Science of the Total Environment 409 (2011) 2244–2251

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Pooled versus separate measurements of tree-ring stable isotopes

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ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 30 December 2010
Accepted 7 February 2011
Available online 12 March 2011

Keywords:
Dendrochronology
Tree rings
Stable isotopes
Pooling
Carbon-13
Oxygen-18

ABSTRACT

δ^{13}C and δ^{18}O of tree rings contain time integrated information about the environmental conditions weighted by seasonal growth dynamics and are well established as sources of palaeoclimatic and ecophysiological data. Annually resolved isotope chronologies are frequently produced by pooling dated growth rings from several trees prior to the isotopic analyses. This procedure has the advantage of saving time and resources, but precludes from defining the isotopic error or statistical uncertainty related to the inter-tree variability. Up to now only a few studies have compared isotope series from pooled tree rings with isotopic measurements from individual trees. We tested whether or not the δ^{13}C and the δ^{18}O chronologies derived from pooled and from individual tree rings display significant differences at two locations from the Iberian Peninsula to assess advantages and constraints of both methodologies. The comparisons along the period 1900–2003 reveal a good agreement between pooled chronologies and the two mean master series which were created by averaging raw individual values (*Mean*) or by generating a mass calibrated mean (*MassC*). In most of the cases, pooled chronologies show high synchronicity with averaged individual samples at interannual scale but some differences also show up especially when comparing δ^{18}O decadal to multi-decadal variations. Moreover, differences in the first order autocorrelation among individuals may be obscured by pooling strategies. The lack of replication of pooled chronologies prevents detection of a bias due to a higher mass contribution of one sample but uncertainties associated with the analytical process itself, as sample inhomogeneity, seems to account for the observed differences.

1. Introduction

Among the characteristics of wood that may be correlated with environmental parameters, stable isotopes in tree rings are valuable sources for studies on past climate and other proxy-based reconstructions (e.g., Danis et al., 2006; Gagen et al., 2007; Treydte et al., 2009).

Apart from changes in the source value (δ^{13}C of atmospheric CO_2, δ^{18}O of soil water), the variability of isotope records from tree rings is closely dependent on the impact of environmental changes on plant physiological processes, mainly photosynthesis and transpiration (e.g., McCarroll and Loader, 2004). Furthermore, tree-ring isotope records are affected by the intra-annual growth dynamics, as well as post-photosynthetic processes related to seasonal changes in carbon allocation or oxygen isotope exchange between sugar and xylem/phloem water prior to cellulose formation (e.g., Helle and Schleser, 2004). Due to the continuous formation of wood during the vegetation period, information about the plant ecophysiological processes is integrated over time and stable isotope analyses become very attractive as a tool for ecophysiological studies, e.g., investigations on the dynamics of intrinsic water-use efficiency of trees (Saurer et al., 2004; Seibt et al., 2008).

Besides the positive attributes of tree-ring series such as the annual resolution, the accurate dating and the wide geographical distribution of trees; stable isotopes in tree rings offer additional advantages. One of the most relevant is that stable isotope chronologies, after a short “juvenile increase”, do not seem to contain any long-term age related trends and, therefore, do not require any statistical detrending (McCarroll et al., 2009; McCarroll and Loader, 2004), and therefore low-frequency fluctuations are fully preserved and the problem of the “segment length curse” is avoided (Cook et al., 1995; Gagen et al., 2007). This statement is nowadays being questioned (Esper et al., 2010) but no agreement has been reached

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doi:10.1016/j.scitotenv.2011.02.010

yet. Compared to ring width or wood density, individual stable isotope series display a stronger common signal between trees which reduces the effective sample size needed to have a representative site signal. In general, the amount of samples needed to build a reliable isotope chronology is much lower than for any other tree-ring parameter and usually 4 to 5 trees are enough (Gagen et al., 2004, 2007; Leavitt, 2010; Leavitt and Long, 1984; Treydte et al., 2001).

To date, annually resolved stable isotope chronologies are built in three ways. The first method follows the standard procedure of tree-ring width chronology development by measuring individual tree-rings and creating a mean site value (Gagen et al., 2006, 2007; Treydte et al., 2001). The second strategy is to pool the tree rings of one year from a chosen number of trees prior to the chemical extraction of cellulose and isotope mass spectrometry determination, obtaining one single representative annual value (Leavitt and Long, 1984; McCarroll and Pawellek, 1998; Treydte et al., 2001, 2006, 2009). The third approach involves serial pooling of shifted tree-ring blocks for building of isotope chronologies which produces a quasi-annual time series (Boettger and Friedrich, 2009). Although annual and quasi-annual approaches may yield similar information about tree response to environmental dynamics, in this study we focus on comparing the first two methods, which rely on annual dissection of individual tree-rings.

The pooling of all material of a particular year to a composite sample before chemical treatment and isotopic analysis is normally done regardless of the mass contribution of each tree ring. This produces a mass-weighted mean chronology which is, theoretically, close to the ring-width weighted mean (Borella et al., 1998; Leavitt, 2008; Treydte et al., 2001). However, it may be different compared to averaging the δ^{13}C and/or δ^{18}O values from individual series. The pooling techniques always suffer from unequal mass contribution of the samples to the pool and therefore to the mean isotopic value. If the contribution to the pool would be of equal masses of wood or cellulose from each tree, the result should not be different to the average of the individual series (unweighted mean). Furthermore, in standard dendrochronological procedures, an equal contribution of every tree to the final chronology is ensured by indexing the individual series before averaging them (Cook and Kairiuskstis, 1990). When working with isotope series, high variability among the mean values of the individual series composing a chronology would bias the result towards the samples with the highest mean isotopic values.

Generally, studies using pooled samples instead of individual tree-ring analysis are more common because of savings in resources and time, especially when the number of samples is very high due to a long time-span or high replications. Cellulose extraction is relatively time-consuming and is still a non-automatized process that needs to be done individually for every sample. Pooling may produce representative series, which is an advantage when there are time and cost constraints. Among the disadvantages is the impossibility to identify any possible non-climatic (e.g., age-related) trends in the isotopic series and the loss of any information related to inter tree variability. Therefore, it prevents calculation of statistics that ensure the reliability and site representativeness of the resulting stable isotope chronology (e.g.: expressed population signal, EPS; Wigley et al., 1984) as well as to establish confidence intervals around the mean.

