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Paper:

McCarroll, D. (in press). A simple stable carbon-isotope method for investigating changes in the use of recent versus old carbon in oak. *Tree Physiology*

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A simple stable carbon-isotope method for investigating changes in the use of recent versus old carbon in oak

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Summary

Stable carbon isotope ratios from early-wood (EW) and latewood (LW) are used to test competing models of carbon storage and allocation, providing a cost-effective alternative to measuring and dating non-structural carbohydrates in mature temperate broad-leaf forest trees growing under natural conditions. Annual samples of EW and LW from seven mature oaks (*Quercus robur* L.) from Scotland, covering AD 1924 to 2012, were pooled, treated to isolate alpha-cellulose and pyrolysed to measure the carbon isotope ratios. LW values are strongly correlated with summer temperature of the year of growth and EW contains the same signal offset by one year. After a warm summer, isotopic ratios of EW are similar to those of the preceding LW, but following cold summers they are relatively enriched. The results conflict with established models of isotopic variation within oak tree rings but support ‘two-pool’ models for storage of non-structural carbohydrates, with EW formation, which occurs prior to bud-burst, preferentially using young reserves accumulated in the previous summer. Under poor growing conditions trees access older reserves. Slight average isotopic enrichment of EW may be explained by preferential accumulation of reserves during warmer summers rather than by isotopic enrichment during starch formation in non-photosynthetic tissue.

Key words

carbon cycling, carbon dynamics, carbon isotopes, non-structural carbohydrates, tree ring, oak, *Quercus*.

Introduction

The importance of including non-structural carbohydrates (NSCs) in models of the carbon dynamics of forest trees has become increasingly clear (Kozlowski, 1992, Dietze et al., 2014, Palacio et al., 2014). Carbon cycle models that do not deal realistically with the way that NSCs are used are likely to be misleading because growth is not an inevitable consequence of carbon assimilation (Fatichi et al., 2014). The sugars and starch that mainly comprise NSCs are likely to serve multiple functions in trees. They may serve several osmotic purposes, such as maintaining turgor and long distance vascular integrity in xylem and phloem (Sala et al., 2012, Dietze et al., 2014). However they are also used for maintenance respiration during winter (Ögren, 2000) and to aid survival during periods of low accumulation or high sink demand resulting from, for example, environmental stress or structural damage (Dunn et al., 1990, Canham et al., 1999). Modelling carbon dynamics in forest trees is important not least because of the potential impacts of climate change and the attendant feedbacks on the carbon and water cycles (Bodin et al., 2013). An understanding of carbohydrate storage is also essential for modelling the resilience of trees to climate change and extreme events.

However, it is difficult to test models because of the paucity of field evidence on the size and age of non-structural carbohydrate pools in mature trees (Barbaroux and Bréda, 2002, Richardson et al., 2013). Measuring the amount of NSC stored by mature trees is difficult, and estimates based only on the stem wood may be misleading, because NSC is also stored in coarse roots, branches and leaves (Barbaroux et al., 2003, Hoch et al., 2003, Würth et al., 2005, Gough et al., 2009). Also measuring and dating the NSC of wood provides a static picture of NSC status, and monitoring over time is expensive and typically limited to a few years at most.

A cost-effective alternative to monitoring NSC dynamics is to use the carbon isotope ratios in different plant components as tracers of carbon accumulation, storage and use. Recent work by Kimak and Leuenberger (2015), for example, suggests that carbohydrate storage strategies of broad-leaf trees are traceable by early- to late-wood carbon isotope differences. They report that under very harsh growing conditions, such as the European Little Ice Age, oak early-wood (EW) is dependent upon young reserves but that under normal or favourable conditions this dependence is not apparent. Under the good growing conditions of recent decades Kimak and Leuenberger (2015) found no significant correlation between the carbon isotope ratios of EW and those of the latewood (LW) of the previous season. They argue that

this is due to either spring growth becoming rapidly autotrophic (Keel et al., 2007), so that reserves contribute in a limited way to EW formation or to rapid mixing of carbon storage so that the signal of the previous year is not preserved. These results are in broad agreement with very detailed analysis of changes in carbon isotope ratios within individual tree rings (Helle and Schleser, 2004), which also suggests that EW cells are generally formed from well-mixed stores of NSC, so that environmental signals are not carried over from one year to the next.

The carbon isotope results of Kimak and Leuenberger (2015) contrast with the results of Richardson et al. (2013) who monitored the seasonal dynamics of NSC in North American forests and also dated the NSC stored in the outer 2cm of the stem wood using the radiocarbon bomb pulse. Whereas the carbon isotope studies suggest that under good growing conditions EW is produced from well-mixed reserves, so that there is no correlation between values obtained from the LW of one season and the EW of the next, the detailed monitoring study of Richardson et al. (2013) concludes that trees preferentially draw on very young reserves, so that the EW is formed primarily from reserves accumulated in the previous year. They argue for a ‘two-pool’ model of carbon storage, where an easily accessed pool dominated by very young reserves is linked to a well-mixed pool of older reserves that can be called upon at need.

A potential weakness of the stable isotope approach, as it has been applied so far, is that conclusions have been extrapolated from very detailed studies of just a few individual rings (Ogle and McCormac, 1994, Loader et al., 1995, Helle and Schleser, 2004) or by comparing EW and LW taken from a single tree (Switsur et al., 1995; Kimak and Leuenberger, 2015). Here we use an alternative approach, adapted from methods developed for extracting strong climate signals from stable isotopes in tree rings (Gagen et al., 2012, Woodley et al., 2012, Young et al., 2012, 2015, Loader et al., 2013), and compare the EW and LW carbon isotope ratios of several trees by pooling the samples prior to analysis (Borella et al., 1998). We argue that although some intra-annual variability is lost by coarsely sub-dividing each tree-ring into EW and LW, by measuring the average response of several trees over a long period we can efficiently provide a more representative picture of stand carbon dynamics and critically test the competing models of carbon storage and allocation in mature temperate broad-leaf forest trees.

Materials and methods

Fifteen straight-growing and mature oak trees (*Quercus robur* L.) were selected from a north facing slope 4km south of Aviemore, Scotland (57° 9'15"N 03° 50'40"W, 300m above mean sea level) and cored using standard methods to produce a ring width chronology. Further details of sampling strategy and the mean ring widths are included in the Supplementary Data. Climate records from Braemar (57° 0' 40"N 03° 23' 49"W, 325m elevation), show that mean annual temperature is 6.5°C and average annual precipitation is 900mm for the period AD 1959 – 2012. Seven cores were chosen for isotopic analysis and their mean ring widths suggest they are representative of the full chronology (Supplementary Figure 1). Accurate separation of EW and LW was achieved by cutting each ring into thin slivers using a razor blade and dissecting microscope. The first 50 rings of each tree were not processed to avoid possible juvenile effects (McCarroll and Loader, 2004; Labuhn et al. 2013, Kilroy et al., in press), resulting in an 89-year chronology covering AD 1924 – 2012, constructed from a pooled series with a minimum sample depth of six trees. The wood was extracted to alpha-cellulose using standard methods (Loader et al., 1997), freeze-dried and 0.30 to 0.35mg weighed into silver capsules and pyrolysed over glassy carbon at 1090°C (Young et al., 2011). Pyrolysis was conducted using a Europa ANCA GSL elemental analyser interfaced with a Europa 20/20 isotope ratio mass spectrometer, with precision from a standard laboratory cellulose of 0.1‰ for $\delta^{13}\text{C}$ (σ_{n-1} n=10).

The raw $\delta^{13}\text{C}$ series were corrected for changes in the atmospheric carbon isotopic ratio ($\delta^{13}\text{C}$) resulting from the burning of isotopically-depleted fossil fuels by simple addition of the difference between smoothed atmospheric $\delta^{13}\text{C}$ values and a pre-industrial datum value of -6.4‰ for AD1849 (McCarroll & Loader, 2004). Regional meteorological data were obtained for northern Scotland from the UK Meteorological Office.

Results

The corrected EW and LW $\delta^{13}\text{C}$ values (Fig 1a) reveal a clear temporal offset. Although the correlation between EW and LW within individual rings (Fig. 1b) is positive and statistically significant ($r = 0.43$, $p < 0.01$), it is caused by the rising trend since the late 1980s in both time series. When all trends are removed by first-differencing (current year minus previous year) the correlation drops to zero (Fig 1d). When the EW $\delta^{13}\text{C}$ values are compared with the LW

values of the previous year (Fig. 1c) the correlation is much stronger ($r = 0.75$) and remains high ($r = 0.65$) after first-differencing (Fig. 1e).

