Stable isotopes in tree rings

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Abstract

Stable isotopes in tree rings could provide palaeoclimate reconstructions with perfect annual resolution and statistically defined confidence limits. Recent advances make the approach viable for non-specialist laboratories. The relevant literature is, however, spread across several disciplines, with common problems approached in different ways. Here we provide the first overview of isotope dendroclimatology, explaining the underlying theory and describing the steps taken in building and interpreting isotope chronologies.

Stable carbon isotopes record the balance between stomatal conductance and photosynthetic rate, dominated at dry sites by relative humidity and soil water status and at moist sites by summer irradiance and temperature. Stable oxygen and hydrogen isotopic ratios record source water, which contains a temperature signal, and leaf transpiration, controlled dominantly by vapour pressure deficit. Variable exchange with xylem (source) water during wood synthesis determines the relative strength of the source water and leaf enrichment signals. Producing long Holocene chronologies will require a change in emphasis towards processing very large numbers of samples efficiently, whilst retaining analytical precision. A variety of sample preparation and data treatment protocols have been used, some of which have a deleterious effect on the palaeoclimate signal. These are reviewed and suggestions made for a more standardised approach.

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1. Introduction

Trees are wonderful things. They stand fixed in the landscape, often for centuries, taking carbon from the air, and oxygen and hydrogen from soil water, and combine these components into layers of wood laid down in annual succession. The width of the annual rings often varies sufficiently to allow the patterns in many different trees to be compared and cross-checked, so that with care it is possible to determine the exact year in which each ring was laid down. Occasionally the trees fall into bogs or shallow lakes, or are buried in river gravel, preserving the timber in good condition for, in some cases, many thousands of years. By carefully piecing together the pattern of ring widths in living trees, and then extending that record back in time using standing and fallen dead wood, building timbers, and sub-fossil logs (Fig. 1) it is possible to produce annually resolved chronologies that stretch back for thousands of years.

In northern Europe pine chronologies now extend more than 7000 years (Eronen et al., 2002; Grudd et al., 2002) and work on larch promises even longer records (Hantemirov and Shiyatov, 2002). In Ireland, Britain and mainland Europe there are oak chronologies extending more than 8000 years (Leuschner et al., 2002; Spurk et al., 2002), and attempts have been made to extend these further still using pines (Becker, 1993). These chronologies and others, including those based on exceedingly long-lived trees, such as the bristlecone pines of California, provide the basis for dendrochronological dating (Baillie, 1995) and were also used to calibrate the radiocarbon timescale (Suess, 1970; Pilcher et al., 1984; Becker, 1993; Stuiver and Reimer, 1993). Perhaps their greatest potential, however, is as archives of the carbon, hydrogen and oxygen extracted from the environment each year and fixed in the annual rings. Air and water sampled by a tree are subtly modified by the tree as it responds to the varying environment in which it lives, and these small changes, expressed as variations in isotopic ratios, provide an archive for reconstructing past environments.

There are many such natural archives of palaeoclimatic information, including ocean and lake sediments,

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Fig. 1. Examples of living, dead and sub-fossil trees used to construct long chronologies. (a) Sampling an ancient oak in Eastern Britain. The physical size, structure and tendency of many old oaks to become hollow make the retrieval of long cores for isotope analyses difficult. This tree is in excess of 350 years old (Photograph © J. Waterhouse). (b) Living Scots pine (*Pinus sylvestris* L.) amongst standing dead trees in NW Norway. At this site living trees reach ages in excess of 400 years and standing deadwood dating to the 11th century has been recovered (© D. McCarroll). (c, d) Standing dead wood (snag) and fallen log from the same site in NW Norway (© D. McCarroll). (e) The long Fennoscandian chronologies are based mainly on pine trunks recovered from cold, shallow lakes, where they may remain in good condition for millenia. In this example from northern Finland a disk is being cut from a pine trunk (© R. Jalkanen).

peat bogs and ice cores, but none have the two great advantages of tree rings. First, the exact dating of each ring provides perfect annual resolution. Second, each part of the chronology is represented by several overlapping trees, so it is possible to define the variability of a measurement as well as the average value, allowing confidence limits to be calculated. Trees are also widespread, so that it is possible to examine geographical variations in the climate of the past, which may be of more interest for predicting the consequences of future climate change than estimates of global or hemispherical conditions. Isotope ratios in tree rings have the added advantage that the physiological controls on their variation are reasonably well understood and relatively

simple in comparison to the myriad factors controlling annual growth increment. There is thus potential, in addition to the use of inferential statistics, for mechanistically modelling the influence of climate, even at sites where growth is not controlled by a single factor.

Improvements in sample preparation techniques, and particularly in the way that samples are combusted and analysed, have meant that generating isotope ratios from wood samples is becoming faster, easier and less expensive. Presently restricted to a few specialist laboratories, it is an approach that could be used much more widely and applied to large numbers of samples, giving access to very long chronologies with perfect annual resolution, yielding quantitative estimates of palaeoclimate parameters with statistically defined confidence limits.

The literature relating to stable isotopes in tree rings is, however, spread across a wide range of disciplines, and different groups are approaching common problems often in very different ways. There is presently no single source that outlines the basic principles of isotope dendroclimatology, reviews recent advances, or that describes the various steps that need to be taken in producing and interpreting an isotope chronology. This paper aims to gather that information from the various specialist fields and present it in a form accessible to a more general scientific audience.

2. Stable isotopes

The three main elements in wood (carbon, oxygen and hydrogen) all have more than one stable (non-radioactive) isotope. Carbon, for example, has two stable isotopes, ^{12}C and ^{13}C, each with six protons but with either six or seven neutrons. These isotopes have almost identical chemical properties but the difference in mass allows physical, chemical and biological processes to discriminate against one of them, thereby imparting an environmental signal. By convention, the ratio of ^{13}C to ^{12}C is expressed in delta (δ) notation with reference to a standard material for which the isotopic ratio is known (Eq. (1)). The carbon isotope ratio ($\delta^{13}C$) is expressed, in parts per thousand (‰), as

$$\delta^{13}C = (R_{sample}/R_{standard} - 1)1000, \qquad (1)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in a sample and standard, respectively. In the case of carbon the chosen standard was a fossil belemnite from the Pee Dee formation of South Carolina, which has since been exhausted and replaced by 'Vienna-PDB' or VPDB (Coplen, 1995). Using this nomenclature the carbon isotopic ratio of CO_2 in air is about -8‰. As the ratio of ^{13}C to ^{12}C declines, indicating that the material is depleted in ^{13}C, then $\delta^{13}C$ values also decline.

Oxygen has three natural stable isotopes, each with eight protons but between 8 and 10 neutrons, giving a mass of between 16 and 18. It is the ratio of ^{18}O to ^{16}O that has traditionally been used in the environmental sciences. The ratios of the stable isotopes of oxygen are also expressed using the δ notation relative to a standard. For carbonates the PDB or more recently VPDB standard may be used, but for water, ice and plant material the standard is standard mean ocean water (SMOW), now replaced by Vienna-SMOW or VSMOW. The difference in mass dictates that water containing the lighter isotopes of oxygen will evaporate more easily than that containing the heavier isotopes, so source water is isotopically heavier than the moisture that evaporates from it. The effect is temperature dependent, so that cold air masses collect moisture that is isotopically lighter than that which evaporates under warmer conditions. When moisture vapour condenses the opposite is true, with the heavier isotopes condensing most readily, and again the isotopic ratio depends on the temperature at which condensation takes place. Global variations in the $\delta^{18}O$ of precipitation are thus linked in a complex way to climate (Dansgaard, 1964).

Hydrogen has two stable isotopes, the heavier one being called deuterium (^2H or D). The ratio of ^2H to ^1H, or the D/H ratio, is also expressed relative to SMOW or VSMOW using the δ notation as δD (‰). Moisture vapour is depleted in deuterium relative to the source. The δD ratio of precipitation also varies globally and is linked in a complex way to climate. Variations in $\delta^{18}O$ and δD of precipitation are also strongly correlated but differ in magnitude along a global meteoric water line (Gat, 1980; IAEA, 1981).

3. Environmental physiology

Although the carbon of each annual tree ring has its origin in the CO_2 of air, and O and H come from soil water and thus precipitation, the isotopic ratios in wood are very different to those in either air or water, so trees do not passively collect and store these elements. The primary value of the isotopic records in tree rings is not simply as samples of ancient air or water, but as sensitive bioindicators of the way that the components of air and water have been changed by the trees in response to the environments in which they lived. This is a branch of environmental physiology and significant advances have been made in understanding precisely what controls the change in isotopic ratios.

3.1. Carbon isotope theory

Plants have evolved different photosynthetic pathways for carbon fixation, which results in large differences in the way that carbon isotopes behave

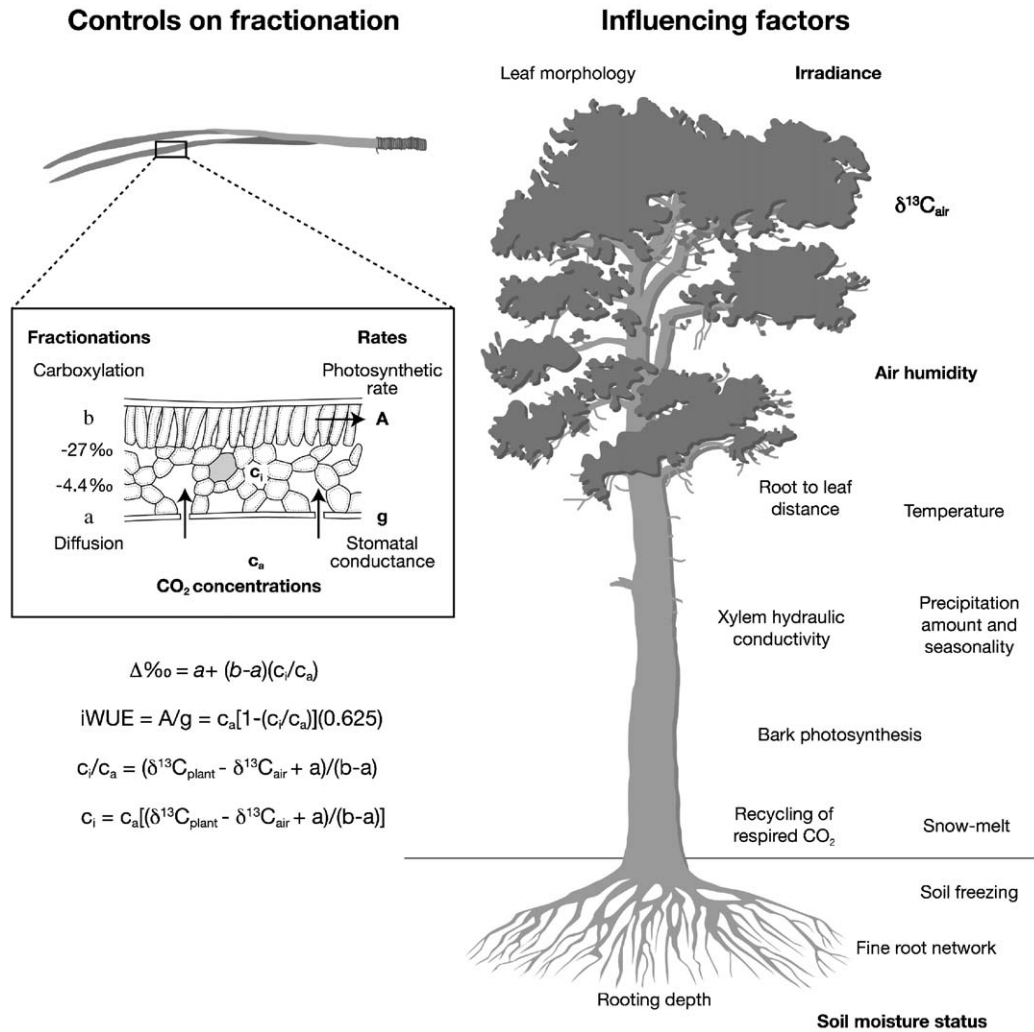**Controls on fractionation**

Influencing factors

Fig. 2. Diagram of a needle-leaf tree showing the main controls on the fractionation of carbon isotopes and the environmental factors that influence them. The equations are explained in the text.

(Ehleringer and Vogel, 1993). All of the trees used in dendroclimatology belong to the C3 group (meaning that the first photosynthetic product contains three carbon atoms), so the others (C4 and CAM) will not be considered here. The ratio of ^{13}C to ^{12}C in the carbon dioxide of air currently yields a $\delta^{13}C$ value of about $-8‰$ (VPDB). The leaves and wood of trees, however, yield much lower values ($-20‰$ to $-30‰$), demonstrating that trees are depleted in ^{13}C relative to air. This change in ratios from a source to a product is known as fractionation, and the degree of fractionation is controlled to some extent by the response of the tree to its environment.

Molecules of H_2O are smaller than those of O_2 or CO_2, so membranes that allow gas exchange are also permeable to water. Land plants have evolved water-proof coatings and internalised their gas exchange surfaces, so that air enters the leaf through pores called stomata, which can be constricted by guard cells to

reduce moisture loss. Carbon gain and moisture loss are thus intimately related. The carbon dioxide in the internal air can move into solution and becomes available to the photosynthetic enzymes that use sunlight to produce sugars. During this transition from external air to leaf sugars there are two main points at which carbon isotopic fractionation occurs.

When air diffuses through the stomata, the carbon dioxide molecules that include the lighter isotope of carbon are able to diffuse more easily than those including the heavier isotope, simply because as molecules bounce off each other the lighter ones bounce furthest. The net effect is that internal air is depleted in ^{13}C relative to ambient air, resulting in a 'fractionation due to diffusion' of $-4.4‰$ (Fig. 2). If the stomatal opening is extremely small ($\leqslant 0.1\,\mu m$), collisions with guard cells become important and fractionation is much higher, but this is only likely to occur in species with a high frequency of very small stomata such as citrus trees

(Farquhar and Lloyd, 1993). The second point of fractionation occurs when internal CO_2 is utilised by the photosynthetic enzyme. Biological processes tend to use ^{12}C in preference to ^{13}C, and this 'net fractionation due to carboxylation' is estimated to be about $-27‰$.

Discrimination against ^{13}C during carbon fixation by trees (and other C3 plants) can be expressed as

$$\Delta‰ = a + (b - a)(c_i/c_a), \qquad (2)$$

where a is the discrimination against $^{13}CO_2$ during diffusion through the stomata ($\approx -4.4‰$), b is the net discrimination due to carboxylation ($\approx -27‰$), and c_i and c_a are intercellular and ambient CO_2 concentrations (Farquhar et al., 1982).

The fractionations that occur due to diffusion and carboxylation are constant. However, fractionations are additive, the net effect depending on the isotopic ratio of the source gas; so if internal CO_2 becomes enriched in ^{13}C, more ^{13}C will be assimilated in leaf sugars. In trees, therefore, the $\delta^{13}C$ value of leaf sugars is controlled dominantly by the ratio $c_i : c_a$. If c_i is high relative to c_a, then stomatal conductance is much higher than the rate of photosynthesis and there will be a strong carboxylation discrimination against ^{13}C, yielding low $\delta^{13}C$ values. If the rate of stomatal conductance is low compared to the rate of photosynthesis, then the internal concentration of CO_2 will drop and there will be less carboxylation discrimination against ^{13}C, leading to higher $\delta^{13}C$ values. The dominant environmental controls on the stable carbon isotopic ratios in tree rings, therefore, should be those that control the rate of stomatal conductance and the rate of photosynthesis.

When leaf sugars are used to make the various components of trees there are further fractionations, so that cellulose and lignin, for example, always yield lower $\delta^{13}C$ values than leaf sugars. In some trees carbon may also be contributed by photosynthesis in the bark (Cernusak et al., 2001), but this does not apply to the trunks of mature trees with thick bark.

3.2. Oxygen isotope theory

The source of water for trees is soil moisture, so part of the signal in the water isotopes (H and O) of trees will come from the isotopic signature of precipitation. However, there are several potential fractionations before the water isotopes become fixed in wood components (Fig. 3). The first occurs within the soil, where evaporation can alter the original isotopic ratio. There are also seasonal variations in the $\delta^{18}O$ of precipitation, and in the depth from which roots access water (Dawson, 1993; Dawson and Pate, 1996), so the residence time of the soil water is important (Buhay and Edwards, 1995). When tree roots take-up water there is no fractionation (Wershaw et al., 1966), so the critical

site of fractionation is the leaf, where evaporation (transpiration) leads to a loss of the lighter isotopes and a consequent enrichment in ^{18}O, which can be as much as 20‰ (Saurer et al., 1998a, b).

The level of enrichment of leaf water above source water at the sites of evaporation ($\Delta^{18}O_e$) is given by

$$\Delta^{18}O_e = \varepsilon^* + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)e_a/e_i, \qquad (3)$$

where ε^* is the proportional depression of water vapour pressure by the heavier $H_2^{18}O$, ε_k is the fractionation as water diffuses through the stomata and leaf boundary layer, $\Delta^{18}O_v$ is the oxygen isotope composition of water vapour in the atmosphere (relative to source water), and e_a and e_i are the ambient and intercellular vapour pressures (Craig and Gordon, 1965; Dongmann et al., 1974; Barbour et al., 2001, 2002). ε^* varies slightly with temperature, but leaf enrichment depends mainly on the difference between the isotopic compositions of the source water and the ambient moisture vapour, and on the ratio of vapour pressure inside to that outside of the leaf. At constant temperature, and where source (soil) water and atmospheric vapour have the same isotopic signature, the degree of enrichment due to evaporation is linearly dependent on $1 - e_a/e_i$ (Barbour et al., 2001). This model explains the isotopic enrichment of water at the site of evaporation, however, backward diffusion of the enrichment is opposed by the convection of isotopically lighter source water to the sites of evaporation (a Péclet effect: Barbour and Farquhar, 2000; Barbour et al., 2001). The $\delta^{18}O$ of leaf water is, therefore, less depleted than that at the site of evaporation, so that Eq. (3) over-estimates the net effect of enrichment due to transpiration.

Sucrose formed in the leaf reflects the isotopic signature of leaf water, but with 27‰ enrichment. The mechanism responsible is exchange of carbonyl oxygen in organic molecules with water (Sternberg et al., 1986). Tree-ring cellulose forms from sugars transported down the trunk, where exchange is with xylem (source) water. If all of the oxygens were to exchange, tree-ring cellulose would record directly the isotopic ratio of source (xylem) water. If there is no exchange, the isotopic ratios reflect both the source water and the degree of enrichment due to evaporation in the leaf. Clearly, if the potential palaeoenvironmental signals are to be interpreted, it is critical to determine what proportion of the oxygen atoms exchange (Roden et al., 2000; Anderson et al., 2002).

