring likely causes the remobilization of stored carbohydrates from previous years, especially in deciduous species, and thus mixes the substrates used for cellulose biosynthesis in a way that will, once again, potentially uncouple trends in $\delta^{13}C$ in tree rings from the shorter-term trends predicted by leaf-scale fractionation models (Helle and Schleser 2004). In contrast, Marshall and Linder (2013) found no carryover of photosynthates from one year to the other in Norway spruce tree rings, which might point to differences in the importance of stored carbohydrates between evergreen and deciduous species.

It is not only that remobilized starch contains isotopic information from previous years (see also section 'In the final assessment of gaps across temporal scales: the ultimate link between isotope fractionation and the long-term tree-ring archive'), but the process of storage and remobilization within the plastids of heterotrophic tissues (as opposed to the chloroplasts of leaves) might cause its own type of fractionation that alters the isotopic composition of the carbohydrates used in tree-ring cellulose synthesis, compared with fresh assimilates (cf. Damesin and Lelarge 2003, Gottlicher et al. 2006, Gessler et al. 2009*c*). In contrast to the chloroplast, where ^{13}C enrichment in starch can be explained by the Calvin cycle aldolase reaction (see section 'Day and night—Diel variations in $\delta^{13}C$ as a consequence of transitory starch storage and remobilization'), no such explanation exists for amyloplasts. Rather, for non-photosynthetic tissues that contain stored carbohydrate reserves, we need to assume that the starch in plastids originates from transported sucrose (Fischer and Weber 2002) and there is no evidence for kinetic or equilibrium isotope effects for the reactions that produce starch from sucrose in non-photosynthetic tissues. One biochemical pathway could, however, explain some level of fractionation during starch synthesis, and also allow consolidation of some divergent reports in the literature: it is known that nitrite reduction and other nutrient reduction processes in non-photosynthetic tissues rely on NADPH from the oxidative pentose phosphate pathway (OPPP) (Heldt and Piechulla 2011). This reaction also uses glucose-6-phosphate as a primary substrate and the produced triose phosphates are re-transported to the cytosol where they can either be used for respiration or undergo a reaction with aldolase to produce fructose-1,3-bisphosphate. The latter compound can be converted to glucose-6-phosphate, which after transport into the plastid is again available for either the OPPP or starch synthesis (Fischer and Weber 2002, Heldt and Piechulla 2011). This cycle with a metabolic branch at the stage of triose-phosphate utilization, and given the known isotope effect of the aldolase reaction (Gleixner and Schmidt 1997), might explain some of the past reports of ^{13}C enrichment in the stored starch of some plants. When we assume that the potato tubers assessed by Gleixner et al. (1998) are not as physiologically active as plant roots, stems and twigs (Damesin and Lelarge 2003, Gottlicher et al. 2006, Gessler et al. 2009*c*), and thus their OPPP activity is lower, we might also be able to explain the observed differences in ^{13}C enrichment among these past studies.

Another metabolic factor that has the potential to change the $\delta^{13}C$ of heterotrophic organic matter is the (re-)fixation of CO_2 by phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) (Cernusak

et al. 2009). Phosphoenolpyruvate carboxylase-derived malate is ^{13}C enriched as compared with CO_2, as a consequence of the net fractionation against ^{12}C of the PEPC fixation reaction (for a review, see Farquhar et al. 1989*a*). Products that are formed in reactions downstream from the formation of malate (such as several amino acids (Melzer and O'Leary 1987)) are thus also enriched in ^{13}C. However, there is no obvious direct line from malate to cellulose or phenylpropanoid pathway metabolites (which are used to form lignin) without decarboxylation of the ^{13}C enriched carbon; thus, the effect of PEPC activity on the $\delta^{13}C$ of tree rings remains uncertain.

The same uncertainty holds true for the possible effects of respiratory isotope fractionation (reviewed in detail by Werner and Gessler (2011)). The reactions of pyruvate dehydrogenase (PDH; EC 1.2.4.1) as well as of the two Krebs cycle enzymes isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase could theoretically result in kinetic isotope effects, but the degree to which these effects are expressed in vivo is not clear (Werner et al. 2011). It is theoretically feasible for variable kinetic isotope effects from these enzyme-catalysed reactions to affect the $^{13}C/^{12}C$ ratio of lignin, depending on the commitment of products from the PDH reaction and the Krebs cycle to the shikimate pathway, which produces lignin precursors. The effects of both PEPC activity and respiratory fractionation on whole-wood tree-ring isotope ratios is an area in need of future research.

Only recently, Cernusak et al. (2013) estimated that 11% of the wood of branches of *Eucalyptus miniata* A.Cunn. ex Schauer were produced from bark photosynthesis. It is generally acknowledged that bark photosynthesis rates in various species amount to up to 75% of leaf photosynthesis rates, that 60–90% of stem-respired CO_2 can be re-fixed by young twigs and branches (Pfanz et al. 2002) and that it significantly affects $\delta^{13}C$ of the bark (Cernusak et al. 2001). Bark photosynthesis mainly uses trunk-respired CO_2 as the substrate, which is depleted in ^{13}C compared with tropospheric CO_2 (used for leaf photosynthesis). Moreover, bark photosynthesis discriminates against ^{13}C and the discrimination is proportional to the re-fixation rate (respiration rate/photosynthesis rate) (Cernusak et al. 2001); as a consequence, the newly fixed carbon is depleted compared with leaf assimilates. Given the importance of bark photosynthesis for the wood production of *E. miniata* (Cernusak and Hutley 2011), the exploration of its contribution in other tree species is needed urgently, and especially in tree trunks as they are mainly used for tree-ring studies.