Stable carbon isotope ratios of latewood cellulose from oak trees growing under moist conditions in the UK are strongly correlated with summer temperature and sunshine (Young et al., 2012), probably because photosynthetic rate, rather than stomatal conductance, dominates fractionation in the leaf. The relative strength of correlation with the temperature of different months (Fig. 2) should therefore help to elucidate the controls on fractionation of the carbon that is incorporated into EW and LW. As expected, there is a strong correlation between LW $\delta^{13}\text{C}$ and temperature of the current summer, with July yielding the highest values for a single month ($r = 0.58$) and July and August as the strongest combination of months ($r = 0.63$). April temperatures are also positively and significantly correlated with LW $\delta^{13}\text{C}$ ($r = 0.35$) and this is not an artefact of any significant correlations between April temperature and the temperature of the summer (not shown).

The pattern of correlations for EW $\delta^{13}\text{C}$ (Fig. 2) is virtually identical to that for LW but the correlations are slightly lower and are with the temperatures of the previous year. The highest value for a single month is July ($r = 0.52$) and the highest for a combination of months is the mean temperature of July and August ($r = 0.57$). Spring temperatures of the current year are not correlated with EW $\delta^{13}\text{C}$. The weak correlations between EW cellulose $\delta^{13}\text{C}$ and the temperature of the current year summer months, after EW formation has ceased, are unlikely to reflect any mechanistic link; they are caused by autocorrelation in the instrumental temperature data.

As well as being offset by one year it is apparent (Figure 1) that the EW values are, on average, higher and less variable than those of the LW. The mean $\delta^{13}\text{C}$ values for the EW and LW series respectively are -25.45‰ and -25.67‰ and although the difference is small, at 0.22‰ , given the large sample size it is statistically significant even using a two-tailed test (independent samples t-test: $p = 0.03$). The variability of the EW values ($\text{SD} = 0.57$) is muted relative to LW ($\text{SD} = 0.75$) giving a variance ratio of 1.73, which is statistically significant (Hartley's test, $p = 0.01$). First order autocorrelation in the LW ($r = 0.21$) is identical to the first order autocorrelation in the mean JA temperature data. In the EW, by contrast, first order autocorrelation is much higher ($r = 0.46$, $p < 0.001$). In the earliest 31 years of the time-series the first order autocorrelation in the JA temperature data is zero and that in the LW is also

very low ($r = 0.10$) whereas the EW still shows significant autocorrelation ($r = 0.42$, $p < 0.05$). Neither series shows significant autocorrelation at lags of more than one year. The higher autocorrelation in EW may reflect use of reserves, whereas the results provide no evidence for use of reserves for LW cellulose formation.

The relationship between the LW values of one year and the EW values of the next appear to be related to the summer temperatures of the year of LW formation (Fig. 3). In the warmest third of summers over the study period ($n = 29$, JA mean temperature 12.75°C to 14.30°C) the correlation between the LW and EW values is very strong ($r = 0.80$, $p < 0.0001$) and there is no tendency for EW to be enriched (ratio enriched to unenriched is 14:15). The mean values of LW and EW are very similar (-25.17‰ and -25.13‰) and a paired t-test returns no significant difference (two-tailed $p = 0.67$). This suggests that after a warm summer the isotopic signal is transferred from one year to the next without isotopic enrichment. For the coolest one third of summers (10.7°C to 11.85°C), in contrast, the correlation is much weaker ($r = 0.39$, $p = 0.04$) and in 24 of these 29 years the EW values are enriched. A paired t-test is strongly significant ($p < 0.0001$). For the intermediate summers the correlation is significant ($r = 0.68$, $p < 0.0001$) and so is the 0.25‰ offset (LW = -25.69‰ , EW = -25.44‰ , $p = 0.002$) with 22 of the 30 EW values showing enrichment. The relationship between summer temperature and isotopic enrichment of EW is explored further in the Supplementary data.

Discussion

Kimak and Leuenberger (2015) pooled material from four cores of one tree, arguing that “the high natural variability of tree ring records might lead to cancelling effects of intra-annual variations when pooling across cores of multiple trees”. Our results, however, show that the common signals are enhanced, by using material from several trees, rather than cancelled, producing a strong and consistent correlation between LW $\delta^{13}\text{C}$ and summer temperature suggesting that pooling wood from several trees prior to isotope analysis produces reliable $\delta^{13}\text{C}$ time series (Borella et al., 1998, Dorado-Liñan, 2011, Woodley et al., 2012). Therefore the concern that remobilization of stored NSCs might decouple trends in $\delta^{13}\text{C}$ in tree rings from the shorter-term trends predicted by leaf-scale fractionation models and thus overwrite seasonal influences of climate (Helle & Schleser, 2004, Offermann et al., 2011, Gessler et al., 2014) appears to be unfounded. On the contrary, at this site, EW carries a signal virtually identical to that of the preceding LW.

Our multi-decadal results contrast with those based on high-resolution sampling across individual rings. Helle and Schleser (2004), for example, found a common tri-phase pattern typified by an early increase to maximum $\delta^{13}\text{C}$ values in EW, a decline that persists until close to the end of the latewood and a final increase. The high values are attributed to remobilization of carbon stored as isotopically-enriched starch. However, these results are based on only two oak rings from a single tree, though the general tri-phase pattern was replicated in two rings each of beech (*Fagus sylvatica*), and mulberry (*Morus alba*) and four rings of poplar (*Populus nigra*), all from central Europe.

The tri-phase model prediction that LW $\delta^{13}\text{C}$ values should be consistently lower than those of the EW of the same ring is true in only 61% of rings in our study. The prediction that LW and the EW of the following year should not be correlated also conflicts with our results. It seems that at this coarser sampling resolution the tri-phase model, with EW consistently more enriched than LW, may not be as widely applicable as has been assumed.

It is unsurprising that oak EW $\delta^{13}\text{C}$ does not carry a climate signal from the current spring because, as a ring-porous species, EW growth in *Q. robur* is rapid and must begin in advance of budburst (Bréda and Granier, 1996, Sass-Klaasen et al., 2011); because winter embolism of the large xylem cells requires the water flow pathway to be restored before the onset of transpiration (Essiamah and Eschrich, 1986, Barbaroux and Bréda, 2002, Michelot et al., 2012). Our results clearly indicate that EW cellulose is formed preferentially from reserves accumulated in the previous growing season rather than from a well-mixed pool of older NSCs. The $\delta^{13}\text{C}$ values of EW are very strongly correlated with those of the preceding LW and carry an identical climate signal. EW $\delta^{13}\text{C}$ values are, on average, slightly enriched, inter-annual variance is lower, and EW displays significant first order autocorrelation, not seen in LW. These differences indicate that EW formation can, at need, access older isotopically-mixed reserves. The clear implication is that there are two pools of carbohydrate that can be drawn upon to support EW formation and that it is the younger of these pools that dominates, particularly under good growing conditions.

These results strongly support the proposal by Richardson et al. (2013) that carbon cycle models should include a 'two-pool model structure' that takes account of non-structural carbohydrates. Using the FöBAAR (FOrest Biomass, Allocation, Assimilation and Respiration) model (Keenan et al., 2012) they demonstrated that model structures that

assumed either no pool of NSC or a single pool were inconsistent with the amount and age of NSC measured in tree stems and with measured changes in woody biomass increment. The best model assumed that spring growth preferentially accesses a ‘fast pool’, dominated by NSCs produced in the previous season, which exchanges with a ‘slow pool’ of much older carbohydrates. The older pool includes both starch and sugars, so they do not divide the two pools on this basis, preferring a ‘last in first out’ model of NSC dynamics whereby the most recently added starch molecules are the first to be converted back to sugars (Lacointe et al., 1993). Recent work supports the two pools concept and confirms that although the young pool is more readily used, much older reserves can still be accessed at need (Carbone et al., 2013, Richardson et al., 2015). Although Richardson et al. (2013) did not measure carbon isotope ratios, their two-pool model would predict the strong correlation between EW and LW of the previous year. The much smaller contribution from the well-mixed older pool explains the muting of the climate signal, reduction in variance and autocorrelation in the EW.

