Contents lists available at ScienceDirect

Chemical Geology

journal homepage: www.elsevier.com/locate/chemgeo

CrossMark

A remarkable relationship of the stable carbon isotopic compositions of wood and cellulose in tree-rings of the tropical species *Cariniana micrantha* (Ducke) from Brazil

G.H. Schleser [a,b,*], D. Anhuf [c], G. Helle [a,**], H. Vos [d]

[a] Helmholtz-Centre Potsdam, GFZ German Research Centre for Geoscience, Section 5.2 Climate Dynamics and Landscape Evolution, 14473 Potsdam, Germany
[b] FZJ Research Center Jülich, Institute of Bio- and Geosciences, Agrosphere (IBG-3), 52425 Jülich, Germany
[c] Department of Physical Geography, University of Passau, Innstraße 40, 94032 Passau, Germany
[d] FZJ Research Center Jülich, Institute for Energy and Climate Research, Stratosphere (IEK-7), 52425 Jülich, Germany

ARTICLE INFO

ABSTRACT

The stable isotopes of carbon were analysed in total wood and cellulose from the tree-rings of the tropical wood species *Cariniana micrantha* (Ducke). The aim was to examine the isotopic relationship between total wood and its cellulose over the last two and a half centuries. Although the correlation for the whole time period is very high ($r = 0.96$) it is remarkable that different sub-periods deviate strongly from this close relationship. Consequently, a good correlation from the subset of a longer isotopic record cannot necessarily prove its validity for the whole record. The study indicates that changes of the carbon isotopes of cellulose and of total wood show sometimes during short sub-periods different isotope patterns presumably caused by different environmental effects. Thorough calculations indicate that strong variations within the isotopic record especially changes of the isotopic level along a chronology lead to high correlations between $\delta^{13}C_{wood}$ and $\delta^{13}C_{cel}$. Contrary thereto subsections with low isotopic variability lead to low correlations. The results imply that long term trends provide similar patterns. Therefore, if long term trends are of interest such as e.g. in climate reconstruction then total wood can be analysed in favour of cellulose, thus saving a tremendous amount of work. However, if short term aspects from a longer record are of interest, cellulose and total wood may sometimes provide different information. In addition it is hypothesized that during intervals of low isotopic variability the proportions of the various wood components may change relative to each other, leading for certain time intervals to different isotope patterns.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

In recent decades, numerous carbon isotope studies in tree-rings have been performed with the objective to reconstruct climate variations of the past. Tree-ring chronologies represent unique terrestrial archives with annual resolution which can absolutely be dated and go back for hundreds, sometimes thousands of years (e.g. Freyer and Belacy, 1983; Pilcher et al., 1984; Briffa et al., 1990; Lipp et al., 1991; Zetterberg et al., 1996; Lindholm et al., 1999).

In the early days of stable carbon isotope investigations, use was made of total wood (e.g. Craig, 1954; Farmer and Baxter, 1974; Libby et al., 1976). However, with the observation that total wood and cellulose and in general the various wood components differ in their isotopic composition (Wilson and Grinstead, 1977; Yildiz, 1995; Borella et al., 1998; Zimmermann, 1998; Loader et al., 2003; Harlow et al., 2006; Szymczak et al., 2011; Gori et al., 2013), most investigators started to extract cellulose for the purpose of climate reconstruction. The main argument was, to prevent any distortion of the isotopic records due to changing proportions of the different wood components relative to each other. As such if any individual component is analysed a climate signal should remain uninfluenced by changing proportions of the tree-ring components. This raises the question of whether or not the proportion of the different wood components is generally constant over the life span of a tree. Up to now this has tacitly been assumed in most isotope investigations in which total wood has been used although this is not proven.

The main wood components, namely carbohydrates, comprising holocellulose (including α-cellulose and hemicelluloses) and lignin, show differences in their carbon isotopes of several permil (Wilson and Grinsted, 1977; Borella et al., 1998; Loader et al., 2003). Although α-cellulose and hemicelluloses represent by far the largest proportion of wood (on average 65–75%), the high carbon content of lignin as compared to carbohydrates which is almost 50% higher (Kürschner and

* Correspondence to: G.H. Schleser, GFZ German Research Centre for Geosciences, Section 5.2: Climate Dynamics and Landscape Evolution, 14473 Potsdam, Germany. Tel.: +49 2461 61 3102.
** Corresponding author. Tel.: +49 331 288 1377.
E-mail addresses: Schleser@gfz-potsdam.de (G.H. Schleser), ghelle@gfz-potsdam.de (G. Helle).

Popik, 1962; Nikitin, 1966; Pettersen, 1984) results in a considerable contribution of carbon from lignin.

Extractive components in wood other than α-cellulose, hemicelluloses and lignin, comprise numerous additional compounds and ash. These compounds represent an extra contribution of carbon which shows a wide range of $\delta^{13}C$ values (O'Leary, 1981; Schmidt and Gleixner, 1998). In wood species from temperate climates, they constitute 4–10% of dry weight. However, they may represent as much as 20% for tropical trees (Pettersen, 1984). These additional extractives comprise a large variety of compounds, such as fats, waxes, alkaloids, proteins, phenolics, simple sugars, pectins, gums, resins, terpenes, starches, glycosides, saponins and essential oils. However, their contribution to the overall $\delta^{13}C$ value is mostly negligible because these extractives make up only a small percentage of the total wood and the $\delta^{13}C$ values of these fractions are mainly in the cellulose to lignin range. They can, however, frequently be related to particular environmental incidents such as fire or drought which may induce for example resin production or may act as part of the trees' defence mechanism against microbial attack. Although resins may not play a prominent role in tropical trees, special organic extractives such as for example residual organic crystals may occasionally be found which show drastically different $\delta^{13}C$ values (O'Leary, 1981; Van de Water, 2002). Such extractives might contribute a significant percentage to the overall isotope value, provided their contribution is relatively large.

Any shift in the ratio of cellulose to lignin or other wood constituents could indicate that total wood and cellulose may not lead to the same variations in their isotopic composition, especially with regard to long isotope records. It is known that extractives vary considerably in content between e.g. heartwood and sapwood and that the lignin-to-cellulose ratio varies not only across one ring, i.e. in late wood as compared to early wood but also radially across the trunk (Narayanamurti and Das, 1955). Presently the different temporal intra-annual origins of photoassimilates for cellulose and lignin are ignored which may sometimes record different seasonal weather events. During xylogenesis, lignification of the wood cell walls generally occurs after the formation of primary cellulose structures (Rossi et al., 2012 and refs. therein), however, it is assumed that cellulose and lignin are largely formed within the same vegetation period, i.e. year. A stable, i.e. constant ratio between lignin and cellulose should then raise the question of whether or not carbon isotopes of total wood are adequately suited to trace the climate variation in the vicinity of a tree. This question is rather important because the extraction of cellulose from wood is quite time consuming and labour intensive.

