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Quantifying uncertainty in isotope dendroclimatology

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Abstract

To maximise the potential of the tree-ring isotopic signal for palaeoclimate research it is essential to understand and characterise the natural variability between individual trees. This study explores the nature of inter-tree isotopic variability and evaluates the implications for developing robust palaeoclimate reconstructions. We confirm levels of natural inter-tree variability similar to those reported in previous studies, but demonstrate, using a large data set of isotopic measurements determined from individual rings of 100 trees, that to obtain a representative regional environmental signal and to reduce problems when combining records, higher levels of replication than those typically adopted in isotope dendroclimatology may need to be considered.

Keywords

carbon, dendrochronology, paleoclimatology, *Pinus sylvestris*, stable isotope, tree-ring

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Rationale

The development of millennial-length palaeoclimate reconstructions from tree-ring stable isotopes requires the compilation of data from multiple sample series. In ring width- or density-based dendroclimatology, series are routinely detrended to remove growth trends and maximise environmental signals, prior to cross-correlation to assess expressed signal strength (Wigley et al., 1984), calibration and independent verification against instrumental climate data (e.g. Cook and Kairiukstis, 1990; Esper et al., 2012; Helama et al., 2002; McCarroll et al., 2013; Schweingruber et al., 1988; Wilson et al., 2012a).

These protocols provide a ‘framework’ within which to develop robust palaeoclimate reconstructions. Tree-ring stable isotope ratios may be compiled and analysed in a similar manner, however, evidence to support an absence of long-term age-dependent trends offers potential to capture a greater proportion of low-frequency environmental information as the data often do not require standardisation (Cook et al., 1995; Gagen et al., 2008; Young et al., 2011a). The ability to retain such low-frequency information is important in the study of climatic change as a reconstruction that retains environmental information across all temporal scales provides a more challenging target for the evaluation of climate model data (Hind et al., 2012; Loader et al., 2013). It also provides the spatio-temporal perspectives on isotopes in the natural environment required to supplement the limited observational data sets used to develop and evaluate the performance of isotope-enabled Earth system models (Daley et al., 2011; Saurer et al., 2012; Shaffer et al., 2008; Tindall et al., 2009; Treydte et al., 2007).

If tree-ring isotopes contain no long-term age-related trends, then where sufficient individuals can be sampled, we hypothesise that resulting isotope series may be combined and interpreted without the need for statistical detrending. Isotopic variability arising from natural inter-tree variability will be reduced (through replication) to yield palaeoenvironmental data with quantifiable uncertainty. This paper explores the statistical nature of such inter-tree isotopic variability, and proposes a revised protocol for sampling in isotope dendroclimatology.

Leavitt and Long (1984) argued that isotope data from tree cores averaged from four to five trees would be sufficient to provide a representative environmental signal determined using the expressed population signal (EPS) (Wigley et al., 1984). Their study was based upon the measured inter- and intra-tree carbon isotope analysis of co-located *Pinus flexulis* J. trees growing in the southwestern USA and has subsequently become an unofficial ‘standard’ approach for many isotope studies. To date, more than 25 multicentennial stable isotope chronologies have been developed following this approach (e.g. Andreu-Hayles et al., 2011; Etien et al., 2008; Grießinger et al., 2011; Hilasvuori et al., 2009; Kress et al., 2010; Liu et al., 2012; Loader et al., 2008; Rinne et al., 2010; Seftigen et al., 2011; Shi et al., 2012; Szymczak et al., 2012; Tardif et al., 2008; Treydte et al., 2006, 2007; Xu et al., 2011; Young et al., 2012).

Whilst it has been demonstrated that isotopic series developed from as few as four to five trees can yield an EPS >0.85 (Leavitt, 2010), this measure only reflects inter-series correlation (inter-annual coherence) and whilst appropriate for determining signal strength in standardised data, it ignores offsets in the absolute isotope values. This sampling strategy was criticised by McCarroll and Pawellek (1998) who suggested that larger samples would be required to provide acceptable confidence limits around mean isotope ratios. However, the carbon isotope data used by McCarroll and Pawellek (1998) was obtained from modern juvenile

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trees, with no corrections made for the isotopic composition or concentration of atmospheric carbon dioxide, and so their study may have overestimated natural inter-tree variability.