Although EW $\delta^{13}\text{C}$ values are, on average, slightly enriched (0.25‰) relative to LW this may not represent the use of isotopically enriched NSC stored as starch. Leaf starch is isotopically enriched relative to leaf sugars, but Gessler et al. (2014) note that “there is no evidence for kinetic or equilibrium effects for the reactions that produce starch from sucrose in non-photosynthetic tissue”. Although Gessler et al. (2014) go on to identify a biochemical pathway that might explain some fractionation, no such explanation is required here. The small offset between LW and EW carbon isotope ratios can be explained very easily using the two-pool model by assuming that the older pool of NSC is built mainly from reserves accumulated during favourable growth years, which in Scotland equates to warm summers. Simply assuming that the coldest one third of summers do not contribute carbon to the older pool, and all others contribute a similar amount, would give a mean isotope value for the long lived pool that is equal to the mean isotope value for all of the EW. There is no need to invoke any fractionation due to storage. This conflicts with the conclusions of Eglin et al. (2010) who could only model oak EW $\delta^{13}\text{C}$ by including a fractionation factor during starch synthesis.

Conclusions

Our results demonstrate that carbon isotope ratios of EW cellulose are very strongly correlated with the values obtained from the LW of the previous year. LW $\delta^{13}\text{C}$ values are strongly correlated with summer temperature, probably via variations in sunshine and therefore photosynthetic rate, and we see identical climate signals shifted by one year in the EW, with no evidence of response to current spring climate. The generally accepted ‘tri-phase’ model of $\delta^{13}\text{C}$ variation in broad leaf tree rings, which suggests that EW is enriched relative to LW, due to reliance on well-mixed reserves derived from isotopically enriched starch, is not supported by our results. In more than 30% of rings the EW was less enriched than the LW. In the warmest one third of summers the $\delta^{13}\text{C}$ values of EW are not significantly different from those of the preceding LW and there is no evidence of enrichment. We are also able to allay the concerns raised by Gessler et al. (2014) that remobilisation of well-mixed reserves might decouple the isotope values measured in tree rings from the environmental controls and physiological processes that operate at the leaf level. On the contrary, the environmental signals imparted in the LW of one season are carried very strongly into the EW of the next.

Our results also contrast with those of Kimak and Leuenberger (2015) who conclude that oak trees only rely on young reserves for EW formation when assimilation is low and that under normal conditions they either use reserves that are isotopically well-mixed or access carbohydrates directly from the new leaves. We find that under the good growing conditions of most of the 20th century, Scottish oak trees have consistently relied upon reserves produced in the previous summer to form EW cellulose, with no discernible contribution from young leaves. Following warm summers there may be no contribution from older carbohydrates but following cold summers these isotopically well-mixed reserves become much more important. The small average offset between LW and EW can be explained by the effect of summer temperature on isotopic ratios of NSCs that are stored for more than one year. They are biased towards higher than average LW values because there is more surplus material available in warmer years. There is no need to invoke any fractionations during conversions between sugar and starch in non-photosynthetic tissues.

The results we present here, although based on a completely different approach, fit very well with the ‘two-pool’ model of carbon dynamics proposed by Richardson et al. (2013). The strong correlation between LW $\delta^{13}\text{C}$ and summer temperature in the Scottish oaks is as predicted by theory and in accordance with other studies on UK oak. The same signal is

carried over from the LW of one year to the EW of the next, virtually unchanged. Clearly, the EW cells are being formed using NSCs that are drawn preferentially from a young pool that accumulated during the previous summer. A smaller contribution from a well-mixed, and presumably much older pool of NSCs that accumulates mainly in warmer summers is reflected in a small isotopic offset, slight muting of the climate signal and a reduction in variance in the EW.

The new approach proposed here provides a powerful and cost-effective way of exploring the response of mature trees, growing under natural conditions, to a wide range of past environmental changes and extreme events. It is unusual in terms of scale of analysis, since although it involves dividing individual tree rings into early and late-wood portions, the wood samples obtained from several trees are pooled prior to isolation of cellulose and mass spectrometry. By greatly reducing the cost, time and effort that would be required to chemically treat and then measure every individual sample, it allows changes in carbon storage and allocation to be viewed at the stand scale and over, potentially, very long timescales (hundreds of years). Measuring the carbon isotope ratios in early- and late-wood does not provide a quantitative measure of the amount of carbon that is stored, so it is not an alternative to measuring and dating NSCs within living trees, but it greatly extends the number of trees that can be studied and particularly the length of time over which measurements can be derived.

The results presented here are from oak trees growing towards the cooler end of their natural range, so the trees respond to summer temperature. However, the same method could be applied to other broadleaf trees and to those that are subject to other potentially growth-limiting factors, such as drought or disease, in order to investigate how the use of stored carbon changes over time. The method could also be used in post-mortem studies, to compare the behaviour of a cohort of dying trees to the behaviour of exactly the same set of trees when they were in good health. A suitable target would be trees that are suffering from, or have been killed by acute oak decline syndrome. Also, there has been considerable discussion about how atmospheric increases in carbon dioxide might influence carbon storage and use in trees, and therefore their resilience to environmental stressors. The new method could provide unique insights in this regard, by comparing the recent behaviour of trees with their behaviour under similar climate conditions before the rapid rise in carbon dioxide.

Acknowledgements

We thank Robert Wilson and the Scot-2k project for fieldwork access and assistance.

Funding

We thank the Leverhulme Trust (RPG-2014-327) for research support.

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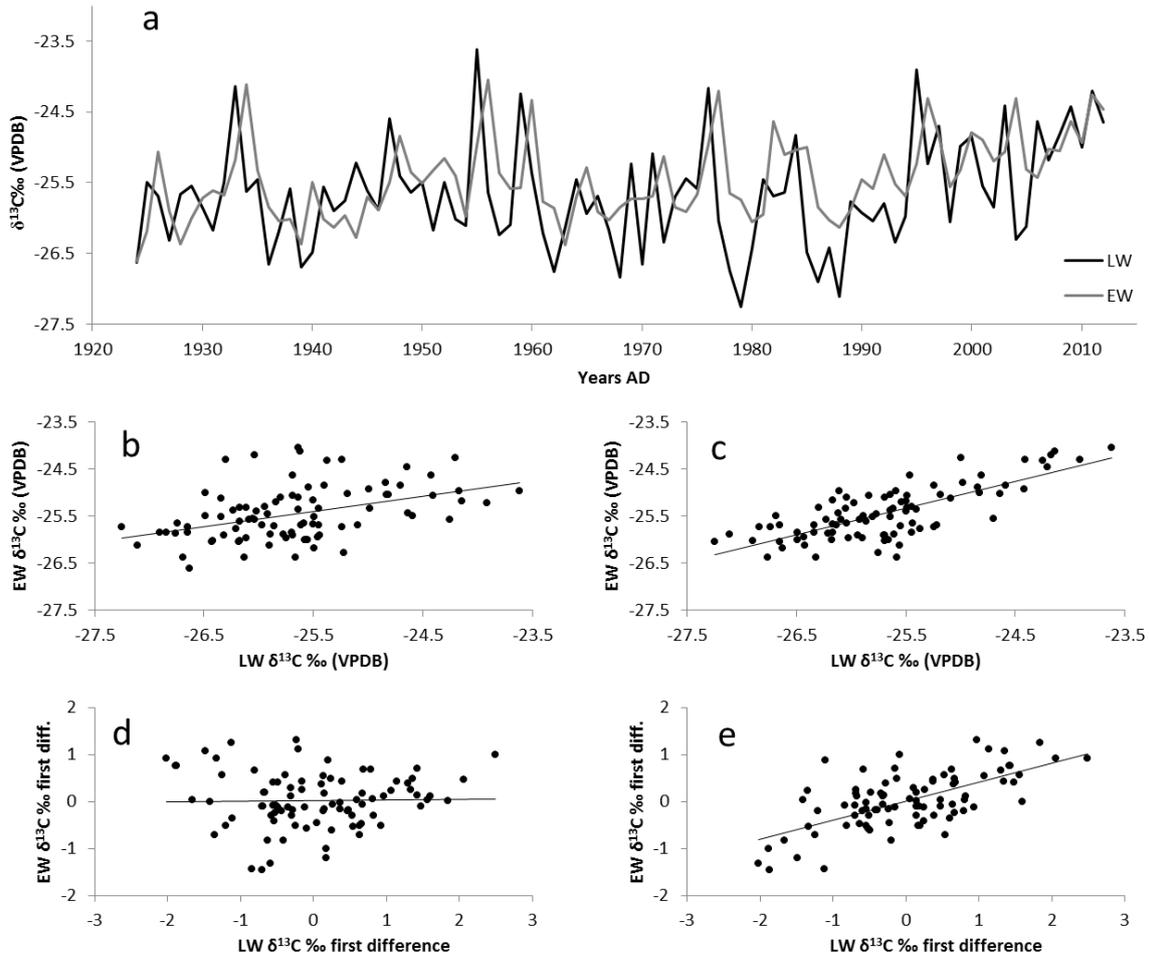


Figure 1. 1a: Time series of early-wood (EW) and late-wood (LW) $\delta^{13}\text{C}$ of cellulose obtained from a pool of seven Scottish oak trees. Note the clear one-year offset. 1b: Relationship between EW and LW $\delta^{13}\text{C}$ values obtained from the same ring. 1c: Relationship between the LW $\delta^{13}\text{C}$ of one year and the EW $\delta^{13}\text{C}$ of the following year. 1d: As for 1b but after first-differencing the data to remove all trends. 1e: as for 1c but after first-differencing the data to remove all trends.