To form cellulose, sucrose cleaves to form hexose phosphates, allowing 20% of oxygen to re-exchange with water. A proportion of the hexose phosphate, however, does not immediately form cellulose, but passes through a futile cycle to triose phosphates, which allows further exchange (Hill et al., 1995). The total proportion of oxygen atoms available for exchange is thus dependent on the number of cycles through triose
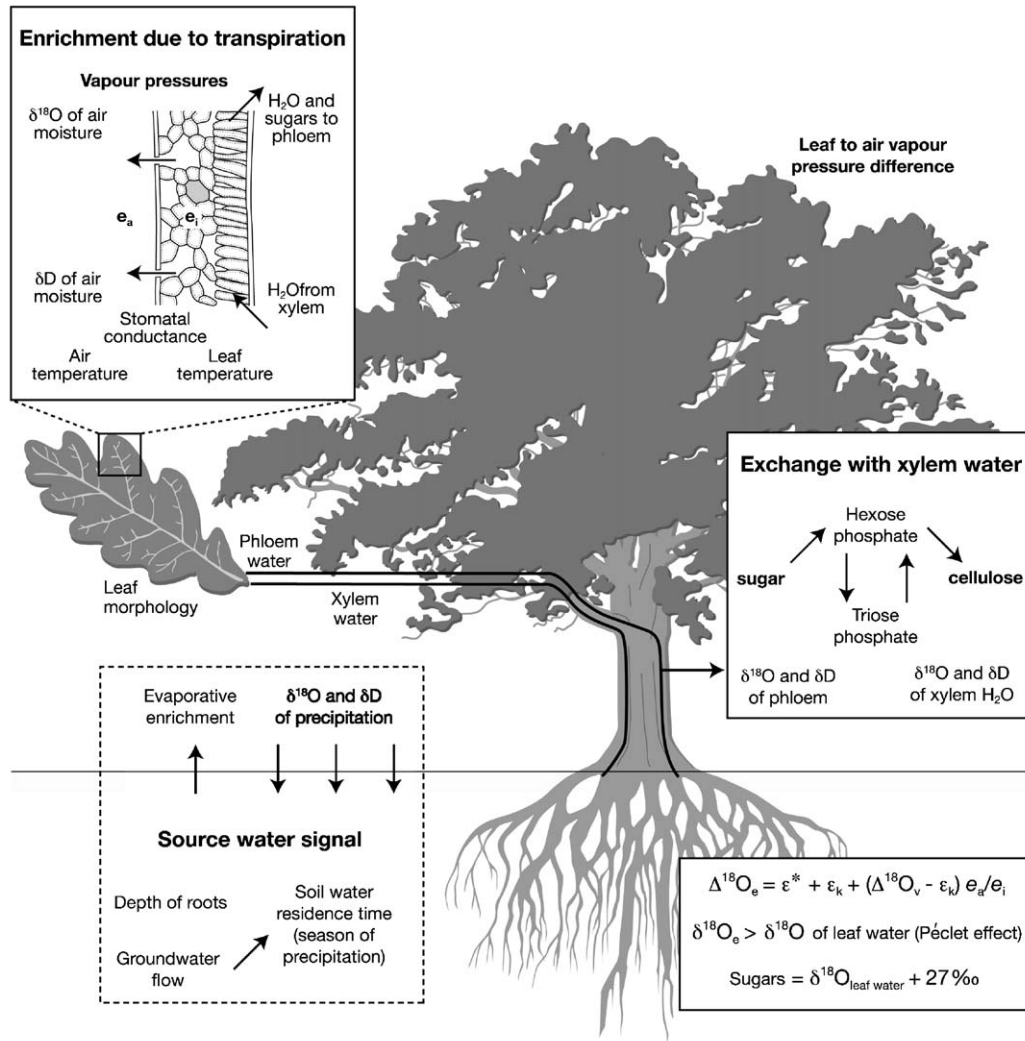
Fig. 3. Diagram of a broad-leaved tree showing the main controls on the fractionation of the water isotopes and the environmental factors that influence them. The equations are explained in the text.

phosphate. This is difficult to predict and likely to vary with the rates of cellulose synthesis and sucrose import, with slow fluxes leading to high exchange (Barbour and Farquhar, 2000). Isotopic fractionation during lignin synthesis is more complex, with more potential for exchange with xylem water (Barbour et al., 2001).

Tree-ring $\delta^{18}O$ is not a direct measure of the $\delta^{18}O$ of the source soil water, because evaporation raises the $\delta^{18}O$ of leaf water. The amount of evaporation depends on stomatal conductance and vapour pressure deficit, both of which are linked to relative humidity (RH), and the resulting fractionation is moderated by the $\delta^{18}O$ of moisture vapour outside the leaf (Roden et al., 2000). As tree rings form, the signal imparted in the leaf is dampened to a variable extent by exchange of oxygen with xylem water. The dominant environmental signals in tree-ring $\delta^{18}O$ are thus likely to be the $\delta^{18}O$ of precipitation and summer humidity, but the relative strength of these two signals will vary.

3.3. Hydrogen isotope theory

Hydrogen isotopes in tree rings remain difficult to measure and limited data are available. The behaviour of hydrogen has, however, been modelled (Roden et al., 2000), and its fate shares much in common with oxygen. The hydrogen in wood comes from soil water and hence precipitation. The δD of precipitation is dependent on condensation temperature and varies both spatially and seasonally. When the water reaches the leaf, evaporation leads to a preferential loss of the lighter isotopes, so the δD of leaf water is enriched relative to the source. Enrichment of δD at the site of evaporation is controlled by the same factors that control the enrichment of $\delta^{18}O$. The fractionation factors are different for hydrogen and oxygen, but the same general model applies (Eq. (3)). As for oxygen, the Péclet effect dampens the evaporative signal, so that δD of total leaf water is lower than that at the site of evaporation.

During photosynthesis there is a marked discrimination against D, so that resulting leaf sugars are isotopically lighter than leaf water. The magnitude of this fractionation is uncertain (−100‰ to −171‰), but when these sugars are used to produce tree rings there is an uncertain but similarly large fractionation in the opposite direction (+144‰ to +166‰; Yakir, 1992). As with oxygen isotopes, there is potential for exchange with xylem water, which will push δD of tree rings back towards that of the source water. Roden et al. (2000) estimate that the degree of dampening of the evaporative-enrichment signal for hydrogen isotopes, due to a combination of the Péclet effect and exchange with xylem water, is likely to be similar to that for oxygen.

On the basis of theory, therefore, the environmental factors influencing hydrogen isotopes in wood should be the same as those influencing oxygen. The dominant signals should be the δD of precipitation and growing season (summer) RH.

4. Measuring isotope ratios

In order for the stable isotope ratios of a tree-ring sample to be determined, it must first be converted to a gaseous form suitable for mass spectrometry. This first stage may be carried out "off-line", typically as single samples or small batches, or "on-line" as part of an integrated system involving the quantitative combustion or pyrolysis of the sample. The stable carbon, hydrogen and oxygen isotope ratios of the resulting sample gases are determined using a stable isotope ratio mass spectrometer (IRMS). An aliquot of sample gas is admitted to the mass spectrometer where it is ionised. The charged particles are accelerated by high voltage and pass down a flight tube where a magnetic field deflects them according to differences in mass towards 'Faraday cup' detectors. The ratios between the detectors are compared with the results from standards of known isotopic composition.

For hydrogen isotope analysis, hydrogen gas (H_2) is used and the mass spectrometer is tuned to detect masses, which represent the isotopic combinations HH, and HD. For carbon and oxygen isotope analysis, traditionally carbon dioxide (but more recently carbon monoxide) has been used as the sample gas and the ratios of the masses 44, 45 and 46 (28, 29 and 30) are measured. These masses represent the isotopic combinations of carbon and oxygen in carbon dioxide: [44], $^{12}C^{16}O^{16}O$; [45], $^{13}C^{16}O^{16}O$, $^{12}C^{17}O^{16}O$, $^{12}C^{16}O^{17}O$; [46], $^{12}C^{18}O^{16}O$, $^{12}C^{16}O^{18}O$, $^{13}C^{17}O^{16}O$, $^{13}C^{16}O^{17}O$, $^{12}C^{17}O^{17}O$. The contribution from radioactive ^{14}C can be neglected. Reference materials of known isotopic compositions relative to an international standard are used to calibrate the sample isotopic compositions.

These are then expressed relative to the relevant international standard (VPDB or VSMOW).

In traditional dual-inlet machines, each sample is run separately or as part of a small batch (ca 16) by manifold operation. Small samples can be analysed following cryogenic concentration. The pressure of the sample gas is "balanced" against that of the reference gas prior to each analysis, ensuring high precision and reliability. Unfortunately, manual dual-inlet analyses are relatively time consuming and although they provided the data for many of the initial studies in this field, their limitations and low sample throughput constrained what could realistically be achieved.

A more significant limitation, however, is the preparation required to convert organic samples into a form suitable for mass spectrometry (CO_2/CO for carbon/oxygen isotopes, H_2 for hydrogen isotopes). This requires a range of lengthy "off-line" preparation, combustion/pyrolysis and purification stages. Only those approaches routinely used for the isotopic analysis of tree rings are described here, though a wider range of preparative techniques and their applications are reviewed elsewhere (Sofer, 1980; Boutton et al., 1985; Wong and Klein, 1986).

4.1. Carbon isotopes (off-line)

The off-line preparation of carbon dioxide from tree-ring samples is based on combustion of the sample in a sealed Pyrex tube with an excess of copper oxide providing oxygen (Buchanan and Corcoran, 1959; Sofer, 1980; Boutton et al., 1985; Field, 1994). The tube is sealed under vacuum then heated to 450°C for 18 h, combusting cellulose to form H_2O (removed cryogenically) and CO_2. Each tube is analysed individually, although batches may be processed together. Standard samples are included to ensure continuity between batches. This method is both robust and reliable, regularly exhibiting precision ≈0.1‰ [(σ_{n-1}) $n = 12$ (0.5 mg α-cellulose)].

4.2. Oxygen isotopes (off-line)

Progress in the analysis of oxygen isotopes in tree rings has been limited by the time-consuming and potentially hazardous nature of the preparative techniques. To obtain reliable isotopic information it is essential that either all the oxygen is in a stable equilibrium with related reaction products or that the element under scrutiny is in a single chemical form and any chemical reactions or transfers have proceeded to completion. Recent reviews illustrate the range of techniques available (Wong and Klein, 1986; Mullane et al., 1988; Farquhar et al., 1997; Loader and Buhay, 1999). Off-line techniques used for the analysis of tree

rings may, however, be considered to fall into two main groups: mercury$_{(II)}$ chloride (mercuric chloride) pyrolysis or nickel tube pyrolysis.

The method developed by Rittenberg and Ponticorvo (1956) and modified more recently (Dunbar and Wilson, 1983; Field et al., 1994; Sauer and Sternberg, 1994) involves pyrolysis of dry cellulose (3–4 mg) in vacuo with mercury$_{(II)}$ chloride. The products, which include mercury, hydrochloric acid, carbon monoxide and carbon dioxide, are purified off-line in a vacuum system. The carbon monoxide is converted to carbon dioxide using an electrical discharge and combined with any carbon dioxide formed during pyrolysis prior to isotopic measurement. This process is very time consuming and involves regular exposure to highly toxic substances. Considerable potential exists for fractionation during the vacuum line manipulation of the pyrolysis products and conversion of CO to CO_2. In spite of these limitations the method yields reliable results and has been adopted by a number of laboratories. Typical precision of standard materials is $\approx 0.2‰$.

A less hazardous method is based upon the pyrolysis of the sample in sealed high purity nickel tubes or "bombs" (Thompson and Gray, 1977; Brenninkmeijer and Mook, 1981). At high temperatures the pyrolysis products inside the tubes comprise hydrogen and carbon monoxide. Permeability of the nickel to hydrogen at temperatures above 950°C permits the diffusion of the hydrogen gas from the tube preventing subsequent reaction with the oxygen bound in the CO. As the tube is cooled the CO disproportionates to CO_2 according to the Boudouard equilibrium to form predominantly CO_2, traces of CO, carbon and nickel carbonyl. The tube is then opened under vacuum, nickel carbonyl thermally degraded and remaining traces of CO converted to CO_2 using electrical disproportionation. The sample, now all in the form of CO_2 is cryogenically combined prior to isotopic analysis. This method has the advantage that the hydrogen gas can also be trapped and analysed (Gray et al., 1984; Motz et al., 1997), however, it is sensitive to contamination from oxidation of the high purity nickel tubes which have a limited lifetime and cannot be used on compounds containing nitrogen. This method also yields precision of $\approx 0.2‰$.

A number of other approaches have been devised and tested, including a pyrolysis train capable of sample preparation and purification on a flow of helium (Ferhi et al., 1983), RF induction heating (Aggett et al., 1965), reaction with guanidine hydrochloride (Wong et al., 1987), contributive oxidation (Taylor and Chen, 1970) and a pyrolysis method in which the sample was converted to CO prior to analysis (Bunton et al., 1956). A novel method developed by Mullane et al. (1988) converts cellulose into benzoic acid and has potential, through derivitisation, for measuring isotopic ratios of oxygen atoms occupying different positions in the glucose rings of cellulose, some of which are unambiguously linked to leaf water (Waterhouse, pers. comm.).

4.3. Hydrogen (off-line)

Up to 30% of the hydrogen atoms in cellulose can exchange with hydrogen from external sources (atmospheric moisture, etc.), so these hydroxyl hydrogens must first be either replaced with nitro (NO_2) groups by nitration or equilibrated with water of known isotopic composition. Hydrogen gas is prepared by in vacuo combustion of dry samples in sealed quartz tubes with excess copper oxide. The resulting carbon dioxide and water are separated cryogenically and, theoretically, this method produces samples for both carbon and hydrogen isotopes (Robertson et al., 1995). The water is reacted with pure Indiana zinc to form zinc oxide and hydrogen gas from which the ratio of deuterium to hydrogen is determined (Stump and Frazer, 1973; Northfelt et al., 1981; Coleman et al., 1982; Heaton and Chernery, 1990). The nickel tube pyrolysis method has also been used to purify samples of hydrogen for isotopic measurement by adding a silica sleeve around the "bomb" to collect the hydrogen (Gray et al., 1984; Motz et al., 1997). High levels of reproducibility can be obtained using this modified approach, but its application is limited to samples of pure (equilibrated) cellulose.

These and other off-line methods are still in use and yield reliable results, but the cost in time and resources required for reliable analyses of the supra-long chronologies has proven prohibitive to the development of isotope dendroclimatology.

5. On-line (continuous flow) methods

The development of continuous flow stable isotope mass spectrometry has reduced dramatically the cost and time required for isotopic analyses so that replicated analysis of millennial sequences is now possible. The modern continuous flow IRMS method was developed by interfacing an elemental analyser with a mass spectrometer for carbon isotope analysis (Matthews and Hayes, 1978; Preston and Owens, 1983, 1985). This enabled the "on-line" preparation, purification and transfer of a sample directly to the mass spectrometer on a continuous flow of carrier gas. The high degree of automation and rapid sample throughput enables large numbers of samples to be measured cost effectively. Complete systems including elemental analyser, interface and IRMS are now commercially available.

Continuous flow methods offer great potential for tree-ring isotope studies, yet a number of method-specific problems must also be considered and routinely addressed by laboratories to ensure reliable and

comparable results. Sustained analyses over many hours using continuous flow methods can lead to variations in carrier gas flow/combustion efficiency that can cause a drift in isotopic measurements. Frequent standard samples must be run in order that such effects can be identified and corrected for. Unlike dual-inlet methods, the reference gas pressure is also not matched to that of the sample gas, so large variations in sample size within a run can also adversely influence results, particularly with very small samples. Standards of different sizes should be run to determine the range for which reliable results can be obtained and the nature of any correction required. Precision of early continuous flow systems was low ($\approx 0.3‰$ ^{13}C), but they are now comparable to off-line methods.

5.1. Carbon isotopes on-line

Continuous flow measurement of carbon isotopes offers a robust and reliable method for quantitative conversion of cellulose, wood or lignin to CO_2 for isotopic measurement. Samples are placed into tin foil capsules and dropped into a tube heated to $1000°C$ through which a stream of helium is passed. Combustion of tin releases heat, ensuring full combustion of the sample. A pulse of oxygen is admitted on the carrier gas and the combustion products pass over a series of combustion and reduction reagents. Traces of water are removed, typically using a chemical trap, and the resulting gases, a mixture of CO_2 and N_2, resolved to separate peaks by a gas chromatography column. The sample gas is admitted into the mass spectrometer via a capillary tube and open split where the carbon dioxide (and/or nitrogen) can be analysed. The results are compared with standard reference gases and samples of known isotopic composition. Addition of an auto-sampler enables many samples (100+) to be prepared and run as a batch. High levels of precision (0.1‰) with rapid sample throughput (8 min per sample) can typically be obtained on samples of $100–200 \mu g$ C. Improving analytical capabilities of these systems also permit the analysis of smaller samples ($<50 \mu g$ carbon) with a slightly reduced precision.

5.2. Oxygen isotopes on-line

Systems capable of oxygen isotope analysis by continuous flow methods are still under development. Most are based upon the complete thermal degradation of samples to carbon monoxide (cf. Santrock and Hayes, 1987; Begley and Scrimgeour, 1996, 1997; Werner et al., 1996; Farquhar et al., 1997; Koziet, 1997; Saurer et al., 1998a, b; Loader and Buhay, 1999), although no definitive method has yet emerged. Systems vary slightly in combination of reagents used, reactor temperatures, or hardware, but a typical system for the analysis of cellulose can be described. Samples in silver foil capsules are dropped into a pyrolysis tube containing vitrified carbon at ca $1100°C$ and the products (ideally H_2 and CO although there may be traces of N_2, CO_2 and water) purified during passage through a water trap and a CO_2 trap on a stream of dry helium, and resolved by passage through a GC column containing a $5 Å$ molecular sieve (Werner et al., 1996; Begley and Scrimgeour, 1997; Farquhar et al., 1997; Saurer et al., 1998a, b). CO has the same mass as N_2 so the system must be leak-free and the two gas peaks separated fully. The samples are admitted to the mass spectrometer and results compared against reference gases and materials of known isotopic composition. A typical sample run lasts 10–20 min depending upon the time taken for elution of the gas peaks. Variants to this method include the use of higher temperatures and ceramic tubing (to prevent isotopic exchange of oxygen with silica at high temperatures) and the use of nickelised carbon as a pyrolysis catalyst (Farquhar et al., 1997; Koziet, 1997). The analytical precision (typically 0.3‰) now approaches that of the off-line method.

5.3. Hydrogen isotopes on-line

There are several practical obstacles to developing reliable continuous flow techniques for hydrogen isotopes, including differentiation of the sample from the He carrier gas, effective separation of the hydrogen ions in the mass spectrometer and amount effects at the detector (Prosser and Scrimgeour, 1995; Tobias et al., 1995; Tobias and Brenna, 1996; Brenna et al., 1998). These issues have been largely surmounted (Kelly et al., 1998) and it is now possible for on-line hydrogen isotopic measurements to be performed through pyrolysis of samples of organic matter (ca 2 mg) with precision approaching 2.5‰.

The high temperature pyrolysis approach is similar to that described for the on-line determination of oxygen isotopes. Dry samples in silver foil capsules are pyrolysed in a stream of helium at $1100°C$ (or above). Traces of water and CO_2 are removed and the remaining pyrolysis products (H_2, CO and N_2) resolved using gas chromatography prior to mass spectrometry. (The impact of these traces of water and CO_2 on the precision of both on-line oxygen and hydrogen isotope analysis has yet to be addressed.) Hydrogen remains the most difficult of the three isotopes to measure, either on- or off-line, and this has limited its application in isotope dendroclimatology.

Further to the methods described above, several other continuous flow IRMS applications may also offer future potential. These include analysis of specific compounds by 'combustion/pyrolysis gas chromatography isotope ratio mass spectrometry' (GC-IRMS) (Gleixner and Schmidt, 1998), use of position-specific

derivitisation of cellulose (Waterhouse, pers. comm.; Mullane et al., 1988), or hydrolysis/pyrolysis methods. The complex chemical preparation required for each sample currently prevents their use in developing replicated climate reconstructions from long time-series, but they may be useful for more detailed studies requiring fewer samples.

