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Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings

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Abstract

The stable carbon isotope ratios ($\delta^{13}C$) of whole wood, cellulose and acid-insoluble lignin from annual latewood increments of *Quercus robur* L., from modern and sub-fossil wood, were measured and their potential use as palaeoenvironmental indicators examined. The resulting time series demonstrate a very high degree of coherence, with $\delta^{13}C$ of cellulose isotopically enriched by approximately 3‰ compared to $\delta^{13}C$ of lignin. The $\delta^{13}C$ values of all three components are influenced by the climate of July and August. Modern whole wood retains the strongest climate signal, perhaps because its composition is closest to that of leaf sugars. In sub-fossil wood there is no evidence that differential decay leads to fractionation of carbon within either cellulose or lignin, but differential decay can alter the cellulose to lignin ratio.

Keywords: stable carbon isotope; climate; tree-ring; dendroclimatology; cellulose; lignin

1. Introduction

Instrumental climatic records are short, rarely extending back beyond the last century, so to evaluate numerical models of climate change, establish levels of 'baseline variability' and quantify the past frequency of extreme events requires natural archives of palaeoclimate information. Long tree-ring chronologies (e.g. Becker et al., 1985; Pilcher et al., 1984; Zetterberg et al., 1996; Lindholm et al., 1999; Briffa, 2000; Lindholm and Eronen, 2000) provide unrivalled terrestrial archives of environmental history because they are absolutely dated, provide annual resolution and extend back continuously for millennia.

There are a variety of ways in which climate information can be extracted from the tree-ring archives, the simplest being to measure the width of the rings. However, ring width tends to vary markedly between trees and between sites, and there are long-term trends that relate to disturbance events and to tree age that are difficult to extract without degrading the climate signal (Cook et al., 1995; Briffa et al., 1990, 1992). Extracting climate information from ring widths,

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therefore, usually requires very large samples, and the climate signal that remains often relates to the high-frequency, inter-annual fluctuations rather than the lower-frequency climatic changes of primary interest. In conifers the relative density of rings may provide a stronger climate signal, which can retain more of the low-frequency variability, but density appears to provide no additional climatic information for hardwoods such as oak (Schweingruber and Briffa, 1996).

Several studies have demonstrated the potential of tree-ring stable isotope ratios for high-resolution climatic reconstruction, using a variety of both hardwood and softwood species (McCarroll and Pawellek, 2001; Hemming et al., 1998; Robertson et al., 1997; Loader and Switsur, 1996). The ratio of the stable isotopes of carbon, in particular, appears to provide a strong proxy indicator of past climate that requires fewer trees than the conventional 'growth proxies', such as ring widths and density, and is less influenced by differences between trees and between sites. Stable carbon isotope ratios also have the advantage that the mechanisms responsible for fractionation are well understood, so that there is potential for mechanistic modeling of the effects of climate, as well as for more conventional approaches based on correlation and statistical inference.

Early work on stable isotope ratios in tree-rings used whole wood (Craig, 1954; Libby et al., 1976; Farmer and Baxter, 1974), 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. It has recently been argued, in light of the considerable time required for preparation of cellulose, that if whole wood contains the same isotopic record as cellulose then analysis of whole wood may be acceptable (Barbour et al., 2001; Leuenberger et al., 1998).

A potential problem with using either whole wood or cellulose to extract climate information from long sub-fossil tree-ring chronologies, however, is differential degradation of the wood components over time. Under both aerobic and anaerobic conditions, the polysaccharide components of vascular plants (mainly cellulose and hemicelluloses) tend to degrade more quickly than the lignin component (Schleser et al., 1999; Benner et al., 1991; Spiker and Hatcher, 1987; Suberkropp and Klug, 1976). If there are variations in the cellulose to lignin ratio that are related to degradation, then this may impart a low-frequency signal in whole wood stable carbon isotope ratios that is unrelated to climate. It is also uncertain whether partial decay results in a change in the isotopic signature of cellulose, which would also degrade the palaeoclimate signal. A potential solution to these problems would be to analyse the stable carbon isotope ratios of lignin.

Compound-specific lignin biomarkers have been used to reconstruct palaeovegetation dynamics (Goñi and Eglinton, 1996), but there has been little research into the $\delta^{13}C$ values of tree-ring lignin. Lignin is a compound produced by trees through secondary metabolic processes to provide strength to their cellular structure and in certain cases as a response to damage, nutrient stress or disease (Okuyama et al., 1998; Gindl et al., 2000). In one of a limited number of early studies, Gray and Thompson (1976) concluded that whilst an environmental signal was contained within the cellulose fraction of *Picea glauca* tree-rings, owing to difficulties of carrying out direct lignin $\delta^{18}O$ analysis it did not represent an alternative proxy of palaeoclimatic change. Wilson and Grinsted (1977) later found that although offset by approximately 3‰, $\delta^{13}C$ values of lignin demonstrated similar general trends to the $\delta^{13}C$ trends of cellulose analysed across two annual rings of *Pinus radiata*. They identified a significant time lag between the two trends and proposed that this represented differences in the period of cellulose deposition and lignification.

More recently, Turney et al. (1999) were able to demonstrate a link between the $\delta^{13}C$ of cladodes lignin, sampled across a network of sites, and vapour pressure deficit. Barbour et al. (2001) examined the oxygen isotope composition of cellulose, lignin and whole wood samples collected across a wide geographical area. Both *Pinus* and *Quercus* species exhibited positive correlations with modeled source waters and demonstrated that for both lignin *and* cellulose some re-exchange of oxy-

gen occurred during biosynthesis. A close correlation between cellulose, lignin and whole wood was observed, and the necessity for extraction of individual components from whole wood for isotopic analysis of plant macrofossils discussed. Their data suggested that no climate information was lost when analyzing whole wood 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.