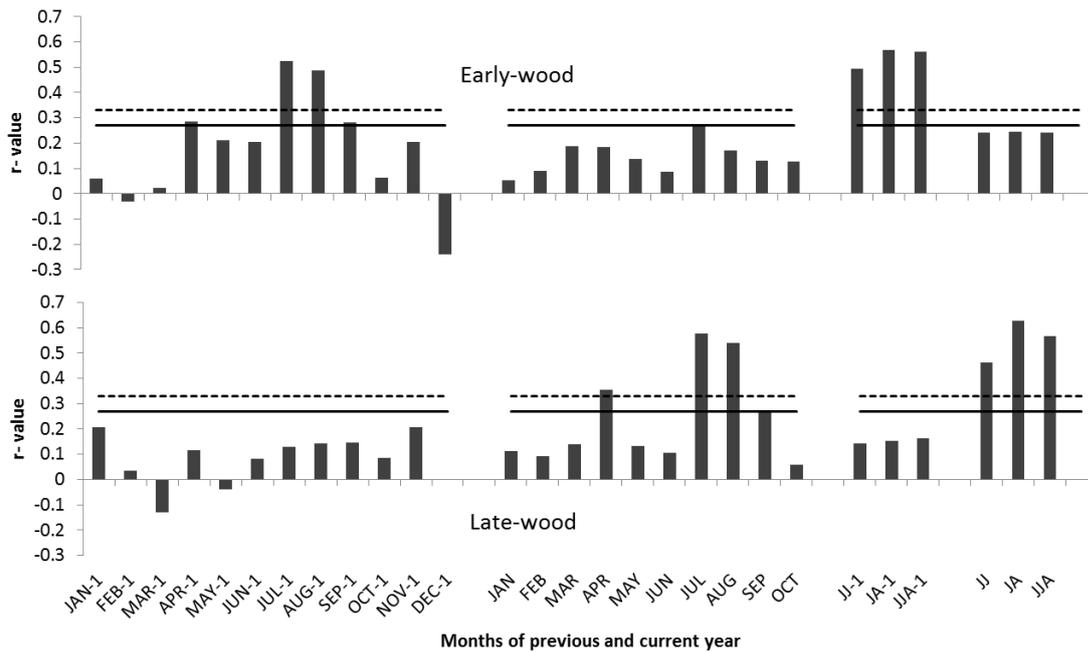


Figure 2. Pearson’s correlation coefficients (r-values) for early wood and late-wood $\delta^{13}\text{C}$ compared with monthly mean temperatures for the current and previous years. The solid black horizontal line is the critical value for $p=0.05$ for a single test and the dashed line represents the critical value ‘Bonferroni’ corrected to take account of family-wise error due to multiple testing.

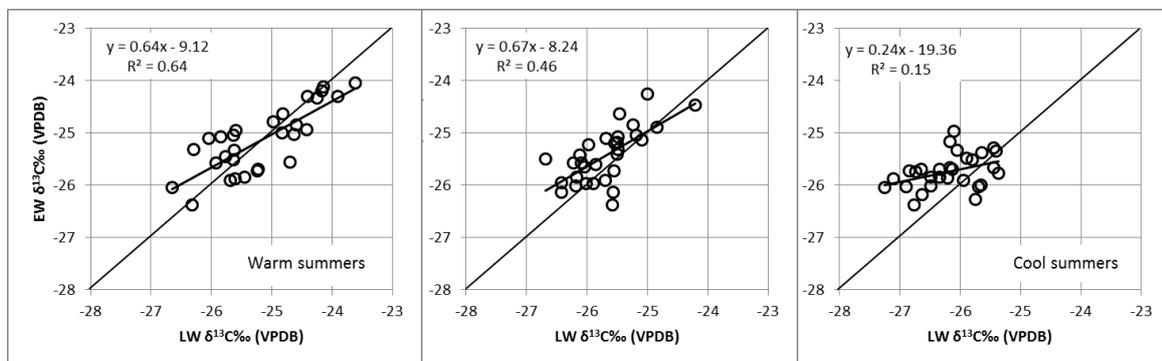


Figure 3. Correlation between the LW $\delta^{13}\text{C}$ value of one year and the EW $\delta^{13}\text{C}$ value of the next separated according to summer temperature. In the warmest one third of summers ($n=29$) the LW and EW values are strongly correlated and there is no offset whereas in the coolest one third of summers the correlation is weak and EW values are isotopically enriched.

Supplementary data:

A simple stable carbon-isotope method for investigating changes in the use of recent versus old carbon in oak

Danny McCarroll, Matthew Whitney, Giles H.F. Young, Neil J. Loader and Mary H. Gagen

Sampling strategy

Fifteen straight-growing and mature oak trees (*Quercus robur* L.) were selected from a north facing slope, at 300m above sea level, 4km south of Aviemore, Scotland (57° 9'15"N 03° 50'40"W). The trees appeared to be largely of an even (but not identical) age based upon physical size and even dominance in the stand. Given local (historic) forestry practice, it is possible that these trees may have formed part of a small plantation, however, this is difficult to test conclusively without a complete assessment of the age structure of the entire stand. Cores were collected with a 5mm diameter increment borer at breast height, air dried and sanded to a smooth surface. Ring widths were measured and trees cross-dated using the TSAP dendrochronology program (www.rinntech.com) with measurement precision of 0.01mm. Of the 15 trees sampled, 13 cross-dated well and these were used to generate a site chronology (Table 1) covering the period AD 1859 to 2012.

Seven cores were selected for isotopic analysis, mainly based upon suitability for cutting through avoidance of large woody rays capable of off-setting ring boundaries within a core. Accurate separation of EW and LW was achieved by cutting each ring into thin slivers using a razor blade and dissecting microscope. The first 50 rings of each tree were not processed to avoid possible juvenile effects resulting in an 89-year chronology covering AD 1924 – 2012, constructed from a pooled series with a minimum sample depth of six trees.