The development of continuous flow technology effectively provides access to research questions that require large numbers of samples, including palaeoclimate reconstructions using long tree-ring chronologies. The systems are now available 'off the shelf', and need not be restricted to specialist laboratories. However, with this greater accessibility comes a responsibility to continue to look beyond the mass spectrometer as a 'black box' and to preserve good practices essential to the generation of high-quality data. A thorough scientific approach to sample collection, preparation and analysis remains essential, even when very large numbers of samples are being analysed. There also remains a pressing need for the development and distribution of dedicated international cellulose standards for stable carbon, hydrogen *and* oxygen isotope analysis.

5.4. Sample preparation

For carbon and oxygen isotope analysis wholewood, lignin or cellulose may be analysed. Where cellulose is the preferred sample material it is isolated from wholewood through a series of chemical steps. For softwoods and samples containing a high proportion of extractives, resins are removed from the wood shavings using a Soxhlet apparatus and a mixture of toluene:ethanol (2:1). The lignin component is then removed through oxidation in an acidified sodium chlorite solution to yield holocellulose. The hemicellulose component is further removed through washing with sodium hydroxide to produce homogeneous α-cellulose which is washed thoroughly and dried prior to analysis. Variants upon these methods have been refined for particular species, although the principal procedures remain (Leavitt and Danzer, 1993; Loader et al., 1997; Brendel et al., 2000). Further methodological details and comparisons are reviewed in Green (1963), Sheu and Chiu (1995) and van de Water (2002).

Preparation of samples for hydrogen isotope analysis is complicated further because 30% of the hydrogen atoms comprising the cellulose molecule, the hydroxyl hydrogens, are capable of isotopic exchange with environmental moisture. Complete exchange of these hydrogen atoms, during cellulose processing or storage for example, can potentially mask or even eliminate the environmental signal preserved in the non-exchangeable carbon bound, carboxyl hydrogens. To eliminate the influence of exchangeable hydroxyl hydrogen, cellulose samples must either be nitrated or equilibrated prior to analysis. Nitration replaces the exchangeable hydrogen with nitro (NO_2) groups, whereas equilibration exchanges all the hydroxyl hydrogen with hydrogen from water of known isotopic composition (a mass balance being employed to determine the δD value of the carbon bound component). A number of methods have been proposed relating to the nitration of cellulose or wholewood samples (Bennett and Timmell, 1955; Green, 1963; Ramesh et al., 1986). Standard procedures rely upon a slow reaction with nitrating mixture, often at reduced temperatures, followed by a purification of the final product from non-nitrated residue by dissolution of the cellulose nitrate in acetone and subsequent reprecipitation of the pure product in cold distilled water. The equilibration method has also been developed for wood and cellulose samples using two procedures. Both rely upon the equilibration of the exchangeable hydrogen with water of known isotopic composition, but the nature and duration of this exchange is modified such that variants include a cold freeze-thawing method and a high temperature equilibration with steam (Schimmellman, 1991; Feng and Epstein, 1994).

Both the nitration and equilibration of cellulose are time consuming and potentially hazardous. This additional processing requirement, in conjunction with reduced H/D analytical availability, is reflected in the restricted length and number of hydrogen isotope time-series published. By its nature equilibrated cellulose is not isotopically stable under standard laboratory conditions. Cellulose nitrate is explosive and should be stored in a freezer to prevent decomposition. Technological and resource limitations have prevented extensive investigation into the signal preserved in the resin-extracted wholewood or lignin fractions compared to the cellulose-based methods above. It is hypothesised that the large isotopic shifts observed in hydrogen isotope biochemistry during cellulose formation will also be reflected in the processes of lignification. Consequently, it is not possible to make any firm recommendations regarding the potential or relative value for hydrogen isotope analysis of equilibrated wholewood over cellulose for palaeoclimate reconstruction. New, more rapid methods of sample preparation through equilibration or derivitisation are currently under development that may provide an insight into or solution to these problems in the near future (Huber and Leuenberger, 2003; Leuenberger, pers. comm.; Waterhouse, pers. comm.).

6. Sampling strategies

On the basis of isotope theory, $\delta^{13}C$, $\delta^{18}O$ and δD in tree rings should vary in response to the climatic conditions of the year in which the ring was formed,

suggesting a potential source of palaeoclimate information. However, some care is needed in the design of sampling schemes, and in the treatment of data if that potential is to be fully realised. A range of sampling strategies and data treatment protocols have been applied, making it difficult to compare results directly. The aim here is to describe the choices that must be made in collecting and analysing tree-ring isotope data and to clarify the implications of those choices for the type of palaeoclimate information that is likely to be produced.

6.1. Tree species

Stable isotopes have been measured in many tree species (Table 1) and between-species differences in isotope ratios and environmental signals are to be expected. The water transport system in conifers, for example, is less efficient than that in ring-porous broad-leaved species such as oak, and hydraulic conductivity affects stomatal conductance, which influences all three isotopes. Conifers, therefore, tend to yield higher $\delta^{13}C$ ratios than angiosperms (Stuiver and Braziunas, 1987; Leavitt and Newberry, 1992; Leavitt, 2002). The differences in leaf morphology are also important, and Barbour et al. (2002) have suggested that this may lead to a difference in the environmental controls on fractionation of oxygen. Needle temperatures remain close to, or parallel, that of the air, so the dominant control on evaporative enrichment is vapour pressure deficit. Flat leaves are less strongly coupled to the atmosphere, because of the greater potential for evaporative cooling, so stomatal conductance becomes more important. Between-species differences in rooting depth will influence moisture availability, and hence stomatal conductance (Warren et al., 2001), and also the residence time of the soil water that is accessed, and so the isotopic composition of source water. Shallow-rooting trees may retain a better record of annual variations in the $\delta^{18}O$ of precipitation, whereas the more consistent source water signal in deep-rooted, ground-water-tapping trees might be an advantage in isolating the evaporative-enrichment signal. Position in the canopy will determine radiation received, strongly influencing carbon isotope ratios.

Although some studies have compared isotope ratios of different species (e.g. Ramesh et al., 1985; Hemming et al., 1998), some care is required in interpreting such differences where sample sizes are small. The few papers that have reported results from large samples of individual trees suggest that differences within a species, even growing at the same site, can be as large as those between species (Leavitt and Long, 1984; Leavitt and Lara, 1994; McCarroll and Pawellek, 1998; Brendel et al., 2002). Similarity in response to climate, however, is more important than absolute differences in isotopic

ratios. Warren et al. (2001), for example, were able to combine $\delta^{13}C$ from many different conifers for comparison with climatic and altitudinal gradients, and Gagen et al. (2004, in press) suggest that $\delta^{13}C$ signals obtained from two pine species are so similar that they can be regarded as equivalent, despite large differences in ring widths and densities. Tang et al. (2000) found that between-species variation in δD was less than within-site variation of the same species.

From a Quaternary perspective, the most important tree species are those that form the long chronologies, which in Europe is mainly oak and pine. There is some logic, therefore, in focusing effort on these groups, and data are now available for several species of oak and many of pine (Table 1).

6.2. Choice of site

Stomatal conductance is controlled by soil moisture status as well as RH, so trees from dry sites will produce different ratios and be more sensitive to variations in moisture regime than trees from moist sites. The difference may be largest for carbon isotopes, where stomatal conductance is often the strongest control, but will also influence oxygen and deuterium through the level of leaf enrichment.

Since moist and dry sites will yield different results, the choice of site needs to be tailored to the research question. In sampling trees for ring-width studies, the traditional advice is to seek 'sensitive sites', where the trees are under stress and therefore most likely to respond to variations in climate. If the aim is to reconstruct moisture regime then dry sites are desirable (e.g. Saurer et al., 1995, 1997), though where precipitation is seasonal the isotopic signature of source water may relate to winter whilst degree of leaf enrichment relates to summer (Robertson et al., 2001). Where, however, the aim is to use modern trees to understand (calibrate) the signal retained in long chronologies, the aim should be to sample trees that grow under similar conditions to those that comprise the sub-fossil chronology. On the basis of isotope theory, we can predict that deliberately sampling dry sites, as is common practice, could give very misleading results.

6.3. Sampling resolution

Samples may be acquired by felling trees and removing a disk, or using increment borers. Most studies use samples taken from near breast height. The weight of sample required depends on the wood fraction to be analysed and the type of mass spectrometer used. Most early studies used blocks of several rings (Table 1), but as sample preparation techniques have become more standardised and mass spectrometers have improved, it has become feasible to deal efficiently with small

Table 1
A summary of papers that have exploited tree-ring isotope time-series for palaeoenvironmental research

Reference	Species	Site	Age-range	Wood component	Isotopes	Method	Data treatment	Environmental or other signal
Anderson et al. (1998)	4 *A. alba*	C Switzerland	1913–1995	Pooled whole ring α-cell.	$\delta^{13}C$, $\delta^{18}O$	On-line	None and (first differences)	Temp. 0.27‰/°C (0.23‰/°C), prec. and RH
Anderson et al. (2002)	4 Fir *A. alba*	C Switzerland	1913–1995	Pooled whole ring α-cell.	$\delta^{18}O$	On-line	None	Modelled $\delta^{18}O$ of prec. (May–September) correct for T, RH and ring width index
Becker et al. (1991)	*Quercus* sp. and *P. sylv*	S central Europe	Lateglacial–Holocene	10-year blocks. Cell. nit.	$\delta^{13}C$, δD	Off-line	None	Qualitative to Lateglacial–Holocene transition
Bert et al. (1997)	10 *A. alba*	France	1860–1980	5-year blocks. Holocellulose	$\delta^{13}C$	Off-line	Discrimination calculated	Possible age related trend, changing iWUE response to CO_2
Buhay and Edwards (1995)	Elm, pine maple	Ontario Canada	1610–1990	10-year blocks. Cellulose	$\delta^{18}O$, δD	Off-line, NiTP, cell. nit.	None	Modelled $\delta^{18}O$ of prec. and air RH, qualitative reconstruction
Burk and Stuiver (1981)	Various	N America	Spatial study	3 years + blocks. Cellulose	$\delta^{18}O$	Cellulose	None	RH and temp.
Craig (1954)	*Sequioa gigantea*	N America	1027 BC–AD 1649	Wholewood	$\delta^{13}C$	Off-line	None	Link to ^{13}C in wood and the atmosphere
Dubois (1984)	*Pinus sylvestris*	United Kingdom	Recent and ancient	Bulk cellulose nitrate	δD	Off-line	None	Link to deuterium content of prec. and RH enrichment. Correlation with altitude along a humidity gradient
Dupouey et al. (1993)	*F. sylv.*	France	1950–1990	Cellulose annual	$\delta^{13}C$	Off-line with CuO	Ci calculated	Extractable soil moisture (July) and ^{13}C ($t-1$). Response to CO_2, correlation with basal area increment
Duquesnay et al. (1998)	*F. sylv.*	NE France	1850–1990	Pooled 10-year cellulose	$\delta^{13}C$	Off-line with CuO	Δ, Ci and WUE	Age effects and long-term trends, no climate correlations
Edwards et al. (2000)	19 Fir *A. alba*	S Germany	1004–1980	LW cellulose	$\delta^{13}C$, δD	Off-line nitrate	De-trended and shifted	RH and temp., same series as Lipp et al. (1991)
Epstein and Krishnamurthy (1990)	1 *P. aristata* (+ 22 species)	California (and Global)	990–1990	3–5-year blocks. Cell. nit.	$\delta^{13}C$, δD	Off-line	25-year moving average	Qualitative link to temperature
Epstein and Yapp (1976)	Various (incl. *P. aristata*)	Scotland and N America	1841–1970 970–1974	Wholewood 10-year blocks	δD	Off-line nitrated	40-year running mean	Winter temperature. Qualitative link to other climate proxies/archives
Farmer and Baxter (1974)	*Q. robur Larix decidua*	United Kingdom	1892–1972	Wholewood	$\delta^{13}C$	Off-line	10-year running mean	Identification of changing atmospheric ^{13}C
February and Stock (1999)	6 *Widdringtonia cedarb*	S Africa	1900–1976	Whole ring cellulose	$\delta^{13}C$	Off-line with CuO	Corrected not de-trended	Air $\delta^{13}C$, not prec. Other climate data not available
Feng et al. (1999)	2 *Picea*	NE China	1967–1996 10,040 BP	5-year blocks. Cell. nit.	δD	Off-line	None	Qualitative comparison of monsoon influence; recent vs. ancient period
Feng and Epstein (1995a)	Pine, juniper, oak	N America	1840–1990	5-year blocks. Cell. nit.	$\delta^{13}C$	Off-line combustion	Polynomial, 15-year running average	High frequency = precipitation. Long term = atmospheric ^{13}C and pCO_2
Feng and Epstein (1995b)	7 various	N America	1840–1990	5-year blocks. Cell. nit.	δD	Off-line combustion	25-year running average	+ 5.3‰/°C to + 17‰/°C
Freyer (1979a)	26 various	Northern Hemisphere	1850–1975	2–5-year blocks. Cellulose	$\delta^{13}C$	Off-line	None	Identification of trends in atmospheric ^{13}C during the last 150 years
Freyer (1979b)	10 various	Germany	1890–1975	2-year blocks	$\delta^{13}C$	Off-line	None	Influence of pollution on $\delta^{13}C$
Freyer and Belacy (1983)	12 *Q. robur* and *Pin. sylv*	Germany and Sweden	1480–1979	Annual and 10-year blocks. Cellulose	$\delta^{13}C$	Off-line	None (calculation of 1st differences)	Northern Hemisphere "industrial effect" and temp., prec. 0.13‰/°C mean monthly temperature of April and June
Gray and Se (1984)	3 *Pic. glacua* (+ 5 var.)	Canada	1883–1975	5-year blocks. Cell. nit.	δD	Off-line (Ni vessel)	None	Temperature and source water; precipitation-derived groundwater
Gray and Thompson (1976)	1 *Picea glauca*	Canada	1880–1969	5-year blocks. Cellulose	$\delta^{18}O$	Off-line (NiTP)	None	1.3 ± 0.1‰/°C

Reference	Species	Location	Period	Sample	Isotope	Method	Treatment	Results
Gray and Thompson (1977)	*Picea glauca*	Canada	1882–1969	5-year blocks. Wholewood, lignin and cell	$\delta^{18}O$	Off-line (NiTP)	None	Signal strength with temperature (September–August): cellulose > wholewood > lignin
Hemming et al. (1998)	*F. sylv., Pin. sylv., Q. rob*	United Kingdom	1900–1994	Various	$\delta^{13}C$, $\delta^{18}O$, δD	On-line (C), Off-line $HgCl_2$(O), CuO(H)	Corrected and first differences	RH > temp. > prec. > sunshine
Jedrysek et al. (1998)	2 *Quercus* + fragments	Poland	1850–1970 10th–20th centuries	1- and 5-year LW cell. nit.	$\delta^{13}C$, δD	Off-line	Running average (15 years)	^{13}C May–July prec. (−1‰/m–30 mm). No correlation with δD and rainfall/temperature
Kitagawa and Matsumoto (1995)	*Cryptomeria japonica* (12)	S Japan	1862–1991 1846 years	5- and 10-year blocks, α-cell.	$\delta^{13}C$	Off-line	None	Elevation trend interpreted as temp.– 0.29‰/°C long-term record contains Medieval Warm Period and LIA
Krishnamurthy (1996)	1 *Juniperus phoenica*	Sinai Peninsula	1550–1950	5-year wood blocks	$\delta^{13}C$	Unspecified	Ratio internal to ambient CO_2	Air $\delta^{13}C$ and climate, possible moisture
Krishnamurthy and Epstein (1985)	1 *Juniperus procera*	Kenya	1834–1979	5-year blocks. Cell. nit.	δD	Off-line combustion	None	Comparison with lake levels, and inferred water stress
Lawrence and White (1984)	2 *Pinus strobus*	N America	1960–1980	Annual (C-bound H)	δD	Off-line combustion	None	May–August precipitation amount and δD source
Leavitt (1993)	56 *Pinus edulis*	N America	1780–1990	5-year blocks	$\delta^{13}C$	Off-line	None	Regional treeing ^{13}C and moisture stress signals
Leavitt and Lara (1994)	5 *Fitzroya cupressoides*	Chile	1700–1900	5-year groups holocellulose	$\delta^{13}C$	Off-line	Corrected and c_i/c_a	Evidence for "anthropogenic effect" in Southern Hemisphere
Leavitt and Long (1985)	10 *Juniperus* sp.	N America	1930–1979	5-year blocks. Cellulose (full circumference)	$\delta^{13}C$	Off-line (microcombustion system)	None	Reconstruction of atmospheric ^{13}C. Mean December temp. (−0.27‰/°C) and prec. (−0.04‰/mm)
Libby and Pandolfi (1974)	*Quercus petraea*	Germany	1712–1954 1530–1800	3–4-year blocks. Wholewood	$\delta^{13}C$, $\delta^{18}O$, δD	Off-line	^{13}C corrected for Suess effect. 9-year running average	$\delta^{13}C$ 2.73‰/°C, δD 89.5‰/°C $\delta^{18}O$ 5.29‰/°C (winter temp.)
Libby et al. (1976)	*Q. pet., A. alba, Cryyptomeria japonica*	Germany, Japan	1350–1950 1660–1950 137–1970	Wood blocks, about 5 years	$\delta^{18}O$, δD	Off-line	Smoothed by eye	Temperature
Lipp and Trimborn (1991)	*Picea abies, A. alba*	Southern Germany	1004–1980	LW cellulose	$\delta^{13}C$, δD	Off-line nitrate	Unclear	$\delta^{13}C$ 0.48‰/°C δD 2.2‰/°C
Lipp et al. (1991)	*A. alba*	Germany	1004–1980	LW cell. nit.	$\delta^{13}C$, δD	Off-line nitrate	De-trended and shifted	$\delta^{13}C$ August temperature 0.33‰/°C δD no clear signal
Liu et al. (1996)	4 *P. tabulaeformis*	N China	1885–1990	Annual pooled multiple radii	$\delta^{13}C$	Off-line (CuO)	Discrimination calculated	Correlation with June temperature and May–June precipitation
Loader and Switsur (1996)	3 *Pinus sylvestris*	United Kingdom	1760–1991	1–10-year cellulose	$\delta^{13}C$	Off-line (CuO)	None and (first differences)	Correlation with summer (June–August) temperature
McCarroll and Pawellek (2001)	36 Scots pine *P. sylvestris*	N Finland, 4 sites	1961–1995	LW cellulose	$\delta^{13}C$	Off-line with CuO	Corrected and de-trended	Summer sun or precipitation. Site-dependent
McCormack et al. (1994)	360 *Q. robur* and *Q. petraea*	United Kingdom	4890 BC–AD 1980	10–20-year holocellulose	$\delta^{13}C$	Through-flow, pre-charred	None	Identified isotopic difference between land- and bog-grown oaks
Okada et al. (1995)	3 *Chamae-cyparis*	Japan	1680–1989	4 radii, 5-year blocks. Cellulose	$\delta^{13}C$	Off-line combustion	None	No direct external forcing identified
Pearman et al. (1976)	*Athrotaxis selaginodies*	Australia	1895–1970	Wholewood 5-year blocks	$\delta^{13}C$	Off-line combustion	Running mean	February max. temp. (deviation from *average*). 0.24–0.48‰/°C
Pendall (2000)	*P. edulis*	SW USA	1989–1996	E and L wood α-cell.	δD	Off-line nitrate	None	RH dominates. LW more sensitive than EW
Ramesh et al. (1985)	*Abies pindrow*	India	1903–1932	Cell. and cell. nit.	$\delta^{13}C$, δD, $\delta^{18}O$	Off-line	None	Identified common forcing between radii
Ramesh et al. (1986)	*Abies pindrow*	India	1903–1932	Cell. and cell. nit.	$\delta^{13}C$, δD, $\delta^{18}O$	Off-line	None	$\delta^{13}C$ RH and cloud, δD prec. and temp.; 6.6‰/°C, $\delta^{18}O$; RH 0.19‰/%
Robertson et al. (1997a, b)	10 oak *Q. robur*	SW Finland	1895–1995	LW α-cell.	$\delta^{13}C$	On-line	Standardised by filtering	Prec. > RH > temp.
Robertson et al. (2001)	4 oak *Q. robur*	E England	1895–1994	LW α-cell.	$\delta^{18}O$	Off-line with $HgCl_2$	No de-trending	$\delta^{18}O$ of winter prec. and summer RH