This paper presents results of a pilot study into the nature of stable carbon isotope variability in the lignin, cellulose and whole wood fractions of annually resolved latewood sequences of oak (*Quercus robur* L.) from a site in eastern England. The aims of this pilot study were to:

(1) quantify the difference in $\delta^{13}C$ values obtained from whole wood and its main constituents cellulose and lignin;

(2) determine whether lignin $\delta^{13}C$ values are as strongly related to climate as those obtained from whole wood and cellulose;

(3) investigate whether the climate signal retained by lignin $\delta^{13}C$ relates to the same part of the growing season as the signal contained in the cellulose, or whether there is a temporal offset; and

(4) compare results from modern oak trees with those from a sample of sub-fossil (c. 4350 years old) bog oak from the same area, to see whether differential decay of the wood constituents might degrade the climate signal retained by $\delta^{13}C$ values obtained from whole wood, cellulose or lignin.

2. Methods

Samples of *Quercus robur* L. were collected from two trees growing near Sandringham Park, East Anglia, UK (52°50′N, 0°30′E). The site is in close proximity to several long-standing meteorological stations and experiences a climate typical of the southeastern UK (1951–1980 mean annual temperature 9.5°C; mean July temperature, 15.9°C; mean total annual precipitation, 693 mm; mean total July precipitation, 60 mm; mean annual relative humidity, 74.1%; mean July relative humidity, 66.4%).

Cores were collected using 12 mm diameter increment borers. Each tree-ring sequence was absolutely dated against local site and regional dendrochronologies using the TSAP software package (Frank Rinn Associates, Heidelberg, Germany) and a 55-year sequence (1946–2000) isolated for isotopic analysis. During core extraction, preparation and cross-dating no lubricants, polishes or pencil marks were used which might influence the isotopic composition. A 20-year sequence of rings from a bog oak specimen from the British Isles oak chronology (Pilcher et al., 1984), which grew in close proximity to the present study site, was also obtained for this study. This sample was dated absolutely by dendrochronology, and covered the period 2340–2361 BC.

Since earlywood $\delta^{13}C$ values are influenced by the climate of the previous year (Hill et al., 1995), latewood samples were removed from each sequence using a chiropodic raspe.

Table 1
Correlation matrix (R) demonstrating the statistical relationship between individual wood components of the core samples analysed

	SP07 Cellulose	**SP07** Whole wood	**SP10** Lignin	**SP10** Cellulose	**SP10** Whole wood
SP07 Lignin	0.936**	0.966**	0.606**	0.475**	0.482**
	SP07 Cellulose	0.981**	0.513**	0.387**	0.373**
		SP07 Whole wood	0.537**	0.407**	0.395**
			SP10 Lignin	0.938**	0.870**
				SP10 Cellulose	0.876**
	FEN Cellulose	**FEN** Whole wood			
FEN Lignin	0.836**	0.893**			
FEN Cellulose		0.965**			

**$P < 0.01$ (two-tailed test).

Cellulose was isolated from the resulting homogeneous whole wood powder using techniques modified for small samples by Loader et al. (1997) and 'Klason' lignin (produced with the minimum degree of modification) isolated by carbohydrate dissolution with 72% sulphuric acid (TAPPI, 1988). Each component was dried thoroughly prior to isotopic analysis.

Whole wood, cellulose and lignin stable carbon isotope ratios for the modern (SP07 and SP10) and archaeological (FEN) samples were determined using continuous flow stable isotope mass spectrometry techniques at the Environmental Dynamics Institute, University of Wales Swansea. Results are expressed using the conventional δ notation as ‰ deviations from the Vienna Pee