Ring widths

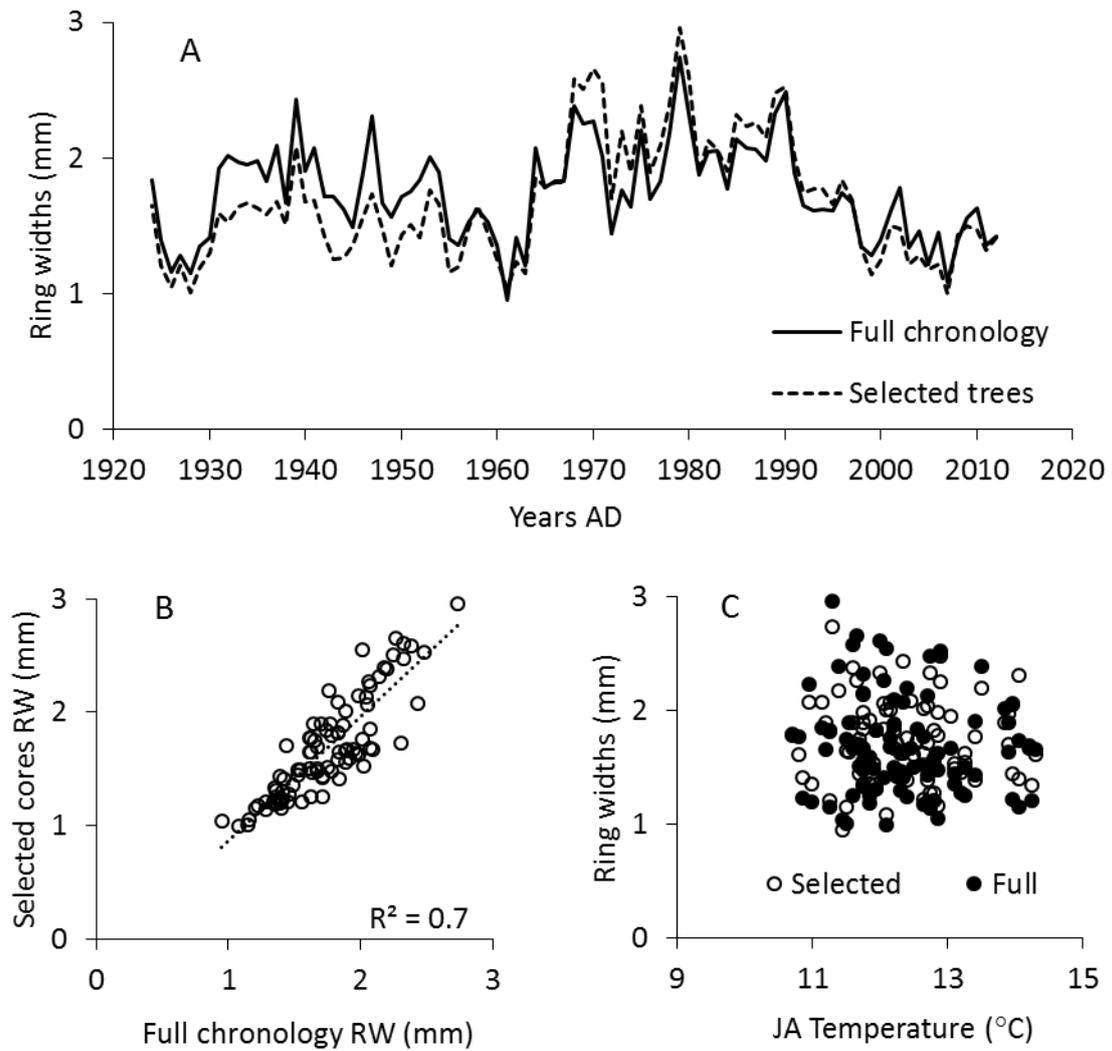
The mean ring widths from the 7 cores chosen for isotopic analysis correlate very strongly with the full site chronology ($r = 0.87$, $p < 0.0001$) suggesting that the cores chosen for isotopic analysis are representative of the trees growing at this site (Supplementary Figure 1). The mean ring widths do not correlate significantly with mean JA temperatures over the period AD 1924 to 2012 (selected cores, $r = -0.10$, full chronology $r = -0.03$) even after first-differencing (value of the current year

minus the value of the preceding year) to remove all trends and low-frequency variability (selected cores $r = 0.01$, full chronology $r = 0.08$). Correlations with JA temperatures of the preceding summer are slightly stronger but still not statistically significant. There is a statistically significant negative correlation between the mean ring widths and the pooled latewood stable carbon isotope ratios (selected cores $r = -0.36$, $p < 0.001$, full chronology $r = -0.33$, $p = 0.002$). First-differencing substantially weakens these correlations, suggesting they are partly caused by lower frequency trends in the two data sets (selected cores $r = -0.25$, $p = 0.02$, full chronology $r = -0.17$, $p = 0.1$).

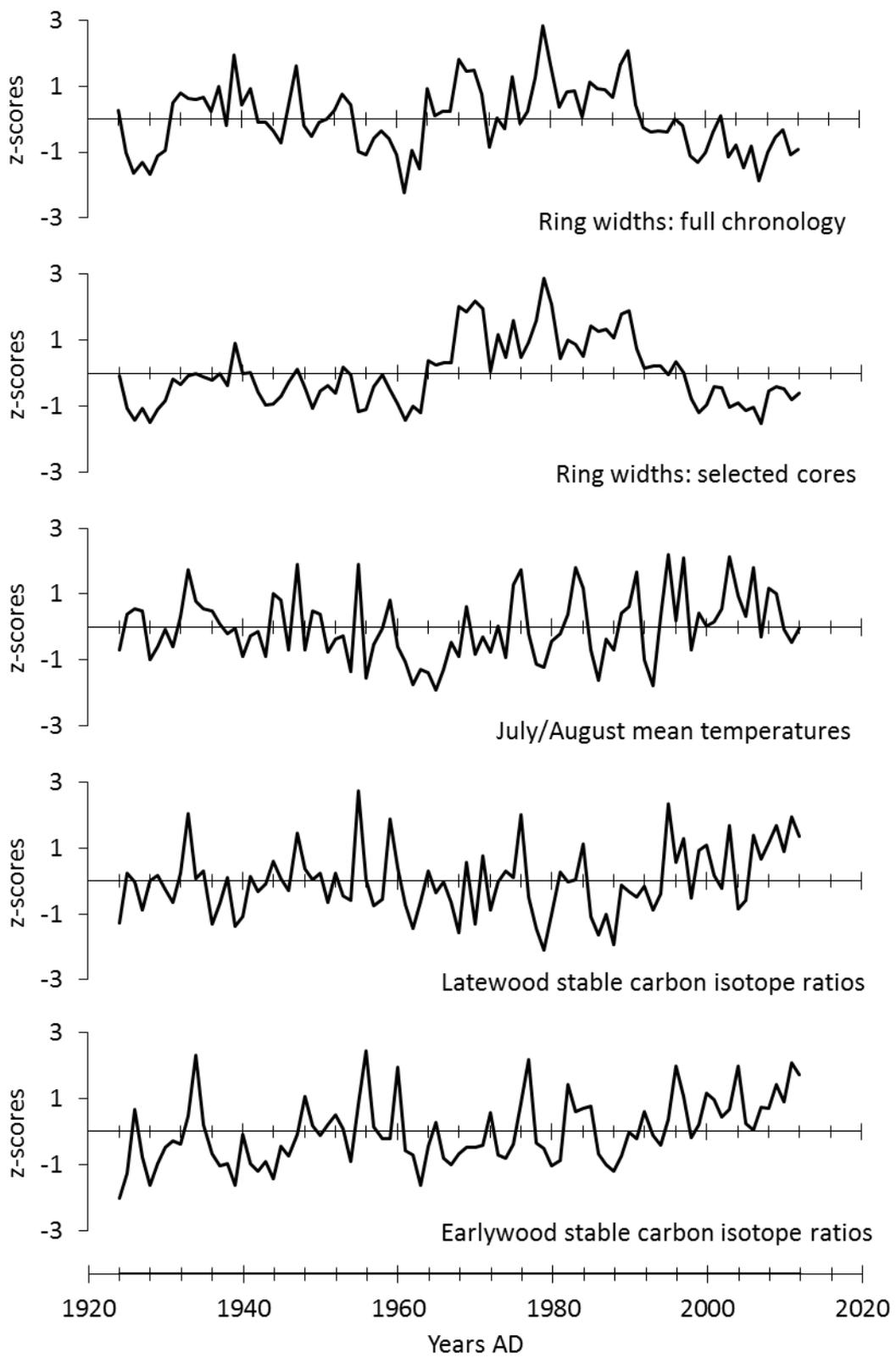
The raw and first-differenced data for ring widths, mean JA temperature and LW and EW stable carbon isotope ratios are presented in Supplementary Table 1 and for ease of comparison they are plotted as z-scores (translated into standard deviation units by subtracting the mean and dividing by the standard deviation) in Supplementary Figure 2. The ring widths of the individual cores included in the isotope pool are presented in Supplementary Table 2.