Changing ratios of cellulose to total wood could be the result of particular environmental conditions in the surroundings of a tree, such as weather anomalies, tree competition, and changing length in seasonality. Consequently, the interpretation of isotope data from cellulose and total wood could in part point to different environmental signals.

Many investigators report very high correlations for the carbon isotopes of total wood and cellulose (Livingston and Spittlehouse, 1996; Borella et al., 1998; Zimmermann, 1998; Dietz, 2006; Gori et al., 2013). In these cases it would make sense to discard the cellulose extraction and restrict the carbon isotope investigations to total wood. However, some questions have to be raised with regard to such an approach because the currently available investigations are mostly restricted to rather short time periods, normally not extending over much more than 50 years:

1. Is a high correlation deduced from a particular time window of an isotope chronology stable in time? In other words, is it justified to extrapolate the correlation properties from a time interval to any other time interval without considering a weakening of the degree of the relationship? The issue at stake might be related to uniformitarianism.
2. What may be the reason if a correlation of the stable carbon isotopes between cellulose and total wood is changing in time?

3. What plant physiological reactions may be responsible for the decoupling of a correlation in a particular time period? Are the two components, i. e. cellulose and total wood (presumably lignin), responding differently to certain environmental changes and as such provide in part independent information? For example, does the proportion of cellulose to total wood change as a tree matures?
4. What are the implications of a non stable correlation between the carbon stable isotopes of cellulose and total wood for climate reconstructions?

The present study is a first approach by which in a tropical tree the correlation between cellulose and wood will be investigated for a longer time period, namely a period of about 253 years. This investigation addresses primarily points 1, 2 and 4 of the above listed points.

2. Material and methods

The investigation centred on tree material which was collected from the 'terra firme', the inundation free tropical rain forest, north of Manaus, Brazil. For the present study, a tree species was selected which shows frequently far more than 200 years of age. *Cariniana micrantha* (Ducke) is known to reach ages of up to 500 years (Anhuf et al., 2013) and, most important, shows tree-rings although they are not always distinct. In some cases they become incoherent or indistinguishable and may thus cause big challenges for trustworthy chronological reconstructions. Therefore, thin section investigations of some time intervals were added for which the corresponding $\delta^{13}C$ values were measured in order to substantiate tree-ring borders (Verheyden et al., 2004; Pons and Helle, 2011). We are, however, aware that the exact number of tree-rings cannot be given here especially with regard to false rings and wedging rings. A sufficient number of individuals for successful dendrochronological cross-dating could not be sampled. Nevertheless, tree rings have been counted repeatedly and the period covered is well in the order of two and a half centuries. In view of the question discussed, namely whether or not total wood and cellulose do always show the same isotopic pattern, the exact length of the chronological sequence is irrelevant.

2.1. Material

Samples of *C. micrantha* (Ducke) were collected within the Reserve of Cuieiras (Reserva Biológica do Cuieiras, better known as ZF2) some 60 km north of Manaus. Cores were collected at breast height (≈ 1.3 m) using a 12 mm diameter increment borer. The trees are growing in the 'terra firme', an area which shows a distinct dry period of 2 to 3 months with less than 100 mm precipitation per month. This dry period occurs generally between June and November and is sufficient in length to bring the cambial activity to a standstill (Worbes, 1999). Accordingly, growth is interrupted for a short time, thus leading mostly to visible tree-rings.

First tree-ring inspections were undertaken at the INPA (Instituto National das Pesquisas na Amazônia) at Manaus which indeed demonstrated the visibility of tree-rings. On a scale from 0 to 3, characterizing the quality of tree-ring visibility, with the best visibility given by 3, the clarity of tree-rings for *C. micrantha* (Ducke) is given by 1.5. Consequently, the recognition of tree-ring borders is partly difficult, justifying the additional analysis of isotopic thin section investigations.

For the present investigation a period with 253 counted tree rings, i.e. years, was chosen encompassing the time interval from 1750 to 2007. This represents the outer part of the tree, i.e. the sap wood including a restricted part of the heartwood. The age of the investigated tree was well above 253 years.

C. micrantha (Ducke) has a diffuse porous wood structure, like the European beech (*Fagus sylvatica*). Contrary to the situation for diffuse porous species of temperate climate zones, the number of large vessels does not vary much along a tree ring. Hence, density across the tree-

rings of *C. micrantha* (Ducke) is practically constant. Therefore, a differentiation in early wood (EW) and late wood (LW) was not possible. Moreover, no anatomy-based definition for early wood and late wood exists for diffuse porous broadleaf tree species in contrast to the clear definition formulated for coniferous species (Mork, 1928; Denne, 1989). Thus, no effort was made to define any increment which might symbolize the EW section of a tree-ring, although the initial wood development and subsequently the associated $\delta^{13}C$ value may be influenced by the environmental conditions of the previous year (Hill et al., 1995). Even highly resolved intra-annual increments on the basis of isotope values do not allow defining the border between EW and LW. Thus, the investigation was based on wood material consisting of the whole annual increment. This implies that the carry-over of stored photoassimilates, i.e. of reserves, from the previous year may induce isotopic distortions of the current year affecting the corresponding time series. In view of the currently discussed problem, this should, however, be of no relevance.

2.2. Isolation of cellulose and isotope analyses

The wood material of each tree-ring was dissected using a UV-laser dissection technique (Schollaen et al., 2014). Wood samples were homogenized with an ultra centrifugation mill (Retsch, ZM1, mesh size 0.05 mm), while the extracted α-cellulose was homogenized with an ultrasonic device (Laumer et al., 2009). The extraction of α-cellulose was carried out as specified by Boettger et al. (2007) utilizing the device described by Wieloch et al. (2011).

Samples were measured using an elemental analyser interfaced to a continuous flow isotope ratio mass spectrometer (Thermo Fisher Scientific, Delta Plus XL IRMS). The ratio of the carbon isotopes is expressed in the delta (δ) notation with reference to the Vienna Pee Dee belemnite (VPDB) standard (Craig, 1957; Coplen, 1995):

$$\delta^{13}C \ (\text{‰}) = \left(R_{sample}/R_{standard} - 1 \right) \times 1000,$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in a sample and a laboratory standard, respectively, the latter normally being calibrated to the international VPDB standard. The reproducibility of the method, including cellulose extraction, is better than 0.05‰. For isotope analyses of total wood the reproducibility was 0.07‰. No source correction for atmospheric $^{13}CO_2$ changes was applied because the objective of this study was merely to investigate the behaviour of cellulose relative to total wood.