Sources of variability for the $\delta^{18}O$ of tree rings: a round-trip circuit through the tree

Turning away from the topic of carbon isotope fractionation, the oxygen isotope composition of tree-ring whole wood or cellulose is determined during three main steps (Figure 1b, Table 2). First, oxygen in primary assimilates synthesized in the leaf is ultimately derived from leaf water. Thus, the first source of variation for $\delta^{18}O$ in plant matter is that of the $\delta^{18}O$ of source water entering the leaf, generally through xylem flow from the soil. Water in the leaf becomes isotopically enriched during transpiration, and this enriched ratio is carried into intercellular CO_2, and ultimately photosynthates. During assimilation oxygen atoms exchange between leaf water (which is the reaction water for biochemical processes) and carbonyl groups, thus imprinting leaf water enrichment on the assimilates. Assimilates are then exported to plant sinks through the phloem, and exposed to further opportunities for isotopic effects and exchange with internal plant waterpools. As a consequence, the signature of $\delta^{18}O$ in tree rings reflects source water variations, evaporative processes at the leaf level and mixing of water that might exchange oxygen with organic matter associated with phloem transport and synthesis of wood constituents. Disentangling these different signals carried in the tree rings is still a challenge but at the same time a prerequisite for accurate interpretation of this environmental archive.

Water uptake, xylem transport and leaf evaporative enrichment

One important source of environmental variation in tree rings is the $\delta^{18}O$ of source water, which reflects the wide range of variation in meteoric water and soil water (Dawson et al. 1993, Tang and Feng 2001, West et al. 2007). Contrary to what was originally assumed in early isotope studies, xylem water is not a direct reflection of meteoric water, particularly in seasonally dry climates, where rooting depth and its relation to sub-surface water storage are complex (Tang and Feng 2001, Brooks et al. 2010). As a further complication in describing seasonal patterns of xylem water, during periods of tree dormancy (e.g., winter rest) and/or low transpiration rates (e.g., summer drought), the turnover time for water in the trunk is longer (Brandes et al. 2007), allowing for evaporative fractionation of water accumulated in the xylem (Dawson and Ehleringer 1993). Since this phenomenon only occurs during periods of limited growth, it is unlikely to be reflected significantly in wood or tree-ring cellulose.

Once in the leaf, the original isotopic composition of xylem water is changed as a consequence of evaporative enrichment during transpiration (Farquhar and Lloyd 1993). The steady-state isotopic enrichment in ^{18}O in the leaf ($\Delta^{18}O_L$) over source water has been described using a form of the Craig–Gordon model that is corrected for advection of non-enriched xylem water as opposed by back-diffusion of enriched water from the sites of evaporation, i.e., for the Péclet effect (Craig and Gordon 1965, Dongmann et al. 1974, Farquhar and Lloyd 1993). The Craig–Gordon model adapted by Dongmann et al. (1974) to plant leaves describes the evaporative enrichment ($\Delta^{18}O_e$) at the sites of evaporation:

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_\kappa + (\Delta_v - \varepsilon_\kappa)\frac{e_a}{e_i}, \quad (2)$$

Table 2. Processes and their environmental drivers influencing the oxygen isotope signature of organic compounds from H_2O uptake to cellulose synthesis, going through evaporative enrichment at the leaf level. *A* and *E*, assimilation and transpiration rates; VPD, vapour pressure deficit; T_{air}, air temperature; T_{leaf}, leaf temperature; T_{soil}, soil temperature; PAR, photosynthetic active radiation; g_s, stomatal conductance.

Tissue	Isotope fractionation or isotopologue mixing process	Underlying physical, physiological or biochemical mechanism	Environmental drivers	Effect on $\delta^{18}O$ of organic matter
Water uptake and xylem transport				
Meteoric water	Variations in meteoric water (Dansgaard 1964)	Fractionation processes associated with phase changes	T_{air}, RH, VPD precipitation (amount and distribution), altitude, continentality	*Temporal*: seasonal and inter-annual variation of $\delta^{18}O$ in source water: affects the water media for primary assimilates (leaf water) and cellulose biosynthesis (stem water)
Soil/roots	Water uptake from different depths (Ehleringer and Dawson 1992) and/or pools of water (Tang and Feng 2001)	Existence of evaporative gradients in the soil Pools of water with different availability can be filled with distinct rain events	Seasonal variations in soil moisture and water potential	*Temporal*: seasonal and inter-annual variation of $\delta^{18}O$ in source water
Stems	Enrichment in xylem water along the stem and branches (Dawson and Ehleringer 1993)	Evaporation through the bark and/or exchange with phloem water	Low *E* associated with drought or low temperatures. Conditions of limited growth: unlikely stored in organic matter	*Spatial*: not observed in organic matter. Opposite basipetal trends have been reported (Cernusak et al. 2005, Gessler et al. 2009*a*, Voltas et al. 2013)
Leaves	Leaf water uptake of fog or dew (Dawson 1998)	Adsorption of condensed water through stomata (reversed sap flow)	Low soil water availability together with fog or dew	*Temporal*: seasonal variation of $\delta^{18}O$ in source water
Biosynthesis of primary assimilates				
Leaf mesophyll	Water enrichment at the site of evaporation (Craig and Gordon 1965, Dongmann et al. 1974)	Fractionation associated with CO_2 diffusion, carboxylation dark-/photorespiration	Major drivers: Direct (positive) effect of T_{air} and RH on e_a/e_i Radiation and g_s, as affecting T_{leaf} (e_i) Minor drivers: Direct effect of g_s on e_a/e_i Wind speed as affecting leaf boundary layer and thus e_a/e_i	*Temporal*: short-term variation of $\delta^{18}O$ in new assimilates during the day, day-to-day and seasonal variations *Spatial*: potentially decreased $\delta^{18}O$ in the lower canopy. Opposite basipetal trends have been reported (Cernusak et al. 2005, Gessler et al. 2009*a*, Voltas et al. 2013)
Leaf mesophyll	Radial Péclet effect: (Farquhar and Lloyd 1993)	Back diffusion of heavy isotopologues from the site of evaporation to veins, opposed to mass flow driven by transpiration. Causes a depletion in leaf $\delta^{18}O$ as compared with the site of evaporation	Negative effect of *E*: T_{air}, T_{leaf} and RH (VPD), water availability $\delta^{18}O$ negatively related to the effective path length *L*: drought stress and light	*Temporal*: seasonal changes in transpiration and hydraulic conductivity of the mesophyll. *Spatial*: gradients in leaf water enrichment within the leaf
Leaf mesophyll	Incorporation of oxygen from leaf water with an equilibrium fractionation factor (ε_{wc}) (DeNiro and Epstein 1979, Sternberg et al. 1986, Farquhar et al. 1998, Schmidt et al. 2001)	Direct incorporation of oxygen atoms into 3-phospho-D-glycerate 1-indirectly (through exchange with CO_2) 2-directly from water	Assumed to be constant	*Temporal*: short-term variation of $\delta^{18}O$ in new assimilates during the day, day-to-day and seasonal variations
Leaf mesophyll	Partial exchange with leaf water during carbohydrate biosynthesis	Direct incorporation of oxygen from water Indirect effect of exchange with CO_2	Turnover time of assimilates: *A*, day length, sink strength (export)	*Temporal*: reduction of day–night and day-to-day differences in $\delta^{18}O$ of sugars as compared to leaf water