Summer temperature and carbon storage

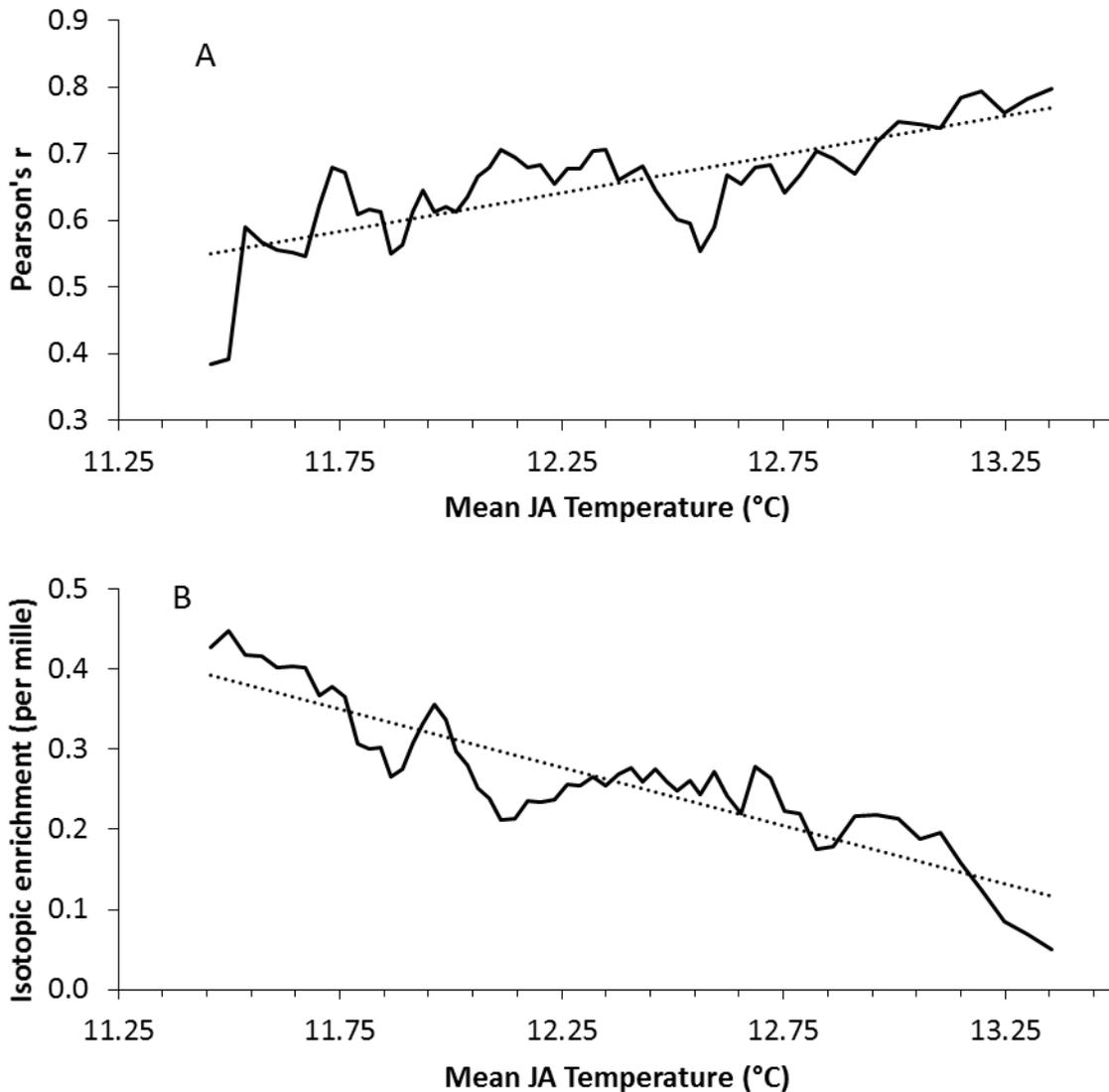
The relationship between summer temperature and the carbon isotope ratios was further explored by first shifting the EW $\delta^{13}\text{C}$ ratios back by one year and then re-casting the chronology in rank order of mean JA temperature. The influence of JA temperature on the strength of the correlation between EW and LW $\delta^{13}\text{C}$ can now be examined using a window of 30 years moved incrementally across the chronology (Supplementary Figure 3A). The results clearly support the conclusion that the correlation between the LW of one year and the EW of the next is weak during cool summers and strong during warm summers. The same procedure can be used to examine the influence of summer temperature on the degree of enrichment of EW relative to the preceding latewood, using a moving 30-year average (Supplementary Figure 3B). The results strongly support the conclusion that the degree of enrichment increases as summer temperatures decline. The correlation between mean JA temperature and enrichment at the annual level is also statistically significant ($r = -0.32$, two-tail $p = 0.002$). Our interpretation of these results is that in warm summers abundant NSC is allocated to the short-term storage pool and this is used to form EW cellulose in the following spring, with little or no isotopic enrichment. In colder summers insufficient NSCs are added to the short-term storage pool so that EW must rely partly or fully on older stores of NSC accumulated under warmer conditions, so that EW is enriched relative to the preceding LW.



Supplementary Figure 1. Ring widths for the full Aviemore chronology and for the trees that were selected for isotopic analysis plotted through time (A) and against each other (B). Neither is significantly correlated with mean July/August temperature at nearby Braemar (C).



Supplementary Figure 2. Ring widths, mean JA temperatures and stable isotope ratios expressed as z-scores.



Supplementary Figure 3. After shifting EW values back by one year the chronology has been re-cast in rank order of mean JA temperature. The correlation between EW and LW $\delta^{13}\text{C}$ values (A) and the mean isotopic enrichment of EW relative to preceding LW (B) is then calculated for a 30-yr window moved across the chronology one year at a time. The values are plotted relative to the mean temperature of each window. These data are not suitable for regression analysis, because the points are not independent of each other, so the best-fit lines are included simply to illustrate the trends.

Supplementary Table 1. Raw mean ring widths ($\text{mm} \times 100$) for the full site chronology (Full RW) and for the cores selected for isotopic analysis (Selected RW), mean July/August temperature at Braemar ($^{\circ}\text{C}$), and the stable carbon isotope ratios obtained from the pooled LW and EW samples.

Year	Full RW	Selected RW	JA Mean T	Latewood $\delta^{13}\text{C}$	Earlywood $\delta^{13}\text{C}$
2012	142	141.2	12.4	-24.6	-24.5
2011	136	131.5	12.0	-24.2	-24.3
2010	163	147.0	12.3	-25.0	-24.9
2009	155	149.7	13.3	-24.4	-24.6
2008	139	143.7	13.4	-24.8	-25.1
2007	108	99.7	12.1	-25.2	-25.0
2006	145	121.5	14.0	-24.6	-25.4
2005	122	118.0	12.7	-26.1	-25.3
2004	146	127.8	13.2	-26.3	-24.3
2003	134	121.2	14.3	-24.4	-25.1
2002	178	148.0	12.9	-25.8	-25.2
2001	161	150.2	12.5	-25.5	-24.9
2000	139	124.8	12.4	-24.8	-24.8
1999	128	113.8	12.8	-25.0	-25.3
1998	135	133.3	11.8	-26.1	-25.6
1997	167	169.0	14.2	-24.7	-24.8
1996	174	183.7	12.6	-25.2	-24.3
1995	161	165.3	14.3	-23.9	-25.2
1994	162	177.3	12.7	-26.0	-25.7
1993	161	176.8	10.8	-26.3	-25.5
1992	165	174.5	11.5	-25.8	-25.1
1991	189	201.5	13.9	-26.0	-25.6
1990	248	252.5	12.9	-25.9	-25.5
1989	233	247.5	12.8	-25.8	-25.9
1988	198	214.8	11.8	-27.1	-26.1
1987	206	226.5	12.1	-26.4	-26.0

1986	207	223.5	11.0	-26.9	-25.8
1985	214	231.8	11.8	-26.5	-25.0
1984	177	190.2	13.4	-24.8	-25.0
1983	205	206.7	14.0	-25.6	-25.1
1982	204	213.2	12.7	-25.7	-24.6
1981	187	188.2	12.2	-25.5	-26.0
1980	233	260.8	12.0	-26.4	-26.1
1979	274	296.0	11.3	-27.3	-25.7
1978	218	238.8	11.4	-26.7	-25.7
1977	183	209.2	12.2	-26.0	-24.2
1976	170	189.2	13.9	-24.2	-25.0
1975	220	238.5	13.5	-25.6	-25.7
1974	164	189.3	11.6	-25.4	-25.9
1973	176	219.3	12.4	-25.7	-25.9
1972	144	170.0	11.7	-26.3	-25.1
1971	201	255.2	12.1	-25.1	-25.7
1970	227	265.3	11.7	-26.7	-25.7
1969	225	250.3	12.9	-25.2	-25.7
1968	238	258.3	11.6	-26.8	-25.8
1967	183	182.2	12.0	-26.2	-26.0
1966	183	181.4	11.3	-25.7	-25.9
1965	178	179.1	10.7	-25.9	-25.3
1964	207	185.3	11.2	-25.5	-25.7
1963	121	114.9	11.3	-26.1	-26.4
1962	141	123.3	10.9	-26.8	-25.9
1961	95	103.9	11.5	-26.2	-25.8
1960	136	125.7	11.9	-25.4	-24.3
1959	153	144.6	13.1	-24.3	-25.6
1958	162	165.1	12.4	-26.1	-25.6