The presence of offsets between individual trees is not particularly problematic where average series are produced using a single generation of living trees (Bale et al., 2011). However, producing long chronologies usually involves joining together different cohorts of living, dead and subfossil trees. If samples are not sufficiently large then the offsets between trees can be reflected in offsets in the mean series where different cohorts join (Loader et al., 2013). The presence of such steps is most likely to reflect the inability of four to five trees to represent fully the inter-tree variability of a specific population (Etien et al., 2008; Gagen et al., 2011, 2012; Hangartner et al., 2012).

Stable isotopes from individual trees certainly contain non-climatic information reflecting, perhaps, differences in micro-habitat, genetics or environmental disturbance which may be expressed as short-lived or more prolonged perturbations. Where such perturbations are sporadic in nature they may be identified and resolved through replication (McCarroll and Loader, 2004), and if individual measurements are available, the inter-tree variability allows confidence limits to be placed around the mean isotope value for each year. Uncertainty in the mean can be combined with calibration error to produce a quantified uncertainty for climate reconstructions (McCarroll et al., 2013), which is essential for proxy-model comparison. If tree-ring isotopes are to be used in this way then it would be useful to know the level of replication required to yield a representative mean signal. This is especially important if a pooling strategy is adopted, combining the rings from several trees prior to analysis, because without values for each tree it is not possible to calculate directly the uncertainty around the mean value for each year (Woodley et al., 2012).

Method

To explore the range of natural inter-tree variability, 100 mature (non-juvenile) Scots pine trees (*Pinus sylvestris* L.) were sampled from native pine woodland in the Cairngorm Mountains, UK. Trees were selected as randomly as possible and to simulate the recovery and selection of sample material from antiquity (Wilson et al., 2012b). This approach includes the caveats that trees exhibiting obvious significant signs of damage or disease or apparently very young trees (<50 years in age) were rejected as these would not normally be used in isotope dendroclimatology. Cores were collected using a 10 mm diameter increment borer from trees growing across an area of c. 3.5 km^2 and from a range of woodland micro-environments typical of the region (variable slope, soil depth, drainage, light availability, canopy density, understorey vegetation, core orientation, etc.; Figure 1). Tree-rings were precisely dated and wood from the year 2009 manually excised using a scalpel for cellulose preparation and isotopic analysis by pyrolysis at 1400°C (Laumer et al., 2009; Loader et al., 1997; Rinne et al., 2005; Young et al., 2011b). Analytical precision of standard cellulose was 0.1 per mille (‰) (δ^{13}C) and 0.3‰ (δ^{18}O) σ_{n-1} n=10.

The resulting data set of isotopic observations from 100 trees covered a range of tree ages and environments. It was deemed to represent the isotopic composition of the forest growing across the study area. The resulting carbon and oxygen isotope data sets were explored using a replicate resampling (bootstrap) method (Canty and Ripley, 2009; Quenouille, 1949) to determine how different levels of replication affected the uncertainty in the mean. By sampling at different levels of replication (with replacement), based upon subsamples of single ring measurements from 1 to 20 trees it becomes possible to determine the relative confidence intervals associated with the natural variability within the data, to assign uncertainty estimates around the mean and to propose levels of sample replication to attain reasonable uncertainty estimates.

Since the Cairngorm data represent variability between trees for only a single year, there is the possibility that a single year may not adequately represent the true inter-tree variability across a range of climatic conditions. We therefore test this relationship through comparison of our 100-tree single ring data with summary data from independently developed data sets of stable carbon and oxygen isotopes analysed from ten individual Scots pines (AD 1900–2003) growing in northern Sweden (Loader et al., 2013).

Results

An analysis of the distribution of the stable carbon and oxygen isotope data from the 100 trees indicates that both isotopes exhibit a near-normal distribution (Shapiro-Wilks normality test W_{CARBON} = 0.99, p-value = 0.82, W_{OXYGEN} = 0.99, p-value = 0.62). The isotopic range for the 100 trees is 2.93‰ for carbon and 2.35‰ for oxygen (Figure 2). This range is somewhat large when compared with natural climate-driven isotopic variability over time, but not unexpected considering previously reported intra-tree variability and the non-selective sampling protocol employed here. As the AD 2009 ring falls within the recent period of post-industrial increased atmospheric CO$_2$ concentration it is also possible that changes in tree response (intrinsic water-use efficiency) resulting from changing CO$_2$ concentrations will lead to a wider range of variability than observed in pre-industrial times. Therefore these values may be considered as providing a conservative estimate of inter-tree isotopic coherence (McCarroll et al., 2009; Waterhouse et al., 2004).

