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Paper:

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Key Points:
- No age-related trends are found in stable isotopes from oak tree rings based on a case study in the UK
- The conventional method of trend identification can produce spurious age trends with grossly inflated probabilities
- Age-related trends may occur in other species or in other regions, but should be reassessed using the "mean of the slope" approach

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Absence of Age-Related Trends in Stable Oxygen Isotope Ratios From Oak Tree Rings

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Abstract The potential for age-related trends in the stable oxygen isotope ratios of latewood alpha cellulose was investigated in samples of living oak trees and historic building timbers from the UK. When the series are examined individually, it is clear that the strongest trends in individual trees and timbers reflect concurrent trends in climate. Nonclimatic trends are very small and represent random noise that can be removed by averaging. If the same data are analyzed using the more conventional approach of aligning the series by ring number and fitting a regression line, so that the magnitude of the age trend is based on the slope of the mean and the statistical significance on the correlation coefficient, the results are very different. We demonstrate that this conventional approach regularly produces spurious age trends with grossly inflated probabilities, because of offsets in the mean values of series of different length. We conclude that there is no need to detrend stable oxygen isotope series from individual trees or timbers of oak from the UK and that to do so would remove important climatic information. Long isotope chronologies can safely be constructed by combining data from multiple individual trees, or by pooling material from trees prior to chemical treatment and isotopic measurement. Age-related trends may occur in other species or in other regions, but where they have been identified using the conventional "slope of the mean" approach they should be reassessed using the "mean of the slope" approach.

1. Introduction

Tree rings provide one of the most valuable archives of paleoclimatic information, but climate reconstructions based on morphological growth proxies, such as ring widths and relative densities, rely on trees whose growth is limited by a single climatic control (Bradley, 2014). The method works best for species growing close to their environmental limits, at high latitudes and altitudes for temperature signals (Frank & Esper, 2005; McCarroll et al., 2013) and in seasonally dry areas for precipitation and related signals (Cook et al., 1999). As a result of these methodological limitations, hemispheric and regional reconstructions of paleoclimate are biased toward areas where few people live, and the heavily populated, history-rich, less climatically stressed midlatitudes are poorly represented (Luterbacher et al., 2016).

Perhaps the greatest potential for climate reconstruction in the moist midlatitudes lies in the use of oak (*Quercus* spp.). In NW Europe the two native oak species (*Q. robur* L. and *Q. petraea* Liebl.) are widespread, their timber is a common component of both historic buildings and archaeological remains, and oak stems are also abundant in river gravels and preserved in peat (Edvardsson et al., 2016; Haneca et al., 2009). Many long oak ring width chronologies have been constructed for archaeological dating purposes (Baillie, 1982; Pilcher et al., 1984), and to calibrate the radiocarbon time scale (Becker, 1993), but it has unfortunately not proved possible to translate these ring width records into reliable palaeoclimate reconstructions. Even very well-replicated chronologies, compiled using the best available methods (Cooper et al., 2013; Wilson et al., 2013), fail to pass calibration and verification thresholds of reliability (National Research Council, 2007). The problem is that oak trees over most of Europe are not sufficiently limited by a single environmental factor, so that even where a common growth signal can be extracted, it is not clearly linked to a single climate parameter that can then be reconstructed.

A possible solution lies, not in measuring morphological features, but in the wood chemistry (McCarroll & Loader, 2004). The stable isotopes of carbon and oxygen in UK oak trees have been shown to carry very strong signals of summer temperature and precipitation amount (Loader et al., 2008; Rinne et al., 2013; Young et al., 2012, 2015). The oxygen isotope signal reflects mainly the isotopic signature of summer

rainfall, which depends on the dominant circulation pattern (Young et al., 2015). The frontal rainfall that dominates wet summers has lower oxygen isotope ratios than the anticyclonic-derived rainfall that dominates in dry summers (Darling & Talbot, 2003), and the oak trees record this signal passively, even when their growth rate is not constrained by climate. The oxygen isotope ratios in the latewood (summer wood) of UK oak trees and timbers thus record past changes in the amount of May to August rainfall (Young et al., 2015). Oaks in France appear to record a similar signal (Daux et al., 2018; Labuhn et al., 2016).