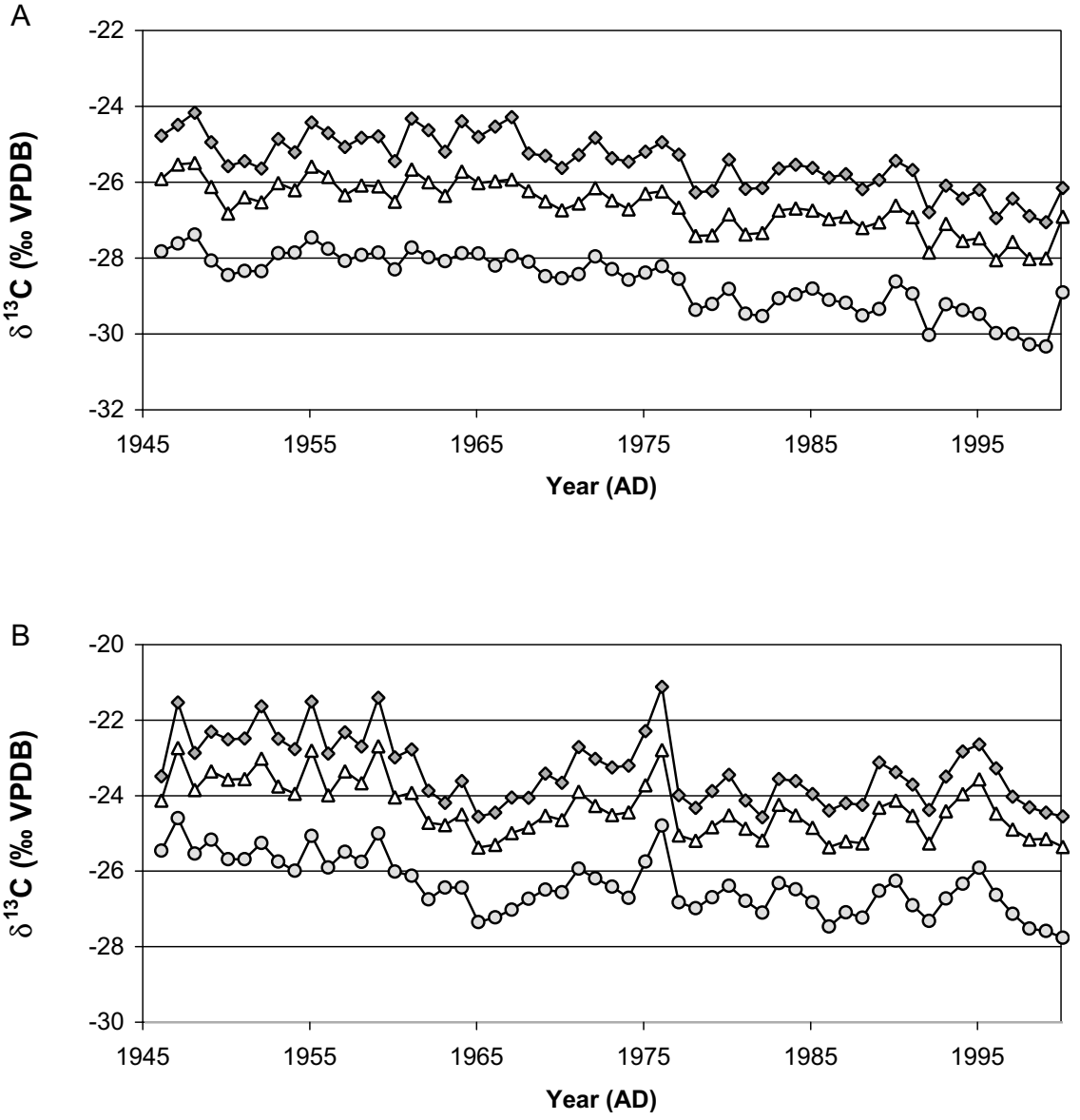


Fig. 1. Stable carbon isotope time series 1946–2000 for the tree sequences (A) SP07, (B) SP10. Cellulose, grey rhomboids; whole wood, white triangles; lignin, grey circles.

Dee belemnite (VPDB) standard (Coplen, 1995; Craig, 1957).

3. Results and discussion

3.1. The nature of the tree-ring lignin signal

For each tree there is a very strong correspondence but consistent offset between the $\delta^{13}C$ values of the individual wood constituents (Table 1, Fig. 1). The whole wood results were expected to follow those of the cellulose and lignin, since they are its principal components (usually c. 90% C in oak; Leuenberger et al., 1998; Borella et al., 1998), but the very close correspondence of the cellulose and lignin curves is surprising.

For the modern trees, the mean offset between

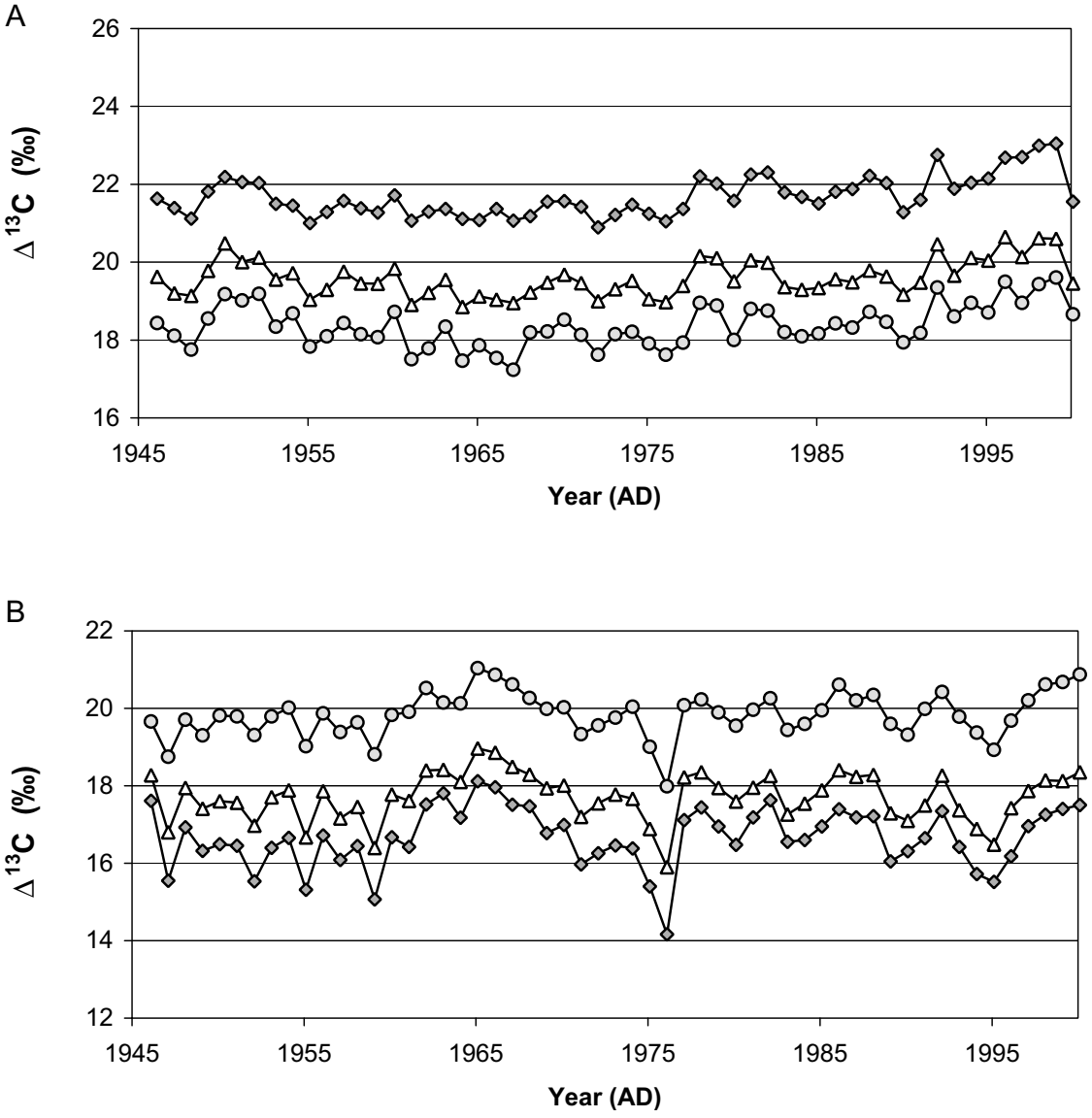
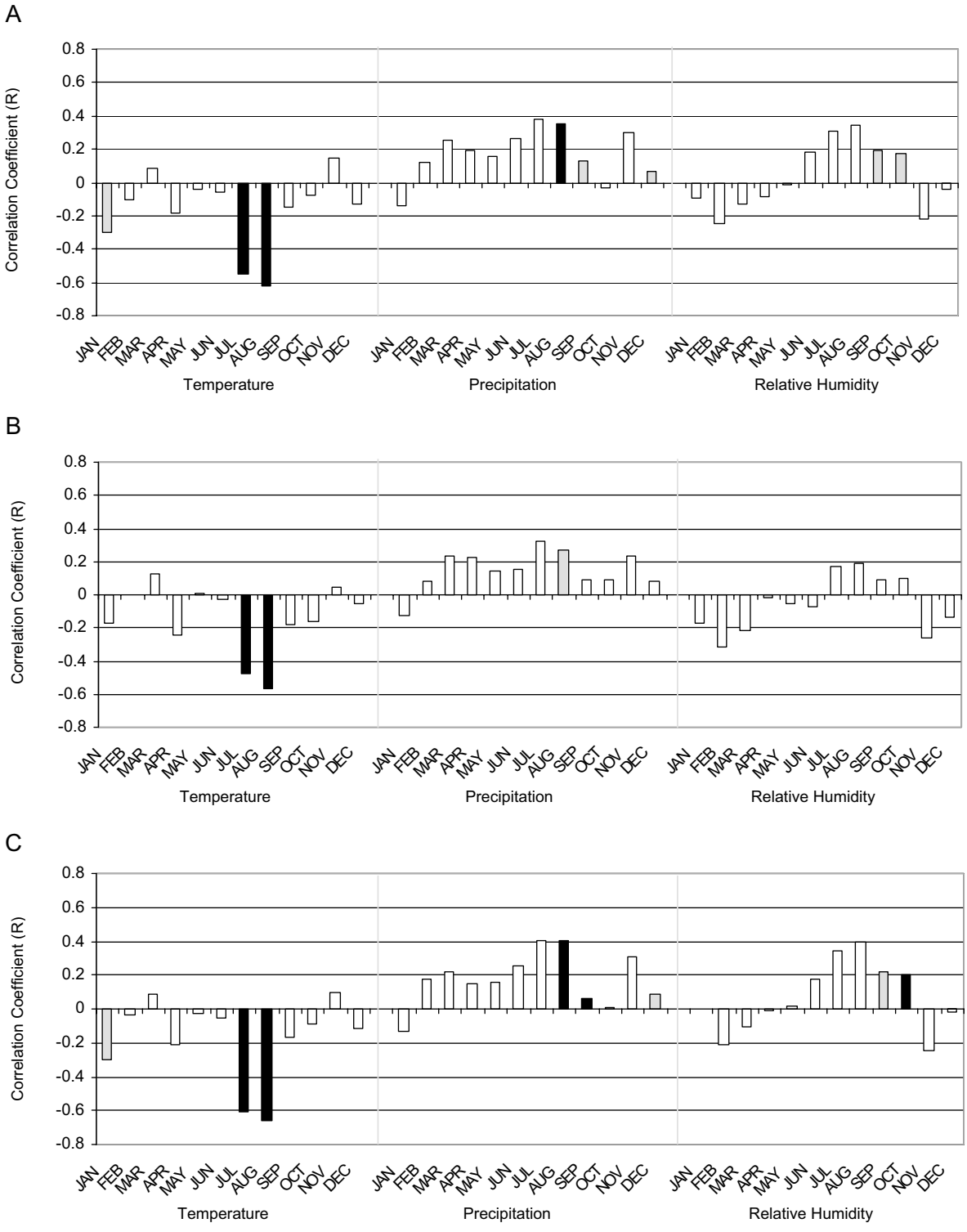