Only few studies have tested the representativeness of pooled isotope series in comparison to the weighted and unweighted mean of individual series and most of them were performed using δ^{13}C. Borella et al. (1998) compared weighted and unweighted means of two sites and concluded that in general terms, there is no need of mixing the same amount of wood material, but care should be taken if a period with a high growth variation is detected in any tree. Treydte et al. (2001) showed that pooling of multiple cores display similar results to those obtained from individual series, despite significant differences in some years.

This paper goes one step further and compares pooled chronologies of two stable isotopes (δ^{13}C and δ^{18}O) from two sites at the Iberian Peninsula with the individual stable isotope tree-ring series of the same individuals that compose the pool. Our comparison is purely made on real values rather than on a combination of empirical and simulated data, and therefore, promises to shed more light on the crucial question to what extent pooling is comparable to the average of individual measurements. The test includes a comparison of the pooled chronologies (*Pool*) with the mass-calibrated mean chronologies (*MassC*), defined as the theoretical result of a pool if the isotopic value of every single tree and its contribution in mass to the pool is considered. It is also compared to the arithmetic average of the single series (*Mean*).

2. Material and methods

2.1. Site and sampling

During the European project ISONET, we extracted wood cores (5 mm diameter) of *Pinus uncinata* Ramond ex DC. in Lam. et DC at the Sierra de Cadí-Pedraforca in the Pyrenees and *Pinus nigra* Arn. spp. *salzmanii* (Dunal) at Sierra de Cazorla, Segura y las Villas during summer of 2003. The sites were selected because of their long-lived forests placed in climatic ecotones, where individuals are expected to be highly sensitive to climate. Specifically, Parque Natural de Sierra de Cazorla, Segura y las Villas (NCZ) is characterised by an oromediterranean dry–humid climate (Rivas-Martínez, 1983) with a recurring summer drought that mainly limits tree growth. The Cadí-Pedraforca Range (UPF) is located at the Pre-Pyrenees, just on the natural boundary between Mediterranean to Eurosiberian climatic regions (Rivas-Martínez, 1983) where low winter temperatures limit the annual growing period.

Several dominant trees were sampled at each site taking two cores per tree from different radial directions for establishing the tree-ring chronologies. Cores were sanded and visually crossdated (Stokes and Smiley, 1968). Ring-width measurements and correct dating were checked with the programme COFECHA (Holmes, 1983). Four trees showing high correlations of the ring widths series with the other trees and a small number of missing rings were selected at each site for stable isotope analysis (δ^{13}C and δ^{18}O).

In the context of the European project MILLENNIUM, the same sites were sampled again during the summer of 2006, with the aim of increasing the length of the chronologies back in time and performing individual measurements for establishing confidence intervals around the mean. Among the trees selected were the ones used for the pool in the ISONET project. At least two samples (12-mm cores) were taken from every tree and visually crossdated. Finally one core per tree was selected for the isotope analyses.

2.2. Stable isotope and wood density measurements

For the pooled isotope chronologies, two 5 mm cores per tree were taken and the tree rings separated with a scalpel before pooling the material of all trees year by year according to Treydte et al., 2001. Prior to cellulose extraction composite wood samples were milled with an ultra-centrifugal mill (Retsch, Germany) to mesh size $<$0.7 mm (Andreu et al., 2008; Treydte et al., 2001).

For setting up the individual isotope chronologies, we took one 12 mm core per tree of the same four trees used for the pooled chronologies. Each of the 12 mm cores came from the same orientation as one of the two 5 mm cores used previously for the pool chronologies. The wood cores were dated, then split year by year and cut into small pieces for cellulose extraction.

After cellulose extraction, individual and pooled samples were homogenised using an ultra-sonic device (Laumer et al., 2009).

We extracted α-cellulose from individual and pooled samples to avoid additional isotope variability due to varying proportions of wood components such as starch, lignin, resin and fatty acids, which display different isotopic signatures of up to 4‰ (Gleixner et al., 1993). The extraction of cellulose was done chemically using sodium chlorite and sodium hydroxide (Boettger and Friedrich, 2009; Loader et al., 1997; Sohn and Reiff, 1942).

Carbon isotopes for both pooled and individual series were measured at the GFZ-Potsdam Dendro Laboratory by combusting α-cellulose to CO_2 in an elemental analyser (Fisons NA 1500 NC) coupled via an open split to an isotope ratio mass spectrometer (IsoPrime, GV Instruments, Manchester, UK) operated in continuous flow mode. The reproducibility is better than 0.1‰. Oxygen isotopes from pooled samples were measured on CO at the Physics Department of the University of Bern using a TC/EA (Thermo Finnigan) pyrolysis furnace coupled online to a Delta Plus XL mass spectrometer. Similarly, individual oxygen isotope samples were measured at the GFZ-Potsdam Dendro Laboratory utilising a TC/EA pyrolysis furnace and a mass spectrometer a Delta V Advantage (Thermo Scientific, Bremen, D) with a standard precision of better than ±0.2‰ (replicate measurements of homogenised reference material (cellulose powder and sucrose (Merck KGaA, Darmstadt, D))) and reproducibility of ±0.25‰ (replicate measurements of samples). The isotope ratios are given in the conventional delta (δ) notation, relative to the standards VPDB ($\delta^{13}C$) and VSMOW ($\delta^{18}O$).

MILLENNIUM 12 mm cores used for isotopic analyses were also measured for density using a WALESCH 2003 X-ray densitometer (cf. Eschbach et al., 1995; Polge, 1965).

Earlywood mean density (EWmD), latewood mean density (LWmD), were used together with earlywood width (EWw) and latewood width (LWiw) for the calculation of mass-calibrated mean isotope chronologies (*MassC*).

2.3. Data analysis

The $\delta^{13}C$ tree-ring series are affected by the depletion in atmospheric $^{13}CO_2$ due to fossil fuel burning and deforestation since industrialization (ca. AD1850). The resulting changes in the carbon isotope source value introduces a decreasing trend that is not related to tree-physiological response to climatic or environmental change and needs to be removed from the raw $\delta^{13}C$ tree-ring series. The most common way is to subtract annual changes in $\delta^{13}C$ of atmospheric CO_2, obtained from ice cores and direct measurements, from each tree-ring stable isotope value (Elsig et al., 2009; Leuenberger et al., 1992). We applied this atmospheric correction to the $\delta^{13}C$ ISONET pool chronology (NCZ$\delta^{13}C_{Pool}$ and UPF$\delta^{13}C_{Pool}$, respectively for the two sites studied) and the MILLENNIUM individual series (see Leuenberger, 2007 and McCarroll and Loader, 2004 for details and values). Long-term changes of the atmospheric $\delta^{13}C$ source value affect all trees equally, however, individual trees may respond differently to changing CO_2 concentrations. Hence, no correction was adopted for potential impacts of the increasing atmospheric CO_2 concentration on the carbon isotope fractionation during photosynthesis such as the PIN correction (McCarroll et al., 2009; Treydte et al., 2009).