3. Results and discussion

3.1. Tree-ring boundaries of C. micrantha (Ducke)

The investigation started with a thorough check of the tree-ring boundaries. Although they were mostly visible a decision was not always unanimously clear. Therefore, thin section investigations were used for subsets to substantiate the correct choice of tree-ring boundaries. Test measurements revealed a clear intra-annual isotope pattern with an initial increase in $\delta^{13}C$ values up to a maximum followed by a gradual decrease towards the end of the tree-ring. In general, the increase is rather steep at the start of a new tree ring which facilitates the recognition of boundaries. Detailed information about thin section investigations is given in Helle and Schleser (2004) and Schleser et al. (1999b). As an example, Fig. 1 shows the continuous intra-annual isotope variations of the investigated *C. micrantha* (Ducke) for a period of 8 years (from 1779 to 1787). The tree-ring boundaries as recognized by visual, i.e. microscopic analyses are indicated by arrows which match perfectly with the isotope thin section results. These results corroborate the investigations by Pons and Helle (2011) who realized that $\delta^{13}C$ values of thin sections are well suited for the verification of
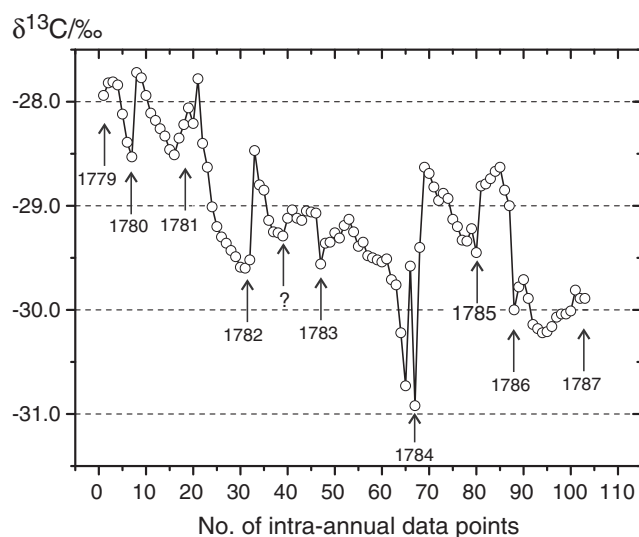

Fig. 1. The intra-annual sequence of $\delta^{13}C$ values from *C. micrantha* (Ducke) for a period of 8 years. Samples of 140 μm thickness were separated. The start or end of the tree-ring boundaries is indicated by arrows. Due to the different tree-ring widths the arrows are not equidistant to each other. For details cf. text.

indistinct or almost invisible tree-ring boundaries of tropical trees (see also Verheyden et al., 2004). The selected sequence was chosen to demonstrate that thin sections do sometimes also raise questions regarding tree-ring boundaries. The interval between 1782 and 1783 could well represent two years with an additional boundary indicated by the question mark in Fig. 1, although the $\delta^{13}C$ increase is not very strong (merely 0.3‰). An additional tree-ring was, however, ruled out by additional careful visual inspections. Moreover, the start of the 1784 boundary is questionable. Unfortunately additional measurements were not possible due to the very low amount of available material. Nevertheless, there is no doubt about the existence of the 1784 boundary at the data point no. 67, and thus the uncertainty for the start of the 1784 ring is 280 μm.

Consequently, the tree-ring boundaries of *C. micrantha* (Ducke) were reasonably recognized by the microscopic and the intra-annual isotopic analyses, allowing the further discussion of the inter-annual variability over the last two and a half centuries.

3.2. $\delta^{13}C$ values of total wood and its cellulose

As known from many investigations the $\delta^{13}C$ values of cellulose are enriched by 1‰ to almost 4‰ relative to total wood (Schleser, 1992; Marshall and Monserud, 1996; Borella et al., 1998; Zimmermann, 1998; Loader et al., 2003; Ferrio and Voltas, 2005; Dietz, 2006; Roden and Farquhar, 2012). Fig. 2 documents the $\delta^{13}C$ difference between total wood and the corresponding cellulose for the investigated *C. micrantha* (Ducke) covering the last two and a half centuries. The mean difference between the two data sets is 1.5 ± 0.36‰ by which total wood is depleted relative to cellulose, corroborating published data of trees from the temperate zones. It is, however, remarkable that the variations are not statistically distributed around the mean. For more than 100 years of the initial part of the chronology an unusual almost sinusoidal like pattern is observed which is superseded by an erratic phase and a minimum during the period from 1900 to 1950 followed by a slight increase for the last 50 years. The solid black line represents a 2-year moving average.

The similarity of the trends between cellulose and total wood was tested by correlating the two data sets. In view of the question raised at the beginning, this could give an answer as to whether or not cellulose and total wood can equally well be used for reconstructions of past environmental changes. The relationship between $\delta^{13}C$ values of
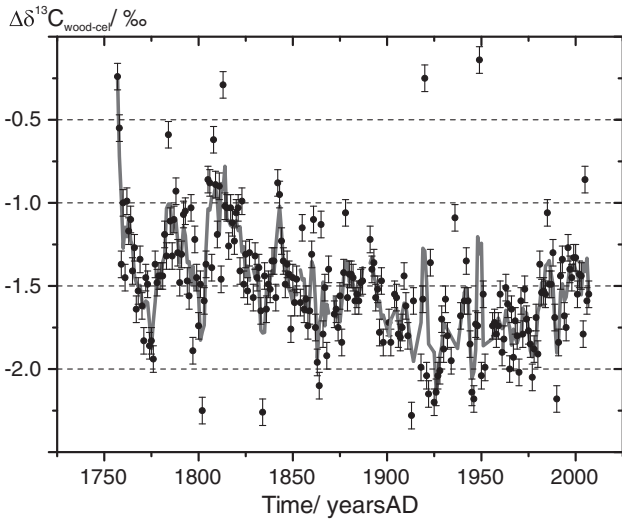
Fig. 2. The difference of the $\delta^{13}C$ values from total wood and cellulose presented as $\Delta\delta^{13}C_{wood-cel}$ for *C. micrantha* (Ducke) covering the time period from 1755 to 2007. On the average total wood is depleted by 1.5 ± 0.36‰ relative to its cellulose. Solid line represents a 2-year moving average.

cellulose and total wood of the whole chronology is shown in Fig. 3 yielding a correlation coefficient of r = 0.96. This is rather convincing for preferring total wood to cellulose for climate reconstructions.

3.3. Stability of the correlation between total wood and cellulose for selected time windows

For reconstructing the environment of the past it is common practice to calibrate a set of isotope data from a longer record with available meteorological data (see e.g. Schollaen et al., 2013) and try to validate this result by taking the succeeding data set of the chronology, reconstruct the accompanying meteorological data by using the calibration results and compare them with the corresponding yet existing environmental data. In most regions, records of accessible meteorological data sets are rather short. More than 100 to 120 years for calibration and validation are rarely available. The main point is that in isotope research a
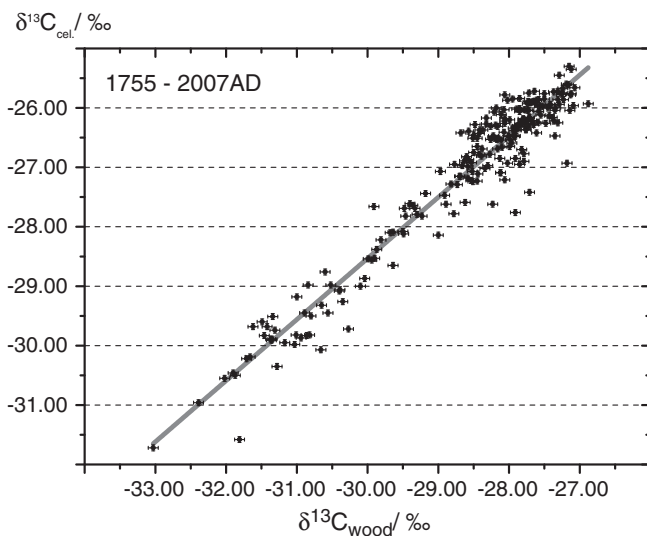
successfully i.e. reasonably well correlated sequence although limited in length is normally assumed to be valid for the whole record under investigation. Logically, similar considerations have to be accepted for the relationship between total wood and cellulose: A satisfying, statistically sound correlation, although short in length, should be valid for a whole record preferably for the entire life span of a tree.