Fig. 2. Discrimination ($\Delta^{13}C$) for cellulose, whole wood and lignin series (A) SP07, (B) SP10. Cellulose, grey rhomboids; whole wood, white triangles; lignin, grey circles.


A
B
C
Correlation Coefficient (R)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
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Temperature
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lignin and cellulose is approximately 3‰ ($\sigma_{n-1} = 0.3$, two sequences), which agrees with earlier estimates (Wilson and Grinsted, 1977). An overall decrease of approximately 1.2‰ occurs between 1946 and 2000, reflecting changes in the local carbon isotope ratio of atmospheric CO_2 as a consequence of industrialisation (Freyer and Belacy, 1983). The isotopic discrimination for each component of the modern sequences was then calculated using Eq. 1 prior to their inclusion into mean series and correlation with climate variables (Fig. 2A,B).

$$\Delta^{13}C_P = \frac{\delta^{13}C_{ATM} - \delta^{13}C_P}{1 + (\delta^{13}C_P/1000)} \quad (1)$$

where $\Delta^{13}C_P$ is the discrimination during photosynthesis (‰ VPDB), $\delta^{13}C_{ATM}$ is the carbon isotopic composition of the atmosphere (after Hemming, 1999) and $\delta^{13}C_P$ is the carbon isotope ratio of the plant material.