Although there is clear potential for constructing long, well-replicated isotope chronologies using oak, this work can be constrained by the high cost of separating the alpha cellulose from individual latewood samples and measuring the samples individually by mass spectrometry. A cost-effective solution is to pool the wood from several trees prior to the chemistry and mass spectrometry (Borella et al., 1998; Dorada-Liñan, 2011; Lavergne et al., 2017; Leavitt, 2008; Liu et al., 2015; Szymczak et al., 2012). However, doubts have been raised about the wisdom of such an approach, as it has been argued that the isotope ratios from the rings of individual trees are influenced by tree age as well as by climate, and therefore require statistical detrending to remove these nonclimatic trends (Esper et al., 2010; Helama et al., 2015). Detrending individual tree ring isotope series would, however, severely constrain the building of long, well-replicated chronologies. Detrending would also negate one of the greatest advantages of isotope-based climate reconstructions, which is that they have the potential to retain climate information at all temporal frequencies (Gagen et al., 2007), thus circumventing the "segment length curse" that makes it difficult to extract long-term climate information from tree growth proxies (Cook et al., 1995).

Testing for the presence of age trends requires isotope data from many trees, preferably of very different calendar age, so that trends in climate are not confused with parallel trends due to increasing age. Given the cost involved, suitable data sets are sparse and are also heavily biased toward conifers growing at high latitudes and altitudes. Several authors have argued for the presence of long-term trends in tree ring $\delta^{18}O$ (Esper et al., 2010; Treydte et al., 2006; Yamada et al., 2018). However, in perhaps the most comprehensive single study, Young et al. (2011) identified no such effect in tree line conifers. No similarly detailed studies have been conducted on European oaks, but the limited evidence that is available suggests that there are no long age trends in oak tree ring $\delta^{18}O$ (Duffy et al., 2017; Li et al., 2015; Young et al., 2015).

The presence of age trends is sometimes only inferred from differences in the mean values of young and older cohorts of trees (Treydte et al., 2006). When the magnitude is quantified it is usually based on the slope parameter of a regression of isotope ratios against ring number (Esper et al., 2010; Gagen et al., 2008; Helama et al., 2015; Loader et al., 2013; Young et al., 2011). The statistical significance, if it is calculated, is typically based on the correlation coefficient, with degrees of freedom defined by the full length of the mean chronology, irrespective of the length of the component series. Testing the significance of the slope parameter (e.g., Young et al., 2011) gives identical results (McCarroll, 2017, p. 273). Helama et al. (2015) use error bars on cohorts arranged by ring number. We argue here that the common approach of averaging the isotope values of trees and timbers aligned by ring number is flawed, and can lead to the identification of spurious age trends with wildly exaggerated probabilities. We introduce a simple new method based upon measuring trends in the individual samples and compiling them. The average trend can be furnished with statistically defined confidence limits, using degrees of freedom based on the number of trees rather than the number of years. The two approaches (conventional "trend of the mean" and new "mean of the trends") give very different results.