(Continued)

Table 2. Continued

Tissue	Isotope fractionation or isotopologue mixing process	Underlying physical, physiological or biochemical mechanism	Environmental drivers	Effect on $\delta^{18}O$ of organic matter
Phloem transport and wood formation				
Phloem	Partial exchange with (unenriched) phloem water during unloading/ retrieval of sugars	Metabolic conversion in accompanying cells may offer opportunities for exchange with cell water	Not known. Potentially associated with phloem turnover, and thus with assimilation and transport rates	*Spatial:* depletion in $\delta^{18}O$ of phloem organic matter (Gessler et al. 2013), although no changes or even opposite trends have been observed *Temporal:* divergences between leaf and phloem changes over time depending on phloem turnover
Xylem/wood	Partial exchange with (unenriched) xylem water during cellulose biosynthesis (Hill et al. 1995, Roden and Ehleringer 1999, Sternberg et al. 2006)	Exchange rate depends on the portion of hexose phosphates that cycle through triose phosphate before being incorporated into cellulose (Barbour and Farquhar 2000). The exchange rate is normally assumed to be 0.42 (Cernusak et al. 2005) but intra-annual variations have been shown (Gessler et al. 2009*a*)	The exchange rate is assumed to be not temperature dependent (Sternberg and Ellsworth 2011); however, intra-annual variations have been observed but it is not clear if they are due to environmental drivers (Gessler et al. 2009*a*)	*Temporal:* if the exchange rate is affected by environmental drivers the relative influence of source water on tree-ring $\delta^{18}O$ might change with the driver on the seasonal, inter-annual, multi-decadal scale

where ε^+ is the equilibrium fractionation between liquid water and vapour dependent on temperature (Bottinga and Craig 1969), ε_κ accounts for the kinetic fractionation during the diffusion of water vapour from the leaf to the atmosphere, $\Delta^{18}O_v$ is the isotopic difference of atmospheric water vapour compared with source water, and e_a and e_i represent the water vapour pressure in the atmosphere and the leaf intercellular air space, respectively.

Following Eq. (2) the evaporative enrichment is expanded to the whole leaf lamina:

$$\Delta^{18}O_L = \Delta^{18}O_e \frac{1 - e^{-\wp}}{\wp} \text{ with } \wp = \frac{EL_{eff}}{CD}, \quad (3)$$

where $\wp$ (Péclet number) is the dimensionless ratio of forward viscous flow to back-diffusion, E stands for transpiration rate (mol m^{-2} s^{-1}), L_{eff} stands for the scaled effective path length (m), C is the molar concentration of water (55.56×10^3 mol m^{-3}) and D is the tracer diffusivity (m^2 s^{-1}) of heavy water isotopologues (either $H_2{}^{18}O$ or $^2H^1HO$) in 'normal' water. According to this model, the actual leaf water enrichment would be inversely correlated to transpiration rates, thus reinforcing the original effect of transpiration and stomatal conductance through its cooling effect (Farquhar and Lloyd 1993, Wang and Yakir 2000). However, there is increasing evidence that L_{eff}, which was originally thought to be a species-specific parameter, can vary in response to environmental conditions, modulating the expected changes in $\Delta^{18}O_L$ in response to E (Ripullone et al. 2008, Ferrio et al. 2009, 2012, Zhou et al. 2011, Song et al. 2013).

Transfer of the ^{18}O isotopic signal from leaf and stem water into organic matter

During the first step of carbon fixation, two oxygen atoms are directly incorporated into organic matter from water, and a third one is derived from CO_2 (Farquhar et al. 1998, Schmidt et al. 2001, see Figure 1b). However, given that oxygen atoms from CO_2 inside the leaf exchange very fast with those in H_2O, they share the same $\delta^{18}O$ trend. Moreover, nearly every metabolic step involves a certain degree of exchange of oxygen atoms between carbonyl groups and water (DeNiro and Epstein 1979, Farquhar et al. 1998). Consequently, carbohydrates in the leaf carry a time-integrated ^{18}O signature of leaf water (Barnard et al. 2007*b*, Gessler et al. 2007*b*) and with an equilibrium fractionation factor (ε_{wc}) resulting in carbonyl oxygen being ~27‰ more enriched than source water (Sternberg and Deniro 1983, Yakir and DeNiro 1990). Recently, Sternberg and Ellsworth (2011) observed that ε_{wc} was temperature dependent below 20 °C. As a first approximation, one might assume that average temperatures within temperate and boreal forest stands are often <20 °C during the growing season (e.g., Clinton 2003, Lemenih et al. 2004), and thus temperature

variations might additionally affect the transfer of the leaf water isotope signal to organic matter via its effects on ε_{wc}.