Table 1 (*continued*)

Reference	Species	Site	Age-range	Wood component	Isotopes	Method	Data treatment	Environmental or their signal
Saurer and Siegenthaler (1989)	4 *F. sylv.*	C Switzerland	1935–1986	3-year block. Cellulose	$\delta^{13}C$	Off-line with CuO	None	$\delta^{13}C$ temp. and prec.
Saurer et al. (1995)	12 *F. sylv.*	C Switzerland	1934–1989	3-year block. Cellulose	$\delta^{13}C$	Off-line with CuO	None	$\delta^{13}C$ soil moisture status, total precipitation.
Saurer et al. (1998a, b)	*F. sylv.*	C Switzerland	1935–1990	3-year block. Cellulose	$\delta^{13}C$, $\delta^{18}O$	On and off-line Ni pyrolysis	Standardised, c, $c_{a-}c_i$, c_i/c_a	$\delta^{13}C$ temp. and prec. $\delta^{18}O$ of source
Saurer et al. (2002)	*Larix, Picea, Pinus*	Eurasia	1861–1890 1961–1990	30-year blocks. Wholewood	$\delta^{18}O$	On-line pyrolysis	None	Spatial study, comparison with precipitation $\delta^{18}O$
Schiegl (1974)	Picea	Germany	1785–1970	5-year blocks. Wholewood	δD	Off-line	None	Correlation with summer temperature.
Schleser et al. (1999b)	5 *Picea abies*	Germany	1957–1992	EW and LW, Cell. and wood	$\delta^{13}C$	On-line	None	July temp. (0.12‰/°C), mean annual temp. (0.10‰/°C)
Sheu et al. (1996)	*Abies kawakamii*	Taiwan	1873–1992	Annual (cellulose)	$\delta^{13}C$	Off-line with CuO	None	May–October temp. ($\delta^{13}C$–0.46‰/°C)
Sonninen and Jungner (1995)	1 *Pinus sylvestris*	Finland	1841–1990	Annual (cellulose)	$\delta^{13}C$	Off-line	None	July temp. 0.1‰°C
Stuiver and Braziunas (1987)	19 conifers	N America	1100–1850	10-year cellulose	$\delta^{13}C$	No details	Standardised	Latitudinal trend. RH > temp. (0.32‰/°C)
Switsur et al. (1994)	*Quercus robur*	United Kingdom	1890–1990	Annual, EW and LW cellulose	$\delta^{13}C$, $\delta^{18}O$, δD	Off-line	None	$\delta^{13}C$ July *T* and RH $\delta^{18}O$ July *T*, July/August RH
Switsur et al. (1996)	1 *Quercus robur*	E England, UK	1869–1993	LW α-cell.	$\delta^{13}C$, $\delta^{18}O$, δD	Online, CuO, HgCl₂, cell. nit.	None	Temp. (D, $^{13}C^{18}O$), RH (^{13}C, ^{18}O), prec. (^{18}O)
Tang et al. (1999)	*Pinus longaeva*	California, USA	1795–1993	~Every 5th ring, cellulose	$\delta^{13}C$	Off-line	De-trended, calculated WUE	WUE increases with CO_2
Tang et al. (2000)	*Pseudotsuga menziesii*	NW USA	1934–1996	Annual cellulose nitrate	δD	Off-line CuO and Zn	None	Source-water signal dominates, temperature signal is weak
Tans and Mook (1980)	3 oak, 1 beech	Netherlands	1855–1977	Annual wood, cellulose.	$\delta^{13}C$	Off-line	Corrected for $\delta^{13}C$ trend	Mean summer temp. 0.39 (R) mean annual temp. 0.27 (R)
Treydte et al. (2001)	Spruce *Picea abies*	Swiss Alps	1946–1995	Pooled LW α-cell.	$\delta^{13}C$	On-line	Corrected for $\delta^{13}C$ and CO_2	Late summer temp., prec. and RH
Waterhouse et al. (2000)	5 *Pinus sylvestris*	N Russia	1898–1990	Annual LW α-cell.	$\delta^{13}C$	On-line	3-year running mean	Correlation with flow of river Ob (via moisture stress)
Yapp and Epstein (1982)	Various (25)	N America	Spatial 1961–1975	Cellulose nitrate	δD	Off-line	Corrected for outliers	Correlation with mean annual temp. 5.80‰/°C
Zimmermann et al. (1997)	*Juniperus cf. tibetica*	Tibetan Plateau	1200–1994	5-year blocks and averages cell.	$\delta^{13}C$	On-line	Corrected for $\delta^{13}C$ (polynomial)	Inferred soil moisture status

Abbreviations: Q = *Quercus* (oak); *Q. pet* = *Qiercis petraea* (oak); *A. alba* = *Abies alba* (fir); *F. sylv.* = *Fagus sylvatica* (Beech); LW = latewood; EW = earlywood; α-cell. = α-cellulose; cell. nit. = cellulose nitrate; NiTP = nickel tube pyrolysis; Δ = discrimination; c_i = internal CO_2 concentration; c_a ambient CO_2 concentration; WUE = water use efficiency; *T* or temp. = temperature; prec. = precipitation; RH = relative humidity.
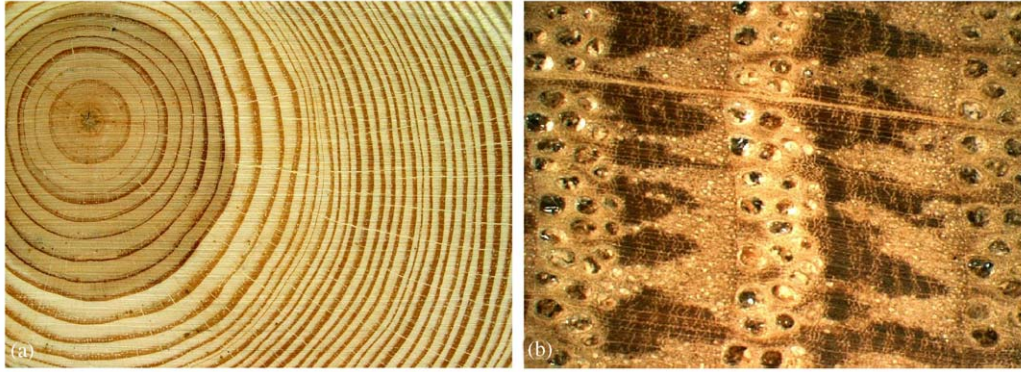
Fig. 4. Tree rings usually include both 'earlywood', comprising large, thin-walled cells formed in the spring and 'latewood' made from smaller, denser cells formed during the summer. Since earlywood may be produced using stored photosynthates, to achieve true annual resolution in isotopic analysis it is necessary to ensure that only the latewood is used. (a) Section of a pine tree showing the difference in colour (and density) of the earlywood and latewood of each ring (© R. Jalkanen). (b) Thin section of oak. Large vessels of the earlywood are clearly visible, separated and sometimes offset radially by medullary rays (© G. Helle).

samples, and many studies now attempt to obtain annual resolution.

Tree rings (Fig. 4) usually comprise large, thin-walled cells of earlywood, formed in spring and manufactured, at least partly, using stored photosynthates and smaller thicker-walled cells of latewood formed during summer. To obtain true annual resolution in the isotopic record, it is necessary to take only the latewood (Switsur et al., 1995). Power tools are avoided because they may cause charring and fractionation, so the work is usually done by hand using a scalpel or small chisel. This currently limits the minimum size of tree rings that can be utilised. Isotopic signals vary around the circumference of a tree (Ramesh et al., 1985), so Leavitt and Long (1984) recommend pooling samples from four radii. However, the variability between trees is much greater than the variability within rings, so better precision of the sample mean is achieved by sampling more trees (McCarroll and Pawellek, 1998).

6.4. Individual trees or pooled samples?

Analytical and cost constraints forced early studies to pool samples from several trees prior to analysis, and many studies still do this. Leavitt and Long (1984) suggested pooling four cores from four trees will yield an accurate absolute $\delta^{13}C$ value, though this has been disputed (McCarroll and Pawellek, 1998). Treydte et al. (2001) have demonstrated that the pooling of multiple cores can yield results similar to those obtained from preparation and combination of individual sequences. Their findings support the use of the pooling approach where study aims require mean time-series or where resources are limited. However, pooling samples prior to isotopic analysis severely constrains the utility of tree-ring isotope series. Defining variability, and thus precision, by placing confidence limits around mean

values and resulting reconstructions is a great advantage of tree rings over other natural archives, most of which yield a single number for each time increment. Pooling samples also prevents removal of any non-climatic (e.g. age) trends in the individual trees. Given the technology available, the usual reason for pooling samples is now cost of analyses. One solution might be to sacrifice temporal resolution instead. The fact that it is possible to obtain annual resolution using tree rings does not mean that is always the best sampling strategy, given financial or other constraints. With long time-series, the lower-frequency variations, that may represent variations in climate, are usually of more interest than inter-annual variability.

If blocks of several rings are taken from each tree, it is still possible to identify trends in individual trees and to place statistically defined confidence limits around mean isotope values, and ultimately around palaeoclimate estimates. For carbon isotopes inclusion of the early-wood is acceptable, though it would be logical to begin each block with latewood (current years sugars) and end with earlywood (stored photosynthates). For stable oxygen and hydrogen isotopes this strategy may be more problematic because of potential for depletion of xylem water during winter. Exchange with highly enriched xylem water during earlywood cellulose and lignin synthesis could have a very distorting effect (Field, 1994). More research is required into the isotopic characteristics of earlywood before this strategy can be recommended.

A simple alternative would be to sample latewood of individual trees, but not from every ring. Sampling every fourth ring, for example, would cost the same as pooling wood from four trees, producing high (not annual) resolution chronologies without any of the dampening effects of pooling wood or mixing adjacent years. Such data could be compared directly with annually resolved

chronologies. This is an unusual strategy (Tang et al., 1999), but we recommend it as preferable to the common practice of pooling samples.

6.5. Wood component

Early work on stable isotope ratios in tree rings used wholewood (Craig, 1954; Farmer and Baxter, 1974; Libby et al., 1976), but since Wilson and Grinsted (1977) demonstrated that different components of wood differ isotopically, most studies have concentrated on analysis of cellulose, as the dominant and most easily isolated component of wood. Wood is a complex material comprising a range of chemical components, including cellulose, lignin, hemicelluloses, resins, tannins, etc. Since the biochemical processes used in the formation of each component from the initial photosynthate are known to differ, so too do the stable isotope ratios of the resulting components (Barbour et al., 2002; Loader et al., 2004, in press). The reasons for this shift away from wholewood towards α-cellulose addressed several important methodological issues. Firstly, the cellulose of each annual increment could be unambiguously linked to a specific growth period since it is cellulose that forms the cellular framework of the tree ring. Secondly, the isolation of a single chemical component, in this case cellulose, reduced problems associated with variability in the lignin:cellulose ratio that could occur inter-annually, between individuals or (diagenetically) within a sequence through time. A third reason for the extraction of α-cellulose was the greater level of homogeneity that is attained during the purification process. Large isotopic variability has been demonstrated to occur within individual tree rings (Loader et al., 1995; Swistur et al., 1995; Schleser et al., 1999a) consequently homogeneity of sample material remains an important consideration, particularly when pooling material from many trees or blocks of annual rings.

The $\delta^{13}C$ values obtained from lignin and cellulose are offset by about 3‰, with no apparent temporal offset in the climate signal (Loader et al., 2004, in press). In theory, the isotopic signals of the water isotopes should be similar for cellulose and lignin, though Barbour et al. (2001) note that there may be more potential exchange with xylem water during lignin synthesis, which would dilute that part of the signal imparted by evaporative enrichment in the leaf. It would seem therefore, that either of the main components of wood can be used to provide a palaeoclimate signal. Given on-line combustion techniques, however, the extraction of cellulose has become a limiting step in some laboratories, and extraction of lignin is even more laborious, so several authors have suggested considering again the use of wholewood (e.g. Leuenberger et al., 1998; Schleser et al., 1999a; Barbour et al., 2001).

Isolating a single chemical component of the wood removes variability due to differences in the proportion of different components between rings and between trees, but this is not the main source of variability in isotopic ratios. On the contrary, there is clear evidence of a great deal of variability between trees (McCarroll and Pawellek, 1998; Ponton et al., 2001), and little evidence that isolating the cellulose significantly reduces the variability. Indeed, $\delta^{13}C$ variability between individuals growing under very similar conditions seems to be characteristic of C3 plants in general. For example, Van de Water et al. (2002) found that, for a range of species, variability in leaf $\delta^{13}C$ of plants growing at the same site commonly equals differences measured along a substantial altitudinal gradient. Also, Van de Water (2002) compared wholewood, holocellulose and α-cellulose and found that although C3 plants showed isotopic enrichment with increased removal of labile carbon compounds, the variability of the results was not improved by any of the treatments.

Loader et al. (2004, in press) found that oak tree-ring cellulose ^{13}C was more strongly correlated with climate variables than lignin, but that the strongest correlations were obtained using wholewood. Borella and Leuenberger (1998) found no significant difference in the climate signals of cellulose and wholewood $\delta^{13}C$ for oak and beech, concluding that cellulose extraction is unnecessary. Warren et al. (2001) report that wholewood of conifers was consistently 0.87‰ more negative than cellulose. Borella et al. (1999) compared $\delta^{18}O$ of wood and cellulose from 15 rings of a single oak tree and concluded that some of the desired signal was lost in wholewood analysis. However, Barbour et al. (2001) compared tree-ring cellulose and lignin $\delta^{18}O$ of oak and pine trees from around the world, concluding that cellulose and wood record essentially the same information and that no climate information was lost when analysing wholewood over α-cellulose and, depending upon local conditions, extraction of α-cellulose from wood samples may be unnecessary for isotope studies looking at correlations with site parameters.

Even if there is some doubt about whether there is a slight loss of signal when using wholewood rather than cellulose, this will be offset by the advantage of allowing a larger number of trees to be analysed. In palaeoclimate studies it is the average isotope signal of the whole sample that is of interest, not the exact value of any individual tree or ring, so the aim should always be to maximise the precision of the estimate of the mean, not the precision of the individual measurements. The absolute values obtained from trees, even growing close together, varies so much that the precision of the average depends much more on the number of trees in the sample than on the precision of each individual measurement.

A potential problem of wholewood analysis is differential decay, since under most conditions where wood is preserved, cellulose decays more quickly than lignin (Suberkropp and Klug, 1976; Spiker and Hatcher, 1987; Benner et al., 1991; Schleser et al., 1999b). Although the isotopic signature of the remaining cellulose may be unaffected (Schleser et al., 1999b; Loader et al., 2004, in press), the change in cellulose to lignin ratio would change the isotopic ratio of the remaining wood. However, given a mean offset in $\delta^{13}C$ between cellulose and wholewood of 1‰, and an initial cellulose/lignin ratio of 2:1, half of the cellulose would need to be lost to change the ratio in the decayed wood by 1‰. This is smaller than the typical offset between trees (> 2‰). The use of wholewood, or resin-extracted conifer wood, seems promising.

7. Data treatment

Tree-ring isotopic data are rich in palaeoclimate information, but may also contain trends and variability unrelated to past climate. The aim of data treatments for palaeoclimatic studies should be to remove this extraneous 'noise' without weakening the palaeoclimate signal. This can be surprisingly difficult to achieve, and a wide range of approaches has been used. Inappropriate data treatment can have a very deleterious effect.

7.1. Atmospheric $\delta^{13}C$ trend

Coal and oil are of organic origin and hence depleted in ^{13}C, so anthropogenic increases in the concentration of CO_2 in the atmosphere have resulted in a lowering of the $\delta^{13}C$ value of air by about 1.5‰ since industrialisation. Since fractionation is additive, this trend should be reflected in tree rings. The trend is apparent in most tree-ring $\delta^{13}C$ series (e.g. Freyer and Belacy, 1983; Epstein and Krishnamurthy, 1990; Leavitt and Lara, 1994; Feng and Epstein, 1995a, b; February and Stock, 1999; Treydte et al., 2001), though not all (e.g. Stuiver, 1978; Tans and Mook, 1980; Francey, 1981; Robertson et al., 1997a, b; Anderson et al., 1998; Duquesnay et al., 1998).

The simplest way to remove the atmospheric decline is statistical de-trending. However, this cannot distinguish trends produced directly by the decline in the $^{13}C/^{12}C$ ratio in the atmosphere and those due to climate or other environmental controls. This is particularly problematic because the period over which the $\delta^{13}C$ of the atmosphere declines witnessed many other changes that may have influenced tree growth and function (Briffa et al., 1998a, b), not least increases in the atmospheric concentration of CO_2. Statistical de-trending will remove the direct effect of declining $\delta^{13}C$ of the air, but also any physiological response to increased atmospheric CO_2 concentrations, and any change in climate that has occurred as a result of the CO_2 rise. Unless the aim is to focus only on very high-frequency variability, as in reconstructing the frequency of extreme events such as droughts for example, statistical de-trending is undesirable.

Two approaches avoid the problems of statistical de-trending: removing the trend mathematically or expressing isotope ratios in terms of discrimination against ^{13}C, rather than relative to a standard. Both require reasonable annual estimates of the $\delta^{13}C$ of the atmosphere. Ice cores provide evidence for the early and pre-industrial period and air samples more recent measurements. The incomplete data show a non-linear decline but with marked scatter of the points, so that some generalisation and extrapolation are required to extract annual values. A range of approaches to fitting a line through the data have been used, including splines, and various orders of polynomials, each giving slightly different results, making corrected data difficult to compare directly.

A key criterion for a common approach might be that values should not change as new atmospheric $\delta^{13}C$ data accrue each year. This precludes polynomials, because adding a few points has an unpredictable affect on the rest of the curve.

A simple yet effective solution has been proposed by Saurer et al. (1997) who used a series of straight segments with inflection points rather than a curve. This approach has the advantage that accruing data does not change earlier values. Also, if future research discovers that one segment is seriously in error, only values from that age range need be amended. Francey et al. (1999) have compiled a high precision record of atmospheric $\delta^{13}C$ based on Antarctic ice cores which, for the purposes of correcting tree-ring data, can be summarised by two straight segments between 1850 and 1961, with an annual decline of 0.0044‰ and between 1962 and 1980 with a steeper annual decline of 0.0281‰. If the latter is extrapolated to the end of the last century the estimated values fit well with those obtained from firn (compacted snow) in the same area. The estimated value for the year 2000 is –7.99‰. Although there are other data sets from elsewhere in the world that could be used, and which might give more precise estimates of local atmospheric $\delta^{13}C$, we propose that these data are sufficiently precise to provide a standard method of removing the atmospheric decline in $\delta^{13}C$ from tree-ring data. These estimated values are presented in Table 2.