Using the 100-tree (single ring) data set, we can assess how the size of the uncertainty around the mean isotope value changes with differing levels of replication. This can be tested in two ways; if we accept normality then we may simply use the 95% confidence limits, with the t-distribution multiplier for a small sample. However, an alternative is to bootstrap sample the large data set and to assess how variable these limits actually are. If the population is exactly normally distributed, and replication is appropriately high, the two approaches will be equivalent. In this study the data set was sampled 1000 times (with replacement) and with levels of replication (n) rising from 1 to 20. This produces smooth distributions and we can calculate from these the proportion of values falling beyond any predefined limits. Table 1 provides the full data distribution and the effect of changing replication (n) upon the 95% and 90% confidence intervals.

For a sample size of 10 we can expect 90% of mean values to fall within a range of 0.57‰ and 0.56‰ (carbon and oxygen isotopes, respectively), and 95% of mean values to fall within a range of 0.68‰ and 0.67‰. Raising sample size to 20 (95%) gives ranges of 0.50‰ and 0.46‰. For a sample of 5 (95% and 90% confidence intervals) the values are 0.98 and 0.86‰ for carbon and 0.92 and 0.79‰ for oxygen. The mean 90% and 95% confidence intervals for the sample of ten Swedish *Pinus sylvestris* sampled over the 20th century (Loader et al., 2013) are 0.26‰ and 0.32‰ δ^{13}C and 0.32‰ and 0.40‰ for δ^{18}O, respectively (analysed over 104 years AD 1900–2003 σ_{n-1} n=10 trees) which agrees favourably with the estimate calculated from the Cairngorms trees indicating that these results do not depend upon a specific year or location (Figure 2). Since these data are all sampled from tree growth within the recent 'industrial period' an equivalent analysis conducted on the carbon isotope data set corrected for post-industrial changes in atmospheric δ^{13}C and CO$_2$ concentration yields a reduced 95% confidence interval of 0.31‰ suggesting that inter-tree carbon isotopic variability may be only slightly greater during recent decades as a consequence of environmental modification.