Stable-isotope chronologies do not usually need standardisation since they are assumed not to be affected by age related trends after the juvenile growth phase (Gagen et al., 2008). Our trees are all older than 500 years and thus are considered not to be affected by juvenile isotope effects during the chosen period of investigation: AD1900–AD2003.

At both sites, we developed three chronologies for each stable isotope series: (1) *Pool* mass weighted series resulting from pooling all material before measuring; (2) *MassC* theoretical mass-weighted series (see details of its calculation below); (3) *Mean*, $\delta^{13}C_{Mean}$ and $\delta^{18}O_{Mean}$ mass-unweighted chronologies generated by arithmetically averaging individual $\delta^{13}C$ and $\delta^{18}O$ series.

As the series did not show heteroscedascity, the $\delta^{13}C$ and $\delta^{18}O$ series were indexed by differencing from the mean and so, variability within each series remained unaltered (McCarroll and Pawellek, 1998; Saurer et al., 2008).

Mass calibrated mean chronologies (*MassC*) were calculated as follows:

$$MassC_t = \frac{\sum_{i=1}^{n}\left(\delta_{i,t} * m_{i,t}\right)}{\sum_{i=1}^{n} m_{i,t}}$$

where

$MassCt$	is the calibrated mean isotope value for year t
$\delta_{i,t}$	is either the $\delta^{13}C$ or the $\delta^{18}O$ value for sample i in year t
$m_{i,t}$	is the mass value in mg of sample i in year t calculated as:

$$m_{i,t} = (v_{EWi} * \rho_{EWi}) + (v_{LWi} * \rho_{LWi})$$

being:

V_{EWi}	volume of early wood on i
ρ_{EWi}	mean density for early wood portion on i
V_{LWi}	volume of late wood on i
ρ_{LWi}	mean density for late wood portion on i

Volumes were calculated as cylinder

$$V_{xwi} = \pi * r^2 * h_{xwi}$$

being:

V_{XWi}	volume of early or latewood portion on i
π	*pi* constant
r^2	radius of the core (6 mm)
h_{xwi}	width of early or latewood portion on i

The *Pool* chronologies $\delta^{13}C_{Pool}$ and $\delta^{18}O_{Pool}$ were compared directly to the $\delta^{13}C_{Mean}$, $\delta^{18}O_{Mean}$, $\delta^{13}C_{MassC}$ and $\delta^{18}O_{MassC}$ chronologies for the common period (1900-2003). They were transformed into anomalies (by subtracting from the mean) and then compared in different frequency domains.

The variability among the different isotope series was assessed by the range of maximum and minimum values, as well as the standard deviations of each series. The first partial autocorrelation coefficients (*PAC1*) were used to quantify the existence of dependency on lag processes.

The strength of the linear relationships between the tree-ring master series *Pool*, *Mean* and *MassC* were assessed by Pearson's correlation coefficients (r). High-frequency coherence was measured using the Gleichläufigkeit (GLK) which is a non-parametric statistic that measures the number of times two series show the same upward or downward trend in relation to the preceding year (Eckstein and Bauch, 1969).

3. Results

3.1. Individual trees

The $\delta^{13}C$ mean values of individual trees vary within a total range of 1.23 and 1.27‰ at NCZ and UPF respectively (Table 1). Stable oxygen isotopes series at UPF likewise differ from the mean by 1.28‰, while at NCZ differences among the means of the individual trees

Table 1

Statistics for the individual trees and master series derived from them (*MassC* and *Mean*) and *Pool* for the period 1900–2003 for the two studied sites (UPF and NZC). Includes number of tress/cores; mean ± SD = mean and standard deviation; Min = minimum value; Max = maximum value; range of values and *PAC1* = first order autocorrelation.

Chronology ID	Tree 1D	First ring measured	Trees/ cores	$\delta^{13}C$(‰)$_{corrected}$					$\delta^{18}O$(‰)				
				Mean ± SD	Min	Max	Range	PAC1	Mean ± SD	Min	Max	Range	PAC1
	NCZ_{002}	1605	1/1	−21.56 ± 0.40	−22.60	−20.64	1.96	0.52	32.92 ± 0.97	29.79	35.06	5.27	0.34
	NCZ_{015}	1600	1/1	−21.71 ± 0.61	−23.21	−20.08	3.13	0.44	34.58 ± 1.32	30.64	37.21	6.57	0.07
	NCZ_{026}	1413	1/1	−20.48 ± 0.37	−21.46	−19.65	1.81	0.42	34.14 ± 1.18	31.20	36.30	5.10	0.51
	NCZ_{027}	1472	1/1	−20.97 ± 0.67	−23.53	−19.88	3.65	0.32	31.12 ± 0.91	29.03	33.09	4.06	0.39
NCZ_{mean}			4/4	−21.28 ± 0.37	−22.47	−20.49	1.98	0.34	33.36 ± 0.77	31.44	34.97	3.53	0.35
NCZ_{MassC}			4/4	−21.34 ± 0.40	−22.49	−20.33	2.16	0.36	33.59 ± 0.87	31.84	35.67	3.83	0.27
NCZ_{Pool}			4/8	−21.13 ± 0.35	−21.98	−19.76	2.22	0.40	33.61 ± 0.89	31.46	35.33	3.87	0.41
	UPF_{003}	1552	1/1	−22.59 ± 0.68	−23.97	−21.29	2.68	0.69	31.38 ± 0.73	29.90	33.23	3.33	0.37
	UPF_{004}	1391	1/1	−21.71 ± 0.48	−23.89	−21.84	2.05	0.55	30.36 ± 0.79	28.76	32.64	3.88	0.38
	UPF_{005}	1491	1/1	−22.88 ± 0.47	−23.89	−20.73	3.16	0.58	30.28 ± 0.83	28.15	32.45	4.30	0.53
	UPF_{009}	1507	1/1	−21.61 ± 0.45	−22.87	−20.63	2.24	0.52	30.10 ± 0.85	27.61	32.16	4.55	0.53
UPF_{mean}			4/4	−22.20 ± 0.36	−23.17	−21.44	1.73	0.47	30.53 ± 0.60	29.29	31.70	2.41	0.40
UPF_{MassC}			4/4	−22.16 ± 0.38	−23.13	−21.4	1.73	0.49	30.42 ± 0.55	28.9	30.32	1.42	0.26
UPF_{pool}			4/8	−21.28 ± 0.48	−23.67	−20.61	3.06	0.24	30.89 ± 0.88	28.29	32.85	4.56	0.22