2. Materials and Methods

Two sets of samples are used in this study: 17 living or recently felled trees and 32 oak timbers, from historic buildings of the tenth to nineteenth centuries, obtained from the Oxford Dendrochronology Laboratory archive. The samples are representative of the material available for constructing a long oxygen isotope chronology for the UK. Very few long ring sequences are available or suitable for isotope chronology construction, because the decline in ring widths with age makes it increasingly difficult to separate the latewood, so all series are trimmed to a maximum of 100 years. Modern and preindustrial trees are considered separately because of the potential for anthropogenic trends in climate, atmospheric chemistry, air pollution, and forest management to influence the results of the modern trees.
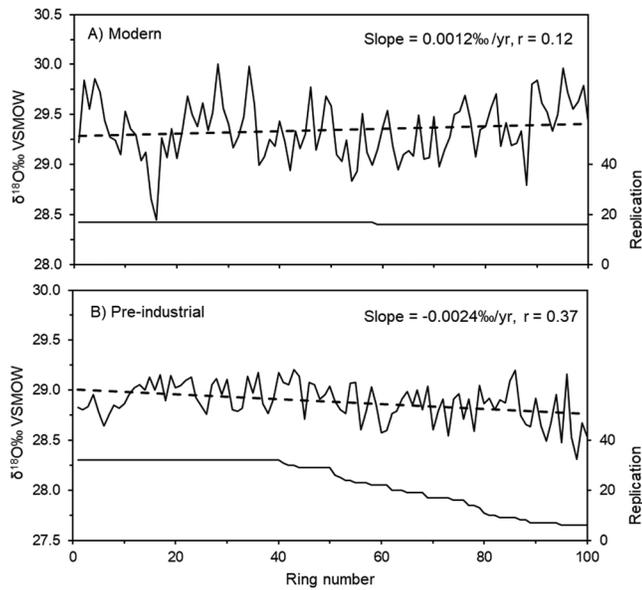
Figure 1. The mean of the (a) modern and (b) preindustrial samples aligned by ring number with apparent age trends identified by fitting a linear regression line. This is the conventional "slope of the mean" approach.

The latewood of each annual ring was cut into thin slivers (approximately 40 μm thick) under magnification and the alpha-cellulose extracted (Loader et al., 1997), homogenized, and freeze-dried. Between 0.30 and 0.35 mg was weighed into silver capsules and pyrolyzed for stable oxygen isotope analysis by mass spectrometry. Results are expressed as per mil (‰) relative to Vienna Standard Mean Ocean Water using the delta notation (δ^{18}O; Coplen, 1995).

We investigate possible linear age trends in the two groups, first using the conventional "slope of the mean" method of fitting a simple linear regression line through the mean of the age-aligned (by ring number) series. However, since the aim is to determine whether there are consistent age-related trends, a more direct approach is to examine those trends in the individual trees, and then to compile them in a way that allows them to be compared and averaged. We achieved this by fitting simple linear regression lines to the individual series (rather than the mean series) and plotting the regression lines for all trees aligned at a common point of origin ("mean of the slope" method). For the mean of the slope method statistical significance is assessed by using the binomial test to determine the probability of the split of positive and negative slopes occurring by chance and by calculating the 95% confidence limits around the mean of the slope values. We also use second-order polynomials to test whether nonlinear regression lines would provide a much better fit to potential age trends. All probabilities are two-tailed.

Trends in the isotope data from the modern trees are compared with those in the England and Wales precipitation record for May to August (Alexander & Jones, 2001; Young et al., 2015). Trends in the preindustrial series are compared with relevant sections of a long stable oxygen isotope "master chronology" based on pooled latewood cellulose from 10 separate trees (Loader et al., 2019). The master chronology was constructed by overlapping the trees so that they are not aligned by cambial age and timbers enter and leave the pools individually rather than in cohorts. Any age-related trends should thus be minimized in the master chronology, which can be used as a proxy for May to August precipitation amount (Young et al., 2015). None of the trees or timbers used in this study are included in the master chronology.

To test whether the contrasting approaches can produce spurious age trends, when there are no real age trends in the constituent series, we generated time series of random numbers. These series have a similar range of mean values to the preindustrial timbers used in this study and we varied the length of the series to exactly reflect the change in replication, a procedure that was repeated 100 times. The conventional slope of the mean approach was then applied by fitting a linear regression and the mean of the slope value was also calculated.