Owing to the combined effects of fractionation and the $\delta^{13}C$ of the product being directly related to that of the source, changing atmospheric $\delta^{13}C$ may be corrected for by simply adding the difference between the atmospheric value (for each year) and a standard value to the $\delta^{13}C$ of each tree ring. A logical standard value is the 'pre-industrial' $\delta^{13}C$ of the atmosphere, for which

Table 2
Estimated annual values for the $\delta^{13}C$ of atmospheric CO_2 together with the correction factor (add Δ) necessary to quote tree-ring $\delta^{13}C$ values relative to a pre-industrial standard value of $-6.4‰$

Year	$\delta^{13}C$	Δ	CO_2	Year	$\delta^{13}C$	Δ	CO_2	Year	$\delta^{13}C$	Δ	CO_2
1850	−6.41	0.01	285.2	1901	−6.64	0.24	297.0	1952	−6.86	0.46	312.8
1851	−6.42	0.02	285.3	1902	−6.64	0.24	297.3	1953	−6.87	0.47	313.2
1852	−6.42	0.02	285.4	1903	−6.65	0.25	297.6	1954	−6.87	0.47	313.6
1853	−6.43	0.03	285.5	1904	−6.65	0.25	297.9	1955	−6.88	0.48	314.1
1854	−6.43	0.03	285.6	1905	−6.66	0.26	298.2	1956	−6.88	0.48	314.6
1855	−6.43	0.03	285.7	1906	−6.66	0.26	298.5	1957	−6.89	0.49	315.1
1856	−6.44	0.04	285.8	1907	−6.66	0.26	298.9	1958	−6.89	0.49	315.7
1857	−6.44	0.04	285.9	1908	−6.67	0.27	299.2	1959	−6.90	0.50	315.8
1858	−6.45	0.05	286.0	1909	−6.67	0.27	299.6	1960	−6.90	0.50	316.8
1859	−6.45	0.05	286.2	1910	−6.68	0.28	299.9	1961	−6.90	0.50	317.5
1860	−6.46	0.06	286.3	1911	−6.68	0.28	300.2	1962	−6.92	0.52	318.3
1861	−6.46	0.06	286.5	1912	−6.69	0.29	300.5	1963	−6.95	0.55	318.8
1862	−6.47	0.07	286.6	1913	−6.69	0.29	300.9	1964	−6.98	0.58	319.4
1863	−6.47	0.07	286.8	1914	−6.70	0.30	301.2	1965	−7.01	0.61	319.9
1864	−6.47	0.07	287.0	1915	−6.70	0.30	301.5	1966	−7.03	0.63	321.2
1865	−6.48	0.08	287.2	1916	−6.70	0.30	301.8	1967	−7.06	0.66	322.0
1866	−6.48	0.08	287.4	1917	−6.71	0.31	302.2	1968	−7.09	0.69	322.9
1867	−6.49	0.09	287.6	1918	−6.71	0.31	302.5	1969	−7.12	0.72	324.5
1868	−6.49	0.09	287.8	1919	−6.72	0.32	302.9	1970	−7.15	0.75	325.5
1869	−6.50	0.10	288.0	1920	−6.72	0.32	303.2	1971	−7.17	0.77	326.2
1870	−6.50	0.10	288.2	1921	−6.73	0.33	303.5	1972	−7.20	0.80	327.3
1871	−6.51	0.11	288.4	1922	−6.73	0.33	303.9	1973	−7.23	0.83	329.5
1872	−6.51	0.11	288.7	1923	−6.74	0.34	304.2	1974	−7.26	0.86	330.1
1873	−6.51	0.11	288.9	1924	−6.74	0.34	304.6	1975	−7.29	0.89	331.0
1874	−6.52	0.12	289.1	1925	−6.74	0.34	304.9	1976	−7.32	0.92	332.0
1875	−6.52	0.12	289.4	1926	−6.75	0.35	305.2	1977	−7.34	0.94	333.7
1876	−6.53	0.13	289.7	1927	−6.75	0.35	305.6	1978	−7.37	0.97	335.3
1877	−6.53	0.13	289.9	1928	−6.76	0.36	305.9	1979	−7.40	1.00	336.7
1878	−6.54	0.14	290.2	1929	−6.76	0.36	306.2	1980	−7.43	1.03	338.5
1879	−6.54	0.14	290.5	1930	−6.77	0.37	306.5	1981	−7.46	1.06	339.8
1880	−6.55	0.15	290.8	1931	−6.77	0.37	306.8	1982	−7.48	1.08	341.0
1881	−6.55	0.15	291.1	1932	−6.78	0.38	307.1	1983	−7.51	1.11	342.6
1882	−6.55	0.15	291.4	1933	−6.78	0.38	307.4	1984	−7.54	1.14	344.3
1883	−6.56	0.16	291.7	1934	−6.78	0.38	307.7	1985	−7.57	1.17	345.7
1884	−6.56	0.16	292.0	1935	−6.79	0.39	308.0	1986	−7.60	1.20	347.0
1885	−6.57	0.17	292.3	1936	−6.79	0.39	308.3	1987	−7.62	1.22	348.8
1886	−6.57	0.17	292.6	1937	−6.80	0.40	308.5	1988	−7.65	1.25	351.3
1887	−6.58	0.18	292.9	1938	−6.80	0.40	308.8	1989	−7.68	1.28	352.8
1888	−6.58	0.18	293.1	1939	−6.81	0.41	309.1	1990	−7.71	1.31	354.0
1889	−6.58	0.18	293.4	1940	−6.81	0.41	309.3	1991	−7.74	1.34	355.5
1890	−6.59	0.19	293.7	1941	−6.82	0.42	309.5	1992	−7.77	1.37	356.3
1891	−6.59	0.19	294.0	1942	−6.82	0.42	309.8	1993	−7.79	1.39	357.0
1892	−6.60	0.20	294.3	1943	−6.82	0.42	310.0	1994	−7.82	1.42	358.9
1893	−6.60	0.20	294.6	1944	−6.83	0.43	310.2	1995	−7.85	1.45	360.9
1894	−6.61	0.21	294.9	1945	−6.83	0.43	310.5	1996	−7.88	1.48	362.7
1895	−6.61	0.21	295.2	1946	−6.84	0.44	310.8	1997	−7.91	1.51	363.8
1896	−6.62	0.22	295.5	1947	−6.84	0.44	311.0	1998	−7.93	1.53	365.5
1897	−6.62	0.22	295.8	1948	−6.85	0.45	311.3	1999	−7.96	1.56	367.0
1898	−6.62	0.22	296.1	1949	−6.85	0.45	311.7	2000	−7.99	1.59	368.5
1899	−6.63	0.23	296.4	1950	−6.86	0.46	312.0	2001	−8.02	1.62	370.0
1900	−6.63	0.23	296.7	1951	−6.86	0.46	312.4	2002	−8.05	1.65	371.5
								2003	−8.07	1.67	373.0

Note: Data are interpolated using the high precision records of atmospheric $\delta^{13}C$ obtained from Antarctic ice cores (Francey et al., 1999). Estimated values for the atmospheric concentration of CO_2 (ppm) are taken from Robertson et al. (2001).

−6.4‰ is a reasonable estimate. This is close to values around AD 1850 and also prior to the Little Ice Age (Francey et al., 1999). The values that need to be added in order to quote industrial tree-ring $\delta^{13}C$ values relative to pre-industrial atmospheric $\delta^{13}C$ are also presented in Table 2.

Treydte et al. (2001) have suggested that the correction for changes in $\delta^{13}C$ should also account for the

response of trees to the increase in atmospheric concentration of CO_2. They propose a correction of 0.007‰ in $\delta^{13}C$ per unit increase in atmospheric CO_2 (ppmv). We would caution against the current use of such correction factors until existing uncertainties are resolved. The response of trees to increasing atmospheric concentrations of CO_2 may vary and is in itself an important and ongoing field of study (Feng and Epstein, 1995a; Kürschner, 1996; Marshall and Monserud, 1996; Picon et al., 1996; Feng, 1998, 1999; Luomala et al., 2003). Nevertheless, Table 2 includes reasonable annual values for atmospheric CO_2 concentrations taken from Robertson et al. (2001). The values for 1998–2003 are based on linear extrapolation of the 1980–1997 estimates.

The other approach to removing the atmospheric decline in $\delta^{13}C$ is to express the $^{13}C/^{12}C$ ratio in terms of discrimination against ^{13}C using the equation

$$\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1 - \delta^{13}C_p/1000), \qquad (4)$$

where Δ is discrimination against ^{13}C, $\delta^{13}C_a$ is the carbon isotope ratio of the air (the source) and $\delta^{13}C_p$ is the carbon isotope ratio of the product (leaf sugars). Discrimination is commonly used in environmental physiology, but is less attractive for long chronologies because early and pre-industrial variations in $\delta^{13}C_a$ are small and not known with sufficient resolution.

Stable carbon isotope ratios can also be expressed in terms of changes in water use efficiency, since the ratio of carbon fixed to water lost is, like carbon isotope discrimination, controlled by stomatal conductance and photosynthetic rate (Farquhar et al., 1982; Ehleringer et al., 1993; Leavitt et al., 2003). Intrinsic water use efficiency iWUE is defined as

$$iWUE = A/g = c_a[1 - (c_i/c_a)](0.625), \qquad (5)$$

where A is the rate of CO_2 assimilation, g is stomatal conductance and c_i and c_a are intercellular and atmospheric CO_2 concentrations, respectively. The c_i/c_a ratio can be obtained from

$$c_i/c_a = (\delta^{13}C_{plant} - \delta^{13}C_{air} + a)/(b - a). \qquad (6)$$

In this case, $\delta^{13}C_{plant}$ refers to the ratio of sugars fixed in the leaf, and if wood or cellulose is used a correction factor needs to be added to account of the difference between leaf sugars and the component analysed.

Some studies have used tree-ring $\delta^{13}C$ to reconstruct long-term changes in the internal concentration of CO_2:

$$c_i = c_a[(\delta^{13}C_{plant} - \delta^{13}C_{air} + a)/(b - a)]. \qquad (7)$$

Presenting results simply as $\delta^{13}C$ values has the advantage that it is possible to use these to calculate discrimination, water use efficiency, c_i or c_i/c_a. However, if only these derivative values are quoted, it is not possible to convert back to $\delta^{13}C$ without clear information on the annual values for atmospheric CO_2

concentrations and the $\delta^{13}C$ values for air that were used in the calculations. For convenience, reasonable values are presented in Table 2.

7.2. Effect of tree age

One of the enduring problems of extracting palaeoclimate information from tree-ring-width data derives from the fact that as trees age, ring widths decline. There is a substantial literature dealing with de-trending tree-ring data, the aim in all cases being to remove the age-trend whilst retaining as much of the palaeoclimate signal as possible. However, if a tree series in statistically de-trended, it is inevitable that any climatic signals that have a temporal frequency equal to or longer than the life-span of the tree will be removed. The temporal frequency of the palaeoclimate signal derived from statistically de-trended tree-ring series, therefore, cannot be longer than the life-span (segment length) of the trees used to construct it. This has become known as the 'segment length curse', making it difficult to use tree-ring widths to reconstruct absolute changes in climate over long timescales (Cook et al., 1995).

One of the key questions facing isotope dendroclimatology is whether there are age-related trends in the isotope ratios obtained from trees, and if so how they might be removed. Unfortunately, the evidence is equivocal and obscured in part by the sampling strategies and data treatment protocols often adopted in isotope studies. If, for example, wood from several trees is pooled prior to analysis, it is impossible to examine any potential age-trends in the individual trees.

Several studies that have used individual trees, as opposed to pooled samples, report age-related trends in carbon isotopic ratios (e.g. McCarroll and Pawellek, 2001). Duquesnay et al. (1998) reported a clear age effect in high forest beech trees by sampling trees in different age classes for the same years, though the rise in $\delta^{13}C$ was followed by a decline. There are insufficient water isotope data available from individual trees to determine whether there are similar, though less pronounced age-trends.

One interpretation of the age-related trend in $\delta^{13}C$ is a 'juvenile effect' caused by recycling of respired air, already depleted in ^{13}C, by young trees growing close to the forest floor (Schleser and Jayasekera, 1985). However, the age-trend has been identified even in Alpine tree-line situations where tree cover is sparse and slopes open and windy, so that recycling of respired air is unlikely to be (at least wholly) responsible. Even in dense tropical forest, recycling does not account for the observed height gradient in $\delta^{13}C$ (van der Merwe and Medina, 1991). Cernusak et al. (2001) have suggested that a decreasing contribution of photosynthate from bark refixation of respired CO_2 may be responsible, since bark thickens and access to light declines as the

tree grows. An alternative explanation relates to changes in the hydraulic conductivity of trees as they age (Ryan and Yoder, 1997; McDowell et al., 2002). Leaf water potential decreases with tree height due to gravity and because, at a given transpiration rate, the hydraulic conductance from soil to leaf declines with increasing path length. A decline in leaf-water potential should cause stomatal conductance to decline as trees grow higher, which would have a marked effect on fractionation of carbon and a more muted effect on the water isotopes. This will be offset as some trees age because the sapwood to leaf area ratio increases, making more water potentially available. Schäfer et al. (2000) have explained the declining stomatal conductance in beech trees (*Fagus sylvatica*) with tree height as a function of these two controls. Monserud and Marshall (2001) correlated carbon discrimination with tree height and showed clear trends for two pine species (*Pinus moniticola* and *Pinus ponderosa*), and in both cases the sapwood to leaf area ratio also increased. Monserud and Marshall (2001) found no such trends for Douglas fir (*Pseudotsuga menziesii*), but for the same species McDowell et al. (2002) have shown a clear trend of declining discrimination and stomatal conductance for three height classes.

Annual tree height increments can often be measured on sub-fossil tree stems (Jalkanen et al., 1998; McCarroll et al., 2004, in press) and hydraulic conductivity can be estimated from measurements of wood anatomy in both angiosperms and conifers (Ponton et al., 2001; McDowell et al., 2002, 2004, in press). There is thus potential for modelling changes in stomatal conductance as trees age, and so removing the age effect seen in carbon, and the predicted smaller effect on water isotope fractionation without recourse to statistical de-trending. More data are required from individual trees, of varying age and longevity, before the problem can be fully addressed. No correction is possible where samples are pooled prior to analysis. The alternative is to use only those rings that formed after any 'juvenile effect' ceased. This limits the length of series that can be used, which may make it more difficult to construct very long chronologies with sufficient replication, but it is feasible, and since there is no statistical de-trending the 'segment length curse' does not apply and no palaeoclimate information is lost.

7.3. Dealing with variability

The reason for large offsets in carbon isotope ratios, even of adjacent trees, is not entirely clear. The limited evidence suggests that differences between trees remain stable over time (McCarroll and Pawellek, 1998), so local microclimate and variability in soil moisture capacity are not likely to be wholly responsible. The differences in fractionation, and therefore water use

efficiency, may be genetically determined (Zhang et al., 1993; Johnsen et al., 1999; Brendel et al., 2002; Dawson et al., 2002), perhaps reflecting differences in hydraulic conductivity, which have explained differences in $\delta^{13}C$ along branches (Walcroft et al., 1996), with tree height and even between species (Ponton et al., 2001, 2002). Low hydraulic conductivity leads to reduced stomatal conductance, which has a large effect on $\delta^{13}C$ and a smaller effect on the water isotopes, though broad-leaved trees may be more sensitive that conifers. Hydraulic conductivity might also influence the rate of supply of water to the sites of evaporation in the leaf, and therefore the magnitude of the Péclet effect.

Irrespective of the reason for the variability between trees, its existence must be taken into account when quoting mean isotope values. The correct way to express variability is to use statistically defined confidence intervals, which define the probability of the true mean lying within a particular range of the sample mean. It is bad practice to simply quote mean values ±one or two standard deviations or standard errors (SE) because ±2SE only approaches the 95% confidence interval when the sample size is large (>25). Where the sample size is small, as is the case for most studies of isotopes in tree rings, the *t*-distribution must be used, so for a sample of four the 95% confidence interval is ±3.18SE and for a sample of 10 it is ±2.26SE.

In dealing with variability it is critical to distinguish between relative and absolute differences, because the inter-annual variability in a sample of trees may be very similar, despite large differences in absolute isotope values. Statistically defined confidence limits around annual mean values reflect the absolute differences between trees rather than the relative coherence in the year-to-year signal. To obtain confidence limits for the latter it is necessary to standardise the data, by expressing the variability of each tree relative to a common mean. Confidence intervals around a standardised mean reflect the degree to which the trees respond in parallel.

Where the primary aim is to examine response to variations in climate, using meteorological records, it is appropriate to use standardised isotope data. Over long timescales, however, we expect climate changes to be reflected in low-frequency variations in the absolute isotopic ratios in trees, so standardising will mask any long-term changes in climate. The between-tree variability in absolute isotopic ratios is a significant but surmountable problem; the solution lies simply in sample size. Until recently, lack of capacity has forced laboratories to pool samples, or build chronologies using little or no replication. If stable isotopes are to be used to reconstruct long-term climatic changes, mean absolute isotope ratios are essential. The ability to place confidence limits around mean isotope ratios from the distant past is one of the great advantages of isotope

dendroclimatology and a prime reason for working with individual tree rings rather than pooled samples.

The number of trees required to provide a representative sample for comparison with climate data depends on the degree to which the trees vary in parallel, and can be calculated using the expressed population signal:

$$\text{EPS}(t) = (tr_{bt})/(tr_{bt} + (1 - r_{bt})), \tag{8}$$

where t is the number of trees and r_{bt} is the mean between-tree correlation. An EPS $\geqslant 0.85$ suggests the sample size is adequate. The available data suggest that the common signal in tree-ring isotopic data is much stronger than that obtained from ring widths or relative densities, so fewer trees (commonly 4 or 5) are required (Robertson et al., 1997a, b; McCarroll and Pawellek, 1998). However, the correlation between trees takes no account of absolute differences, so the EPS is not a reliable indicator of the likely confidence intervals around the absolute mean.

Rather than defining the replication required to build isotope series, and applying that universally, it might be better for individual studies to define the confidence limits that are acceptable, and vary sample sizes accordingly. Chronologies would then have varying replication, with more trees incorporated where variability is high or greater precision is required.

8. Palaeoenvironmental signals

Variations in tree-ring isotope ratios have been interpreted in many different ways (Table 1), with inferred palaeoclimate signals including temperature, RH, antecedent precipitation and the isotopic ratios of source water. Given current understanding of the controls on fractionation, this is not surprising. None of the isotopes has a single controlling factor, so the dominant control will change according to the conditions under which the trees grew.

8.1. Carbon

Although several authors quote increases in $\delta^{13}C$ per mil per degree Celsius of temperature change (Table 1), it is now clear that variability in tree-ring $\delta^{13}C$ cannot be used as a palaeothermometer, nor is it simply a measure of water availability (Stewart et al., 1995). Warren et al. (2001) compiled information from conifers worldwide and concluded that $\delta^{13}C$ is only an indicator of drought stress in seasonally dry climates, and that variation in irradiance and N concentration can have as large an affect on discrimination as water availability.

For carbon isotopes, fractionation is a function of the ratio of internal to external concentrations of CO_2 and is thus a measure of the balance between stomatal conductance and photosynthetic rate. Where moisture stress is limiting, stomatal conductance dominates and the environmental controls are air RH and soil moisture status, giving strong correlations with RH and antecedent precipitation (e.g. Gagen et al., 2004, in press). Where trees are rarely moisture-stressed, the dominant control may be photosynthetic rate, controlled mainly by irradiance and temperature.

Irradiance can influence $\delta^{13}C$ indirectly. For example, in tropical orchids varying $\delta^{13}C$ was not associated with changes in leaf nitrogen, suggesting irradiance influences $\delta^{13}C$ mainly by increasing stomatal limitation to photosynthesis (Zimmerman and Ehleringer, 1990). However, in an experiment using oak seedlings, carbon assimilation rate more than tripled as incident irradiance increased from 8% to 100%, whereas stomatal conductance only doubled, so that variations in water use efficiency (and hence $\delta^{13}C$) must be caused mainly by variations in photosynthetic rate (Ponton et al., 2002). Leaf N concentrations increased with increasing irradiance. Similar conclusions have been reached for variations in $\delta^{13}C$ of tropical shrubs (Jackson et al., 1993) and banana (Israeli et al., 1996). Warren et al. (2001) report increased $\delta^{13}C$ in conifer stands due to increased radiation interception following thinning and McCarroll and Pawellek (2001) report a strong correlation between $\delta^{13}C$ of pine tree rings from moist sites in northern Finland and average hours of summer sunshine.