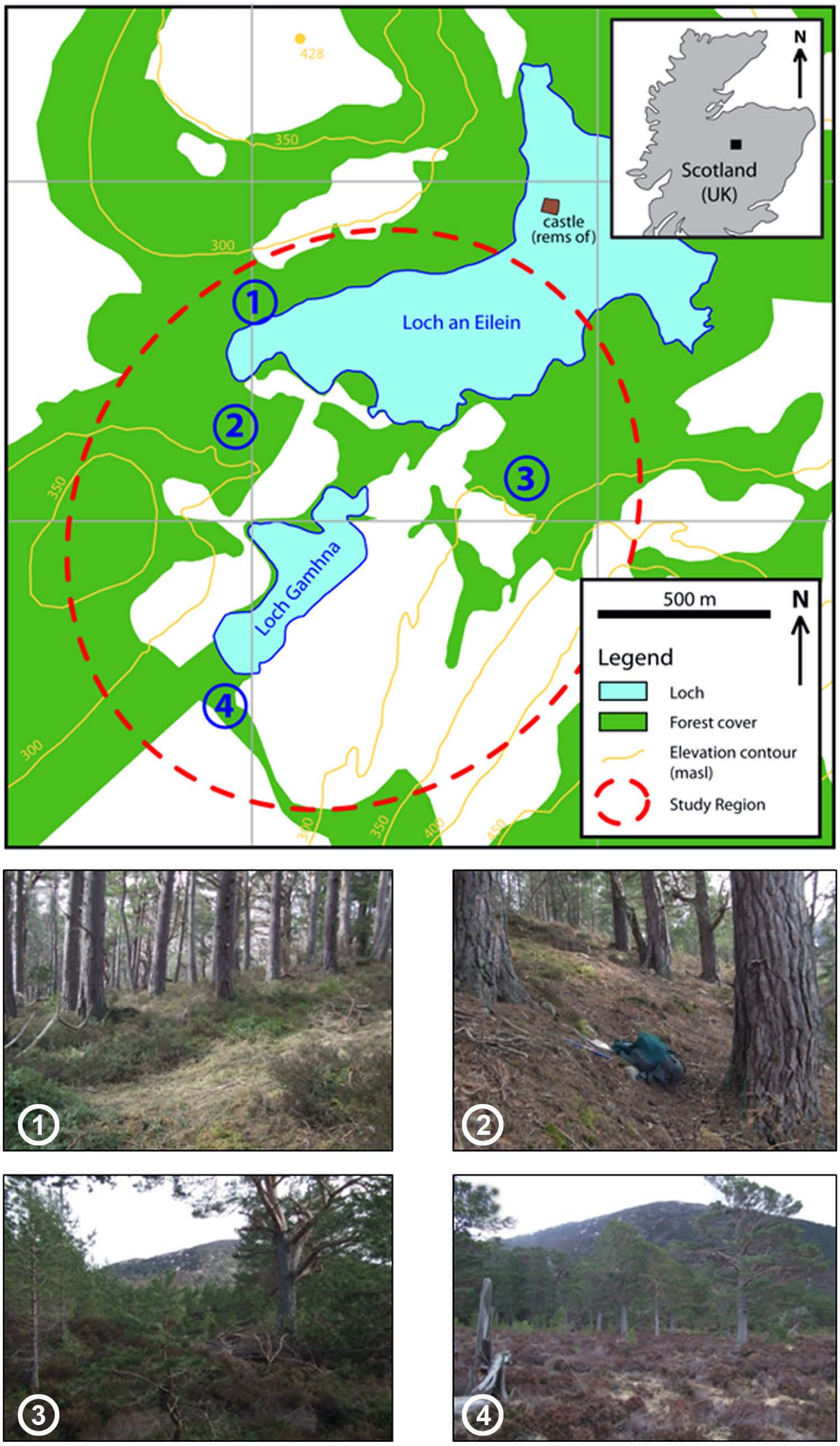

Figure 1. Location of the Rothiemurchus study site and region from which samples were collected for this study (upper panel). The sample area covers approximately 3.5 km^2 and incorporates a range of micro-environments (gradient, stand age and density, aspect, topography, hydrology and altitude). Photographic examples of these environments (1–4) are presented in the lower panels with their location within the sample area located on the accompanying map.

How large an uncertainty is acceptable? This is not a purely statistical question; it depends on how and for what purpose the data are being used. If we take a sample of 10 we can be 90% confident that the true mean lies within ±0.3‰ of our estimate. If these data are to be used in palaeoclimatology then this uncertainty needs to be combined with the uncertainty derived from the calibration equation to determine how uncertain the climate reconstruction will be. If the calibration is very strong we can use