were higher (3.46‰). This is basically due to the fact that one tree, (NCZ$\delta^{18}O_{027}$) displays a considerable low mean value (31.12‰) in contrast to the means of the other three trees, varying within a range of 1.66‰ (32.92‰, 34.58‰, and 34.14‰ for NCZ$\delta^{18}O_{002}$, NCZ$\delta^{18}O_{015}$ and NCZ$\delta^{18}O_{026}$, respectively). The largest range within a single tree is 6.57‰ (minimum value 30.64; maximum value 37.21) for NCZ$\delta^{18}O_{015}$ and 4.55‰ (27.61 to 32.16) for UPF$\delta^{18}O_{009}$, while for stable-carbon isotope series the maximum ranges are reduced: 3.65‰ (−23.53 to −19.88) for NCZ$\delta^{13}C_{027}$ and 3.16‰ (−23.89 to −20.73) for UPF$\delta^{13}C_{005}$.

The dispersion of the data is consistently less in stable-carbon isotope series with a mean standard deviation far below 1‰:0.51‰ (range from 0.37 to 0.67) at NCZ and 0.52‰ (range from 0.45 to 0.68) at UPF while in oxygen isotope series the mean standard deviations are higher, being 0.8‰ (0.73 to 0.85) at UPF and 1.1‰ (0.91 to 1.32) at NCZ.

Individual stable carbon isotope series show a remarkable stronger autocorrelation than $\delta^{18}O$ series. The *PCA1* for $\delta^{13}C$ series range from 0.32 to 0.52 at NCZ and from 0.52 to 0.69 at UPF and display mean values of 0.43 and 0.59, respectively. $\delta^{18}O$ individual series show higher variability in *PCA1* at NCZ with values ranging from 0.39 to 0.07 (not significant) and a mean of 0.33. At UPF$\delta^{18}O$, *PCA1* displays a tighter range of values: 0.37 to 0.53, and a mean of 0.42.

Pearson's correlation coefficients between individual stable isotope series before and after removing autocorrelation are shown in Table 2. Individual raw series of UPF$\delta^{18}O$ display comparable high correlation coefficients except for the pair UPF$\delta^{18}O_{004}$–UPF$\delta^{18}O_{005}$ ($r = 0.14$), which considerably increases when autocorrelation is removed ($r = 0.34$), while the rest of the values shows a moderate rise. Similarly, stable-carbon isotope series at NCZ and UPF display slightly increased correlation coefficients when using residual individual series except for the pairs UPF$\delta^{13}C_{005}$–UPF$\delta^{13}C_{003}$ and UPF$\delta^{13}C_{009}$–UPF$\delta^{13}C_{003}$ where the values consistently increase (from $r = -0.05$ to 0.46 and from r = 0.11 to 0.37 respectively). In some instances, even the sign of the correlation changes, e.g., for the pair NCZ$\delta^{13}C_{027}$–NCZ$\delta^{13}C_{002}$ it changes from $r = -0.17$ to 0.14. A different pattern occurs at NCZ$\delta^{18}O$ where correlation between raw series is low and even negative, however insignificant, as in the case of NCZ$\delta^{18}O_{002}$–NCZ$\delta^{18}O_{026}$ ($r = -0.17$) NCZ$\delta^{18}O_{015}$–NCZ$\delta^{18}O_{026}$ ($r = -0.07$). In this case, the removal of autocorrelation does not improve inter-tree correlations and even leads to a decrease in strength of the linear relationships.

3.2. Pool versus MassC

As described in the introduction, *Pool* values are the outcome of mixing all material of one year and the final result will be a combination of the contribution of mass per sample and its isotopic signature. For comparison, a "mass weighted mean" was calculated for each year taking into consideration the individual-tree isotopic values, ring width and density measurements to produce mass calibrated mean values (*MassC*). The mean values for *Pool* and mass calibrated mean (*MassC*) (Table 1) differed for the NCZ isotope series, only 0.21‰ ($\delta^{13}C$) and 0.02‰ ($\delta^{18}O$). However, mean differences between UPF_{Pool} and UPF_{MassC} are substantially high, that is, 0.88‰ for $\delta^{13}C$ and 0.47‰ for $\delta^{18}O$. The inter-annual variability, *i.e.*, standard deviation is very similar for *Pool* and *MassC* series, only in the case of UPF$\delta^{18}O$ the standard deviations differ notably, being 0.88‰ for UPF$\delta^{18}O_{Pool}$ and 0.55‰ for UPF$\delta^{18}O_{MassC}$. The range between minimum and maximum values in *Pool* and *MassC* is similar for both stable

Table 2

Pearson's correlation coefficients (r) matrix for the individual series of $\delta^{18}O$ and $\delta^{13}C$ at NCZ and UPF. First and second values correspond to Pearson's correlation coefficients for raw measurements and for residual series after removing autocorrelation, respectively. Bold letters indicate correlation coefficient with one or both negative values.