Although several authors report correlations between tree-ring $\delta^{13}C$ and summer temperature (Table 1), at least part of this signal may be indirect, due to correlation between temperature and the direct controlling factors. Hot summers, for example, tend also to be dry, so that temperature is correlated with air RH and soil moisture status, both of which directly control stomatal conductance and therefore $\delta^{13}C$. Summer temperature and sunshine are strongly correlated, and irradiance exerts a strong control on photosynthetic rate, and therefore $\delta^{13}C$. Although temperature is one of the factors influencing photosynthetic rate, and therefore $\delta^{13}C$, in C3 plants at atmospheric concentrations of CO_2 the direct effect may be weaker than is often implicitly assumed (Leegood and Edwards, 1996).

Under most conditions several factors control variability in $\delta^{13}C$, so correlations with a single climatic parameter, and particularly with temperature, are oversimplifications. Using such correlations as transfer functions is perilous, since any changes in the potentially complex chain of real biological links that eventually leads to the observed correlation can become sources of error. The problem is compounded because the only period with high-quality climatic data is the last century, during which time a host of changes have taken place that might change the way trees respond to the environment (Briffa et al., 1998a, b). These include increased atmospheric CO_2, nitrogen deposition,

atmospheric pollution and perhaps cloudiness, as well as changes in forest management.

Edwards et al. (2000) have attempted to address the problem of simple correlations by assuming that tree-ring $\delta^{13}C$ variations are a function of RH and temperature (T), and plotting isotope ratios as 'response surfaces in RH − T space'. They support this using $\delta^{13}C$ of cellulose from bean plants grown under different combinations of T and RH. This bi-variate approach is only a marginal improvement because it also is not based on clear causal links. If, in an experiment, RH and temperature are allowed to vary, and all other factors are held constant, then RH and temperature will of course appear as the strongest controls. This does not imply that these two variables really control the variability of $\delta^{13}C$ in trees, and plotting isotope values in 'RH − T space', although addressing the coupling of these factors, ignores all of the other controls on $\delta^{13}C$. The real axes of the 'space' in which $\delta^{13}C$ varies are stomatal conductance and photosynthetic rate. RH is a poor surrogate for stomatal conductance because it ignores the effect of soil moisture status, and temperature is not the only control on photosynthetic rate.

The search for a single or even a pair of environmental factors that control $\delta^{13}C$ of tree rings worldwide is futile. Where stomatal conductance dominates, the controls include air RH and soil moisture status, which is linked to antecedent precipitation. Where photosynthetic rate dominates, the dominant controls are irradiance and temperature. In areas where the dominant stress factor influencing trees is a combination of high temperature and low precipitation, then $\delta^{13}C$ provides a strong indicator of the severity of those conditions. Under less extreme conditions, and where neither stomatal conductance nor photosynthetic rate are strongly dominant, the $\delta^{13}C$ values will be influenced by all of these factors and will be difficult to interpret. Under such conditions tree-ring $\delta^{13}C$ values, in isolation, are unlikely to provide a powerful tool for palaeoenvironmental reconstruction. The solution may, however, lie in a carefully planned multi-proxy approach.

8.2. Water isotopes

The earliest work on the water isotopes in tree rings described them simply as palaeothermometers, recording the isotopic ratios of source water and therefore of precipitation, which varies with temperature (Libby et al., 1976). Current models predict three major controls on both $\delta^{18}O$ and δD in tree rings: the isotopic signature of source water, evaporative enrichment in the leaf, and exchange with xylem (source) water during cellulose and/or lignin synthesis.

The strong correlations between water isotope ratios and temperature in early and some later studies

(Table 1) probably reflect a combination of the direct effect of temperature on isotopic ratios of precipitation and indirect influences on evaporative enrichment. As with carbon, the degree to which a single climatic parameter controls the isotopic ratios depends on the location in which the tree grew. If the aim is to use tree rings to reconstruct variations in the isotopic composition of precipitation, and therefore temperature, shallow rooted trees growing on thin, well-drained soils are recommended (Waterhouse et al., 2002).

There are some direct temperature effects on evaporative enrichment, including a small effect on the difference in vapour pressure of the water containing heavy and lighter isotopes. In broad-leaved trees, differences in leaf and air temperature may also affect the vapour pressure difference. The dominant control on degree of leaf enrichment, for both $\delta^{18}O$ and δD, however, is leaf to air vapour pressure deficit, which is controlled by air humidity rather than directly by temperature, and influenced also by leaf morphology and boundary layer conditions (Buhay et al., 1996). For both $\delta^{18}O$ and δD there is the added uncertainty produced by exchange with xylem water, which determines the relative strength of the source water and leaf enrichment signals, and the controls on this important factor remain poorly constrained.

Waterhouse et al. (2002) have tested current models of water isotope fractionation using stable isotopes of both oxygen and hydrogen in oak trees from a dry site in eastern England. The models performed well in predicting the annual variation in $\delta^{18}O$ but not δD. They suggest that this may be because of varying abundance of deuterium at different positions in the glucose ring, and therefore differing potential for exchange with xylem water during cellulose synthesis. Robertson et al. (2001) report $\delta^{18}O$ ratios from oak tree rings, also from eastern England and, as predicted, find strong correlations with both summer RH and the $\delta^{18}O$ of precipitation, which at this site is dominated by winter rainfall. Anderson et al. (2002) have used $\delta^{18}O$ from fir trees in central Switzerland to reconstruct past variation in the $\delta^{18}O$ of precipitation by fitting a 'correction factor' based on temperature, RH and tree-ring-width index to remove the effect of leaf enrichment. Pendall (2000) reports RH as the strongest control on δD at dry sites, but found no correlation with inter-annual or seasonal δD of precipitation, suggesting that the leaf enrichment signal dominates.

The limited evidence available from tree rings suggests that current models provide a reasonable explanation for inter-annual variation in $\delta^{18}O$, but perhaps not yet for δD. However, as with carbon it seems that the search for a single environmental signal that can be extracted at all sites is futile. Trees are not buckets and the water isotopes in tree rings are not unadulterated samples of ancient precipitation. As with carbon isotopes, the

greatest potential for interpreting the $\delta^{18}O$ and δD variations in long chronologies may lie in a multi-proxy approach (e.g. Mayr et al., 2003).

9. Potential for a multi-proxy approach

From the perspective of palaeoenvironmental reconstruction, the overwhelming problem with all three isotopes is that they are not controlled by a single environmental variable. Complex mechanistic models that combine several controls are useful for explaining recent variations in each of the three isotopic ratios in tree rings, where they can be tested using instrumental data (Berninger et al., 2000; Hemming et al., 2001; Arneth et al., 2002). However, it is not feasible to invert these models and use the past variations to reconstruct several independent variables using a single isotopic signal. The solution to this dilemma may lie in combining the different isotopes, perhaps together with other palaeoclimate proxies that can be extracted from living, dead and sub-fossil trees. This could potentially be done using traditional statistical approaches to environmental reconstruction, based on transfer functions, or through mechanistic modelling.

The simplest approach is to use several proxies to make independent estimates of a single climate parameter and then combine them by taking a simple or weighted average (McCarroll et al., 2003). If different proxies carry the same 'signal' but have different sources of 'noise', averaging strengthens the signal whilst dampening the noise. The critical condition is that the sources of error (noise) must differ. This may be an effective way of strengthening the palaeoenvironmental signal that can be extracted from trees.

Although progress will be made using statistical approaches, the greatest potential for palaeoclimate research using stable isotopes in tree rings must be through mechanistic modelling. Plant physiologists have made great advances in understanding how climatic and other factors control fractionation of the three isotopes. These models describe the relationship between climate and the isotope ratios mathematically, and so potentially provide a much more robust mechanism with which to interpret past variations. A mechanistic approach to modelling the relationship between proxies and climate is preferable to statistical approaches, based on correlation rather that causation, not least because we know that many of the environmental variables that influence trees have changed over the last century, due to human activities. Recent correlations, therefore, may not be reliable indicators of the strength or nature of relationships in the past (Briffa et al., 1998a, b).

From the palaeoclimatologists' perspective, the problem with the physiological models is that they cannot easily be reversed so that the isotope ratio can be used to reconstruct climate. However, although the models for carbon and the water isotopes differ, they are not independent, because they are all influenced by the stomata. Stomata evolved to control the balance between supply of CO_2 and loss of moisture. The factors that control loss of moisture, and therefore fractionation of the water isotopes in the leaf, are not independent of those that control the fractionation of carbon, but the links are neither simple nor direct. What is required is a model that links together the various controls on fractionation of the three isotopes simultaneously. Rather than using the isotopes individually, in this case it is the combination of isotope values that would be used to interpret the climate of the past. Thus although many different palaeoclimate scenarios might lead to a particular $\delta^{13}C$ value in a tree ring, a much smaller range of factors would account for a specific combination of $\delta^{13}C$ and $\delta^{18}O$, and a smaller range again a particular combination of all three isotopes.

Even if such a model cannot be used to extract a single climate variable, it could at least be used to indicate the conditions under which different sub-fossil trees lived. If the combination of isotopes could indicate whether a tree grew under conditions of abundant soil water or soil moisture stress, for example, it would greatly improve the confidence with which the carbon isotope signal could be interpreted. Given this information we could either exclude unsuitable trees from the sample or apply a range of different transfer functions, based on living trees growing under different conditions.

10. Future potential and priorities

The rate of progress in the young science of stable isotope dendroclimatology has been remarkable. Ten years ago a 3-year project might result in a few hundreds of isotope measurements; 5 years ago an optimist might aim for a few thousand. A well-resourced laboratory can now, potentially, produce hundreds of isotope measurements in a day. At the same time, plant physiologists have made great advances, so that we are now guided by a sound knowledge of the way that climate controls isotopic variations, and need not rely simply on the magnitude of correlations. At last it is possible to look to the long tree-ring chronologies with a realistic possibility of both dealing with the huge numbers of samples and interpreting the results in terms of quantitative estimates of specific palaeoclimate parameters, with statistically defined confidence intervals. That must surely be the greatest potential and most pressing priority for isotope dendroclimatology, for it will allow us to reconstruct the climate of the Holocene, in particular places rather than averaged across huge areas, and with perfect annual resolution.

Although an annual resolution might seem more than adequate, and is rarely achieved in palaeoclimate research, tree rings also present the possibility of extracting seasonal climatic information by measuring isotopic variations within rings. Intra-annual variations in stable isotopes have now been measured in over 20 species worldwide (Wilson and Grinsted, 1975, 1977; Leavitt and Long, 1982, 1991; Leavitt, 1993; Ogle and McCormac, 1994; Loader et al., 1995; Robertson et al., 1996; Sheu et al., 1996; Walcroft et al., 1997; Schleser et al., 1999a; Porté and Loustau, 2001; Warren et al., 2001; Leavitt, 2002; Leavitt et al., 2003; Helle and Schleser, 2003). Initially restricted to a few very thick rings, which were sectioned by hand, the increasing use of microtomes and laser-ablation makes this an exciting field for future research. Similar very high-resolution sampling may hold the key to extracting palaeoclimate information from tropical trees, most of which lack annual rings and the further elucidation of important plant physiological processes.

As well as retaining records of the climate of the past, isotopes in tree rings may hold important guides to the future consequences of greenhouse gas emissions. One of the critical questions facing climate modellers is the degree to which emissions of CO_2 are absorbed by forests, resulting in increased growth and (temporary) storage of carbon (the 'greening effect'). Experiments growing trees under increased CO_2 are usually restricted to seedlings, and even where large trees have been enclosed the experiments are too short to determine the long-term plasticity of response. Stable isotopes in tree rings provide a way of examining the long-term response of trees to increases in CO_2 (Waterhouse et al., 2004).

11. Conclusions

Research on isotopes in marine sediments has had an enormous impact on Quaternary science, demonstrating cyclical variations in climate over a timescale of millennia. Ice cores have much better resolution but record on a global or hemispherical-scale, providing little information on the geography of change. The great beauty of the tree-ring archive is that it provides even better resolution than ice cores, and suitable trees are widespread. It is becoming increasingly apparent that a critical question facing us is not whether global climate will change, but how it will change in different places. Models can predict this, but to test them we need high-resolution and spatially explicit evidence of the way that past climate changes behaved over space, as well as over time. We can obtain that information from stable isotopes in tree rings.

Stable isotope dendroclimatology is a young field but it is advancing very rapidly. Until recently, progress was constrained by the time taken to prepare and analyse

tree-ring samples and by the cost of analyses. However, recent advances in both sample preparation techniques and mass spectrometry have greatly increased the rate at which samples can be analysed, and at the same time reduced the unit cost. Plant physiologists have also made great advances in understanding how climate influences fractionation. At last we can look to the long tree-ring chronologies with a realistic intention of producing and interpreting isotope data for the whole of the Holocene.

To achieve this will require a change in emphasis from isotope dendroclimatologists. Time and cost constraints have severely restricted the number of samples that can be analysed, which has led to an emphasis on analytical precision, with most laboratories first isolating cellulose or α-cellulose, often from several radii from each tree, and then pooling wood from several trees prior to analysis. However, from a Quaternary perspective it is not the analytical precision of each measurement that is critical, it is the confidence limits around the mean value for each year. The variability within trees and between the main wood components, cellulose and lignin, is small compared to the differences between trees, so the most effective way to increase the precision of the mean is to include more trees, but if samples are pooled prior to analysis, the precision of the mean cannot be calculated.

The ability to calculate the precision of the mean for each year is perhaps the most important advantage of isotope dendroclimatology, because the confidence limits of the mean can be used to place confidence limits around the estimates of palaeoclimate. The potential to create place-specific, annually resolved records of past climate, with statistically defined confidence intervals, makes tree rings unrivalled in their potential for palaeoclimate research. However, the full potential can only be realised by analysing each ring from each tree separately. Few laboratories have attempted this for long sequences because of the cost, but in comparison to marine and ice core research the technique is remarkably cost-efficient.

If isotope dendroclimatology is to achieve its potential it will require investment. We need laboratories that can process tens of thousands of samples in a year, not a few hundreds. Each tree should be treated independently and the focus must be on replication as well as precision. Technology has reached the stage where this is achievable now, and we expect to see further advances in the near future.

At the same time as isotopes have become easier to measure, they have become easier to interpret. There are now reliable mechanistic models that explain how climate and other environmental factors determine the isotopic ratios in tree rings. The models are not perfect, but they are a powerful tool and they free us from a reliance on statistical inference. If the plant physiologists

can take one step further, and link carbon and the water isotopes in an integrated model, it will greatly enhance our ability to reconstruct specific climate variables through time.

There are still many problems facing stable isotope dendroclimatology. There may be trends and variability in the isotope signals that are not related to climate, there is substantial variability between trees, different tree species may respond to climate in different ways, and there are still many gaps in our knowledge of precisely what controls isotope variability under different conditions. Although we have good mechanistic models, they demonstrate that none of the isotopes carry a single, simple climatic signal that can be applied universally. However, a similar or more daunting range of problems faced researchers when they began to work on both marine and ice cores. We are confident that stable isotopes, together with other evidence from tree rings, will prove as important for understanding Holocene palaeoclimate as those archives have been for longer timescales.

Acknowledgements

References

Aggett, J., Bunton, C.A., Lewis, T.A., Llewellyn, D.R., O'Connor, C., Odell, A.L., 1965. The isotopic analysis of oxygen in organic compounds and in co-ordination compounds containing organic ligands. International Journal of Applied Radiation and Isotopes 16, 165–170.

Anderson, W.T., Bernasconi, S.M., McKenzie, J.A., 1998. Oxygen and carbon isotopic record of climatic variability in tree ring cellulose (Picea abies): an example from central Switzerland (1913–1995). Journal of Geophysical Research 103/D24, 31625–31636.

Anderson, W.T., Bernasconi, S.M., McKenzie, J.A., Saurer, M., Schweingruber, F., 2002. Model evaluation for reconstructing the oxygen isotopic composition in precipitation from tree ring cellulose over the last century. Chemical Geology 182, 121–137.

Arneth, A., Lloyd, J., Santruckova, H., Bird, M., Grigoryev, S., Kalaschnikov, Y.N., Gleixner, G., Schulze, E.D., 2002. Response of central Siberian Scots pine to soil water deficit and long-term trends in atmospheric CO_2 concentrations. Global Biogeochemical Cycles 16, 1–13.

Baillie, M.G.L., 1995. A Slice through Time: Dendrochronology and Precision Dating. Batsford, London, 176pp.

Barbour, M.M., Farquhar, G.D., 2000. Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. Plant, Cell and Environment 23, 473–485.

Barbour, M.M., Andrews, T.J., Farquhar, G.D., 2001. Correlations between oxygen isotope ratios of wood constituents of Quercus and Pinus samples from around the world. Australian Journal of Plant Physiology 28, 335–348.

Barbour, M.M., Walcroft, A.S., Farquhar, G.D., 2002. Seasonal variation in $\delta^{13}C$ and $\delta^{18}O$ of cellulose from growth rings of Pinus radiata. Plant, Cell and Environment 25, 1483–1499.

Becker, B., 1993. An 11,000-year German oak and pine dendrochronology for radiocarbon calibration. Radiocarbon 35, 201–213.

Becker, B., Kromer, B., Trimborn, P., 1991. A stable isotope tree-ring timescale of the Late Glacial/Holocene boundary. Nature 353, 647–649.

Begley, I.S., Scrimgeour, C.M., 1996. On-line reduction of H_2O for δ^2H and $\delta^{18}O$ measurement by continuous-flow isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 10, 969–973.

Begley, I.S., Scrimgeour, C.M., 1997. High precision δ^2H and $\delta^{18}O$ measurement for water and volatile organic compounds by continuous-flow pyrolysis isotope ratio mass spectrometry. Analytical Chemistry 69, 1530–1535.

Bennett, C.F., Timmell, T.E., 1955. Preparation of cellulose trinitrate. Svensk Pappersidning 58, 281–286.

Berninger, F., Sonninen, E., Aalto, T., Lloyd, J., 2000. Modelling ^{13}C discrimination in tree rings. Global Biogeochemical Cycles 14, 213–223.

Bert, D., Leavitt, S.W., Dupouey, J.-L., 1997. Variations of wood $\delta^{13}C$ and water use efficiency of Abies alba during the last century. Ecology 78 (5), 1588–1596.

Borella, S., Leuenberger, M., 1998. Reducing uncertainties in $\delta^{13}C$ analysis of tree rings: pooling, milling and cellulose extraction. Journal of Geophysical Research 103, 19519–19526.

Borella, S., Leuenberger, M., Saurer, M., 1999. Analysis of $\delta^{18}O$ in tree rings: wood–cellulose comparison and method dependent sensitivity. Journal of Geophysical Research 104, 19267–19273.

Boutton, T.W., Wong, W.W., Hachey, D.L., Lee, L.S., Cabera, M.P., Klein, P.D., 1985. Comparison of quartz and Pyrex tubes for combustion of organic samples for stable carbon isotope analysis. Analytical Chemistry 55, 1832–1833.

Brendel, O., Iannetta, P.P.M., Stewart, D., 2000. A rapid and simple method to isolate pure alpha-cellulose. Phytochemical Analysis 11, 7–10.