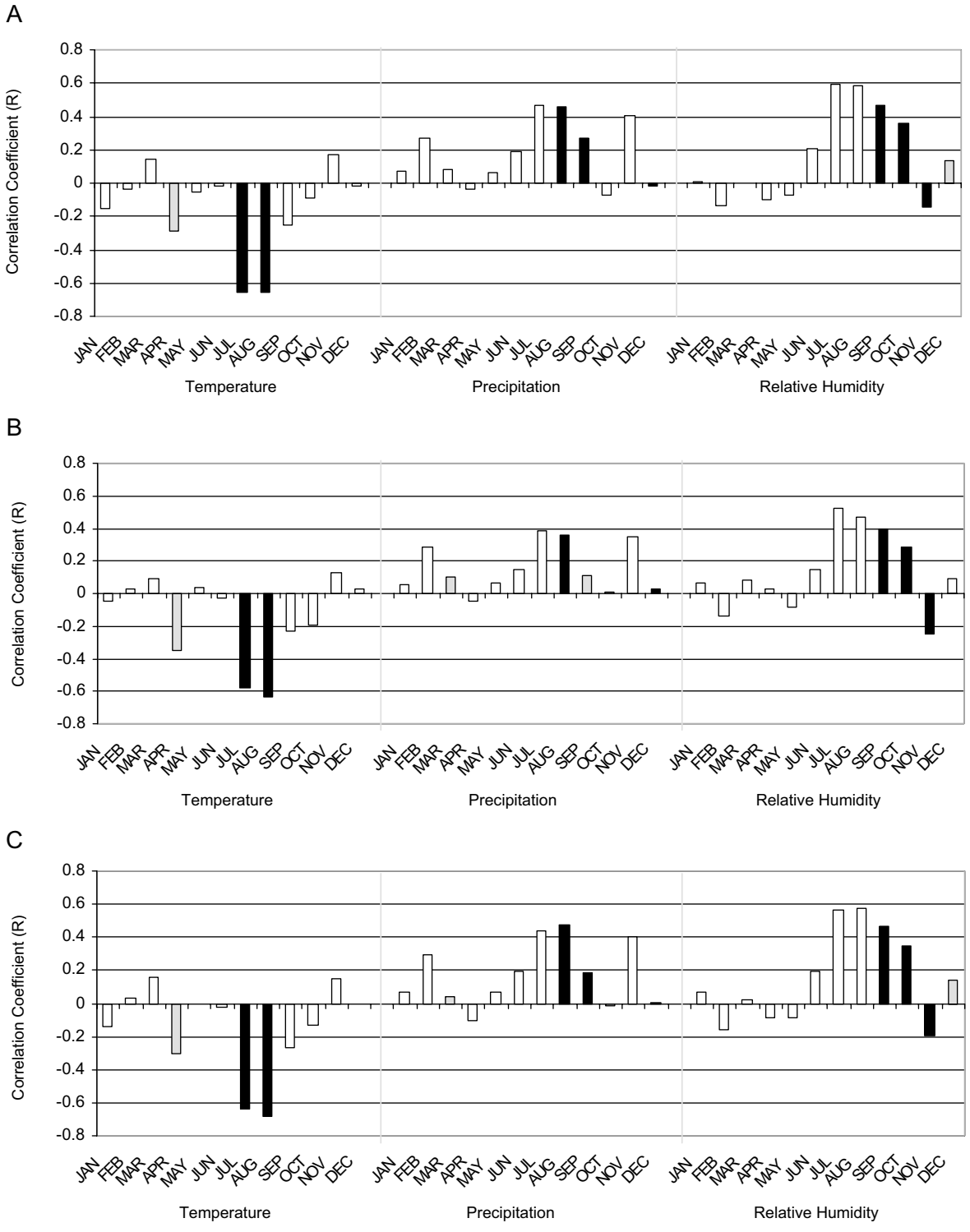
3.2. Climatic associations

The resulting data were used to calculate standard (Pearson product moment) linear correlation coefficients (R) and are presented as composite diagrams (after Fritts, 1976) which display graphically the relationship between the mean isotope time series and monthly meteorological variables (Manley, 1974; Parker et al., 1992; Robertson et al., 1997) (Fig. 3A,B). The climate of high summer (July and August) is most strongly correlated with the latewood $\Delta^{13}C$ of whole wood, cellulose and lignin, with the strength of the correlations declining in that order. In eastern England, high summer is the time when oak trees fix most of their carbon, and the variation in $\Delta^{13}C$ values is a response to differences in the internal concentration of CO_2 when that carbon was fixed. The internal concentration of CO_2 reflects the balance between stomatal conductance and photosynthetic rate, so that hot, dry, sunny summers yield more negative $\Delta^{13}C$ values than those that are cooler and moister (Francey and Farquhar, 1982; McCarroll and Pawellek, 2001). Whole wood may be most strongly correlated with climate because its composition is closest to that of the sugars manufactured in the leaf. As photosynthates are partitioned to form cellulose and lignin there may be some loss of palaeoclimate signal, and the lower variability of the lignin series and the poorer correlation with climate variables suggest that this effect may be more marked in lignin than in cellulose.

The results presented in Fig. 3 demonstrate that $\Delta^{13}C$ values are significantly correlated with climate variables even when they have not been statistically de-trended to remove age-related or physiological trends. Possible 'juvenile trends' were removed indirectly through analysis of only those tree-rings formed during the mature growth phase (post c. 40 years growth). This suggests that they may also retain the lower-frequency palaeoclimate information that is usually lost when ring width measurements are de-trended and averaged (Cook et al., 1995; Briffa et al., 1992). To compare the degree to which $\Delta^{13}C$ values retain high-frequency, inter-annual climate information, the lower-frequency variance was then removed from the isotope and climate data sets by calculating the first differences ($x_n - x_{n-1}$).

The resulting inter-annual correlations with climate (Fig. 4) are even stronger than those obtained using the raw $\Delta^{13}C$ series (Fig. 3). These results suggest that the palaeoclimate signal retained in the latewood $\Delta^{13}C$ values of whole wood, cellulose or lignin is strong and that $\Delta^{13}C$ values may retain climatic information at a wider range of temporal frequencies than more traditional growth proxies. On the basis of the small sample used in this pilot study, it appears that whole wood $\Delta^{13}C$ values are more strongly correlated with climate than those of cellulose, which are in turn stronger than those of lignin ($\Delta^{13}C$ v. mean July and August temperature; $R^2_{\text{whole wood}} = 0.50$, $R^2_{\text{cellulose}} = 0.42$, $R^2_{\text{lignin}} = 0.34$, $n = 48$, $P < 0.01$).