3. Results

All but one of the modern trees has 100 measured rings; however, the preindustrial ring counts range from 40 to 100, typical of the timbers available for chronology construction. Near pith "Juvenile" effects in δ^{18}O of UK oaks are very small and short-lived (Duffy et al., 2017), so we do not need to remove any data relating to the earliest rings of the sampled trees. The mean values of the modern trees range from 28.87 to 30.51‰ and of the preindustrial trees from 28.02 to 29.63‰, giving a maximum level offset of 2.49‰ between the means of individual trees. First-order autocorrelation is generally low, with mean values of 0.12 and 0.11 for the modern and preindustrial. Only four samples have values >0.3 and the maximum is 0.44. Summary statistics for the modern and preindustrial samples are tabulated in the supporting file together with the raw data and supporting graphs with linear and polynomial regression lines.

When potential age trends are investigated using the conventional method of aligning the individual series by ring number and fitting a regression line, the two groups give contrasting results. For the modern trees, there is a small rising trend (0.0012‰/year), and for the preindustrial (PI) trees, the trend is twice as steep but in the opposite direction (−0.0024‰/year; Figure 1). If statistical significance of the trends is assessed,
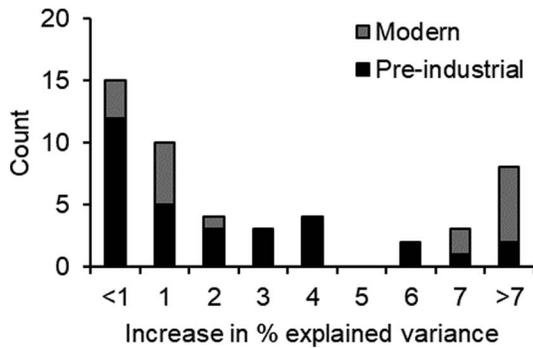
Figure 2. Increase in percentage of variance explained when a straight regression line is replaced with a second-order polynomial curve.

using the correlation coefficients (or significance of the slope coefficient) and assuming degrees of freedom are equal to the full mean series length minus two, the trend in the modern trees is not statistically significant ($r = 0.12$, $t = 1.19$, $p = 0.48$). However, the falling trend in the preindustrial trees is strongly significant ($r = 0.37$, $t = 3.97$, $p < 0.001$).

To test whether linear or nonlinear regression is appropriate for characterizing potential age trends in the individual trees or timbers, both a straight line and a second-order polynomial curve was fitted (see supporting information). Polynomial curves are more flexible than the monotonic curves that are normally fitted to tree ring proxies, including the negative exponential. Therefore, if polynomial curves do not result in a large increase in the amount of variance explained (R^2) it is impossible for monotonic curves to do so. For the 49 individual series examined, the median increase in varian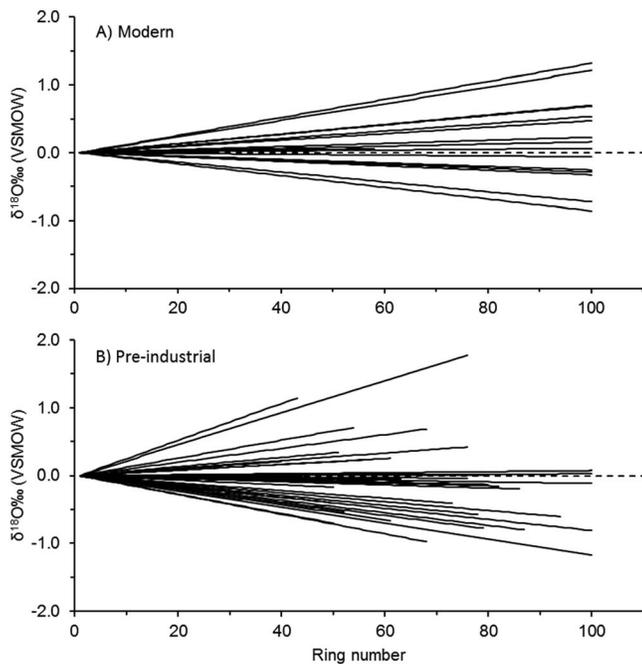ce explained is 1.9%. In only eight cases is the increase larger than 7% (Figure 2). In five of the six cases where the improvement in variance explained is larger than 10% (M2, M5, M17, PI25, PI26) the polynomial fits are not monotonic; they rise and then fall. Polynomial curves always explain more variance than straight lines, but in this case the increases are so small that we conclude that nonlinear regression is not appropriate.