Cernusak et al. (2005) assumed that the time integration of the evaporative signal in organic matter is weighted by assimilation rate, and Gessler et al. (2007*b*) showed that the night-time evaporative enrichment of leaf water is also partially imprinted in sugars released from starch in the dark. Taking these points into account, Barnard et al. (2007*b*) and Gessler et al. (2007*b*) showed that the oxygen isotopic composition of newly assimilated organic matter could be well explained by modelled or measured evaporative enrichment when an additional ε_{wc} of 27‰ was included in the model and when the turnover time of starch to form leaf sugars was considered.

It is still unclear whether further exchange between oxygen in organic compounds and in water occurs during phloem loading and transport. Sucrose is the main exported sugar and it carries no free carbonyl group that could exchange oxygen atoms with water. Only recently, Gessler et al. (2013) studied the transfer of oxygen isotope enrichment from leaf water via leaf sugars to phloem sugars in five tree species. While leaf sugars showed the expected ^{18}O enrichment in all five species, phloem sugars were less enriched than expected from leaf water enrichment in three out of five species, namely in two coniferous gymnosperms (*P. sylvestris* and *Larix decidua* Mill.) and in a broadleaf evergreen angiosperm (*E. delegatensis*). The authors explained the species-specific differences on one hand with oxygen atom exchange with non-enriched water during phloem loading. In conifers, sieve tubes are loaded with sugars not within the mesophyll but in the central cylinder where water for potential oxygen exchange is assumed to be xylem like (i.e., more or less non-enriched). On the other hand, a significant contribution of assimilates from bark photosynthesis in the three species with rather sparse crowns was assumed to explain the observed phloem ^{18}O enrichment patterns. In cambium cells, where woody tissues are formed, these processes may affect the $\delta^{18}O$ of organic compounds used as substrates for cellulose and/or lignin synthesis. As PEPC- or ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39)-mediated assimilation in trunk cambium cells takes place with non-enriched reaction water (Cernusak et al. 2005), the organic matter produced is less ^{18}O enriched than the sugars originating from leaves and might rather reflect the source water signature.

Furthermore, and also depending on physiological conditions, storage and remobilization processes, i.e., the interconversion of soluble carbohydrates and starch, may lead to further exchange of oxygen atoms with water (Gessler et al. 2007*b*). Offermann et al. (2011) found a greater contribution of xylem water $\delta^{18}O$ to the $\delta^{18}O$ of phloem organic matter in spring than in summer and autumn, coinciding with the phase of wood formation, in which most assimilates are likely to derive from remobilized organic matter (Helle and Schleser 2004). As a consequence of these processes, the initial oxygen isotope signal of the photosynthates might—depending on species and environmental conditions—be already altered when they arrive at the trunk cambium.

Mixing of evaporative and source water signals in the $\delta^{18}O$ of tree-ring cellulose

Additional oxygen exchange occurs between sugars and reaction water during cellulose biosynthesis (Sternberg et al. 2006). The following equation describes the relationship between leaf water evaporative enrichment ($\Delta^{18}O_L$) and the ^{18}O enrichment of cellulose ($\Delta^{18}O_c$) taking into account the exchange of carbonyl oxygen with unenriched xylem water (Barbour and Farquhar 2000):

$$\Delta^{18}O_c = \Delta^{18}O_L\left(1 - p_x p_{ex}\right) + \varepsilon_{wc}, \quad (4)$$

where p_{ex} is the proportion of exchangeable oxygen in cellulose formed from sucrose and p_x is the proportion of unenriched (source) water in the developing cambium cell. Note that Eq. (4) only adequately describes the oxygen atom exchange between carbonyl oxygen and water directly associated with cellulose synthesis if no other exchange processes (e.g., associated with phloem transport) occur.

p_{ex} depends on the portion of hexose phosphates that cycle through triose phosphate before being incorporated into cellulose (Hill et al. 1995). However, since only oxygen atoms in carbonyl groups are subject to exchange within a reasonable time (Farquhar et al. 1998), multiple exchange processes do not necessarily result in a total loss of the evaporative enrichment signal produced in the leaf. Nevertheless, it has been observed that the rate of exchange with water increases with the complexity of the metabolic cycles involved, and variable exchange rates from <40% to ~100% have been described (Schmidt et al. 2001, Sternberg et al. 2006). The total exchange of oxygen atoms with surrounding water can only be explained assuming multiple metabolic conversions, causing the exposure to exchange of oxygen atoms that are normally in non-exchangeable positions, and exchange rates significantly higher than ~40% have been mainly observed under high salinity even though the exact mechanisms underlying these high p_{ex} values are not fully understood (Ellsworth and Sternberg 2014). Only recently, Song et al. (2014) observed that the turnover time of carbohydrates and thus potentially the relative strengths of carbon sink versus source in plants can strongly affect p_{ex}. Long residence times of carbohydrates in the trunk might be consequently one reason for some of the high exchange rates observed.

The vast majority of values for p_{ex} is however in the range of 40%. Roden and Ehleringer (1999) estimated the $\delta^{18}O$ of assimilates and calculated the ratio of exchange between

these assimilates and non-enriched stem water during cellulose synthesis to be between 36 and 42%. Similarly Cernusak et al. (2005) compiled values for p_{ex} and found a high consistency across a wide range of species and environmental conditions, with the average being 42%. This finding was independent of the tissue examined: in *E. globulus*, for example, p_{ex} amounted to ~40% in both leaf and stem cellulose. In general, p_{ex} was found not to be influenced by temperature (Sternberg and Ellsworth 2011), though some observations have indicated that the value is not constant during the entire growing season (Gessler et al. 2009*a*, Offermann et al. 2011). The seasonal environmental factors that cause this lack of stationarity have not yet been identified but the results of Song et al. (2014) indicate that precipitation, which is known to modify carbon source–sink relationships (McDowell et al. 2011), might be one of the drivers. Variations in p_{ex} driven by either external or plant-internal signals would change the relative contribution of the leaf water evaporative enrichment signal and the source water signal to the tree-ring $\delta^{18}O$ and would thus complicate its interpretation (Augusti and Schleucher 2007), and high exchange rates with unenriched xylem water in the stem might lead to the observations made recently by Treydte et al. (2014) that the intra-annual tree-ring ^{18}O isotope signal in *L. decidua* mirrored rather trends in source water than in needle water evaporative enrichment. We need more information to definitively determine whether external drivers such as temperature, or internal processes such as storage and remobilization affect p_{ex} so that we can account for all determining influences in tree-ring isotope models.