Brendel, O., Pot, D., Plomion, C., Rozenberg, P., Guehl, J.-M., 2002. Genetic parameters and QTL analysis of $\delta^{13}C$ and ring width in maritime pine. Plant, Cell and Environment 25, 945–953.

Brenna, J.T., Tobias, H.J., Corso, T.N., 1998. High precision deuterium and ^{13}C measurement by continuous flow IRMS: organic and position specific isotope analysis. In: Griffifths, H. (Ed.), Stable Isotopes. BIOS Scientific Publishers, Oxford.

Brenninkmeijer, C.A.M., Mook, W.G., 1981. A batch process for direct conversion of organic oxygen and water to CO_2 for $^{18}O/^{16}O$ analysis. International Journal of Applied Radiation and Isotopes 32, 137–141.

Briffa, K., Schweingruber, F.H., Jones, P.D., Osborn, T.J., Harris, I.C., Shiyatov, S.G., Vaganov, E.A., Grudd, H., 1998a. Trees tell of past climates: but are they speaking less clearly today?

Philosophical Transactions of the Royal Society of London 353B, 65–73.

Briffa, K., Schweingruber, F.H., Jones, P.D., Osborn, T.J., Shiyatov, S.G., Vaganov, E.A., 1998b. Reduced sensitivity of recent tree-growth to temperature at high northern latitudes. Nature 391, 678–682.

Buchanan, D.L., Corcoran, B.J., 1959. Sealed tube combustion for the determination of carbon-14 and total carbon. Analytical Chemistry 31, 1635–1638.

Buhay, W.M., Edwards, T.W.D., 1995. Climate in southwestern Ontario, Canada, between AD 1610 and 1885 inferred from oxygen and hydrogen isotopic measurements of wood cellulose from trees in different hydrologic settings. Quaternary Research 44, 438–446.

Buhay, W.M., Edwards, T.W.D., Aravena, R., 1996. Evaluating kinetic fractionation factors for ecologic and palaeoclimatic reconstructions from oxygen and hydrogen isotope ratios in plant water and cellulose. Geochimica et Cosmochimica Acta 60, 2209–2218.

Bunton, C.A., Lewis, T.A., Lewellyn, D.R., 1956. Oxygen-18 tracer studies in the formation of reactions of organic peracid. Journal of the Chemical Society, pp. 1226–1230.

Burk, R.L., Stuiver, M., 1981. Oxygen isotope ratios in trees reflect mean annual temperature and humidity. Science 211, 1417–1419.

Cernusak, L.A., Marshall, J.D., Comstock, J.P, Balster, N.J., 2001. Carbon isotope discrimination in photosynthetic bark. Oecologia 128, 24–35.

Coleman, M.L., Shepherd, T.J., Durham, J.J., Rouse, J.E., Moore, G.R., 1982. Reduction of water with zinc for hydrogen isotope analysis. Analytical Chemistry 66, 2989–2991.

Cook, E.R., Briffa, K.R., Meko, D.M., Graybill, D.A., Funkhouser, G., 1995. The 'segment length curse' in long tree-ring chronology development for palaeoclimatic studies. The Holocene 5, 229–237.

Coplen, T.B., 1995. Discontinuance of SMOW and PDB. Nature 373, 285.

Craig, H., 1954. Carbon-13 variations in Sequoia rings and the atmosphere. Science 119, 141–144.

Craig, H., Gordon, L.I., 1965. Deuterium and oxygen-18 variations in the ocean and marine atmospheres. In: Tongiorgi, E. (Ed.), Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Palaeotemperatures. Lischi and Figli, Pisa, Italy, pp. 9–130.

Dansgaard, W., 1964. Stable isotopes in precipitation. Tellus 16, 436–468.

Dawson, T.E., 1993. Water sources of plants as determined from xylem-water isotopic composition: perspectives on plant competition, distribution and water relations. In: Ehleringer, J.R., Happ, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon–Water Relations. Academic Press, New York, pp. 465–496.

Dawson, T.E., Pate, J.S., 1996. Seasonal water uptake and movement in root systems of Australian phraeatophytic plants of dimorphic root morphology: a stable isotope investigation. Oecologia 107, 13–20.

Dawson, T.E., Mambeli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable isotopes in plant ecology. Annual Review of Ecology and Systematics 33, 507–559.

Dongmann, G., Nürnberg, H.W., Förstel, H., Wagener, K., 1974. On the enrichment of $H_2^{18}O$ in leaves of transpiring plants. Radiation, Environment and Biophysiology 11, 41–52.

Dubois, A.D., 1984. On the climatic interpretation of the hydrogen isotope ratios in recent and fossil wood. Bulletin de la Société Belge de Géologie 93, 267–270.

Dunbar, J., Wilson, A.T., 1983. Re-evaluation of the $HgCl_2$ pyrolysis technique for oxygen isotope analysis. International Journal of Applied Radiation and Isotopes 34, 932–934.

Dupouey, J.-L., Leavitt, S.W., Choisnel, E., Jourdain, S., 1993. Modeling carbon isotope fractionation in tree-rings based upon effective evapotranspiration and soil-water status. Plant, Cell and Environment 16, 939–947.

Duquesnay, A., Bréda, N., Stievenard, M., Dupouey, J.L., 1998. Changes of tree-ring $\delta^{13}C$ and water-use efficiency of beech (Fagus sylvatica L.) in north-eastern France during the past century. Plant, Cell and Environment 21, 565–572.

Edwards, T.W.D., Graf, W., Trimborn, P., Stichler, W., Payer, H.D., 2000. $\delta^{13}C$ response surface resolves humidity and temperature signals in trees. Geochimica et Cosmochimica Acta 64, 161–167.

Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), 1993. Stable Isotopes and Plant Carbon–Water Relations. Academic Press, New York, 555pp.

Ehleringer, J.R., Vogel, J.C., 1993. Historical aspects of stable isotopes. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon–Water Relations. Academic Press, New York, pp. 9–18.

Epstein, S., Krishnamurthy, R.V., 1990. Environmental information in the isotopic record in trees. Philosophical Transactions of the Royal Society 330A, 427–439.

Epstein, S., Yapp, C.J., 1976. Climatic implications of the D/H ratio of hydrogen in C–H groups in tree cellulose. Earth and Planetary Science Letters 30, 252–261.

Eronen, M., Zetterberg, P., Briffa, K.R., Lindholm, M., Meriläinen, J., Timonen, M., 2002. The supra-long Scots pine tree-ring record for Finnish Lapland: Part 1, chronology construction and initial inferences. The Holocene 12, 673–680.

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

Farquhar, G.D., Lloyd, J., 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon–Water Relations. Academic Press, New York, pp. 47–70.

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. Australian Journal of Plant Physiology 9, 121–137.

Farquhar, G.D., Henry, B.K., Styles, J.M., 1997. A rapid on-line technique for the determination of oxygen isotope composition of nitrogen containing organic compounds and water. Rapid Communications in Mass Spectrometry 11, 1554–1560.

February, E.C., Stock, W.D., 1999. Declining trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide from tree rings of South African Widdringtonia cedarbergensis. Quaternary Research 52, 229–236.

Feng, X.H., 1998. Long-term c_i/c_a response of trees in western North America to atmospheric CO_2 concentration derived from carbon isotope chronologies. Oecologia 117, 19–25.

Feng, X.H., 1999. Trends in intrinsic water-use efficiency of natural trees for the past 100–200 years: a response to atmospheric CO_2 concentration. Geochimica et Cosmochimica Acta 63, 1891–1903.

Feng, X.H., Epstein, S., 1994. Climatic implications of an 8000 year hydrogen isotope time series from Bristlecone Pine trees. Science 265, 1079–1081.

Feng, X., Epstein, S., 1995a. Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration. Geochimica et Cosmochimica Acta 59, 2599–2608.

Feng, X., Epstein, S., 1995b. Climatic temperature records in δD data from tree rings. Geochimica et Cosmochimica Acta 59, 3029–3037.

Feng, X., Cui, H., Tang, K., Conkey, L.E., 1999. Tree-ring delta-D as an indicator of Asian monsoon intensity. Quaternary Research 51, 262–266.

Ferhi, A., Baraic, T., Jusserand, C., LeTolle, R., 1983. An integrated method for the isotopic analysis of oxygen from organic compounds, air, water vapour and leaf water. International Journal of Applied Radiation and Isotopes 34, 1451–1457.

Field, E.M., 1994. Measurement of stable isotope ratios in tree-rings for climatic reconstruction. M.Phil. Thesis, Anglia Polytechnic University, Cambridge, UK.

Field, E.M., Switsur, V.R., Waterhouse, J.S., 1994. An improved technique for determinations of oxygen stable isotope ratios in wood cellulose. Applied Radiation and Isotopes 45, 177–181.

Francey, R.J., 1981. Tasmanian tree rings belie suggested anthropogenic $^{13}C/^{12}C$ trends. Nature 290, 232–235.

Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M., Langenfelds, R.L., Michel, E., Steele, L.P., 1999. A 1000-year high precision record of $\delta^{13}C$ in atmospheric CO_2. Tellus 51B, 170–193.

Freyer, H.D., 1979a. On the ^{13}C record in tree rings. Part 1. ^{13}C variations in Northern Hemispheric trees during the last 150 years. Tellus 31, 124–137.

Freyer, H.D., 1979b. On the ^{13}C record in tree rings. Part 2. Registration of microenvironmental CO_2 and anomalous pollution effect. Tellus 31, 308–312.

Freyer, H.D., Belacy, N., 1983. $^{12}C/^{13}C$ records in Northern Hemispheric trees during the past 500 years: anthropogenic impact and climatic superpositions. Journal of Geophysical Research 88, 6844–6852.

Gagen, M., McCarroll, D., Edouard, J.-L., 2004. The effect of site conditions on pine tree ring width, density and $\delta^{13}C$ series in a dry sub-Alpine environment. Arctic, Antarctic and Alpine Research, in press.

Gat, J.R., 1980. The isotopes of hydrogen and oxygen in precipitation. In: Fritz, P., Fontes, J.Ch. (Eds.), Handbook of Environmental Isotope Geochemistry. Elsevier, Amsterdam.

Gleixner, G., Schmidt, H.-L., 1998. On-line determination of group specific isotope ratios in model compounds and aquatic humic substances by coupling pyrolysis to a GC-C-IRMS. ACS Symposium Series 707, American Chemical Society, Washington, DC.

Gray, J., Se, J.S., 1984. Climatic implications of the natural variation of D/H ratios in tree-ring cellulose. Earth and Planetary Science Letters 70, 129–138.

Gray, J., Thompson, P., 1976. Climatic information from $^{18}O/^{16}O$ ratios of cellulose in tree-rings. Nature 262, 481–482.

Gray, J., Thompson, P., 1977. Climatic information from $^{18}O/^{16}O$ analysis of cellulose, lignin and wholewood from tree-rings. Nature 270, 708–709.

Gray, J., Yonge, C., Thompson, P., 1984. Simultaneous determination of $^{18}O/^{16}O$ and D/H in compounds containing C, H and O. International Journal of Applied Radiation and Isotopes 6, 525–530.

Green, J.W., 1963. Wood cellulose. In: Whistler, R.L. (Ed.), Methods in Carbohydrate Chemistry III. Academic Press, New York.

Grudd, H., Briffa, K.R., Karlén, W., Bartholin, T.S., Jones, P.D., Kromer, B., 2002. A 7400-year tree-ring chronology in northern Swedish Lapland: natural climatic variability expressed on annual to millenial timescales. The Holocene 12, 657–665.

Hantemirov, R.M., Shiyatov, S.G., 2002. A continuous multimillenial ring-width chronology from Yamal, northwestern Siberia. The Holocene 12, 717–726.

Heaton, T.H.E., Chernery, C.A., 1990. Use of zinc turnings in the reduction of water to hydrogen for isotopic analysis. Analytical Chemistry 55, 995–998.

Helle, G., Schleser, G.H., 2003. Seasonal variations of stable carbon isotopes from tree-rings of *Quercus petraea*. In: Schleser, G., Winiger, M., Bräuning, A., Gärtner, H., Helle, G., Jansma, E., Neuwirth, B., Treydte, K. (Eds.), TRACE: Tree Rings in Archaeology, Climatology and Ecology, Vol. 1. pp. 66–70. Forschungszentrum, Julich

Hemming, D., Fritts, H.C., Leavitt, S.W., Wright, W., Long, A., Shashkin, A., 2001. Modelling tree-ring $\delta^{13}C$. Dendrochronologia 19, 23–38.

Hemming, D.L., Switsur, V.R., Waterhouse, J.S., Heaton, T.H.E., Carter, A.H.C., 1998. Climate and the stable carbon isotope composition of tree ring cellulose: an intercomparison of three tree species. Tellus 50B, 25–32.

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

Huber, C., Leuenberger, M., 2003. Fast on-line high-precision determination of hydrogen isotope ratios of water or ice by continuous-flow isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 17, 1319–1325.

International Atomic Energy Agency (IAEA), 1981. In: Gat, J.R., Gonfiantini, R. (Eds.), Stable Isotope Hydrology, Deuterium and Oxygen-18 in the Water Cycle. Technical Reports Series No. 210, IAEA, Vienna.

Israeli, Y., Schwartz, A., Plaut, Z., Yakir, D., 1996. Effects of light regime on $\delta^{13}C$, photosynthesis and yield of field-grown banana (*Musa* sp. Muscaceae). Plant, Cell and Environment 19, 225–230.

Jackson, P.C., Meinzer, F.C., Goldstein, G., Holbrook, N.M., Cavelier, J., Rada, F., 1993. Environmental and physiological influences on carbon isotope composition of gap and understory plants in a lowland tropical forest. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon–Water Relations. Academic Press, New York, pp. 131–140.

Jalkanen, R., Aalto, T., Kurkela, T., 1998. Revealing past needle density in *Pinus* spp. Scandinavian Journal of Forest Research 13, 292–296.

Jedrysek, M.O., Krapiec, M., Skrzypek, G., Kaluzny, A., Halas, S., 1998. An attempt to calibrate carbon and hydrogen isotope ratios in oak tree rings cellulose: the last millennium. RMZ Materials and Geoenvironment 45, 82–90.

Johnsen, F.H., Flanagan, L.B, Huber, D.A., Major, J.E., 1999. Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in *Picea mariana*: analyses from a half-diallel mating design using field-grown trees. Canadian Journal of Forestry Research 29, 1727–1735.

Kelly, S.D., Parker, L.G., Sharman, M., Dennis, M.J., 1998. On-line quantitative determination of $^{2}H/^{1}H$ isotope ratios in organic and water samples using an elemental analyser coupled to an isotope ratio mass spectrometer. Journal of Mass Spectrometry 33, 735–738.

Kitagawa, H., Matsumoto, E., 1995. Climatic implications of $\delta^{13}C$ variations in a Japanese cedar (*Cryptomeria japonica*) during the last two millenia. Geophysical Research Letters 22, 2155–2158.

Koziet, J., 1997. Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter. Journal of Mass Spectrometry 32, 103–108.

Krishnamurthy, R.V., 1996. Implications of a 400 year tree ring based $^{13}C/^{12}C$ chronology. Geophysical Research Letters 23, 371–374.

Krishnamurthy, R.V., Epstein, S., 1985. Treering D/H ratio from Kenya, East Africa and its palaeoclimatic significance. Nature 317, 160–162.

Kürschner, W.M., 1996. Leaf stomata as biosensors of palaeoatmospheric CO_2 levels. LPP Contribution Series 5, 152.

Lawrence, J.R., White, J.W.C., 1984. Growing season precipitation from D/H ratios of Eastern White Pine. Nature 311, 558–560.

Leavitt, S.W., 1993. Environmental information from $^{13}C/^{12}C$ ratios of wood. Geophysical Monographs 78, 325–331.

Leavitt, S.W., 2002. Prospects for reconstruction of seasonal environment from tree-ring $\delta^{13}C$: baseline findings from the Great Lakes area, USA. Chemical Geology 191, 47–58.

Leavitt, S.W., Danzer, S.R., 1993. Method for batch processing of small wood samples to holocellulose for stable carbon isotope analysis. Analytical Chemistry 65, 87–89.

Leavitt, S.W., Lara, A., 1994. South American tree rings show declining $\delta^{13}C$ trend. Tellus 46B, 152–157.

Leavitt, S.W., Long, A., 1982. Evidence for $^{13}C/^{12}C$ fractionation between tree leaves and wood. Nature 298, 742–744.

Leavitt, S.W., Long, A., 1984. Sampling strategy for stable carbon isotope analysis of tree rings in pine. Nature 311, 145–147.

Leavitt, S.W., Long, A., 1985. An atmospheric $^{13}C/^{12}C$ reconstruction generated through removal of climate effects from tree ring $^{13}C/^{12}C$ measurements. Tellus 35B, 92–102.

Leavitt, S.W., Long, A., 1991. Seasonal stable-carbon isotope variability in tree rings: possible palaeoenvironmental signals. Chemical Geology 87, 59–70.

Leavitt, S.W., Newberry, T., 1992. Systematics of stable-carbon isotopic differences between gymnosperm and angiosperm trees. Plant Physiology 11, 257–262.

Leavitt, S.W., Idso, S.B., Kimball, B.A., Burns, J.K., Sinha, A., Stott, L., 2003. The effect of long-term atmospheric CO_2 enrichment on the intrinsic water-use efficiency of sour orange trees. Chemosphere 50, 217–222.

Leegood, R.C., Edwards, G.E., 1996. Carbon metabolism and photorespiration: temperature dependence in relation to other environmental factors. In: Baker, N.R. (Ed.), Photosynthesis and the Environment. Kluwer Academic, Dordrecht, pp. 191–221.

Leuenberger, M., Borella, S., Stocker, T., Saurer, M., Siegwolf, R., Schweingruber, F., Matyssek, R., 1998. Stable isotopes in tree-rings as climate and stress indicators. Final Report NRP 31, VDF, ETH Zurich.

Leuschner, H.H., Saas-Klaassen, U., Jansma, E., Baillie, M.G.L., Spurk, M., 2002. Subfossil European bog oaks: population dynamics and long-term growth depressions as indicators of changes in the Holocene hydro-regime and climate. The Holocene 12, 695–706.

Libby, L.M., Pandolfi, L.J., 1974. Temperature dependence of, isotope ratios in tree rings. Proceedings of the National Academy of Science 71, 2482–2486.

Libby, L.M., Pandolfi, L.J., Payton, P.H., Marshall III, J., Becker, B., Giertz-Siebenlist, V., 1976. Isotopic tree thermometers. Nature 261, 284–290.

Lipp, J., Trimborn, P., 1991. Long-term records and basic principles of tree-ring isotope data with emphasis on local environmental conditions. Paläoklimaforschung 6, 105–117.

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

Liu, Y., Wu, X., Leavitt, S.W., Hughes, M.K., 1996. Stable carbon isotope in tree rings from Huangling, China and climatic variation. Science in China D(39) 2, 152–161.

Loader, N.J., Buhay, W.M., 1999. Rapid catalytic oxidation of CO to CO_2—on the development of a new approach to on-line oxygen isotope analysis of organic matter. Rapid Communications in Mass Spectrometry 13, 1828–1832.

Loader, N.J., Switsur, V.R., 1996. Reconstructing past environmental change using stable isotopes in tree-rings. Botanical Journal of Scotland 48, 65–78.

Loader, N.J., Switsur, V.R., Field, E.M., 1995. High-resolution stable isotope analysis of tree-rings: implications of 'microdendroclimatology' for palaeoenvironmental research. The Holocene 5, 457–460.

Loader, N.J., Robertson, I., Barker, A.C., Switsur, V.R., Waterhouse, J.S., 1997. A modified method for the batch processing of small whole wood samples to α-cellulose. Chemical Geology 136, 313–317.