Fig. 3. Composite correlation diagrams (after Fritts, 1976) describing the statistical relationship between monthly temperature, precipitation and relative humidity (1946–1994) and (A) $\Delta^{13}C_{\text{lignin}}$, (B) $\Delta^{13}C_{\text{cellulose}}$, (C) $\Delta^{13}C_{\text{whole wood}}$. Black bars $P = < 0.01$, grey bars $P = < 0.05$ significance level.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-0.6
-0.8
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3.3. Temporal offset in cellulose and lignin signals

Previous work has suggested that there may be a time lag between the formation of cellulose and lignin in wood cells (Fritts, 1976; Wilson and Grinsted, 1977; Fujita et al., 1983; Gindl and Grabner, 2000; Gindl et al., 2000). If this is the case then the $\Delta^{13}C$ values obtained from these two components might record the climate of different parts of the growing season. This was tested here by examining the correlation between the $\Delta^{13}C$ values and climate data averaged across overlapping temporal windows of varying duration (Aykroyd et al., 2001; Robertson et al., in press). This approach has recently been used to demonstrate the different parts of the growing season that influence palaeoclimate proxies that can be extracted from pine trees (McCarroll et al., in press). It was demonstrated, for example, that height growth is strongly controlled by the mean temperature of about four weeks in mid-summer whereas maximum density records a much longer window covering most of the growth season. The same techniques were applied here, using windows of between 30 and 80 days duration, overlapping by one day at a time to cover the whole potential growing season. The highest correlations for all three components were obtained using a window of 70 days stretching from the last week of June to the first week of September. There is thus no clear evidence in the stable carbon isotope ratios to suggest that inter-annually cellulose, lignin and whole wood carry significantly different or temporally offset climate signals. If the period of lignification starts or finishes later than cellulose formation, the absolute amounts involved are too small to register a difference in the isotopic ratios of the annual latewood components.

Limited work has been carried out on the lignification of hardwoods, in relation to climatic parameters; however, several studies based upon the UV absorption of lignin components in *Quercus mongolica* suggest that a delay between cellulose synthesis and lignification should be observed. Yoshinaga et al. (1997a) propose an offset of approximately 14 days after cell wall thickening during which lignification continues before the cell wall is fully lignified. Their high-resolution analysis of an oak tree-ring, in association with other studies using broad-leaved trees, also identifies that the distribution of lignin throughout the tree-ring is more closely related to cell age and function than to a sequential accumulation (Higuchi, 1990; Yoshinaga et al., 1997a,b).

3.4. Influence of differential decay

Isotopic heterogeneity in the composition of plant macrofossils introduces large isotopic differences independent of those resulting from initial photosynthesis (Epstein and Krishnamurthy, 1990). Most studies, therefore, report stable isotope ratios measured after the isolation of a single chemical component, typically cellulose (Wilson and Grinsted, 1977; Wilson, 1978; Gray and Thompson, 1976; Leavitt and Danzer, 1993; Switsur and Waterhouse, 1998). As stable isotope mass spectrometry has become less time-consuming (Loader and Buhay, 1999; Koziet, 1997; Preston and Owens, 1985), the isolation of cellulose has, in many laboratories, become the rate-limiting step, and so the need for isolation of a single component has further been questioned (Barbour et al., 2001; Rundgren et al., 2000; Borella et al., 1998; Leuenberger et al., 1998; McCarroll and Loader, in press).

Our results suggest that the climate signal in the $\Delta^{13}C$ values of whole wood may be stronger than that of either cellulose or lignin, probably because the isotopic composition of whole wood is most similar to that of the total sugars fixed in the leaf. However, if the intention is to apply stable isotope dendroclimatology to the long sub-fossil tree-ring sequences then some caution is required

Fig. 4. Composite correlation diagrams (after Fritts, 1976) describing the statistical relationship (first differences) between monthly temperature, precipitation and relative humidity (1946–1994) and (A) $\Delta^{13}C_{lignin}$, (B) $\Delta^{13}C_{cellulose}$, (C) $\Delta^{13}C_{whole\ wood}$. Black bars $P = < 0.01$, grey bars $P = < 0.05$ significance levels.

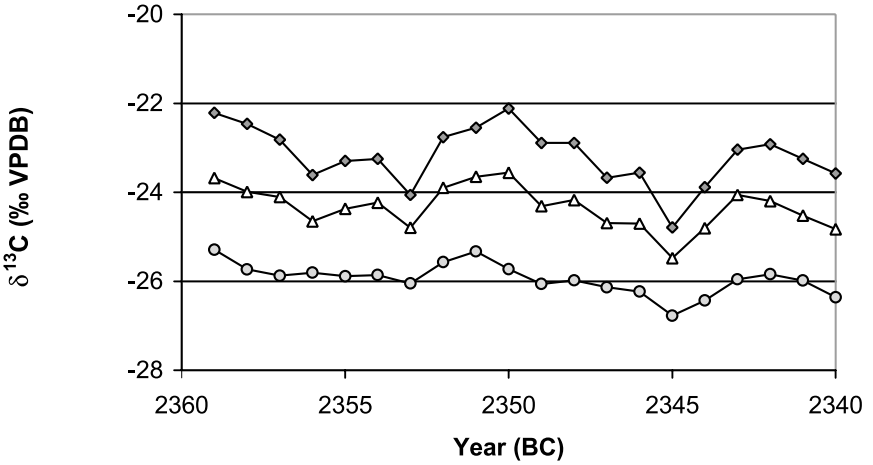

Fig. 5. Stable carbon isotope time series (2359–2340 BC) for the archaeological timber sequence FEN. Cellulose, grey rhomboids; whole wood, white triangles; lignin, grey circles.

because of the potential effects of differential decay of wood components.