A first test was made by subdividing the last 100 years of the available record of *C. micrantha* (Ducke) in two 50 year sequences and comparing their correlations, notwithstanding the fact that the correlation between total wood and cellulose is high for the whole record of the two and a half centuries. The results are shown in Figs. 4 and 5 which document the correlations for the periods of 1955 to 2007 and 1905 to 1955, respectively. It is surprising to see that the correlations differ substantially. While during the period from 1955 to 2007, total wood and cellulose correlate well (Fig. 4, r = 0.92), the correlation for the period from 1905 to 1955 (Fig. 5) breaks almost completely down with a correlation coefficient of only r = 0.05. On the basis of this result, the correlation r was investigated by using a moving interval length of 50 years over the existing 253 years period of the *C. micrantha* (Ducke). The result is shown in Fig. 6 (upper curve) with ages representing the middle of the interval. The correlation intervals of Figs. 4 and 5 are marked as grey circles in Fig. 6 representing two contrasting situations representing very low and very high r-values, respectively. This shows that the correlation of the carbon isotopes between total wood and its cellulose is only constant during specific time intervals. At particular intervals, there seems to exist a decoupling of the two data sets. Two aspects seem to be responsible for these phenomena: Firstly, a strong isotopic shift of the chronology from one isotopic level to another, shown simultaneously for cellulose and total wood. Such level changes are also seen in carbon isotope chronologies by other authors of tropical trees (see e.g. Ballantyne et al., 2011), reasons for this phenomenon are manifold: e.g. penetration of a tree's crown through the canopy into the free atmosphere, or clearing of a tree's surrounding through storm, fire or age (see e.g. van der Sleen et al., 2014). Thus, subsets like the first 80 years of the correlation curve from Fig. 6 (upper curve), showing a highly variable isotopic sequence for cellulose and total wood, characterized by their strong standard deviations (Fig. 6, lower curve) will automatically result in high correlations. Secondly, changes of the chemical composition of various wood components relative to each other along a broadly constant isotopic level of the
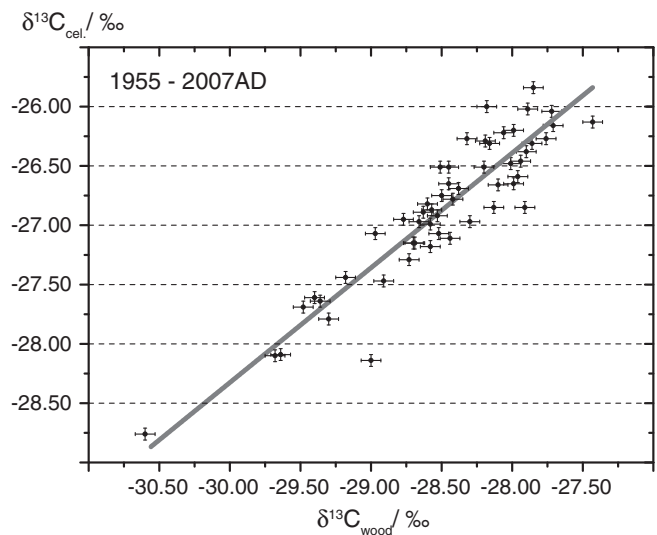

Fig. 3. The relation of $\delta^{13}C$ values between total wood and cellulose of *C. micrantha* (Ducke) for the time period from 1755 to 2007. The correlation coefficient is: r = 0.96.

Fig. 4. The relation of $\delta^{13}C$ values of total wood and cellulose for the period of 1955 to 2007. This data set represents the final values of the isotopic sequence given in Fig. 3. The correlation coefficient is r = 0.92.
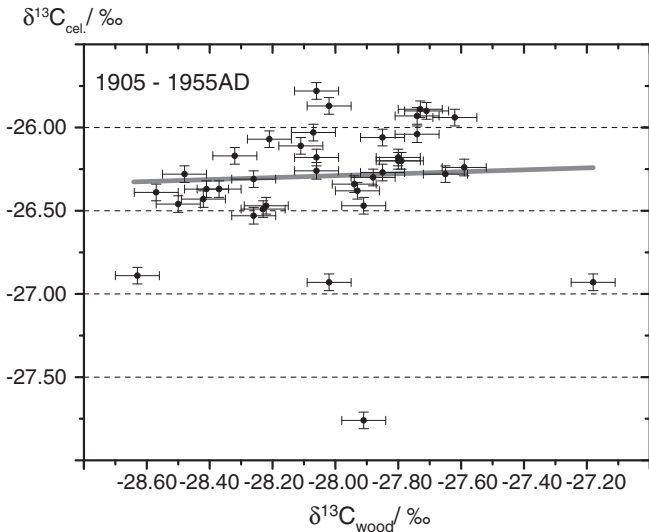
Fig. 5. The relation of $\delta^{13}C$ values of total wood and cellulose for the period of 1905 to 1955. The data set is taken from the long chronology of Fig. 3. The correlation coefficient is r = 0.05.

chronology, showing a low isotopic standard deviation. Such sequences with low moving standard deviations as from 1850 to 1930 (based on interval centres) may inherently include a mixture of the lowest and rather high correlations. Reasons might be changing compositions of wood components relative to each other during the vegetation period or transfer of material across the tree-ring border into the subsequent tree-ring. It is clear that a reconstruction of $\delta^{13}C_{cel}$ based on the $\delta^{13}C_{wood}$ of the interval 1905 to 1955 will not reflect the general picture of the ups and downs of $\delta^{13}C_{cel}$ over the whole time horizon, i.e. the whole isotopic record. Currently it is unclear as to whether or not these are unusual effects which cannot be generalized or whether certain environmental situations may indeed induce such behaviour pattern. The result obtained needs further investigations because it raises a number of questions for the interpretation of carbon isotopes in tree-rings. Total wood and cellulose might as such sometimes record

Fig. 6. Moving correlation r of total wood and its cellulose for *C. micrantha* (Ducke), upper curve, considering an interval lengths of 50 years (ages indicate middle of interval). The two lower curves represent the moving isotopic standard deviation (SD) of $\delta^{13}C_{wood}$ and $\delta^{13}C_{cel}$. For different time windows of the chronology which cover the period from 1755 to 2007 the isotope trends of total wood and its cellulose deviate strongly from each other. For details cf. text.

different environmental episodes, both being of interest for reconstructions.