Variations in isotopic fractionation and oxygen exchange: can we still derive meaningful information from oxygen isotope signals in tree rings with all these uncertainties?

Only recently, Kahmen et al. (2011) showed that both leaf and stem cellulose $\delta^{18}O$ were significantly correlated to leaf-to-air vapour pressure difference along a steep climate gradient in the tropics. Moreover, Helliker and Richter (2008) showed that when cellulose $\delta^{18}O$ is used as a model input and modelled in relation to source water and enriched leaf water, one can use the temperature dependencies of ε^{+} and e_i to derive a photosynthetically weighted canopy temperature, i.e., the canopy leaf temperature at which most of the photosynthate that contributes to tree-ring cellulose production was assimilated. Using this form of inverse modelling, these researchers showed that across broad latitudinal gradients, from the tropics to boreal forests, trees generally assimilate most of the photosynthate used to construct tree-ring cellulose at 20–21 °C (see also Song et al. 2011).

This simple modelling of the temperature–photosynthesis relation from cellulose $\delta^{18}O$ assumes constant values for ε_{wc} and p_{ex}. The potential temperature dependency of ε_{wc} and putative seasonal variations in p_{ex} are complications for the approach of Helliker and Richter (2008). However, this study and that by Song et al. (2011) provide compelling evidence from multiple trees sampled across broad geographical space and covering multi-decadal temporal resolution that tree-ring cellulose may reflect broad convergence in the photosynthetic temperature response. There is considerably more research that needs to be conducted before we can use these relations reliably. However, this research shows that even though there are still many uncertainties about the processes affecting the $\delta^{18}O$ isotope signals on the way from the canopy to the tree ring they are still valuable tools to assess climate and physiology.

Seeing the forest for the trees: position-specific oxygen isotope composition

Owing to the partial exchange of organic oxygen with source water—which itself does not exhibit constant isotope ratios—the oxygen isotopic composition of whole wood or cellulose is determined by the combination of evaporative effects, the isotopic composition of source water and opportunities for post-assimilation exchange.

One promising approach to achieve an empirical partitioning of these influences is the measurement of the isotope abundance at a specific intramolecular position, i.e., the isotopomer distribution of a metabolite (Betson et al. 2006, Sternberg et al. 2006). Intramolecular variations in the isotopic abundance indicate that each position within a chemical compound has a distinct chemical history and carries the signal of independent isotope effects (Augusti and Schleucher 2007). Oxygen 5 and 6 of the glucose monomers of cellulose exhibits evidence of full exchange with source water during cellulose synthesis (Waterhouse et al. 2013). Therefore, the respective isotopomer abundances can be expected to be independent of all leaf-level processes and to depend exclusively on source water isotope abundance (Augusti and Schleucher 2007). On the other hand, the oxygen isotope information related to oxygen 2 and 4 is assumed to represent evaporative enrichment and thus physiological processes at the leaf level (Waterhouse et al. 2013). Position-specific analysis can, thus, provide a way to reconstruct the source water isotope abundance as well as the evaporative enrichment of water in the leaf, allowing precipitation and/or temperature reconstruction and resolving the complication of overlapping environmental and physiological influences (Augusti et al. 2006). Only recently, Ellsworth et al. (2013) have proposed a method to process small amounts of wood cellulose to produce phenylglucosazone, which removes the oxygen attached to carbon 2 and thus preserves mainly the signal from source water. Accordingly, combined analysis of cellulose and phenylglucosazone offers the potential to disentangle source and leaf-level signals in tree-ring records. This type of analysis would be especially useful when combined with the inverse modelling approaches relating

cellulose $\delta^{18}O$ to climate variables, such as temperature (e.g., Helliker and Richter 2008).

The long way down—two case studies on the transfer of the carbon and oxygen isotopic signal from the leaves to the tree ring on a seasonal time scale

There are few studies addressing the transfer of isotopic signals from primary assimilates to tree rings within the time scales of at least one whole growing season (for an overview, see Gessler et al. 2009*a*). To our knowledge, the complete pathways for both oxygen and carbon isotopes have only been assessed for *P. sylvestris* (Gessler et al. 2009*a*), partially based on data from Brandes et al. (2007), and for *Fagus sylvatica* L. from Offermann et al. (2011).

When tracing the $\delta^{13}C$ signal through the tree, clear differences between these two species were observed (Figure 2). In the conifer, $\delta^{13}C$ in phloem sugars increased in the basipetal direction along the transport pathway, and phloem and whole-wood $\delta^{13}C$ showed more or less consistent seasonal courses (Figure 2b). For ^{18}O a comparable seasonal correlation between phloem sugars and cellulose was observed (Figure 2d). When taking into account transport and turnover times, the biochemical oxygen isotope fractionation and the exchange of carbonyl oxygen with xylem water during cellulose synthesis, the leaf water ^{18}O enrichment signal could be traced into the tree ring (Gessler et al. 2009*a*). In *F. sylvatica*, in contrast, the $\delta^{13}C$ of leaf sugars and phloem organic matter showed a comparable pattern only during part of the growing season (Figure 2a) and there was no significant correlation between the $\delta^{13}C$ of phloem sugars and whole wood during the period of radial growth. Moreover, Offermann et al. (2011) found no clear transfer of the evaporative ^{18}O enrichment signal in leaf water and leaf sugars to phloem sugars or to tree rings. Both phloem sugars and tree-ring whole wood showed opposite patterns in ^{18}O enrichment relative to those observed for leaf assimilates, especially at the beginning of the growing season (Figure 2c).