The sample of sub-fossil oak (c. 4350 years old) shows similar trends in both the lignin and cellulose isotope ratios at high frequency, suggesting a high degree of signal preservation (Table 1, Fig. 5). Assuming uniformitarianism, mass balance calculations suggest, however, that compared to the modern series, on average 7% of the cellulose fraction has degraded over time (65% mean cellulose in the modern samples compared with 58% cellulose in the archaeological sample). The isotopic offset, however, remains similar between the cellulose and lignin components of this sample (2.8‰ $\sigma_{n-1} = 0.41$, $n = 20$). These results suggest that differential decay is likely to lead to changes in the cellulose/lignin ratio that will influence $\delta^{13}C$ values obtained from whole wood. The extent and nature of this variation will depend upon sample history and conditions of preservation. Since the isotopic offset between cellulose and lignin remains stable, even after partial decay, either of these components is likely to yield reliable results, but the climate signal in cellulose may be stronger than that in lignin.

4. Conclusions

There is a strong correlation between the stable carbon isotope ratios of acid-insoluble lignin and cellulose preserved in the annual latewood increments of oak (*Quercus robur* L.). The time series are almost parallel, but the variance of the cellulose values is slightly greater than that of the lignin. Carbon isotopic fractionation during cellulose and lignin synthesis imparts an isotopic difference relative to whole wood of approximately 1‰ and −2‰ respectively, which is maintained throughout the records studied. This offset is most likely related to secondary metabolic processes occurring during the cleavage of photosynthates at the site of lignification and cellulose synthesis. Although the mechanisms causing this effect are yet to be fully characterised, the resulting series remain consistent with accepted models of initial carbon isotope discrimination (Francey and Farquhar, 1982; Farquhar et al., 1989; Farquhar and Lloyd, 1993; Beerling, 1994; Marshall and Monserud, 1996).

Of the three indicators analysed, lignin demonstrated generally lower correlations with climate variables and modern whole wood the highest. Whole wood samples may preserve the best climate signal because they represent most closely the total isotopic composition of the sugars produced in the leaf. The $\delta^{13}C$ values of lignin are, however, strongly correlated with climate and could be used in isotope dendroclimatology.

Our results have implications for the protocol

used when using wood or other plant macrofossils for palaeoclimatic or palaeoenvironmental analyses. Although modern whole wood may retain the strongest climate signal, differential decay could lead to changes in the ratio of cellulose to lignin, so that in sub-fossil wood there may be fluctuations in whole wood $\delta^{13}C$ values that are unrelated to climate. We found no evidence that the isotopic offset between cellulose and lignin changes during differential decay, so either component could be used to provide a palaeoclimatic signal, though the climatic signal in cellulose may be stronger than that in lignin.

Our findings are demonstrated using the tree-ring archive but are also applicable to the isotopic analysis of bulk plant macrofossil material. We accept that practical limitations can prevent the isolation of a single sample component and that whole wood may be used to provide evidence of inter-annual variance. However, some lower-frequency variance could be induced by differential decay and should be interpreted with caution.

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References

Aykroyd, R.G., Lucy, D., Pollard, A.M., Carter, A.H.C., Robertson, I., 2001. Temporal variability in the strength of proxy-climate correlations. Geophys. Res. Lett. 28, 1559–1562.

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. Aust. J. Plant Phys. 28, 335–348.

Becker, B., Billamboz, A., Egger, H., Gassmann, P., Orcel, A., Orcel, Chr., Ruoff, U., 1985. Dendrochronologie in der Ur- und Frühgeschichte. Die absolut Datierung von Pfahlbausiedlungen nördlich der Alpen im Jahrringkalender Mitteleuropas. Antiqua 11, Basel.

Beerling, D.J., 1994. Predicting leaf gas exchange and $\delta^{13}C$ responses to the past 30000 years of global environmental change. New Phytol. 128, 425–433.

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

Borella, S., Leuenberger, M., Saurer, M., Siegwolf, R., 1998. Reducing uncertainties in $\delta^{13}C$ analysis of tree rings: Pooling, milling and cellulose extraction. J. Geophys. Res. D 16, 19516–19526.

Briffa, K.R., Bartholin, T.S., Eckstein, D., Jones, P.D., Karlén, W., Schweingruber, F.H., Zetterberg, P., 1990. A 1,400-year tree-ring record of summer temperatures in Fennoscandia. Nature 346, 434–439.

Briffa, K.R., Jones, P.D., Bartholin, T.S., Eckstein, D., Schweingruber, F.H., Karlén, W., Zetterberg, P., Eronen, M., 1992. Fennoscandian summers from AD 500: temperature changes on short and long timescales. Clim. Dyn. 7, 111–119.

Briffa, K.R., 2000. Annual climate variability in the Holocene: interpreting the message of ancient trees. Quat. Sci. Rev. 19, 87–105.

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

Coplen, T., 1995. Discontinuance of SMOW and PDB. Nature 375, 285.

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

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

Epstein, S., Krishnamurthy, R.V., 1990. Environmental information in the isotopic record in trees. Phil Trans. R. Soc. London A 330, 427–439.

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., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 503–537.