	NCZ$\delta^{13}C_{002}$	NCZ$\delta^{13}C_{015}$	NCZ$\delta^{13}C_{026}$		UPF$\delta^{13}C_{003}$	UPF$\delta^{13}C_{004}$	UPF$\delta^{13}C_{005}$
NCZ$\delta^{13}C_{002}$				UPF$\delta^{13}C_{003}$			
NCZ$\delta^{13}C_{015}$	0.39/0.51			UPF$\delta^{13}C_{004}$	0.41/0.46		
NCZ$\delta^{13}C_{026}$	0.24/0.39	0.53/0.53		UPF$\delta^{13}C_{005}$	**−0.05/0.46**	0.40/0.54	
NCZ$\delta^{13}C_{027}$	**−0.17/0.14**	0.20/0.28	0.38/0.39	UPF$\delta^{13}C_{009}$	0.11/0.37	0.51/0.42	0.50/0.60
	NCZ$\delta^{18}O_{002}$	NCZ$\delta^{18}O_{015}$	NCZ$\delta^{18}O_{026}$		UPF$\delta^{18}O_{003}$	UPF$\delta^{18}O_{004}$	UPF$\delta^{18}O_{005}$
NCZ$\delta^{18}O_{002}$				UPF$\delta^{18}O_{003}$			
NCZ$\delta^{18}O_{015}$	0.34/0.31			UPF$\delta^{18}O_{004}$	0.48/0.50		
NCZ$\delta^{18}O_{026}$	**−0.17/−0.1**	**−0.07/−0.006**		UPF$\delta^{18}O_{005}$	0.50/0.46	0.14/0.34	
NCZ$\delta^{18}O_{027}$	0.26/0.39	0.26/0.26	0.31/0.14	UPF$\delta^{18}O_{009}$	0.45/0.35	0.54/0.47	0.45/0.50

isotopes in NCZ series. Considerable differences can be found in the UPF series where the *Pool* chronologies for $\delta^{18}O$ and $\delta^{13}C$ display a consistently wider range than the *MassC* chronologies. Discrepancies also show up when comparing first-order partial autocorrelations (*PCA1*). While *PCA1* is similar for NCZ$\delta^{13}C_{Pool}$ and NCZ$\delta^{13}C_{MassC}$ as well as for UPF$\delta^{18}O_{Pool}$ and UPF$\delta^{18}O_{MassC}$, disparity in the level of autocorrelation appears in UPF$\delta^{13}C$ (0.24‰ *Pool* versus 0.49‰ *MassC*) and, NCZ$\delta^{18}O$ series (0.41‰ *Pool* versus 0.27‰ *MassC*).

3.3. Mass weighted (Pool and MassC) versus unweighted (Mean) chronologies

Average values of the *Mean* master series (Table 1) are found to be closer to the *MassC* than to the *Pool* series, showing the highest discrepancy between UPF$\delta^{13}C_{Pool}$ and UPF$\delta^{13}C_{Mean}$ (0.92‰) coinciding with the smallest difference between UPF$\delta^{13}C_{MassC}$ and UPF$\delta^{13}C_{Mean}$ (0.04‰). The inter-annual variability is found to be generally higher for the *Pool* than for *Mean* and *MassC*. The SD is consistently similar among the three types of master isotope series from NCZ, however at UPF the dispersion of the *MassC* data tends to be more similar to *Mean* than to *Pool*.

MassC isotope chronologies disclose values systematically within one standard deviation (1SD) from the *Mean* (Fig. 1). Stable isotope *Pool* series display values mostly confined within the 1SD of the corresponding *Mean* series, with principally only a few values lying outside 1SD. However, UPF$\delta^{18}O_{Pool}$ exhibits remarkable deviations from the established threshold of 1SD from the UPF$\delta^{18}O_{Mean}$, showing a systematic mismatch of annual $\delta^{18}O$ values in the first half of the series (1900–1953) followed by a decreasing number of values crossing the 1SD line on the second half (1954–2003). Similarly, but less obvious, more values of NCZ$\delta^{13}C_{Pool}$ cross the positive 1SD during the second half of the study period.

Graphical comparison of the *Pool, MassC* and *Mean* series over the entire study period show a high synchronism in interannual and interdecadal variations between *MassC* and *Mean* with some departures of *Pool* series (Fig. 2). Stable-carbon isotope series display a high coherence among all four types of master series. The stable oxygen pool chronologies (UPF$\delta^{18}O_{Pool}$ and NCZ$\delta^{18}O_{Pool}$) show a higher variability and periods with overestimation and underestimation with respect to *MassC* and *Mean* which are the most evident for UPF$\delta^{18}O_{Pool}$.

The correlations of both pairs UPF$\delta^{13}C_{Mean}$–UPF$\delta^{13}C_{Pool}$ and UPF$\delta^{13}C_{Mean}$–UPF$\delta^{13}C_{MassC}$ are 0.778 and 0.734 respectively (Table 3). The agreement between oxygen stable isotope chronologies is slightly lower: correlation coefficients between UPF$\delta^{18}O_{Pool}$ with UPF$\delta^{18}O_{Mean}$ and UPF$\delta^{18}O_{MassC}$, are of 0.636, and 0.587, respectively ($p<0.001$). At NCZ, $\delta^{18}O_{Mean}$ displayed considerably lower correlations with $\delta^{18}O_{Pool}$ ($r=0.666$) and the lowest with NCZ$\delta^{18}O_{MassC}$ ($r=0.497$). This difference does not apply for the stable carbon isotopes, for which NCZ$\delta^{13}C_{Mean}$ and NCZ$\delta^{13}C_{MassC}$ display very similar correlations with NCZ$\delta^{13}C_{Pool}$ ($r=0.745$ and $r=0.749$, respectively).

The GLK revealed high levels of common variance between *Mean* and *MassC* (>90%). When comparing *MassC* and *Mean* with *Pool* at both sites and for both types of isotopes, the GLK values are in the order of 65–70%, with somewhat higher levels of common variance for $\delta^{13}C$ than for $\delta^{18}O$ series.

4. Discussion

Similar to other tree-ring proxies, stable isotopes require adequate replication to ensure data reliability by leading to a representative site chronology with reduced genetic and site specific influences (Fritts, 1976). For this purpose, the first step is to assess and evaluate the common signal for all trees. In our case, most of the individual series means and SD show small differences between trees, especially in $\delta^{13}C$ values. They also exhibit similar dispersion of the data, but some Pearson's correlation coefficients are very low and a considerable number of them increase after removing autocorrelation. The time-related interdependence reveals the influence of previous year on current year growth but this may include climatic and non-climatic information (Cook and Kairiukstis, 1990; Fritts, 1976). The differences in autocorrelation among trees may indicate asynchronicities in