Such isotope patterns in the non-photosynthetic plant parts are related to one of the issues raised by Roden and Siegwolf

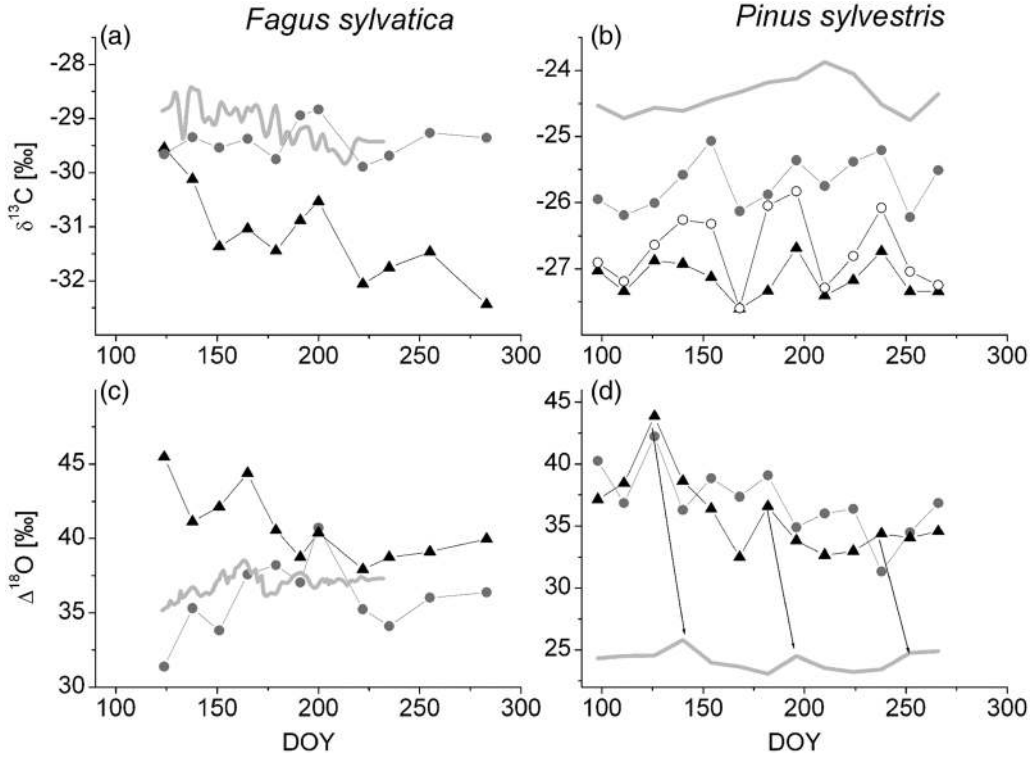

Figure 2. Seasonal patterns of the carbon isotope composition ($\delta^{13}C$; a, b) and of the oxygen isotope enrichment ($\Delta^{18}O$; c, d) in different organic matter pools in *F. sylvatica* and *P. sylvestris*. For *F. sylvatica* (a; c) we show the $\delta^{13}C$ and $\Delta^{18}O$ of leaf water-soluble organic matter (black triangles) as a proxy for new assimilates, of trunk phloem organic matter (grey circles) and of whole wood (light grey bold lines). In *P. sylvestris* $\delta^{13}C$ (b) is displayed for needle water-soluble organic matter, trunk phloem and whole wood. In addition, $\delta^{13}C$ of the phloem of twigs (white circles) from the upper third of the crown, where the needles were also collected, is shown. $\Delta^{18}O$ of *P. sylvestris* (d) is displayed for measured trunk phloem organic matter (grey circles), trunk phloem organic matter modelled from leaf water enrichment (taking into account a fractionation of 27‰ between water and carbonyl oxygen and a time lag of 2 days (Gessler et al. 2009*a*)) (black triangles). The bold light grey line depicts the $\Delta^{18}O$ of tree-ring cellulose. The arrows indicate time lags between the oxygen isotope signal in phloem organic matter and in the tree rings. Data for *F. sylvatica* are taken and adapted from Offermann et al. (2011), and data for *P. sylvestris* from Brandes et al. (2007) and Gessler et al. (2009*a*). DOY, day of year. Note that the scaling of the *y*-axes differs between (a) and (b) as well as between (c) and (d).

(2012) that need to be taken into account when applying the dual-isotope approach for distinguishing the effects of stomatal conductance or assimilation on $\delta^{13}C$ and thus intrinsic water-use efficiency (Scheidegger et al. 2000). The model of Scheidegger et al. (2000) is based on the assumption that on one hand photosynthesis and g_s affect $\delta^{13}C$ and that on the other hand the water pressure deficit of the air—which mainly drives the evaporative enrichment of ^{18}O—drives g_s. If one of the isotope signals is damped or changed in a way different from the other downstream of photosynthesis and leaf evaporation, the interpretation of the model will be erroneous.

With respect to our case studies, two phenomena need closer attention: (i) the cause of the enrichment in ^{13}C during basipetal transport in *P. sylvestris*; and (ii) the cause of the clear differences between the species in the transfer of the leaf and canopy level carbon and oxygen isotope signals to the trunk and, finally into the tree-ring archive.

Basipetal ^{13}C enrichment

Hobbie and Werner (2004) suggested that the enrichment of photosynthate in ^{13}C as it is transported towards the roots is due to the fact that part of the primary assimilates are converted to ^{13}C-depleted compounds such as lignin and lipids, which remain in the leaf. As a consequence, sugars exported from the leaf and loaded into the phloem are enriched in ^{13}C.

During phloem transport to non-photosynthetic plant parts, additional ^{13}C enrichment in phloem-transported sugars has been observed between twigs, the upper trunk (below the canopy) and the trunk base, particularly in *P. sylvestris* (Brandes et al. 2006, 2007, Kodama et al. 2008). Allocation of released sugar to isotopically lighter lignin (cf. Bowling et al. 2008) in the trunk would help in explaining continuous ^{13}C enrichment of sugars along the stem transport path. Only recently, Mauve et al. (2009) observed a small kinetic isotope effect of the invertase reaction in vitro. Invertase catalyses the hydrolysis of sucrose unloaded from the phloem into glucose and fructose (Lim et al. 2006). If the invertase kinetic isotope effect were expressed in vivo, fructose and glucose subjected to further metabolic conversion would become relatively ^{13}C depleted, whereas sucrose that is reloaded without reaction into the phloem would be ^{13}C enriched (Mauve et al. 2009).