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.
Francey, R.J., Farquhar, G.D., 1982. An explanation for the $^{12}C/^{13}C$ variations in tree-rings. Nature 297, 28–31.
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. J. Geophys. Res. 88, 6844–6852.
Fritts, H.C., 1976. Tree Rings and Climate. Academic Press, New York, 567 pp.
Fujita, M., Saiki, H., Harada, H., 1983. Deposition of cellulose hemicelluloses and lignin in the differentiating tracheids. Proc. Int. Symp. Wood Pulp. Chem. 1, 14–19.
Gindl, W., Grabner, M., 2000. Characteristics of spruce [*Picea abies* (L.) Karst] latewood formed under abnormally low temperatures. Holzforschung 54, 9–11.
Gindl, W., Grabner, M., Wimmer, R., 2000. The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width. Trees 14, 409–414.
Goñi, M., Eglinton, T.I., 1996. Stable carbon isotopic analysis of lignin-derived CuO oxidation products by isotope ratio monitoring-gas chromatography-mass spectrometry (IRM-GC-MS). Org. Geochem. 24, 601–615.
Gray, J., Thompson, P., 1976. Climatic information from $^{18}O/^{16}O$ ratios of cellulose in tree-rings. Nature 262, 481–482.
Hemming, D.L., Switsur, V.R., Waterhouse, J.S., Heaton, T.H.E., Carter, A.H.C., 1998. Climate variation and the stable carbon isotope composition of tree-ring cellulose: an intercomparison of *Quercus robur*, *Fagus sylvatica* and *Pinus sylvestris*. Tellus 50B, 25–33.
Hemming, D.L., 1999. Stable Isotopes in Tree-rings: Biosensors of Climate and Atmospheric Carbon-dioxide Variations. Unpublished Ph.D. Thesis, Department of Plant Sciences, University of Cambridge.
Higuchi, T., 1990. Lignin biochemistry: Biosynthesis and biodegradation. Wood Sci. Technol. 24, 23–63.
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 Environ. 18, 931–936.
Koziet, J., 1997. Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter. J. Mass Spectrom. 32, 103–108.
Leavitt, S.W., Danzer, S.R., 1993. A method for batch processing small wood samples to holocellulose for stable carbon isotope analysis. Anal. Chem. 65, 87–89.
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. VDF, Zurich.
Libby, L.M., Pandolfi, L.J., Payton, P.H., Marshall, J., III, Becker, B., Giertz-Siebenlist, V., 1976. Isotopic tree thermometers. Nature 261, 284–290.
Lindholm, M., Eronen, M., Timonen, M., Meriläinen, J., 1999. A ring-width chronology of Scots pine from northern Lapland covering the last two millennia. Ann. Bot. Fenn. 36, 119–126.
Lindholm, M., Eronen, M., 2000. A reconstruction of mid-summer temperatures from ring widths of Scots pine since AD 50 in northern Finland. Geogr. Ann. 82A, 527–535.
Loader, N.J., Switsur, V.R., 1996. Reconstructing past environmental change using stable isotopes in tree-rings. Bot. J. Scotl. 48, 65–78.
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. Chem. Geol. 136, 313–317.
Loader, N.J., Buhay, W.M., 1999. Rapid catalytic oxidation of CO to CO_2. Rapid Commun. Mass Spectrom. 13, 1828–1832.
Manley, G., 1974. Central England temperatures: monthly means 1659 to 1973. Q. J. R. Meteorol. Soc. 100, 389–405.
Marshall, J.D., Monserud, R.A., 1996. Homeostatic gas-exchange parameters inferred from $^{13}C/^{12}C$ in tree rings of conifers. Oecologia 105, 13–21.
McCarroll, D., Loader, N.J. (in press). Stable Isotope Dendroclimatology. Quat. Sci. Rev.
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. Holocene 11, 517–526.
McCarroll, D., Jalkanen, R., Hicks, S., Tuovinen, M., Pawellek, F., Gagen, M.H., Eckstein, D., Schmitt, U., Autio, J., Heikkinen, O., in press. Multi-proxy dendroclimatology: a pilot study in northern Finland. The Holocene.
Okuyama, T., Takeda, H., Yamamoto, H., Yoshida, M., 1998. Relation between growth stress and lignin concentration in the cell wall: Ultraviolet microscopic spectral analysis. J. Wood Sci. 44, 83–89.
Parker, D.E., Legg, T.P., Folland, C.K., 1992. A new daily Central England temperature series, 1772–1991. Int. J. Climatol. 12, 317–342.
Pilcher, J.R., Baillie, M.G.L., Schmidt, B., Becker, B., 1984. A 7,272-year tree-ring chronology for western Europe. Nature 312, 150–152.
Preston, T., Owens, N.J.P., 1985. Preliminary measurements using a gas chromatograph interfaced to an isotope ratio mass spectrometer. Biomed. Mass Spectrom. 12, 510–513.
Robertson, I., Switsur, V.R., Carter, A.H.C., Barker, A.C., Waterhouse, J.S., Briffa, K.R., Jones, P.D., 1997. Signal strength and climate relationships in the $^{13}C/^{12}C$ ratios of tree-ring cellulose from oak in east England. J. Geophys. Res. 102, 19507–19516.
Robertson, I., Loader, N.J., McCarroll, D., Carter, A.H.C., Cheng, L., Leavitt, S.W., in press. $\delta^{13}C$ of tree-ring lignin as an indirect measure of climate change. Water, Air and Soil Pollution: Focus.
Rundgren, M., Loader, N.J., Beerling, D.J., 2000. Variations in the carbon isotope composition of late-Holocene plant macrofossils: a comparison of whole-leaf and cellulose trends. Holocene 10, 149–154.
Schleser, G.H., Frielingsdorf, J., Blair, A., 1999. Carbon isotope behaviour in wood and cellulose during artificial aging. Chem. Geol. 158, 121–130.

Schweingruber, F.H., Briffa, K.R., 1996. Tree-ring density networks for climatic reconstruction. In: Jones, P.D., Bradley, R.S., Jouzel, J. (Eds.), Climatic Variability and Forcing Mechanisms of the Last 2000 Years. Springer, Berlin, pp. 43–66.

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

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

Switsur, V.R., Waterhouse, J.S., 1998. Stable isotopes in tree-ring cellulose. In: Griffiths, H. (Ed.), Stable Isotopes. Bios, Oxford, pp. 303–321.

Technical Association of the Pulp and Paper Industry (TAPPI), 1988. Test Method T222 om-83. TAPPI, Atlanta, GA.

Turney, C.M., Berringer, J., Hunt, J.E., McGlone, M.S., 1999. Estimating leaf to air vapour pressure deficit from terrestrial plant $\delta^{13}C$. J. Quat. Sci. 14, 437–442.

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

Wilson, A.T., 1978. Climatic interpretation of $\delta^{18}O$ and δD in tree-rings. Nature 271, 93.

Yoshinaga, A., Fujita, M., Saiki, H., 1997a. Cellular distribution of guaicyl and syryngyl lignins within an annual ring of oak wood. Mokuzai Gakkaishi 43, 384–390.

Yoshinaga, A., Fujita, M., Saiki, H., 1997b. Secondary wall thickening and lignification of oak xylem components during latewood formation. Mokuzai Gakkaishi 43, 384–390.

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