1957	153	149.0	11.9	-26.2	-25.4
1956	136	119.4	11.0	-25.6	-24.1
1955	140	115.6	14.1	-23.6	-25.0
1954	189	165.7	11.2	-26.1	-26.0
1953	201	176.2	12.2	-26.0	-25.4
1952	184	141.2	12.1	-25.5	-25.2
1951	175	150.8	11.7	-26.2	-25.3
1950	171	143.7	12.7	-25.5	-25.5
1949	156	120.5	12.8	-25.6	-25.4
1948	167	147.8	11.8	-25.4	-24.8
1947	231	173.2	14.1	-24.6	-25.5
1946	189	156.0	11.8	-25.9	-25.9
1945	149	135.8	13.1	-25.6	-25.7
1944	162	125.8	13.3	-25.2	-26.3
1943	171	125.0	11.6	-25.8	-26.0
1942	171	142.0	12.3	-25.9	-26.1
1941	207	168.5	12.2	-25.6	-26.0
1940	190	167.3	11.6	-26.5	-25.5
1939	243	207.8	12.4	-26.7	-26.4
1938	167	150.3	12.2	-25.6	-26.0
1937	209	167.5	12.5	-26.2	-26.0
1936	183	158.5	12.8	-26.7	-25.8
1935	198	162.7	12.9	-25.5	-25.3
1934	195	166.8	13.1	-25.6	-24.1
1933	197	164.2	13.9	-24.1	-25.2
1932	202	152.3	12.7	-25.5	-25.7
1931	192	159.7	11.9	-26.2	-25.6
1930	141	130.0	12.3	-25.9	-25.7
1929	135	118.8	11.9	-25.6	-26.0

1928	115	101.2	11.5	-25.7	-26.4
1927	128	121.0	12.8	-26.3	-25.9
1926	116	104.8	12.9	-25.7	-25.1
1925	139	120.3	12.7	-25.5	-26.2
1924	184	164.5	11.8	-26.6	-26.6

Supplementary Table 2. Individual ring widths of the 7 cores used for isotopic analysis. Rings that are included in the pool are underlined. Units are mm times 100.

Year\tree	#1	#2	#3	#7	#9	#11	#12
2012	<u>69</u>	<u>221</u>	<u>270</u>	<u>108</u>	<u>110</u>		<u>69</u>
2011	<u>58</u>	<u>197</u>	<u>214</u>	<u>132</u>	<u>128</u>		<u>60</u>
2010	<u>65</u>	<u>226</u>	<u>258</u>	<u>104</u>	<u>158</u>		<u>71</u>
2009	<u>59</u>	<u>251</u>	<u>268</u>	<u>83</u>	<u>183</u>		<u>54</u>
2008	<u>62</u>	<u>255</u>	<u>262</u>	<u>112</u>	<u>122</u>		<u>49</u>
2007	<u>34</u>	<u>168</u>	<u>149</u>	<u>78</u>	<u>128</u>		<u>41</u>
2006	<u>51</u>	<u>181</u>	<u>182</u>	<u>101</u>	<u>161</u>		<u>53</u>
2005	<u>52</u>	<u>162</u>	<u>144</u>	<u>166</u>	<u>133</u>		<u>51</u>
2004	<u>52</u>	<u>191</u>	<u>203</u>	<u>117</u>	<u>145</u>		<u>59</u>
2003	<u>51</u>	<u>213</u>	<u>171</u>	<u>135</u>	<u>94</u>		<u>63</u>
2002	<u>77</u>	<u>278</u>	<u>207</u>	<u>107</u>	<u>135</u>		<u>84</u>
2001	<u>65</u>	<u>248</u>	<u>255</u>	<u>119</u>	<u>133</u>		<u>81</u>
2000	<u>79</u>	<u>181</u>	<u>191</u>	<u>89</u>	<u>112</u>		<u>97</u>
1999	<u>81</u>	<u>169</u>	<u>126</u>	<u>82</u>	<u>126</u>		<u>99</u>
1998	<u>64</u>	<u>184</u>	<u>147</u>	<u>129</u>	<u>132</u>		<u>144</u>
1997	<u>78</u>	<u>171</u>	<u>220</u>	<u>117</u>	<u>184</u>		<u>244</u>
1996	<u>73</u>	<u>188</u>	<u>168</u>	<u>173</u>	<u>238</u>		<u>262</u>
1995	<u>83</u>	<u>173</u>	<u>150</u>	<u>146</u>	<u>170</u>		<u>270</u>
1994	<u>86</u>	<u>156</u>	<u>156</u>	<u>154</u>	<u>193</u>		<u>319</u>

1993	<u>82</u>	<u>173</u>	<u>174</u>	<u>153</u>	<u>213</u>		<u>266</u>
1992	<u>93</u>	<u>190</u>	<u>170</u>	<u>170</u>	<u>203</u>		<u>221</u>
1991	<u>119</u>	<u>217</u>	<u>260</u>	<u>176</u>	<u>193</u>		<u>244</u>
1990	<u>161</u>	<u>258</u>	<u>362</u>	<u>176</u>	<u>263</u>		<u>295</u>
1989	<u>141</u>	<u>249</u>	<u>299</u>	<u>276</u>	<u>254</u>		<u>266</u>
1988	<u>155</u>	<u>195</u>	<u>293</u>	<u>222</u>	<u>174</u>		<u>250</u>
1987	<u>143</u>	<u>252</u>	<u>362</u>	<u>171</u>	<u>204</u>		<u>227</u>
1986	<u>167</u>	<u>278</u>	<u>280</u>	<u>189</u>	<u>220</u>		<u>207</u>
1985	<u>193</u>	<u>260</u>	<u>284</u>	<u>220</u>	<u>228</u>		<u>206</u>
1984	<u>122</u>	<u>181</u>	<u>235</u>	<u>214</u>	<u>202</u>		<u>187</u>
1983	<u>133</u>	<u>182</u>	<u>268</u>	<u>220</u>	<u>213</u>		<u>224</u>
1982	<u>188</u>	<u>164</u>	<u>331</u>	<u>212</u>	<u>161</u>		<u>223</u>
1981	<u>151</u>	<u>178</u>	<u>291</u>	<u>191</u>	<u>115</u>		<u>203</u>
1980	<u>250</u>	<u>217</u>	<u>331</u>	<u>350</u>	<u>124</u>		<u>293</u>
1979	<u>277</u>	<u>292</u>	<u>324</u>	<u>328</u>	<u>212</u>		<u>343</u>
1978	<u>191</u>	<u>297</u>	<u>318</u>	<u>232</u>	<u>180</u>		<u>215</u>
1977	<u>155</u>	<u>279</u>	<u>218</u>	<u>236</u>	<u>170</u>		<u>197</u>
1976	<u>164</u>	<u>199</u>	<u>191</u>	<u>201</u>	<u>162</u>		<u>218</u>
1975	<u>222</u>	<u>287</u>	<u>272</u>	<u>212</u>	<u>153</u>		<u>285</u>
1974	<u>196</u>	<u>217</u>	<u>226</u>	<u>151</u>	<u>131</u>		<u>215</u>
1973	<u>165</u>	<u>247</u>	<u>181</u>	<u>160</u>	<u>232</u>		<u>331</u>
1972	<u>162</u>	<u>160</u>	<u>128</u>	<u>104</u>	<u>181</u>		<u>285</u>
1971	<u>211</u>	<u>313</u>	<u>230</u>	<u>137</u>	<u>185</u>		<u>455</u>
1970	<u>176</u>	<u>313</u>	<u>253</u>	<u>185</u>	<u>237</u>		<u>428</u>
1969	<u>213</u>	<u>236</u>	<u>249</u>	<u>145</u>	<u>258</u>		<u>401</u>
1968	<u>235</u>	<u>211</u>	<u>286</u>	<u>126</u>	<u>218</u>		<u>474</u>
1967	<u>157</u>	<u>124</u>	<u>211</u>	<u>95</u>	<u>168</u>		<u>338</u>
1966	<u>182</u>	<u>98</u>	<u>261</u>	<u>82</u>	<u>217</u>	<u>106</u>	<u>324</u>
1965	<u>198</u>	<u>71</u>	<u>299</u>	<u>93</u>	<u>219</u>	<u>106</u>	<u>268</u>