3.4. Correlations from time windows of low isotopic variability

3.4.1. Influence of varying wood compositions in tree-rings during tree growth

It is still open for discussion why during intervals of relatively stable isotopic variations, i.e. low standard deviation of sequences from total wood and cellulose, correlations may either be low or high. One hypothesis is an environmentally induced variable change of various wood components relative to each other.

Since different wood components show partly strong differences in their carbon isotope composition, changing proportions of components will alter the trend of total wood $\delta^{13}C$ values as compared to $\delta^{13}C$ values of any of the wood components. It should be noted that for example late wood of oaks produces a higher content of methoxy groups than early wood, indicating that even within an annual ring the composition of lignin may vary which certainly has an influence on the relative composition of these constituents, i.e. cellulose and lignin (Sitte et al., 1998). This may in part explain the intra-annual variation in $\delta^{13}C$ values (Helle and Schleser, 2004).

In a first simple exercise it will be assumed that wood is just composed of cellulose and lignin. From numerous investigations it is justified to infer a constant isotopic difference between cellulose and lignin of 3.5‰ (e.g. Wilson and Grinsted, 1977), such that $\delta^{13}C_{cel} = \delta^{13}C_{lig} + 3.5‰$. The relative percentage of cellulose and lignin in the corresponding wood will be given by f_{cel} and f_{lig}, respectively. Here cellulose comprises α-cellulose and hemicelluloses because they show largely similar isotopic values. In addition C_{cel} and C_{lig} shall represent the proportions of carbon in either molecule. Under these conditions the overall proportion of carbon mass, P_{cel}, in the corresponding wood regarding cellulose (including hemicelluloses) is $P_{cel} = f_{cel} * C_{cel}$. Similarly the proportion of carbon mass from lignin is $P_{lig} = f_{lig} * C_{lig}$.

For the present assessment it is assumed that wood is composed of 70% cellulose and 30% lignin (e.g. Narayanamurti and Das, 1955; Pettersen, 1984), with cellulose C_{cel} consisting of 44% carbon and lignin C_{lig} consisting of 66% carbon. As such it follows that the proportion of carbon mass of cellulose in the total wood mass is: $P_{cel} = 0.70 * 0.44 = 0.31$ and the carbon mass of lignin from the total wood mass is $P_{lig} = 0.30 * 0.66 = 0.20$, respectively. Thus, the percentage of total carbon in total wood is $P_{cel} + P_{lig} = 31\% + 20\% = 51\%$. With these preconditions $\delta^{13}C_{wood}$ of total wood is given by:

$$\delta^{13}C_{wood} = \left[P_{cel} * \delta^{13}C_{cel} + P_{lig} * \delta^{13}C_{lig} \right] / \left[P_{cel} + P_{lig} \right] \quad (1)$$

$$\delta^{13}C_{wood} = \delta^{13}C_{cel} - 3.5 * \left[P_{lig} \right] / \left[P_{cel} + P_{lig} \right]. \quad (2)$$

$$\Delta\delta^{13}C_{wood-cel} = \delta^{13}C_{wood} - \delta^{13}C_{cel} = -3.5 * \left[P_{lig} \right] / \left[P_{cel} + P_{lig} \right] \quad (3a)$$

leading to a value of:

$$\Delta\delta^{13}C_{wood-cel} = -1.37‰. \quad (3b)$$

Thus, a constant enrichment of 1.37‰ for cellulose relative to wood is inferred. Although this corroborates the above given average result for *C. micrantha* (Ducke) of 1.5 ± 0.36‰, the assumptions used are probably not exactly those of the investigated tree species. The main point is, however, that $\Delta\delta^{13}C_{wood - cel}$ is not constant (see Fig. 2) as would have to be expected according to Eqs. (3a) and (3b). The difference of $\Delta\delta^{13}C_{wood - cel}$ varies substantially between −0.8 and −2.0‰ which is far beyond the error margins of the experimental analyses.

An alternative to this outcome is that the percentages of cellulose and lignin are not constant with tree growth. For the present situation
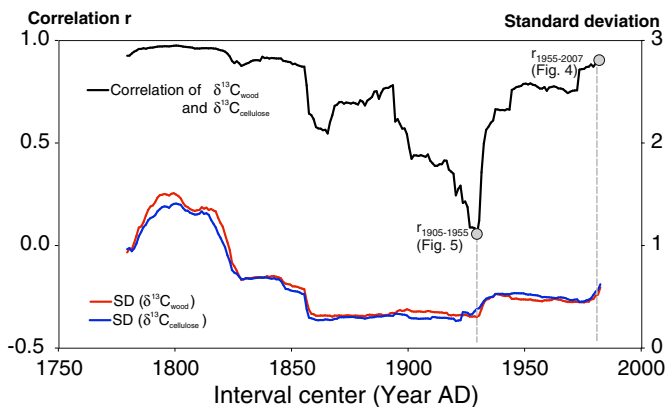
it will be assumed that the cellulose content (α-cellulose and hemicelluloses) of wood f_{cel} will vary between 60% and 80% (Shimizu, 1990). Thus, from Eqs. (1) to (3) it follows, keeping in mind that $f_{cel} + f_{lig} = 1$:

$$\delta^{13}C_{wood} = \left[f_{cel} * C_{cel} * \delta^{13}C_{cel} + (1 - f_{cel}) * C_{lig} * \delta^{13}C_{lig} \right] / \left[f_{cel} * C_{cel} + (1 - f_{cel}) * C_{lig} \right]$$
$$\Delta\delta^{13}C_{wood-cel} = -3.5 * \left[(1 - f_{cel}) * C_{lig} \right] / \left[f_{cel} * C_{cel} + \left(1 - f_{cel} * C_{lig} \right) \right].$$
$$(3c)$$

This leads to $\Delta\delta^{13}C_{wood - cel}$ values ranging from $-1.75‰$ to $-0.95‰$, respectively.

The difference of $\delta^{13}C_{wood} - \delta^{13}C_{cel}$ documented in Fig. 2 as $\Delta\delta^{13}C_{wood - cel}$ varies primarily in the interval as deduced above. Only during the time periods from 1910 to 1925 and roughly 1950 to 1980 the $\Delta\delta^{13}C_{wood - cel}$ values are lower than $-1.75‰$, in some cases being below $-2‰$. Thus, the derivation leads numerically to a satisfactory result, although wood contains hardly up to 40% lignin. 30–35% lignin is generally the upper limit for woody structures (Sakakibara, 1990) despite the fact that up to 36% are reported for some softwood species (Shimizu, 1990).