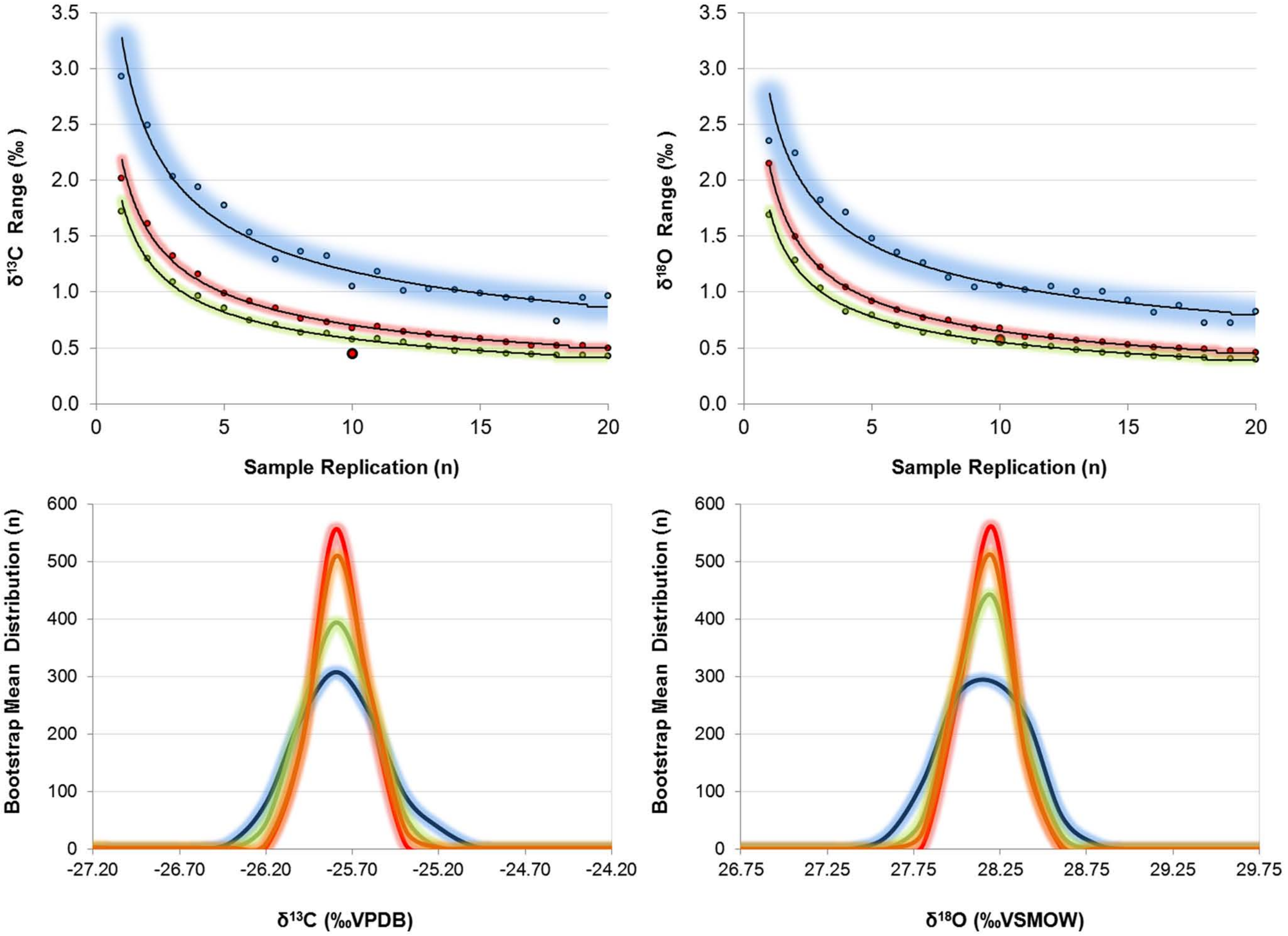

Figure 2. Bootstrapped estimates of uncertainty developed from the Cairngorm data set for carbon and oxygen stable isotopes (upper left and right panels, respectively). Blue line indicates the uncertainty for the whole data set resampled with replacement 1000 times. Red line indicates the 99% confidence interval and green line the 95% confidence interval. Red circle represents the mean annual 99% uncertainty calculated on a pool of ten *Pinus sylvestris* L. trees from northern Sweden analysed as individual trees with annual resolution over a period of 104 years AD 1900–2003. Lower panels show the frequency distribution of the data from the Cairngorms study bootstrapped 1000 times with replacement. Distributions for 5, 10, 15 and 20 tree samples are presented (blue, green, orange and red lines, respectively) for carbon (left panel) and oxygen (right panel) stable isotope ratios.

fewer samples, but if it is weaker we need higher levels of replication. The result of such an approach (McCarroll et al., 2013) may be an increased reconstructed uncertainty, but it should be more representative of the true uncertainty than using only the regression error and importantly provides the probabilistic uncertainty required for statistical evaluation of and comparison with climate model data.

The results presented above suggest that a reliable mean value can be established when c. 10 trees are sampled, although depending upon the aims of the individual study a smaller number may also be acceptable. We recognise that for pilot investigations or where resources are limited, sampling ten trees for isotopic analysis is not always possible. Whilst lower levels of replication may still yield robust estimates of interannual- and decadal-scale variability, dependent to a degree upon segment length (Cook et al., 1995), a more realistic estimation of the regional mean and lower-frequency variability (developed without detrending) may only be attained with sufficient sample replication. A potential solution to such resource limitations is the pooling of sample material to yield annualized chronologies at the expense of the individual series. In such situations, statistical uncertainty may be applied to the isotopic data based upon studies of this kind, in a considered manner, to provide a measure of statistical uncertainty based upon the number of trees within the pool. Care should be taken with any such approach, especially when interpreting longer-term palaeoclimatic variability, or at the points where sample cohorts join. Importantly, these findings confirm that it is highly unlikely that long-term palaeoclimatic changes could faithfully or confidently be reconstructed with only one or two trees.