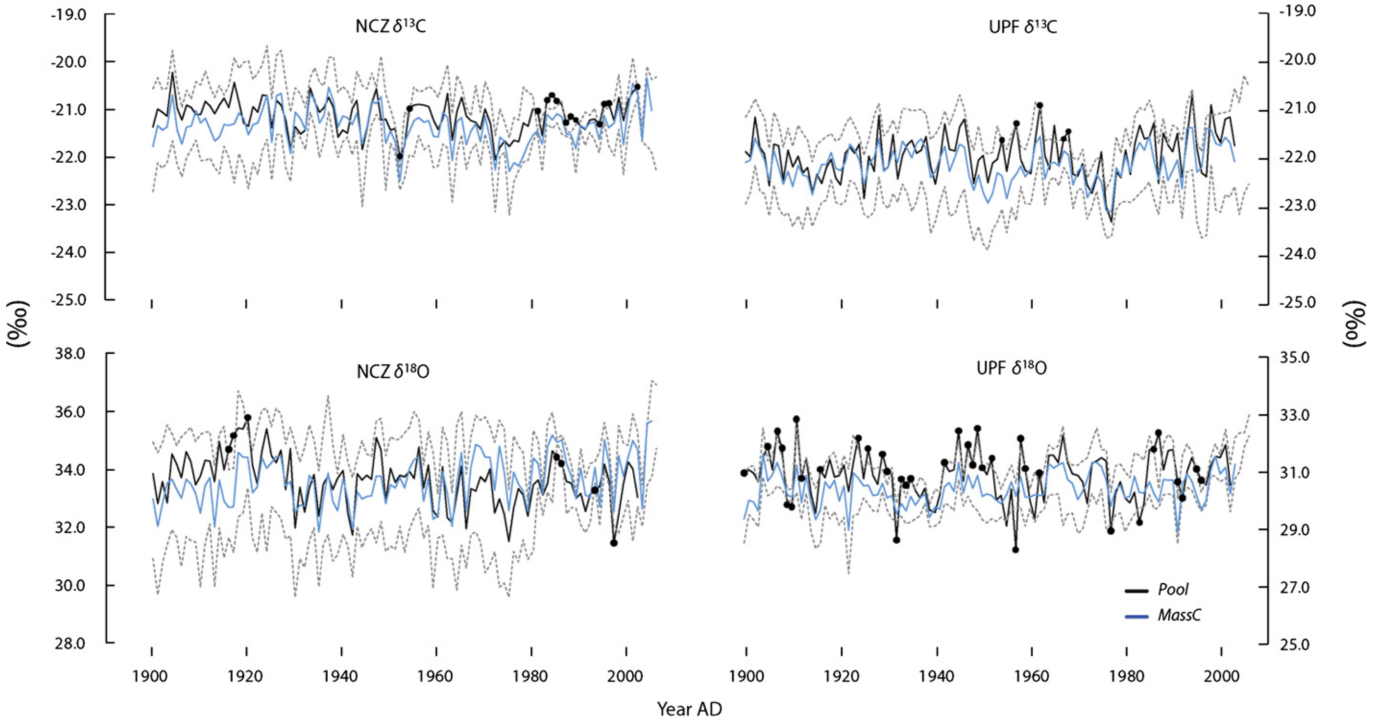

Fig. 1. Comparison of *Pool* (black line) and *MassC* (blue line) master series of $\delta^{18}O$ and $\delta^{13}C$ at NCZ and UPF. Dashed lines indicate 1SD of the *Mean*. Solid circles indicate *Pool* values exceeding 1SD from the *Mean*.

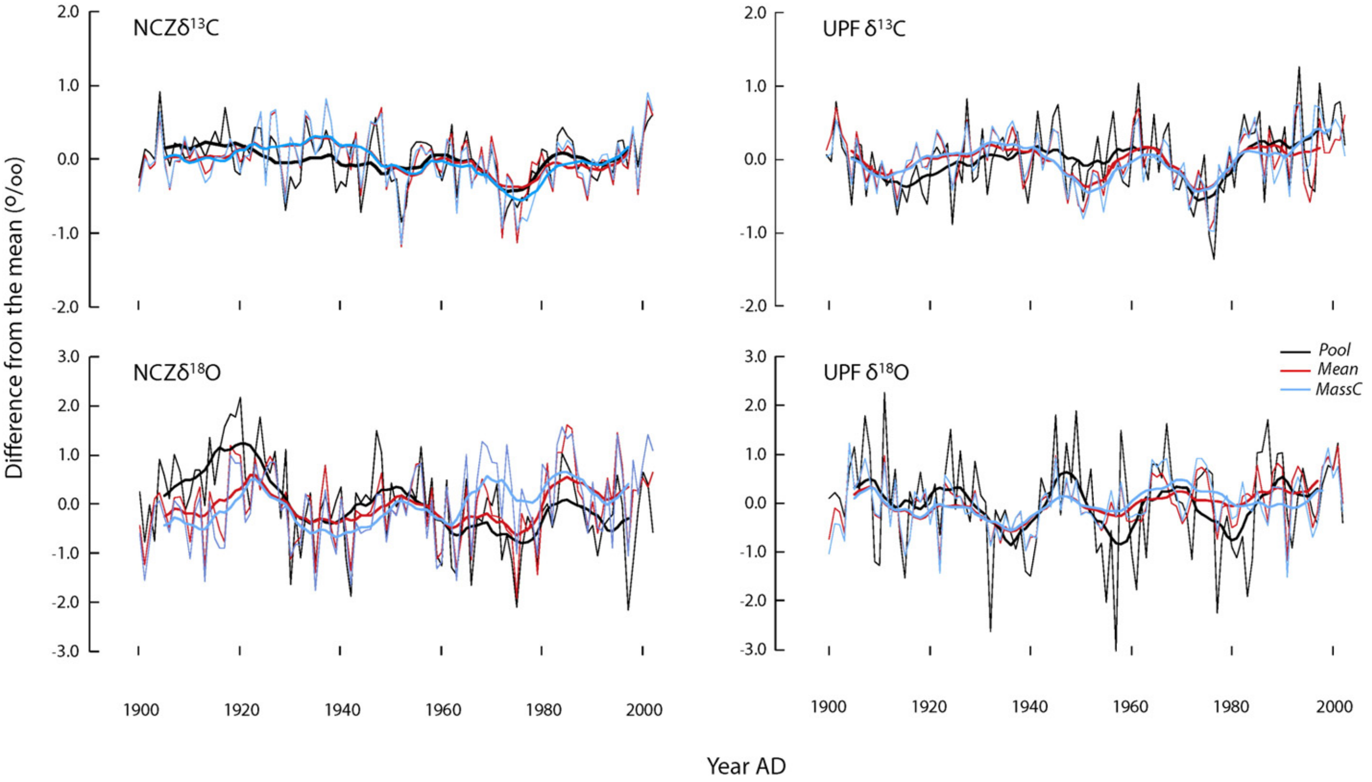

Fig. 2. Comparison of *Pool*, *MassC* and *Mean* master series of δ^{18}O and δ^{13}C. Inter-decadal variations are highlighted by a 10 year window running average.