Tropical wood contains mostly higher lignin content than hardwood of temperate-zones which rarely reaches as much as 30%. In tropical wood even 38% lignin has been measured in some cases (Pettersen, 1984). E.g. *Eperua bijuga* Mart. ex Benth/Muirapiranga from Brazil showed values of 38% for lignin and only 53% for carbohydrates. *Cedrela odorata* L. from Mozambique showed 33% for lignin and 55% for carbohydrates. This indicates that in many tropical trees extractives have to be more important as compared to temperate-zone wood. Up to 20% of extractives were measured for wood of tropical trees (Pettersen, 1984). Additionally, it is known that extractives can even be exchanged between adjacent annual rings such as resins (Tans et al., 1978). Other constituents such as Ca-oxalate are known to show occasionally very low $\delta^{13}C$ values (Rivera and Smith, 1979; Van de Water, 2002).

In general, the chemical composition of wood cannot be defined precisely for a given tree species or even for a given tree (Pettersen, 1984). Type of wood, geographic location, climate and soil conditions determine the chemical composition of a tree. Under the binocular, the investigated *C. micrantha* (Ducke) showed numerous cells filled with small conspicuous black crystal like particles of glittering appearance. For a substantial number of consecutive years, they were rather abundant, while in other subsets of the sequence they were non-existent. Unfortunately their composition could not be determined. It cannot be excluded that these crystal like deposits are either the monohydrate or dihydrate form of Ca-oxalate (Ca(COO)$_2$ × H$_2$O or Ca(COO)$_2$ × 2H$_2$O, respectively), the existence of which has been observed in a number of tree tissues (Van de Water, 2002). The existence of silica (SiO$_2$), the content of which can vary in tropical woods from trace amounts to as much as 9% (Pettersen, 1984), could be excluded. The important point is that Ca-oxalate was sometimes found to be strongly depleted in ^{13}C relative to holocellulose. In *Cowania mexicana* a depletion by more than 7‰ was measured.

A detailed investigation of the chemical composition in combination with the corresponding isotopic signatures with tree age could not be achieved. Therefore, it is impossible to present a realistic explanation of the pattern of the isotope behaviour between total wood and cellulose. It should also be mentioned that the isotopic set-off between cellulose and lignin of 3.5‰ is presumably not constant but varies, especially if the problem of carry-over of stored material is to be considered. However, the presented data of this tropical tree species provide a piece of circumstantial evidence that the observed isotope variations between cellulose and total wood rest on changes of the various wood components relative to each other.

Based on the existing data set and the basics of tropical trees, Eq. (3c) will be expanded by introducing a third wood component termed 'extractives' (subscript: extr) which summarizes all wood components except for cellulose (including hemicelluloses) and lignin. Its value is

defined as $\delta^{13}C_{cel} = \delta^{13}C_{extr} + \beta$. β represents the isotopic difference (in permil) between cellulose and the weighted average value of all extractives present in the corresponding wood:

$$\Delta\delta^{13}C_{wood-cel} = -\left\{ 3.5 * f_{lig} * C_{lig} + \beta * f_{extr} * C_{extr} \right\}$$
$$/ \left[f_{cel} * C_{cel} + f_{lig} * C_{lig} + f_{extr} * C_{extr} \right].$$

For tropical trees a rather broad spectrum of wood compositions is available (Pettersen, 1984). If this spectrum of variations is considered (restricted to tropical woods, partly based on Brazilian species) the following ranges of composition can be defined: $0.53 \leq f(p_{cel}) \leq 0.74$; $0.22 \leq f(p_{lig}) \leq 0.38$ and $0.0 \leq f(p_{extr}) \leq 0.16$. The range of ß values is taken as: $0 \leq ß \leq 7‰$. If possible combinations, as stated by Pettersen (1984), are applied the isotopic difference between wood and cellulose, i.e. $\Delta\delta^{13}C_{wood - cel}$, can bridge the following gap: $-2.2‰ \leq \Delta\delta^{13}C_{wood - cel} \leq -0.9‰$. This difference covers largely the variations as given in Fig. 2, indicating that changes in the wood composition can explain the experimental data. Optical inspections of the tree-ring sequences found no distortions, i.e. changes of cell structures or deformations which could point to unusual environmental influences on the growth behaviour.

Currently it is not possible to specify the exact wood composition of the investigated tree. More precise $\delta^{13}C$ values as a function of the wood composition would need an investigation of wood components as a function of the tree's life including the determination of the corresponding isotope signatures. The current data indicate that the various wood components are not stable in time but tend to change, however, the reasons are currently not clear.

It should be stressed that the situation is different for subfossil or fossil wood since the velocity of degradation associated with this process is different for different wood components (Spiker and Hatcher, 1987; Benner et al., 1991; Schleser et al., 1999a,b).

3.4.2. Problem of carbon transfer across tree-ring boundaries

Since EW was combined with LW it cannot be excluded that there is a significant transfer of certain isotopic signatures by stored photoassimilates (e.g. Hill et al., 1995) over several years of a $\delta^{13}C$ time series. Such a mechanism may have two effects: On the one hand this can lead to changes of the isotopic composition of the preceding year due to changes of its total wood composition caused by transfer of certain chemical compounds (e.g. sucrose) to the current year. On the other hand this transfer will then influence the isotopic composition of the current year.

Evidence from stable isotope ratios in EW and LW of annual growth rings from oak stem tissue indicates that EW bears the isotope signature of stored carbohydrates laid down in the previous year (Hill et al., 1995). This documents that young leaves switch to current photosynthetic products for current cellulose production only if they are becoming net exporters of photosynthates. Therefore, synthesis of EW in deciduous trees from temperate climates occurs at the expense of stored carbohydrates laid down at the end of the previous season. It is commonly assumed that carbon is mainly stored as starch which is then converted to sucrose for transport to the growing wood tissue. In these cases the seasonal isotopic signatures of the preceding year will influence the isotopic signature of the current year, thus distorting the climatic signal of each year. Regarding time series of isotope records from such trees will automatically lead to significant autocorrelation effects which can only be suppressed, at least to a considerable part, if late wood is selected for tree-ring isotope records. Therefore, isotope chronologies of oak trees will normally be restricted to LW. As a matter of fact, investigations do unfortunately not exist which have documented the importance of choosing LW in favour of total ring wood to circumvent contributions of isotopic signature transfer from preceding years. In addition no data exist giving definite proof that LW data are uninfluenced by previous years' environmental signatures. Frequently, except

for oak trees, tree-ring isotope investigations are based on whole ring wood information, especially in case of very narrow rings.

The situation is, however, different for tropical trees because in many cases they keep their leaves all year round, indicating that photosynthates are continuously being produced although the photosynthetic activity may vary during the year. *Cariniana micrantha* (Ducke) belongs to these tree species which are evergreen. Therefore, the question of transfer, i.e. the usage of stored photosynthates for later wood production is difficult to answer especially because it is not possible to determine a border between EW and LW. No studies are presently available which have thoroughly dealt with this problem. At first sight we cannot rule out that at particular seasonal periods a certain mechanism of substrate carry over exists. Nonetheless, it is questionable if a differentiation in EW and LW is meaningful for evergreen tropical trees.