There is not much information about vertical gradients in the $\delta^{13}C$ observed in tree rings. Schleser (1992) observed no vertical gradients in the $\delta^{13}C$ of trunk wood of beech whereas the $\delta^{13}C$ of leaves from twigs inserted along the trunk at the same positions where the wood had been sampled was depleted in ^{13}C. The author assumed that this discrepancy between leaves and trunk was due to a negligible supply of carbon by the lower shaded leaves to the trunk tissue, but we cannot rule out that a ^{13}C enrichment of phloem sugars along the transport pathway balanced the supply with ^{13}C-depleted assimilates from the lower canopy. Only a few authors (Tans and Mook 1980, Leavitt and Long 1986, Nguyen-Queyrens et al. 1998) have looked for patterns in the vertical variation in $\delta^{13}C$ in tree rings along the trunks of trees and no clear trends were observed. However, Tans and Mook (1980) and Leavitt and Long (1986) assessed vertical gradients of only 40 cm and 3 m, respectively, which may have been inadequate to properly assess the issue. A recent study on tree decline in a mature stand of *P. sylvestris* (Voltas et al. 2013) showed an enrichment of up to 2‰ in the $\delta^{13}C$ of wood cellulose from the upper third of the canopy (~4.5 m) downward to breast height, which was particularly strong in declining trees. Most likely, enrichment in the $\delta^{13}C$ of phloem sugars and wood along basipetal gradients in tree trunks varies in response to opportunities for metabolic reaction and subsequent isotope fractionation associated with phloem transport (Gessler et al. 2009*a*), rather than processes that originate at the leaf level.

Differences in the carbon and oxygen isotope signal transfer

There are strong indications that differences in the seasonal carbon storage and remobilization patterns between deciduous and evergreen species are the main reasons for differences in the isotopic coupling observed between tree rings and leaves. By comparing the seasonal variation of $\delta^{13}C$ in (bulk) leaf material and tree-ring cellulose, Helle and Schleser (2004) characterized the influence of remobilized carbon reserves on intra-annual patterns archived in tree rings. The authors identified growth periods, when newly assimilated carbon was transferred to tree-ring cellulose, versus periods when carbon from remobilized starch was transferred to tree-ring cellulose. This is in agreement with the observation for *F. sylvatica* in Figure 2 with relatively high $\delta^{13}C$ and low $\Delta^{18}O$ values in early wood. The initially low $\Delta^{18}O$ values were assumed to be due to high rates of partial exchange of carbonyl oxygen with non-enriched trunk water during starch breakdown (Gessler et al. 2007*b*, 2013), and the high $\delta^{13}C$ values might be a consequence of isotope effects in heterotrophic plastids (see section 'Carbohydrate dynamics in heterotrophic tissues: storage, remobilization, respiration and re-fixation'). The increasing incorporation of newly assimilated organic matter into the tree ring as stem growth proceeded explained the observed increase in $\Delta^{18}O$ together with the decrease in $\delta^{13}C$ (Helle and Schleser 2004, Offermann et al. 2011).

In evergreen coniferous species such as *P. sylvestris*, a tighter coupling between the isotope composition of new assimilates and tree rings is reflected in stronger correlations between leaf and phloem sugars and the tree rings. The rather consistent transfer of the $\delta^{18}O$ and $\delta^{13}C$ signals from the canopy to the trunk phloem organic matter and cellulose or wood during the entire growing season as observed by Gessler et al. (2009*a*) and displayed in Figure 2 is supported by other findings from the literature: Klein et al. (2005) showed in *Pinus halepensis*

Mill. that intra-annual subsections of tree rings indicated rapid responses in $\delta^{13}C$ upon changing environmental conditions and that the responses were comparable in the needles. Moreover, the $\delta^{13}C$ estimated from gas exchange measurements explained the seasonal and inter-annual variations both in the needles and in the tree ring. In addition, Barbour et al. (2002) observed clear and mechanistically explainable intra-annual responses of the isotope signatures ($\delta^{13}C$, $\delta^{18}O$) in the tree-ring archive to variations in water availability in *P. radiata*. We need to assume that in evergreen species the seasonal dynamics of storage and remobilization are less pronounced and thus do not strongly interfere with the isotopic coupling between leaves and tree rings.

In the final assessment of gaps across temporal scales: the ultimate link between isotope fractionation and the long-term tree-ring archive

Many if not all of the fractionation and isotopic mixing processes described in the sections above and summarized in Tables 1 and 2 are effective on short time scales relative to the climate and ecophysiological insight commonly extracted from the isotope composition of tree rings. The overall isotopic signals provided by the shorter-term fractionation processes are damped by the time they are extracted from tree-ring wood or cellulose. Here, we summarize the primary processes that mute the signal during post-leaf assimilation processes and describe other factors that create additional long-term isotopic trends.

Storage and remobilization

In many species, new assimilates are directly incorporated into cellulose and other tree-ring constituents only during particular periods of the growing season (Helle and Schleser 2004). During long time periods, remobilized stored carbohydrates (primarily starch) completely or partially supply substrates for the construction of tree rings. As a consequence, starch-derived parts of the tree ring are coupled to leaf and soil at the time when the starch was produced, possibly many months or even years earlier (e.g., Kagawa et al. 2006*a*, 2006*b*). Richardson et al. (2013) showed by applying ^{14}C analysis that not only starch but also soluble sugars in the outer 2 cm of the stem wood were a decade old in mature trees of different species. The authors hypothesized an intensive and regular inter-conversion between starch and sugars, causing mixing of carbohydrate pools of considerably different age and origin. If true, this would result in a dampening and blurring of the isotopic signals in the tree-ring archive across years to decades and complicate interpretations of seasonal or year-to-year influences of climate on physiology from low-frequency variations in the isotope signal. This is at least partially in contrast to the fast and predictable transfer of carbon and oxygen isotope signals from leaves to the tree ring in *P. sylvestris* (Figure 2b and d). The results from Richardson et al. (2013) are also at odds with the findings of Gaudinski et al. (2009), who observed that the carbon used for bud and root growth in a deciduous oak forest was ~0.4 years old.