1964	<u>133</u>	<u>159</u>	<u>271</u>	<u>154</u>	<u>204</u>	<u>114</u>	<u>262</u>
1963	<u>153</u>	<u>52</u>	<u>138</u>	<u>110</u>	<u>176</u>	<u>79</u>	<u>96</u>
1962	<u>153</u>	<u>68</u>	<u>168</u>	<u>124</u>	<u>194</u>	<u>103</u>	<u>53</u>
1961	<u>127</u>	<u>39</u>	<u>162</u>	<u>101</u>	<u>165</u>	<u>93</u>	<u>40</u>
1960	<u>129</u>	<u>49</u>	<u>212</u>	<u>136</u>	<u>190</u>	<u>124</u>	<u>40</u>
1959	<u>185</u>	<u>60</u>	<u>213</u>	<u>191</u>	<u>201</u>	<u>124</u>	<u>38</u>
1958	<u>222</u>	<u>45</u>	<u>270</u>	<u>238</u>	<u>220</u>	<u>125</u>	<u>36</u>
1957	<u>221</u>	<u>39</u>	<u>256</u>	<u>186</u>	<u>181</u>	<u>117</u>	<u>43</u>
1956	<u>161</u>	<u>51</u>	<u>190</u>	<u>153</u>	<u>149</u>	<u>102</u>	<u>30</u>
1955	<u>116</u>	<u>70</u>	<u>161</u>	<u>121</u>	<u>169</u>	<u>128</u>	<u>44</u>
1954	<u>175</u>	<u>115</u>	<u>212</u>	<u>172</u>	<u>313</u>	<u>127</u>	<u>46</u>
1953	<u>177</u>	<u>116</u>	183	<u>185</u>	<u>335</u>	<u>200</u>	<u>44</u>
1952	<u>116</u>	<u>101</u>	177	<u>195</u>	<u>215</u>	<u>166</u>	<u>54</u>
1951	<u>161</u>	<u>106</u>	203	<u>171</u>	<u>235</u>	<u>192</u>	<u>40</u>
1950	<u>115</u>	<u>95</u>	167	<u>202</u>	<u>229</u>	<u>188</u>	<u>33</u>
1949	<u>168</u>	<u>102</u>	187	<u>120</u>	<u>181</u>	<u>126</u>	<u>26</u>
1948	<u>163</u>	<u>168</u>	213	<u>152</u>	<u>217</u>	<u>145</u>	<u>42</u>
1947	<u>192</u>	<u>150</u>	194	<u>235</u>	<u>249</u>	<u>174</u>	<u>39</u>
1946	<u>167</u>	<u>115</u>	238	<u>181</u>	<u>254</u>	<u>182</u>	<u>37</u>
1945	<u>169</u>	<u>128</u>	256	<u>112</u>	<u>212</u>	<u>166</u>	<u>28</u>
1944	<u>149</u>	<u>105</u>	250	<u>133</u>	<u>161</u>	<u>180</u>	<u>27</u>
1943	<u>153</u>	<u>103</u>	171	<u>146</u>	<u>137</u>	<u>178</u>	<u>33</u>
1942	<u>178</u>	<u>100</u>	179	<u>192</u>	<u>156</u>	<u>185</u>	<u>41</u>
1941	<u>197</u>	<u>113</u>	181	<u>252</u>	<u>188</u>	<u>199</u>	<u>62</u>
1940	<u>185</u>	<u>69</u>	144	<u>266</u>	<u>168</u>	<u>264</u>	<u>52</u>
1939	<u>260</u>	<u>116</u>	278	<u>322</u>	<u>252</u>	<u>232</u>	<u>65</u>
1938	<u>204</u>	<u>91</u>	221	<u>239</u>	<u>167</u>	<u>142</u>	<u>59</u>
1937	<u>167</u>	<u>103</u>	227	<u>299</u>	<u>173</u>	<u>182</u>	<u>81</u>
1936	<u>183</u>	<u>79</u>	211	<u>271</u>	<u>188</u>	<u>167</u>	<u>63</u>

1935	<u>166</u>	<u>110</u>	283	<u>271</u>	<u>196</u>	<u>162</u>	<u>71</u>
1934	<u>185</u>	<u>102</u>	251	<u>305</u>	<u>167</u>	<u>187</u>	<u>55</u>
1933	<u>164</u>	<u>91</u>	227	<u>265</u>	<u>215</u>	<u>194</u>	<u>56</u>
1932	<u>151</u>	<u>112</u>	242	<u>246</u>	<u>171</u>	<u>174</u>	<u>60</u>
1931	<u>168</u>	<u>90</u>	188	<u>270</u>	<u>209</u>	<u>173</u>	<u>48</u>
1930	<u>132</u>	<u>53</u>	145	<u>194</u>	<u>216</u>	<u>158</u>	<u>27</u>
1929	<u>102</u>	<u>44</u>	145	<u>183</u>	<u>190</u>	<u>145</u>	<u>49</u>
1928	<u>83</u>	<u>42</u>	122	<u>178</u>	<u>155</u>	<u>98</u>	<u>51</u>
1927	<u>102</u>	<u>45</u>	125	<u>177</u>	<u>197</u>	<u>147</u>	<u>58</u>
1926	<u>97</u>	<u>35</u>	92	<u>132</u>	<u>170</u>	<u>129</u>	<u>66</u>
1925	<u>122</u>	<u>39</u>	75	<u>168</u>	<u>167</u>	<u>173</u>	<u>53</u>
1924	<u>156</u>	<u>54</u>	170	<u>262</u>	<u>232</u>	<u>220</u>	<u>63</u>
1923	<u>124</u>	55	184	239	<u>149</u>	<u>187</u>	56
1922	<u>123</u>	99	291	290	<u>176</u>	<u>167</u>	85
1921	<u>86</u>	75	228	218	<u>166</u>	<u>187</u>	59
1920	<u>110</u>	86	209	258	<u>174</u>	<u>221</u>	72
1919	<u>166</u>	120	217	321	<u>200</u>	<u>208</u>	65
1918	<u>194</u>	129	264	300	<u>193</u>	<u>220</u>	85
1917	<u>172</u>	131	198	331	<u>198</u>	<u>202</u>	53
1916	<u>152</u>	99	224	327	<u>212</u>	<u>185</u>	60
1915	<u>148</u>	102	219	283	<u>241</u>	<u>180</u>	64
1914	<u>133</u>	77	166	232	<u>204</u>	<u>187</u>	48
1913	<u>103</u>	71	180	234	<u>172</u>	<u>178</u>	53
1912	<u>153</u>	78	181	237	<u>189</u>	<u>184</u>	72
1911	<u>141</u>	76	157	200	<u>187</u>	215	56
1910	<u>193</u>	96	194	264	<u>252</u>	233	60
1909	<u>198</u>	132	240	315	267	201	87
1908	183	113	199	276	243	205	94
1907	192	108	209	280	237	171	123

1906	192	144	164	385	498	215	177
1905	173	116	154	310	436	341	163
1904	166	132	164	292	517	409	169
1903	180	155		320	489	453	150
1902	130	134		237	369	361	139
1901	184	160		288	419	457	174
1900	159	174		364	337	483	170
1899	140	149		305	290	374	152
1898	92	109		225	216	407	92
1897	91	139		328	198	446	138
1896	40	125		291	188	232	136
1895	32	89		189	141	140	76
1894	56	89		210	181	273	132
1893	42	84		160	120	221	82
1892	54	138		266	191	282	178
1891	85	159		209	159	360	200
1890	126	189		266	193	406	197
1889	71	83		104	110	264	118
1888	46	128		132	139	337	151
1887	59	99		76	95	255	105
1886	60	105		117	105	324	118
1885	86	81		104	115	383	121
1884	101	141		151	128	602	167
1883	92	130		90	117	493	186
1882	116	166		87	126	485	168
1881	69	132		92	88	303	101
1880	94	198		114	113	334	157
1879	103	272		168	128	359	192
1878	66	107		118	116	318	131

1877	154	259	258	162	356	217
1876	127	206	181	171	257	166
1875	233	199	307	260	400	254
1874	110	156	275	151	273	117
1873	125			124	228	
1872	172			38	225	
1871	129			126	231	
1870	117			151	231	
1869	102			139	126	
1868	83			53	60	
1867	64			165	133	
1866	21			67	62	
1865	29			71	55	
1864	25			73	102	
1863	54			37	64	
1862	52			45	130	
1861	75			143		
1860	210			190		
1859	191					