4. Conclusion

The carbon isotopes of cellulose and total wood from the tree-rings of the tropical wood species *C. micrantha* (Ducke) were analysed. The investigation covered a time period of about 253 years, ranging from 1755 to 2007. In view of the questions raised it has firstly to be concluded that the carbon isotope correlation between total wood and its cellulose is not stable in time. For *C. micrantha* (Ducke) it is therefore not justified to extrapolate the correlation properties from a particular time interval to any other time interval of the isotope chronology. In general, high correlations will preferably be encountered for chronological subsets in which the isotopic signature varies strongly changing from a certain level to another one. Contrary thereto changes of the chemical composition of wood constituents along relatively stable isotope levels with low standard deviation may result in low correlations although strong correlations may sometimes to be encountered. Secondly, cellulose and total wood presumably respond differently to certain environmental changes. Indications are at hand that the cause might primarily be due to strong changes of the extractives (presumably Ca-oxalate) in the wood of *C. micrantha* (Ducke). This could have consequences for the interpretation of isotope data such that cellulose and total wood record partly different environmental signals.

Up to now studies of the variability of the chemical composition of major tree species relative to climate, soil conditions and tree age are missing. In view of climate reconstruction this would certainly improve the reliability of reconstruction regardless of whether or not trees from the temperate or tropical zone are under investigation. In this respect, a broad study has been started to investigate the relation of carbon isotopes between total wood and cellulose over long time periods. The topic needs further thorough investigation.

Acknowledgements

References

Anhuf, D., Schleser, G.H., Helle, G., 2013. Isotopenproxies aus tropischen Hölzern für Klimarekonstruktionen und Datierungen. Untersuchungen an Standorten in Amazonien. Abschlußbericht GZ (AN 214/8-1 + AN 214/8-2).

Ballantyne, A.P., Baker, P.A., Chambers, J.Q., Villalba, R., Argollo, J., 2011. Regional differences in South American Monsoon precipitation inferred from the growth and isotopic composition of tropical trees. Earth Interact. 15, 1–35.

Benner, R., Fogl, M.L., Sprague, E.K., Hodson, R.E., 1991. Depletion of ^{13}C in lignin and its implication for stable carbon isotope studies. Nature 329, 708–710.

Boettger, T., Haupt, M., Knoller, K., Weise, S.M., Waterhouse, J.S., Rinne, K.T., Loader, N.J., Sonninen, E., Jungner, H., Masson-Delmotte, V., Stievenard, M., Guillemin, M.T., Pierre, M., Pazudur, A., Leuenberger, M., Filot, M., Saurer, M., Reynolds, C.E., Helle, G., Schleser, G.H., 2007. Wood cellulose preparation methods and mass spectrometric analyses of δ^{13}C, δ^{18}O and nonexchangeable δ^{2}H values in cellulose, sugar and starch: an interlaboratory comparison. Ann. Chem. 79, 4603–4612.

Borella, S., Leuenberger, M., Saurer, M., Siegwolf, R., 1998. Reducing uncertainties in δ^{13}C analysis of tree-rings: pooling, milling and cellulose extraction. J. Geophys. Res. 103 (D16), 19519–19526.

Briffa, K.R., Bartholin, T.S., Eckstein, D., Jones, P.D., Karlén, W., Schweingruber, F.H., Zetterberg, P., 1990. A 1400-year tree-ring record of summer temperatures in Fennoscandia. Nature 346, 434–439.

Coplen, T., 1995. Discontinuation of SMOW and PDB. Nature 375–385.

Craig, H., 1954. Carbon-13 variations in *Sequoia* rings and the atmosphere. Science 119, 141–143.

Craig, H., 1957. Isotopic standards for carbon and oxygen correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12, 133–149.

Denne, M.P., 1989. Definition of latewood according to Mork (1928). IAWA Bull. New Ser. 10, 59–62.

Dietz, O., 2006. Stabile Kohlenstoff- und Sauerstoffisotope als saisonale Witterungssindikatoren in tropischen Bäumen. Diplomarbeit, Fachhochschule Aachen, Abt, Jülich.

Farmer, J.G., Baxter, M.S., 1974. Atmospheric carbon dioxide levels as indicated by the stable isotope record in wood. Nature 247, 273–275.

Ferrio, J.P., Voltas, J., 2005. Carbon and oxygen isotope ratios in wood constituents of *Pinus halepensis* as indicators of precipitation, temperature and vapour pressure deficit. Tellus 57B, 164–173.

Freyer, H.D., Belacy, N., 1983. ^{13}C/^{12}C records in Northern Hemisphere trees during the past 500 years: anthropogenic impact and climate superpositions. J. Geophys. Res. 88, 6844–6852.

Gori, Y., Wehrens, R., Greule, M., Keppler, F., Ziller, L., La Porta, N., Camin, F., 2013. Carbon, hydrogen and oxygen stable isotope ratios of whole wood, cellulose and lignin methoxyl groups of *Picea abies* as climate proxies. Rapid Commun. Mass Spectrom. 27, 265–275.

Harlow, B.A., Marshall, J.D., Robinson, A.P., 2006. A multi-species comparison of δ^{13}C from whole wood, extractive-free wood and holocellulose. Tree Physiol. 26, 767–774.

Helle, G., Schleser, G.H., 2004. Beyond CO_2 fixation by RuBiSCO — an interpretation of ^{13}C/^{12}C variations in tree-rings from novel intraseasonal studies of broadleaf trees. Plant Cell Environ. 27, 367–380.

Hill, S.A., Waterhouse, J.S., Field, E.M., Switsur, V.R., Rees, T., 1995. Rapid recycling of triose phosphates in oak stem tissue. Plant Cell Environ. 18, 931–936.

Kürschner, K., Popik, M.G., 1962. Zur Analyse von Hölzern. Mitteilungen zur Chemie, Physik. Biologie und Technologie des Holzes. Holzforschung 16 (1), 1–11.

Laumer, W., Andreu, L., Helle, G., Schleser, G.H., Wieloch, T., Wissel, W., 2009. A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses. Rapid Commun. Mass Spectrom. 23, 1934–1940.

Libby, L.M., Pandolfi, L.J., Payton, P.H., Marshall, J., Becker, B., Sienbenlist, V.G., 1976. Isotopic tree thermometers. Nature 261, 284–288.

Lindholm, M., Eronen, M., Timonen, M., Meriläinen, J., 1999. A ring-width chronology of Scots pine from northern Lapland covering the last two millennia. Ann. Bot. Fenn. 36, 119.126.

Lipp, J., Trimborn, P., Fritz, P., Moser, H., Becker, B., Frenzel, B., 1991. Stable isotopes in tree ring cellulose and climate change. Tellus 43B, 322–330.

Livingston, N.J., Spittlehouse, D.L., 1996. Carbon isotope fractionation in tree ring early and late wood in relation to intra-growing season water balance. Plant Cell Environ. 19, 768–774.

Loader, N.J., Robertson, I., McCarroll, D., 2003. Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. Palaeogeogr. Palaeoclimatol. Palaeoecol. 196, 395–407.