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. Palaeogeography, Palaeoclimatology, Palaeoecology, 196, 395–407.

Luomala, E.-M., Laitinen, K., Kellomäki, S., Vapaavuori, 2003. Variable photosynthetic acclimation in consecutive cohorts of Scots pine needles during 3 years of growth at elevated CO_2 and elevated temperature. Plant, cell and environment 26, 645–660.

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

Matthews, D.E., Hayes, J.M., 1978. Isotope-ratio-monitoring gas chromatography-mass spectrometry. Analytical Chemistry 50, 1465–1473.

Mayr, C., Frenzel, B., Friedrich, M., Spurk, M., Stichler, W., Trimborn, P., 2003. Stable carbon-and hydrogen-isotope ratios of subfossil oaks in southern Germany: methodology and application to a composite record for the Holocene. The Holocene 13, 393–402.

McCarroll, D., Pawellek, F., 1998. Stable carbon isotope ratios of latewood cellulose in *Pinus sylvestris* from northern Finland: variability and signal strength. The Holocene 8, 693–702.

McCarroll, D., Pawellek, F., 2001. Stable carbon isotope ratios of *Pinus sylvestris* from northern Finland and the potential for extracting a climate signal from long Fennoscandian chronologies. The Holocene 11, 517–526.

McCarroll, D., Jalkanen, R., Hicks, S., Tuovinen, M., Pawellek, F., Gagen, M., Eckstein, D., Schmitt, U., Autio, J., Heikkinen, O., 2003. Multi-proxy dendroclimatology: a pilot study in northern Finland. The Holocene, 13, 829–838.

McCormac, F.G., Baillie, M.G.L., Pilcher, J.R., Brown, D.M., Hoper, S.T., 1994. $\delta^{13}C$ measurement from the Irish oak chronology. Radiocarbon 36, 27–35.

McDowell, N., Phillips, N., Lunch, C., Bond, B.J., Ryan, M.G., 2002. An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. Tree Physiology 22, 763–774.

McDowell, N., Brooks, J.R., Fitzgerald, S.A., Bond, B., 2003. Carbon isotope discriminations and growth response of old *pinus ponderosa* trees to stand density reductions. Plant, Cell and Environment, 26, 631–644.

van der Merwe, N.J., Medina, E., 1991. The canopy effect, carbon isotope ratios and food webs in Amazonia. Journal of Archaeological Science 18, 249–259.

Monserud, R.A., Marshall, J.D., 2001. Time-series analysis of $\delta^{13}C$ from tree rings. I. Time trends and autocorrelation. Tree Physiology 21, 1087–1102.

Motz, J.E., Edwards, T.W.D., Buhay, W.M., 1997. Use of nickel-tube pyrolysis for hydrogen-isotope analysis of water and other compounds. Chemical Geology 140, 145–149.

Mullane, M.V., Waterhouse, J.S., Switsur, V.R., 1988. On the development of a novel technique for the determination of stable oxygen isotope ratios in cellulose. Applied Radiation and Isotopes 10, 1029–1035.

Northfelt, D.W., DeNiro, M.J., Epstein, S., 1981. Hydrogen and carbon isotopic ratios of cellulose nitrate and saponifiable lipid fractions prepared from annual growth rings of a Californian redwood. Geochimica et Cosmochimica Acta 45, 1895–1898.

Ogle, N., McCormac, F.G., 1994. High resolution $\delta^{13}C$ measurements of oak show a previously unobserved spring depletion. Geophysical Research Letters 21, 12373–12375.

Okada, N., Fujiwara, T., Ohta, S., Matsumoto, E., 1995. Stable carbon isotopes of *Chamaecyparis obtusa* grown at a high altitude

region in Japan: within and among-tree variations. In: Ohta, S., Fujii, T., Okada, N., Hughes, M.K., Eckstein, D. (Eds.), Tree-Rings: From the Past to the Future. Proceedings of the International Workshop on Asian and Pacific Dendrochronology. Forestry and Forest Products Research Institute Scientific Meeting Report 1, pp. 165–169.

Pearman, G.I., Francey, R.J., Fraser, P.J.B., 1976. Climatic implications of stable carbon isotopes in tree-rings. Nature 260, 771–773.

Pendall, E., 2000. Influence of precipitation seasonality on Piñon pine cellulose δD values. Global Change Biology 6, 287–301.

Picon, C., Guehl, J.M., Aussenac, G., 1996. Growth dynamics, transpiration and water-use efficiency in *Quercus robur* plants submitted to elevated CO_2 and drought. Annales des Sciences Forestieres 53, 431–446.

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.

Ponton, S., Dupouey, J-L., Bréda, N., Feuillat, F., Bodénès, C., Dreyer, E., 2001. Carbon isotope discrimination and wood anatomy variations in mixed stands of *Quercus robur* and *Quercus petraea*. Plant, Cell and Environment 24, 861–868.

Ponton, S., Dupouey, J.-L., Bréda, N., Dreyer, E., 2002. Comparison of water-use efficiency of seedlings from two sympatric oak species: genotype × environment interactions. Tree Physiology 22, 413–422.

Porté, A., Loustau, D., 2001. Seasonal and interannual variations in carbon isotope discrimination in a maritime pine (*Pinus pinaster*) stand assessed from isotopic composition of cellulose in annual rings. Tree Physiology 21, 861–868.

Preston, T., Owens, N.J.P., 1983. Interfacing an automatic elemental analyzer with an isotope ratio mass-spectrometer—the potential for fully automated total nitrogen and ^{15}N analysis. Analyst 108, 971–977.

Preston, T., Owens, N.J.P., 1985. Preliminary ^{13}C measurement using a gas chromatograph interfaced to an isotope ratio mass spectrometer. Biomedical Mass spectrometry 12, 510–513.

Prosser, S.J., Scrimgeour, C.M., 1995. High precision determination of $^2H/^1H$ in H_2 and H_2O by continuous flow isotope ratio mass spectrometry. Analytical Chemistry 34, 1992–1997.

Ramesh, R., Bhattacharya, S.K., Gopalan, K., 1985. Dendroclimatological implications of isotope coherence in trees from Kashmir Valley, India. Nature 317, 802–804.

Ramesh, R., Bhattacharya, S.K., Gopalan, K., 1986. Climatic correlations in the stable isotope records of silver fir (*Abies pindrow*) tree from Kashmir, India. Earth and Planetary Science Letters 79, 66–74.

Rittenberg, D., Ponticorvo, L., 1956. A method for the determination of the [18]O concentration of the oxygen of organic compounds. International Journal of Applied Radiation and Isotopes 1, 208–214.

Robertson, A., Overpeck, J., Rind, D., Mosley-Thompson, E., Zielinski, G., Lean, J., Koch, D., Penner, J., Tegen, I., Healy, R., 2001. Hypothesized climate forcing time series for the last 500 years. Journal of Geophysical Research 106, 14783–14803 (ftp://ftp.ngdc.noaa.gov/paleo/climate_forcing/robertson2001/trace_gas.txt).

Robertson, I., Field, E.M., Heaton, T.H.E., Pilcher, J.R., Pollard, M., Switsur, V.R., Waterhouse, J.S., 1995. Isotope coherence in oak cellulose. Paläoklimaforschung 15, 141–155.

Robertson, I., Pollard, A.M., Heaton, T.H.E., Pilcher, J.R., 1996. Seasonal changes in the isotopic composition of oak cellulose. In: Dean, J.S., Meko, D.M., Swetnam, T.W. (Eds.), Tree Rings, Environment and Humanity. Radiocarbon, 617–628. Radiocarbon, Arizona.

Robertson, I., Rolfe, J., Switsur, V.R., Carter, A.H.C., Hall, M.A., Barker, A.C., Waterhouse, J.S., 1997. Signal strength and climate relationships in $^{13}C/^{12}C$ ratios of tree ring cellulose from oak in southwest Finland. Geophysical Research Letters 24, 1487–1490.

Robertson, I., Switsur, V.R., Carter, A.H.C., Barker, A.C., Waterhouse, J.S., Briffa, K.R., Jones, P.D., 1997a. Signal strength and climate relationships in $^{13}C/^{12}C$ ratios of tree ring cellulose from oak in east England. Journal of Geophysical Research 102, 19507–19519.

Robertson, I., Waterhouse, J.S., Barker, A.C., Carter, A.H.C., Switsur, V.R., 2001b. Oxygen isotope ratios of oak in east England: implications for reconstructing the isotopic composition of precipitation. Earth and Planetary Science Letters 191, 21–31.

Roden, J.S., Lin, G., Ehleringer, J.R., 2000. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. Geochimica et Cosmochimica Acta 64, 21–35.

Ryan, M.G., Yoder, B.J., 1997. Hydraulic limits to tree height and tree growth. Bioscience 47, 235–242.

Santrock, J., Hayes, J.M., 1987. Adaptation of the Unterzaucher procedure for determination of oxygen-18 in organic substances. Analytical Chemistry 59, 119–127.

Sauer, P.E., Sternberg, L.D.S.L., 1994. Improved method for the determination of the oxygen isotopic composition of cellulose. Analytical Chemistry 66, 2409–2411.

Saurer, M., Siegenthaler, U., 1989. $^{13}C/^{12}C$ isotope ratios in tree rings are sensitive to relative humidity. Dendrochronologia 7, 9–13.

Saurer, M., Siegenthaler, U., Schweingruber, F., 1995. The climate–carbon isotope relationship in tree rings and the significance of site conditions. Tellus 47B, 320–330.

Saurer, M., Borella, S., Schweingruber, F., Siegwolf, R., 1997. Stable carbon isotopes in tree rings of beech: climatic versus site-related influences. Trees 11, 291–297.

Saurer, M., Siegwolf, R., Borella, S., Schweingruber, F., 1998. Environmental information from stable isotopes in tree rings of *Fagus sylvatica*. In: Beniston, M., Innes, J.L. (Eds.), The Impacts of Climate Variability on Forests. Springer, Berlin, pp. 241–253.

Saurer, M., Robertson, I., Siegwolf, R., Leuenberger, M., 1998a. Oxygen isotope analysis of cellulose: an interlaboratory comparison. Analytical Chemistry 70, 2074–2080.

Saurer, M., Schweingruber, F.H., Vaganov, E.A., Shiyatov, S.G., Siegwolf, R., 2002b. Spatial and temporal oxygen isotope trends at northern tree-line Eurasia. Geophysical Research Letters 29, 10–14.

Schäfer, K.V.R., Oren, R., Tenhunen, J.D., 2000. The effect of tree height on crown level stomatal conductance. Plant, Cell and Environment 23, 365–375.

Schiegl, W.E., 1974. Climatic significance of deuterium abundance in growth rings of *Picea*. Nature 251, 582–584.

Schimmellman, A., 1991. Determination of the concentration and stable isotopic composition of non-exchangeable hydrogen in organic matter. Analytical Chemistry 63, 2456–2459.

Schleser, G.H., Jayasekera, R., 1985. δ13C variations in leaves of a forest as an indication of reassimilated CO_2 from the soil. Oecologia 65, 536–542.

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

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

Sheu, D., Chiu, C., 1995b. Evaluation of cellulose extraction procedures for stable carbon isotope measurements in tree ring research. International Journal of Environmental Analytical Chemistry 59, 59–67.

Sheu, D.D., Kou, P., Chiu, C.-H., Chen, M.-J., 1996. Variability of tree-ring δ13C in Taiwan fir: growth effect and response to

May–October temperatures. Geochimica et Cosmochimica Acta 60, 171–177.

Sofer, Z., 1980. Preparation of carbon dioxide for stable carbon isotope analysis of petroleum fractions. Analytical Chemistry 52, 1389–1391.

Sonninen, E., Jungner, H., 1995. Stable carbon isotopes in tree-rings of a Scots pine (*Pinus sylvestris* L.) from northern Finland. Paläoklimaforschung 15, 121–128.

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

Spurk, M., Leuschner, H.H., Baillie, M.G.L., Briffa, K.R., Friedrich, M., 2002. Depositional frequency of German subfossil oaks: climatically and non-climatically induced fluctuations in the Holocene. The Holocene 12, 707–715.

Sternberg, L., De Niro, M., Savidge, R., 1986. Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. Plant Physiology 82, 423–427.

Stewart, G.R., Turnbull, M.H., Shmidt, S., Erskine, P.D., 1995. ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. Australian Journal of Plant Physiology 22, 51–55.

Stuiver, M., 1978. Atmospheric carbon dioxide and carbon reservoir changes. Science 199, 253–258.

Stuiver, M., Braziunas, T.F., 1987. Tree cellulose ^{13}C/^{12}C isotope ratios and climate change. Nature 328, 58–60.

Stuiver, M., Reimer, P.J., 1993. Extended ^{14}C data base and revised CALIB 3.0 ^{14}C age calibration program. Radiocarbon 35, 215–230.

Stump, R.K., Frazer, J.W., 1973. Simultaneous determination of carbon, hydrogen and nitrogen in organic compounds. Nuclear Science Abstracts 28, 746.

Suberkropp, K., Klug, M.J., 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57, 707–719.

Suess, H.E., 1970. Bristlecone-pine calibration of the radiocarbon time-scale 5,000 BC to present. In: Olsson, I.U. (Ed.), Radiocarbon Variations and Absolute Chronology. Wiley, Chichester and New York, pp. 303–311.

Switsur, V.R., Waterhouse, J.S., Field, E.M.F., Carter, A.H.C., Hall, M., Pollard, M., Robertson, I., Pilcher, J.R., Heaton, T.H.E., 1994. Stable isotope studies of oak from the English Fenland and Northern Ireland. In: Funnell, B.M., Kay, R.L.F. (Eds.), Palaeoclimate of the Last Glacial/Interglacial Cycle. Natural Environment Research Council Special Publication 94/2, 67–73.

Switsur, V.R., Waterhouse, J.S., Field, E.M., Carter, A.H.C., Loader, N.J., 1995. Stable isotope studies in tree rings from oak—techniques and some preliminary results. Paläoklimaforschung 15, 129–140.

Switsur, V.R., Waterhouse, J.S., Field, E.M., Carter, A.H.C., 1996. Climatic signals from stable isotopes in oak tree-rings from East Anglia, Great Britain. In: Dean, J.S., Meko, D.,M., Swetnam, T.W. (Eds.), Tree Rings, Environment and Humanity Radiocarbon, pp. 637–645. Radiocarbon, Arizona.

Tang, K., Feng, X., Funkhouser, G., 1999. The δ^{13}C of tree rings in full-bark and strip-bark bristle cone pine trees in the White Mountains of California. Global Change Biology 5, 33–40.

Tang, K., Feng, X., Ettle, G.J., 2000. The variations in δD of tree rings and the implications for climatic reconstruction. Geochimica et Cosmochimica Acta 64, 1663–1673.

Tans, P., Mook, W.G., 1980. Past atmospheric CO_2 levels and the ^{13}C/^{12}C ratios in tree rings. Tellus 32, 268–283.

Taylor, J.W., Chen, I., 1970. Variables in oxygen-18 analyses by mass spectrometry. Analytical Chemistry 42, 224.

Thompson, P., Gray, J., 1977. Determination of the ^{18}O/^{16}O ratios in compounds containing C, H and O. International Journal of Applied Radiation and Isotopes 28, 411.

Tobias, H.J., Brenna, J.T., 1996. High precision D/H measurement from organic mixtures by gas chromatography continuous flow isotope ratio mass spectrometry using a palladium filter. Analytical Chemistry 68, 3002–3007.

Tobias, H.J., Goodman, .K.J., Blacken, C.E., Brenna, J.T., 1995. High precision D/H measurement from hydrogen gas and water by continuous flow isotope ratio mass spectrometry. Analytical Chemistry 67, 2486–2492.

Treydte, K., Schleser, G.H., Schweingruber, F.H., Winiger, M., 2001. The climatic significance of δ^{13}C in subalpine spruces (Lötschental, Swiss Alps). Tellus 53B, 593–611.

Van de Water, P.K., 2002. The effect of chemical processing on the δ^{13}C value of plant tissue. Geochimica et Cosmochimica Acta 66, 1211–1219.

Van de Water, P.K., Leavitt, S.W., Betancourt, J.L., 2002. Leaf δ^{13}C variability with elevation, slope aspect, and precipitation in the southwest United States. Oecologia 132, 332–343.

Walcroft, A.S., Silvester, W.B., Grace, J.C., Carson, S.D., Waring, R.H., 1996. Effects of branch length on carbon isotope discrimination in *Pinus radiata*. Tree Physiology 16, 281–286.

Walcroft, A.S., Silvester, W.B., Whitehead, D., Kelliher, F.M., 1997. Seasonal changes in stable carbon isotope ratios within annual rings of *Pinus radiata* reflect environmental regulation of growth processes. Australian Journal of Plant Physiology 24, 57–68.

Warren, C.R., McGrath, J.F., Adams, M.A., 2001. Water availability and carbon isotope discrimination in conifers. Oecologia 127, 476–486.

Waterhouse, J.S., Barker, A.C., Carter, A.H.C., Agafonov, L.I., Loader, N.J., 2000. Stable carbon isotopes in Scots pine tree rings preserve a record of the flow of the river Ob. Geophysical Research Letters 27, 3529–3532.

Waterhouse, J.S., Switsur, V.R., Barker, A.C., Carter, A.H.C., Robertson, I., 2002. Oxygen and hydrogen isotope ratios in tree rings: how well do models predict observed values? Earth and Planetary Science Letters 201, 421–430.

Waterhouse, J.S., Switsur, V.R., Barker, A.C., Carter, A.H.C., Hemming, D.L., Loader, N.J., Robertson, I., 2004. Northern European trees show diminishing response to enhanced atmospheric carbon dioxide concentrations. Quaternary Science Reviews, this issue (doi:10.1016/j.quascirev.2003.06.011).

Werner, R.A., Kornexl, B.E., Roßmann, A., Schmidt, H.-L., 1996. On-line determination of δ^{18}O values of organic substances. Analitica Chimica Acta 319, 159–164.

Wershaw, R.L., Friedman, I., Heller, S.J., 1966. Hydrogen isotope fractionation in water passing through trees. In: Hobson, F., Speers, M. (Eds.), Advances in Organic Geochemistry. Pergamon, New York, pp. 55–67.

Wilson, A.T., Grinsted, M.J., 1975. Palaeotemperatures from tree rings and the D/H ratio of cellulose as a biochemical thermometer. Nature 257, 387–389.

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

Wong, W.W., Klein, P.D., 1986. A review of techniques for the preparation of biological samples for mass-spectrometric measurements of hydrogen-2/hydrogen-1 and oxygen-18/oxygen-16 isotope ratios. Mass Spectrometry Reviews 5, 313–342.

Wong, W.W., Lee, L.S., Klein, P.D., 1987. Oxygen isotope ratio measurements on carbon dioxide generated by reaction of microlitre quantities of biological fluids with guanidine hydrochloride. Analytical Chemistry 59, 690–693.

Yakir, D., 1992. Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant, Cell and Environment 15, 1005–1020.

Yapp, C.J., Epstein, S., 1982. A re-examination of cellulose carbon-bound hydrogen δD measurements and some factors affecting plant–water D/H relationships. Geochimica et Cosmochimica Acta 46, 955–965.

Zhang, J., Marshall, J.D., Jaquish, B.C., 1993. Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. Oecologia 93, 80–87.

Zimmerman, J.K., Ehleringer, J.R., 1990. Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid *Catasetum viridiflavum*. Oecologia 83, 247–249.

Zimmermann, B., Schleser, G.H., Brauning, A., 1997. Preliminary results of a Tibetan stable C-isotope chronology dating from 1200 to 1994. Isotopes in Environmental and Health Studies 33, 157–165.