Of the 17 modern trees, 6 show a declining trend with ring number (slope of the linear regression of isotope ratios against ring number) of which 1 is statistically significant (two-tailed $p < 0.05$). Eleven trees show an increasing trend, six of which are significant (Figure 3a and Table S1 in the supporting information). The division of positive and negative slopes is not statistically significant (binomial test, $p = 0.34$, effect size $r = 0.23$). However, the average of the 17 slope values is positive and significantly different from zero (0.002 ± 0.001‰). The largest slope value is 0.013‰/year for tree M6 ($n = 100$). These results suggest that individual modern trees do not consistently show a rising or falling trend in $\delta^{18}O$ with age (Figure 3a), but that overall the mean of the slopes is positive and statistically significant.

Figure 3. Linear trends of oxygen isotope ratios against ring number fitted to (a) 17 modern trees and (b) 32 preindustrial timbers, shifted to give a common origin. Dashed lines are the horizontal axis. This is the new "mean of the slope" method.

Of the 32 preindustrial timbers, 20 show a decline with increasing ring number, of which 8 are statistically significant ($p < 0.05$). Twelve show an increase, of which 3 are statistically significant ($p < 0.05$). The chance probability of obtaining 20 trends in one direction, from a sample of 32, is more than one in five (binomial test, $p = 0.215$, effect size $r = 0.22$). The average slope value is slightly negative but not significantly different from zero (-0.002 ± 0.003‰/year). The two strongest individual slope values are positive (Figure 3b and Table S2 in the supporting information).

After linear detrending, the strength of the correlation between isotope values and total May to August precipitation increases for eight trees and decreases for nine trees (Figure 4). The average difference in the r values before and after detrending is zero to three decimal places. Sections of the England and Wales precipitation record (May to August totals) that correspond to the same period as each of the sampled trees were collated. As with the isotope results, the mean of the 17 slope values is significantly different from zero (-0.148 ± 0.105 mm/year). This reflects a drying trend over the modern period that is observed as a significant positive mean slope in the oxygen isotope ratios.

Of the 25 preindustrial samples that overlap with the master chronology, only 11 showed an increase in r value after linear detrending and all values lie close to the 1:1 line (Figure 4). The mean correlation between samples and equivalent sections of the master chronology does not change, when the samples are linearly detrended ($r = 0.62$). The preindustrial timbers with the strongest positive (PI3) and negative (PI25) age trends show a decline in the correlation with the master chronology after detrending (PI3: 0.70 to 0.63, PI25: 0.59 to 0.56). However, there is no
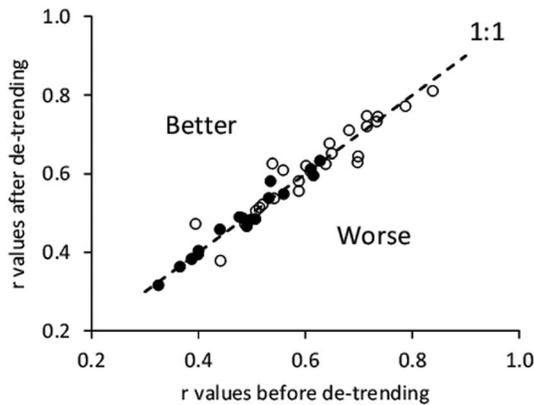
Figure 4. Absolute *r* values, before and after detrending, based on the correlation between δ^{18}O of modern trees (closed circles) and the equivalent sections of the England and Wales precipitation series (May to August sum) and the preindustrial timbers (open circles) and the equivalent sections of a master chronology serving as a proxy for May to August precipitation.

overall correlation between the slope coefficients in the individual timbers and in the equivalent sections of the master chronology (Spearman′s rank correlation, rho = 0.26, $p > 0.05$). It is clear that simple linear detrending does not consistently improve the correlation with the master chronology, as would be expected if there were consistent age-related trends in the individual samples.