Marshall, J.D., Monserud, R.A., 1996. Homeostatic gas-exchange parameters inferred from ^{13}C/^{12}C in tree rings of conifers. Oecologia 105, 13–21.

Mork, E., 1928. Die Qualität des Fichtenholzes unter besonderer Rücksichtnahme auf Schleif- und Papierholz. Papierfabrikant 26, 741–747 (in German).

Narayanamurti, D., Das, N.R., 1955. Die Abhängigkeit der chemischen Zusammensetzung des Holzes einiger indischer Holzarten von seiner Lage innerhalb des Stammes. Holz Roh Werkst. 2, 52–56.

Nikitin, N.I., 1966. The chemistry of cellulose and wood. In: Monson, S. (Ed.), Israel Programme for Scientific Translation.

O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20 (4), 553–567.

Pettersen, R.C., 1984. The chemical composition of wood. In: Rowell, R.M. (Ed.), The Chemistry of Solid Wood American Chemical Society, pp. 57–126 (Washington DC).

Pilcher, J.R., Baillie, M.G.L., Schmidt, B., Becker, B., 1984. A 7272-year tree-ring chronology for Western Europe. Nature 312, 150–152.

Pons, T., Helle, G., 2011. Identification of anatomical non-distinct annual rings in tropical trees using stable isotopes. Trees Struct. Funct. 25 (1), 83–93.

Rivera, E.R., Smith, B.N., 1979. Crystal morphology and ^{13}Carbon/^{12}Carbon composition of solid oaxalate in *Cacti*. Plant Physiol. 64, 966–970.

Roden, J.S., Farquhar, G.D., 2012. A Controlled Test of the Dual-Isotope Approach for the Interpretation of Stable Carbon and Oxygen Isotope Ratio Variation in Tree Rings. pp. 1–14 http://dx.doi.org/10.1093/treephys/tps019.

Rossi, S., Morin, H., Deslauriers, A., 2012. Causes and correlations in cambium phenology: towards an integrated framework of xylogenesis. J. Exp. Bot. 63 (5), 2117–2126.

Sakakibara, A., 1990. Chemistry of lignin. In: Hon, D.N.S., Shiraishi, N. (Eds.), Wood and Cellulosic Chemistry. Marcel Dekker, Inc., New York, Basel, Hongkong, pp. 113–175.

Schleser, G.H., 1992. δ13C pattern in a forest tree as an indicator of carbon transfer in trees. Ecology 73 (5), 1922–1925.

Schleser, G.H., Frielingsdorf, J., Blair, A., 1999a. Carbon isotope behaviour in wood and cellulose during artificial aging. Chem. Geol. 158, 121–130.

Schleser, G.H., Helle, H., Lücke, A., Vos, H., 1999b. Isotope signals as climate proxies: the role of transfer functions in the study of terrestrial archives. Quatern. Sci. Rev. 18, 927–943.

Schmidt, H.-L., Gleixner, G., 1998. Carbon Isotope Effects on Key Reactions in Plant Metabolism and 13C-Patterns in Natural Compounds. In: Griffiths, H. (Ed.), Stable isotopes BIOS Scientific publishers Ltd, Oxford (pp.).

Schollaen, K., Heinrich, I., Neuwirth, B., Krusic, P.J., D'Arrigo, R.D., Karyanto, O., Helle, G., 2013. Quat. Sci. Rev. 73, 170–181.

Schollaen, K., Heinrich, I., Helle, G., 2014. UV-laser-based microscopic dissection of tree rings — a novel sampling tool for δ13C and δ18O studies. New Phytol. 201 (3), 1045–1055.

Shimizu, K., 1990. Chemistry of hemicelluloses. In: Hon, D.N.S., Shiraishi, N. (Eds.), Wood and Cellulosic Chemistry. Marcel Dekker Inc., New York, Basel, Hongkong, pp. 177–213.

Sitte, P., Ziegler, H., Ehrendorfer, F., Bresinsky, A., Strasburger, 1998. Lehrbuch der Botanik. Gustav Fischewr Verlag, Stuttgart, Jena, Lübeck, Ulm, p. 346.

Spiker, E.C., Hatcher, P.G., 1987. The effects of early diagenesis on the chemical and stable carbon isotopic composition of wood. Geochim. Cosmochim. Acta 51, 1385–1391.

Szymczak, S., Joachimski, M.M., Bräuning, A., Hetzer, T., Kuhlemann, J., 2011. Comparison of whole wood and cellulose carbon and oxygen isotope series from Pinus nigra ssp. laricio (Corsica/France). Dendrochronologia 29, 219–226.

Tans, P.P., de Jong, A.F.M., Mook, W.G., 1978. Chemical pretreatment and radial flow of 14C in tree-rings. Nature 271, 234–235.

Van de Water, P.K., 2002. The effect of chemical processing on the δ13C value of plant tissue. Geochim. Cosmochim. Acta 66 (7), 1211–1219.

Van der Sleen, P., Soliz-Gamboa, C.C., Helle, G., Pons, T.L., Anten, N.P.R., Zuidema, P.A., 2014. Understanding causes of tree growth response to gap formation: Δ13C-values in tree rings reveal a predominant effect of light. Trees 28, 439–448.

Verheyden, A., Helle, G., Schleser, G.H., Dehairs, F., Beeckmann, H., Koedam, N., 2004. Annual cyclicity in high-resolution stable carbon and oxygen isotope ratios in the wood oft he mangrove tree Rhizophora mucronata. Plant Cell Environ. 27, 1525–1536.

Wieloch, T., Helle, G., Heinrich, I, Voigt, M., Schyma, P., 2011. A novel device for batch-wise isolation of α-cellulose from small-amount wholewood samples. Dendrochronologia 29 (2), 115–117.

Wilson, A.T., Grinsted, M.J., 1977. 12C/13C in cellulose and lignin as palaeothermometers. Nature 265, 133–135.

Worbes, M., 1999. Annual growth rings, rainfall-dependent growth and long-term growth patterns of tropical trees from the Caparo Forest Reserve in Venezuela. J. Ecol. 87 (3), 391–403.

Yildiz, Ö., 1995. Ist der Isotopengehalt des Holzes aus Jahrringen von Bäumen als Klimaindikator geeignet? Diplomarbeit, Fachhochschule Aachen, Abt, Jülich, Germany

Zetterberg, P., Eronen, M., Lindholm, M., 1996. Construction of a 7500-year tree-ring record for Scots pine (Pinus sylvestris L.) in northern Fennoscandia and its application to growth variation and palaeoclimatic studies. In: Spiecker, H., Mielikainen, K., Kohl, M., Skovsgaard, J.P. (Eds.), Growth Trends in European Forests. European Forest Institute Research Report 5. Springer Verlag, Berlin, pp. 7–18.

Zimmermann, B., 1998. δ13C in tibetischen Wacholdern – Klimaentwicklung der letzten 1600 Jahre. Berichte des Forschungszentrums Jülich 3580, report no.: FZJ-2014-04960, Jül-3580 130 p., http://hdl.handle.net/2128/7987.