the existence and persistence of lagged physiological effects on each individual (Monserud and Marshall, 2001). In this context, the dependency on the growth of the previous season might include some ecological or micro-site specific conditions and its absence could indicate non-sensitive trees, unable to register medium- to low-frequency climate fluctuations. In any case, the climate signal seems in some trees to be altered by a non-random growth forcing and this obscures the climate signal. In general terms, individual corrected δ^{13}C series show first-order autocorrelations at both sampling sites. This memory effect has already been reported by other authors for δ^{13}C series (Boettger and Friedrich, 2009; Monserud and Marshall, 2001) while the δ^{18}O series reflect higher variabilities, especially in NCZδ^{18}O. Different levels of autocorrelation in δ^{18}O series may be due to differences in the water uptake, since individuals relying on water near the surface do not show a memory effect (*i.e.* δ^{18}ONCZ$_{015}$) while trees that take up water stored in the soil profile (e.g., NCZ$_{026}$) might show a certain memory (Marshall and Monserud, 2006; Saurer et al., 2008), which then lead to different levels of autocorrelation that are obscuring the actual climate signal.

Table 3
Matrix of Pearson's correlation coefficients (*r*) and Gelichläufigkeit percentage (GLK) for the different types of master series of δ^{18}O and δ^{13}C (*Pool*, *MassC* and *Mean*). All are significant at the 95% confidence level.

	NCZ$\delta^{13}C_{Pool}$	NCZ$\delta^{13}C_{Mean}$		UPF$\delta^{13}C_{Pool}$	UPF$\delta^{13}C_{Mean}$
NCZ$\delta^{13}C_{Pool}$			UPF$\delta^{13}C_{Pool}$		
NCZ$\delta^{13}C_{Mean}$	0.745/76%		UPF$\delta^{13}C_{Mean}$	0.778/72%	
NCZ$\delta^{13}C_{MassC}$	0.749/72%	0.957/93%	UPF$\delta^{13}C_{MassC}$	0.734/74%	0.901/92%
	NCZ$\delta^{13}C_{Pool}$	NCZ$\delta^{13}C_{Mean}$		UPF$\delta^{18}O_{Pool}$	UPF$\delta^{18}O_{Mean}$
NCZ$\delta^{18}O_{Pool}$			UPF$\delta^{18}O_{Pool}$		
NCZ$\delta^{18}O_{Mean}$	0.666/72%		UPF$\delta^{18}O_{Mean}$	0.636/66%	
NCZ$\delta^{18}O_{MassC}$	0.497/74%	0.854/92%	UPF$\delta^{18}O_{MassC}$	0.587/65%	0.937/95%

In addition to the variable groundwater contribution to tree-ring δ^{18}O values, the overall process leading to the earlywood formation in spring includes the usage of stored carbohydrates from previous-year growth, which mix with current year photosynthetic products (Livingston and Spittlehouse, 1996; McCarroll and Loader, 2004). In this sense, δ^{18}O of one year will include soil moisture isotopic signals from the previous year at least in the spring earlywood formation, while the latewood oxygen content relies more on current-year metabolic activity. In contrast to δ^{13}C, the carry-over effects on tree-ring δ^{18}O are always reduced due to the rapid recycling of triose phosphates during cellulose formation in the trunk, where up to 40% of the oxygen atoms are being exchanged with actual xylem/phloem water (Hill et al., 1995). Previous studies carried out on individuals of *P. nigra* reported dependency of tree growth on the climate conditions of the previous year at NCZ (Martin-Benito et al., 2008) and as we have used the total ring for cellulose extraction, constraints of previous year's growth may be mixed with the growth signal of the current year. In principle, such a memory effect can be minimised by studying the latewood portion of the tree ring, already demonstrated in several studies with suitable material (Danis et al., 2006; Gagen et al., 2004; Roden, 2008; Treydte et al., 2001). However, as for this study, the manual dissection of latewood was not always feasible due to narrow tree rings often less than 0.1 mm.

Theoretically, *Pool* and *MassC* series are supposed to be closely related since the latter is a theoretical approximation of the first one. Simulations carried out by Leavitt, (2008) displayed results consistent with the empirical values from Borella et al. (1998) and Treydte et al. (2001). The authors concluded that even if the isotopic and ring-size differences are large among trees, pooled means obtained by averaging δ^{13}C values of individual trees will be within 1SD of the mean. Thus, it is encouraging, but not a surprise based on the existing literature, that our "weighted means" (*Pool* and *MassC*) do not differ largely from averaged individual measurements (*Mean*).

Pool and *MassC* chronologies are close in mean values but exhibit differences regarding the dispersion of the data, especially in the case of UPF where the range of isotopic values is much wider for *Pool* than for *MassC.* When testing similarities on an annual basis, some differences in δ^{18}O are evident at both sites, e.g., UPFδ^{18}O$_{Pool}$ values are systematically larger than UPFδ^{18}O$_{MassC}$.However, at NCZ this systematic offset is not constant in time, that is, the NCZδ^{18}O$_{Pool}$ values are larger than NCZδ^{18}O$_{MassC}$ during the first half of the series (approx. 1900–1950) and smaller during the second half (1960–2003). One reason may be that for individual analyses and for calculating the *MassC,* only one 12 mm core was used, taken from the same orientation as one of the two 5 mm cores used for *Pool*. Hence, the difference could be due to the intra-tree circumferential variations of isotope values (e.g., Schleser, 1999; Treydte et al., 2001). Previous studies have shown how this circumferential variability of δ^{13}C within a single tree can reach up to 4.5‰ (Tans and Mook, 1980) but most studies report a range between 1 and 2‰ (see review by Leavitt, 2010). Similar investigations for δ^{18}O published so far found a circumferential range of variability between 0.5 to 2‰ (Ramesh et al., 1985). Since the inter-tree variability, as well as the year-to-year variability of δ^{18}O is similar to that of δ^{13}C we argue that the circumferential variation is also of the same order. Thus, two cores from the same tree may differ to some extent in their isotopic signature and therefore the higher values of UPFδ^{18}O$_{Pool}$ could be due to an increased mean isotopic signature of the second 5 mm core, which will enhance its importance on the *Pool* series (Borella et al., 1998). Unfortunately, the isotope values of the second 5 mm core used for the *Pool* were not determined and thus a hypothetical bias introduced by the higher contribution of one sample to the *Pool* cannot be proven in our case.