Assimilation rate and tree internal fluxes

In addition to storage and remobilization, the relative strengths of photosynthate supply and demand by multiple sinks can create competition for the substrate that affects the relation between short-term fractionation processes and the longer-term insight provided in tree-ring isotopes. Especially under stress conditions, photosynthesis is reduced and carbon allocation to different competing sinks can be affected. Dobbertin (2005) postulated that carbon allocation to stem growth might be reduced as an early response to stress at the expense of the more vital carbon sinks such as leaf, bud and root growth. This implies that the carbon and oxygen isotopic signatures imprinted on organic matter during stress are incompletely transferred to the tree ring. In general, tree-ring isotope signals will be dominated by carbon and oxygen isotope signatures imprinted on organic matter during periods of high assimilation rates, resulting in photosynthetic weighting of influences on the cellulose isotope composition.

Long-term changes in atmospheric CO_2 concentration

In the section 'Variability in the isotopic composition of CO_2 and H_2O', we have already referred to the effects of the variability and the long-term changes of the isotopic composition of H_2O and CO_2. Especially the correction of the trend of decreasing $\delta^{13}C$ of atmospheric CO_2 and utilization of the corrected $\delta^{13}C$ records of tree rings rests on the assumption that the remaining high- and low-frequency variations are related to climate-induced changes in photosynthetic carbon isotope fractionation. However, this approach largely ignores the fact that the carbon isotope composition of tree rings is not only determined by the atmospheric $\delta^{13}C$ source value but also by changes in the ratio of c_i/c_a (Farquhar et al. 1982). Consequently, the quasi-exponential decrease in atmospheric $\delta^{13}C$ by ~1.14‰ (±0.15‰) (Friedli et al. 1986) alone is likely not sufficient to account for the overall decrease of tree-ring $\delta^{13}C$. In addition to this effect, some potential increase in leaf internal discrimination against ^{13}C caused by higher atmospheric CO_2 availability probably needs to be considered much more (Treydte et al. 2001, 2009, Gagen et al. 2007, McCarroll et al. 2009) as discussed in the section 'Effects of changing CO_2 concentration on $\delta^{13}C$'. It is, however, still unclear how plants physiologically respond to the higher CO_2 availability (Feng 1998): the response could be passive (increase in c_i/c_a) or active by reducing the stomatal conductance and therefore increasing the water-use efficiency keeping c_i/c_a constant, and might depend on species and also the range of CO_2 concentration change.

Age trends

There is increasing evidence that both the carbon and oxygen isotope compositions of tree rings may undergo age-related variation related to changes in the sources (CO_2 and water) and physiological ageing effects such as development of the root system, increasing hydraulic resistances of tall trees and concomitantly lower stomatal conductance (McDowell et al. 2002). For $\delta^{13}C$ a well-known 'juvenile effect' with depleted but rising values in the first decades after germination has been reported in a number of studies. This trend has been associated with young trees exposed to increased CO_2 levels by soil respiration or reduced direct light influences, which appear to be particularly important in dense forests (Bert et al. 1997, McCarroll and Pawellek 2001, Raffalli-Delerce et al. 2004, Gagen et al. 2008).

An age-related decrease in tree-ring $\delta^{18}O$ records has been reported by Marshall and Monserud (2006) and Treydte et al. (2006) and was explained by the fact that young and old trees may have access to different source water with ageing and development of the root system: young trees may take up relatively more of evaporative ^{18}O-enriched water from the upper soil layers (Drake and Franks 2003), and moreover a slightly inferior water supply could lead to a higher leaf-to-air water vapour pressure gradient with higher ^{18}O enrichment. Other studies partly support this finding (Marshall and Monserud 2006, Esper et al. 2010), but it is under discussion whether these trends are strong enough to bias long-term climate reconstructions from tree-ring isotopes (Esper et al. 2010, Young et al. 2011).

Our mechanistic understanding of isotope fractionation processes is improving, but we still lack a detailed understanding of the processes that determine the isotopic composition of the tree-ring archive over the long term. Dendroclimatological/ecological studies, which tend to be based on statistical models, cover decades to millennia, whereas studies characterizing the effects of plant physiology and environment on isotope fractionation, mixing and oxygen exchange focus on a few growing seasons, at most. Up to now, mechanistic models, considering both leaf-level and translocation fractionations, have been shown to predict inter- and intra-annual variations in tree-ring isotopic composition over several growing seasons with reasonable accuracy (Ogee et al. 2009, Eglin et al. 2010). In this regard, one promising way to exploit the existing mechanistic knowledge for palaeoenvironmental studies is the use of inverse modelling, which takes advantage of the mechanistic models to derive environmental variables from different dendroecological variables, such as stable isotopes (e.g., Song et al. 2011, Boucher et al. 2013). In order to strengthen the link between mechanistic models and dendroecology, and take advantage of new approaches, such as inverse modelling but also intra-annual assessments of isotopes with laser ablation or micro-laser dissection (Schollaen et al. 2013) and compound-specific isotope analysis, we need to expand the assessments of the mechanisms determining the isotope composition of tree rings over longer time periods. This requires a reassessment of already available tree-ring data (isotopes, tree-ring width and density) under the consideration of our current knowledge, along with novel experimental setups but especially with long-term monitoring of the fate of the isotopic signals from the source to the tree-ring archive, not only covering one year but rather aiming to span decades.

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Conflict of interest

None declared.

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