The simulation based on random numbers, with the same series lengths as the preindustrial timbers and realistic variability and offsets, produces very different results for the two approaches used for identifying age trends. The mean slopes are all very small (Figure 5), ranging from −0.001 to +0.001‰/year, but the spread of slopes estimated by the conventional slope of the mean is much larger (−0.005 to +0.004), with 78% statistically significant ($p < 0.05$) and in 67% of cases the significance level is $p < 0.001$. The highest absolute *r* value obtained was 0.84, and 15% of the mean time series gave *r* values beyond ±0.70.

4. Discussion

The conventional method for identifying age trends in tree ring isotopes, where the samples are aligned by ring number (cambial age) and the trend quantified by regression of mean isotope value on ring number, aims to capture the average behavior of the individual samples. A significant trend in the mean is assumed to reflect a significant trend, on average, in the constituent series. However, it is clear from the results presented here that the conventional approach does not capture the average behavior of the individual samples. When the trends in the individual samples are measured and compiled (mean of the slope approach) they produce contrasting results.

In the case of the modern trees, the conventional slope of the mean suggests no significant increase with age, whereas the "mean of the slopes" shows that although the trends are not consistent, the average trend is a statistically significant increase. However, this increase is not noise; it is part of the climate signal carried by the oxygen isotopes. When the equivalent sections of the May to August England and Wales precipitation totals are compiled they also show a statistically significant mean trend. These results suggest that although in each individual modern tree the trend is dominated by random "noise," their average behavior captures a real drying trend in the instrumental climate records. Individually detrending the isotope series prior to constructing a chronology would therefore remove the long-term climate information that is of most interest.

Figure 5. The mean trends of 100 simulations produced by generating sets of 32 time series of random numbers with a similar range of mean values to the preindustrial timbers used in this study and the same differences in series length. (a) The average slopes of the individual series (mean of the slopes) are all close to zero but if the random time series are aligned by ring number, and the slope estimated by fitting a regression line through the mean (the conventional 'slope of the mean' approach) the range of slopes is much greater (B).

In the case of the preindustrial trees, the "slope of the means" (Figure 1a) suggests a declining trend that is strongly statistically significant ($p < 0.001$) whereas the mean of the slopes (Figure 3a) is not significantly different from zero. When each sample is compared with the appropriate section of a master chronology, which provides a preinstrumental proxy for May to August precipitation, the timber with the strongest rising trend covers the period that also has the strongest drying trend. Otherwise, the "age trends" are just random noise, and removing them has no effect on the correlation with (proxy) climate.

The strongly statistically significant "age trend" in the preindustrial trees, identified using the conventional slope of the mean approach, is an artifact of the method and is caused by the offsets in mean isotope values between different timbers and the change in replication with ring number (Figure 1b). To obtain a mean series with no trend, when regressed against ring number, it would be necessary for the average isotope value of the few longest samples (most rings) to be exactly the same as the mean value of all of the samples when replication is high. Even a very slight offset in mean value between the two ends of the time series will inevitably induce
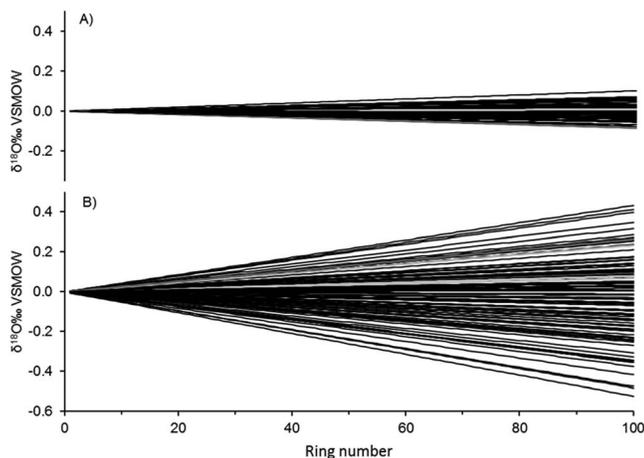
a strongly significant trend in the mean data, because as replication declines the weighting attributed to the longest series increases incrementally. Not only is the value of the slope spurious but also the statistical significance of the slope is grossly exaggerated: by the common assumption that degrees of freedom can be calculated from the full mean series length. This would only be true if adjacent mean values were independent of each other, an assumption which is strongly violated. The offsets between the samples dictate that, as the shorter samples drop out, with increasing cambial age (rising ring number), the mean value must migrate toward that of the longer timbers that remain. This will happen even if all of the samples in the composite have a slope of zero. The same problem has been recognized in quantifying age trends in the mercury content of tree rings (Alvarez et al., 2018). Applying error bars to the mean of cohorts of samples arranged by ring number (Helama et al., 2015) is subject to the same source of error, since the offsets between trees are ignored.