The same considerations can be advanced when comparing the other series with *Mean* master series. We expected *MassC* to be closer to *Pool,* but in fact *MassC* was closer to *Mean* and confined within 1SD from *Mean.* This indicates no substantial differences (except for punctual years) as have been noted for NCZδ^{18}O$_{Pool}$, NCZδ^{13}C$_{Pool}$ and UPFδ^{13}C$_{Pool}$. In contrast, UPFδ^{18}O$_{Pool}$ displays large departures showing a high number of values crossing the upper 1SD of UPFδ^{18}O$_{Mean}$, which suggests a certain degree of systematic overestimation of the annual values. This difference is more obvious when comparing filtered series for which offsets of UPFδ^{18}O$_{Pool}$ and also NCZδ^{18}O$_{Pool}$ related to their respective *MassC* and *Mean* are highlighted. Indeed, *Mean* and *MassC* series display a significantly better coherence, that is, they annually vary in the same way, which is demonstrated by the high GLK coefficients at both NCZ and UPF sites while for *Pool* the coefficients are lower.

The correlation coefficients between *Pool* and *Mean* series found in this study are markedly lower than the ones described by similar studies. For instance, the correlation coefficients between δ^{13}C$_{Pool}$ and δ^{13}C$_{Mean}$ are 0.76 and 0.78 for NCZ and UPF, respectively, while Treydte et al., 2009 reported a correlation of 0.97. Similarly for δ^{18}O series, Treydte et al., 2006 described a correlation coefficient of 0.85, which is consistently higher than the 0.66 and 0.64 for our δ^{18}O series from NCZ and UPF, respectively.

This difference of *Pool* with respect to the other two master series could be due to the mentioned intra-tree variations on the isotopic composition, since *Pool* is derived from a different set of samples compared to *Mean* and *MassC*. But some other sources of uncertainty related to the whole process to obtain the stable isotope analysis also cannot be neglected. In general, we found that differences observed in *Pool* with respect to *MassC* and *Mean* are larger for oxygen than for carbon isotopes. Boettger et al. (2007) also reported larger differences from the mean in oxygen values than in carbon when comparing measurements from different labs. They argued that this might be due to differences in preparation or analytical method used, since for oxygen measurements an incomplete drying has consequences on the final isotopic value. In contrast, they found no evidence that δ^{18}O values depend on cellulose extraction specific details (α-cellulose versus holo-cellulose) as in the case of δ^{13}C. Despite the absence of a general offset between δ^{18}O of α-cellulose and corresponding holo-cellulose, statistical evidence was found that exchange of oxygen does occur in hemi-cellulose and therefore affects the stability of the δ^{18}O signature of holo-cellulose (Wright, 2008).

Borella et al. (1998) already evaluated the consequences of alterations of δ^{13}C in the cellulose extractions and found that an incomplete process can lead to values of about 0.2‰ below the values of pure α-cellulose.

At each site, only one cellulose extraction was performed, and thus the cellulose used to measure both carbon and oxygen isotopes was the same. In the case of UPF, UPFδ^{18}O$_{Pool}$ displays a consistently higher variability in comparison to the other master series but UPFδ^{13}C$_{Pool}$ varies similarly. Since the cellulose was the same for both isotopes, errors associated with the cellulose extraction of the pool samples are unlikely responsible for this difference.

We consider that the higher variability of the *Pool* chronologies may be due to differences in homogeneity of pooled and individual samples. This can be explained by the different methods of homogenization. Pooled samples were grinded prior to cellulose extraction, whereas cellulose of the individual samples was subjected to an ultra-sonic homogenization. Since aliquots of only 0.15 to 0.2 mg of cellulose were taken for the mass spectrometric analyses, a relative inhomogeneity of the pooled samples in relation to the individual tree-ring material may be responsible for the higher variability of *Pool* chronologies as compared to *Mean* chronologies. This, in combination with the lower reproducibility of the mass spectrometric δ^{18}O determination (δ^{18}O$<\pm$ 0.3‰; δ^{13}C$<\pm$0.1‰) makes the more pronounced fluctuations in δ^{18}O very likely.

Independently of the different bunch of cores used for *Pool* and for the individual means (*Mean* and *MassC*), it becomes obvious that other factors are involved in the process of building a stable isotope pool chronology, influencing the quality and reliability of the final series. Previous works have warned about the danger of pooling because of the inability of testing (1) if the replication is enough for building a representative site series and (2) if an error is introduced in any of the methodological steps. Because of that, a mixed approach has been proposed (Leavitt, 2008) in which each tree would be analysed individually with a fix interval (e.g., every tenth year) while the rest of the samples would be pooled. This approach combines the efficiency in the use of time and resources associate to pooling method with the possibility of establishing uncertainties around the mean, of calculating statistics that define signal strength among the samples (e.g., EPS, Wigley et al., 1984) and of detecting inconsistencies due to analytical processes.

5. Conclusions

This study showed that there is good agreement between mean master series created by averaging raw individual values (*Mean*) or generating a mass-calibrated mean (*MassC*). Coherence in the inter-annual up and downward variations are consistent among the different types of master series of the two sampling locations and isotope records. Moreover, variations in the magnitude that lead to some local incongruities in the inter-decadal variations are small in most of the cases.

Only UPFδ^{18}O$_{Pool}$ displays substantial differences when comparing it to the mean of individual measurements and its derivates, which could either be due to an error during analysis or to bias introduced by the contribution of one deviating sample.

Although the comparison carried out in this study is not based in a perfect experimental design (the different master series come from the same trees but from different bunch of samples); the results point out the danger of performing a single measurement per year since the lack of replication prevents detecting such mistakes. Because of this, the most secure way to avoid such uncertainties would be analysing each tree individually. Unfortunately, this is not always possible and if restrictions in sample loads exist, we strongly recommend performing individual measurements of every tree composing the pool with a fixed frequency as already proposed by other authors. This will help to avoid mistakes or help to detect them, while allowing for tests of common signal strengths between the samples and for assessing the representativeness of the final chronology.

Acknowledgments

We thank Marc Fillot and Peter Nyfeler for their contribution to this work, Christoph Küppers for isotope analysis and Björn Günther for performing the density measurements. This research was funded by EU projects ISONET (contract EV K2-2001-00237) and MILLENNIUM (017008–2).

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