Conclusion

This study highlights the need for increased levels of series replication than those typically adopted and initially proposed by Leavitt and Long (1984) if a robust low-frequency signal is to be attained and used to reconstruct palaeoclimates. It helps explain the shifts and differences often observed between overlapping sample cohorts developed from a small number of trees. In areas where the development of long tree-ring stable isotope chronologies may be planned, studies similar to this may be undertaken to assess natural inter-tree variability and to assign more representative confidence limits around reconstructions developed from the combination of several trees. An alternative approach would be to use a small sample of trees but treat the records as one might ring-width or maximum density and to standardise the data prior to combination. This will reduce the uncertainty around the mean indexed values but low-frequency information is likely to be lost.

Although a strong inter-series common signal (EPS>0.85) may be attainable by sampling as few as four trees, this level of replication may be too low to capture adequately the site mean

Table 1. Isotopic range, standard deviation and confidence intervals (‰) calculated for the Cairngorms data set for carbon and oxygen isotope ratios by Bootstrap sampling (1000 samples with replacement for $n = 1$ to 20).

Sample replication (n)	Full range ($\delta^{13}C$)	Standard deviation ($\delta^{13}C$)	95% range ($\delta^{13}C$)	90% range ($\delta^{13}C$)	Full range ($\delta^{18}O$)	Standard deviation ($\delta^{18}O$)	95% range ($\delta^{18}O$)	90% range ($\delta^{18}O$)
1	2.93	0.56	2.02	1.72	2.35	0.51	2.15	1.69
2	2.49	0.41	1.61	1.30	2.24	0.38	1.50	1.29
3	2.03	0.33	1.32	1.09	1.82	0.31	1.22	1.03
4	1.94	0.29	1.16	0.96	1.72	0.26	1.04	0.83
5	1.78	0.26	0.98	0.86	1.48	0.24	0.92	0.79
6	1.53	0.23	0.92	0.75	1.35	0.22	0.84	0.70
7	1.29	0.22	0.85	0.71	1.26	0.19	0.77	0.64
8	1.36	0.20	0.77	0.64	1.13	0.19	0.75	0.63
9	1.32	0.19	0.73	0.63	1.05	0.17	0.67	0.56
10	1.05	0.18	0.68	0.57	1.06	0.17	0.67	0.56
11	1.18	0.18	0.69	0.58	1.02	0.16	0.60	0.52
12	1.01	0.17	0.64	0.55	1.05	0.15	0.60	0.51
13	1.03	0.16	0.62	0.51	1.01	0.14	0.57	0.48
14	1.02	0.14	0.59	0.47	1.01	0.14	0.55	0.46
15	0.99	0.15	0.58	0.47	0.92	0.14	0.53	0.44
16	0.95	0.14	0.55	0.45	0.82	0.13	0.50	0.43
17	0.93	0.14	0.52	0.44	0.88	0.13	0.50	0.42
18	0.74	0.13	0.52	0.43	0.73	0.13	0.49	0.41
19	0.95	0.13	0.52	0.43	0.72	0.12	0.47	0.40
20	0.96	0.13	0.50	0.42	0.82	0.12	0.46	0.40

isotopic signal and associated low-frequency trends. Replicate resampling of a pool of 100 tree-ring samples indicates that higher levels of replication (≥10 trees) should be considered if a more reliable low-frequency signal is sought. We demonstrate this variability and the need for improved consideration of uncertainty using the tree-ring isotope archive, but our approach may be transferrable to other isotope-based palaeoclimate proxies (e.g. *Sphagnum* moss, speleothems or foraminifera) to determine appropriate levels of replication. Achieving this new 'standard' in isotope dendroclimatology using individual trees will require a substantial increase in cost and effort, though this is somewhat offset by recent technological developments in sample preparation and analysis. A realistic alternative is to use pooling strategies to reduce the cost of analysis whilst ensuring that enough trees are included in the sample to provide a reliable signal. The results presented here provide a guide to the likely uncertainty in isotope chronologies built using pools comprising different numbers of trees.

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