The potential for the conventional slope of the mean approach to produce spurious age trends, with wildly exaggerated probabilities, is illustrated by the simulation using random numbers to produce many sets of 32 series, with realistic values and offsets (Figure 5). Even though the mean trend of the individual series is zero, when the random series are arranged by ring number, only 22% of the replicates produced a mean trend that was not statistically significant, and in 67% of cases the spurious trend appears significant at $p < 0.001$. If the randomly generated series had been allowed some autocorrelation, or allocated small random age trends, which would be realistic, the magnitude of errors would be even greater. Normalizing the series prior to analysis would reduce, but not remove, the errors.

5. Conclusions

A great advantage of the isotope approach is that it may not be necessary to statistically detrend the results obtained from individual trees or timbers, prior to compiling mean chronologies to be used for a climate reconstruction. Therefore, the identification of potential age trends in tree ring isotope time series is critically important. Ring widths and density measurements are strongly influenced by tree age, as well as by climate, and so must be detrended, but with the inherent risk of losing some of the long-term climate information (the segment length curse). If isotope series do not have to be detrended individually, long chronologies can be constructed by pooling the wood from several trees prior to the isolation of alpha cellulose and mass spectrometry, hugely reducing the cost and effort involved and facilitating the construction of very long and very well replicated chronologies.

The results presented here, from samples of modern trees and historic building timbers, which represent precisely the material available for building a long isotope chronology for UK oak, clearly demonstrate that there is no consistent relationship between oxygen isotope ratios in the alpha cellulose of latewood rings and ring number. This is demonstrated by measuring the trends in the individual trees, not aligning them by ring number and averaging them first. The strongest individual trends, in both directions, reflect similar trends in the target climate variable (May to August precipitation sum). The rest of the weak trends are simply random noise, and removing them does not improve the correlation with climate.

If the same data are treated using the conventional approach used to identify age trends, where the individual series are first aligned by ring number and the trend estimated by fitting a regression line, very different conclusions are reached. We have demonstrated that this commonly applied method is not appropriate, as the trends do not honestly reflect the real trends in the individual series, but are very strongly controlled by offsets in the mean values of series of different length. Statistical significance values based upon correlation of isotope values with ring number are vastly inflated because the trends are often spurious and the assumed degrees of freedom are much too high, as demonstrated using a random number simulation.

The conventional trend of the mean approach is clearly not appropriate for identifying age trends in tree ring isotopes and can produce very misleading results. Where the aim is to characterize the average behavior of a sample of trees, the appropriate method is to measure the trends in each individual sample and then average them. When calculating the statistical significance of the average slope value, the degrees of freedom should be based on the number of trees, not the maximum series length.

We conclude that there is no evidence for consistent age trends in the $\delta^{18}O$ of the alpha cellulose of latewood rings in the samples of oak available for building long chronologies for the UK. This means that they do not

need to be detrended prior to being compiled and it is appropriate to pool wood samples from several trees prior to chemical treatment and isotope measurement. Where age trends have been identified for other species, or other regions, using the conventional trend of the mean approach, we urge that the data be reassessed using the more appropriate slope of the mean method.

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