

Low-frequency noise in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ tree ring data: A case study of *Pinus uncinata* in the Spanish Pyrenees

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[1] Isotopic discrimination measurements in tree rings are becoming increasingly important estimators of past environmental change. Potential biases inherent to these parameters, including age trend and level offset are, however, not well understood. We here perform measurements on a new millennium-long data set of decadal resolved $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ discrimination from 25 high-elevation pine trees in the Spanish Pyrenees to investigate whether such low-frequency biases exist and how they alter the long-term behavior of derived time series. Alignment of the tree ring data by biological age reveals age trends over the first one to four centuries after germination. On average, isotope values change by -0.089‰ $\delta^{18}\text{O}$ and $+0.064\text{‰}$ $\delta^{13}\text{C}$ per decade over the first 100 years of tree growth. This trend persists into the fourth century after germination for $\delta^{18}\text{O}$ but diminishes to $\sim 0\text{‰}$ over the 100–390 year period for $\delta^{13}\text{C}$. We also find level offsets up to 7‰ $\delta^{18}\text{O}$ and 3‰ $\delta^{13}\text{C}$ between single trees. Analysis of the shape of age trends reveals negative exponential functions as reasonable choices for detrending of $\delta^{18}\text{O}$ and (inverted) $\delta^{13}\text{C}$ time series. The detrended isotope chronologies share low-frequency variance with traditional ring width and density measurements beyond statistical chance, suggesting that the various parameters reflect some of the same environmental forcing. Our results show that $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ from these Spanish pines need to be detrended to assess long-term environmental changes. To evaluate the general applicability of this conclusion, production of (1) well-replicated, (2) nonpooled, and (3) composite chronologies from other species and regions will be required. Increases in measurement speed and technology will make these tests feasible in the coming years.

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1. Introduction

[2] Stable isotope ratios measured from annually dated wood material are becoming an increasingly important proxy in high-resolution palaeoclimatology. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ records are by far most abundant, as these measurements require relatively less laboratory work in comparison to δD , represent the dominant structural components of wood, and contain significant common variance between individual

trees [Leavitt and Long, 1988; McCarroll and Loader, 2004; Treydte et al., 2001]. This coherence is key for utilizing carbon and oxygen measurements for reconstruction purposes, and there are ample publications demonstrating the strength of these data to estimate climate variations within and beyond the period covered by instrumental measurements [e.g., Etien et al., 2008; Gagen et al., 2007; Kress et al., 2010; Treydte et al., 2006].

[3] Between-tree coherence and reconstruction of climate variables are, however, largely restricted to higher-frequency variance and extreme events [Treydte et al., 2007]. Validation of long-term environmental information from tree ring stable isotopes is typically compromised when analyses (1) cover periods too short to address low-frequency variance [e.g., Saurer et al., 2008], (2) represent measurements from just one or two trees [e.g., Berkelhammer and Stott, 2008], or (3) do not utilize time series from individual trees but “pooled” data where the annual rings from multiple trees are milled together before isotope measurement [e.g., Borella

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et al., 1998]. For reconstruction purposes, the pooling method is important as it allows the development of chronologies integrating information from more than one tree per year and can thus help to overcome restrictions set by labor costs. However, the method does not permit analyses of the variance between individual trees, and therefore hinders detection of potentially underlying biases, such as the age trend inherent to traditional tree ring parameters, ring width (TRW) and maximum latewood density (MXD) [Fritts, 1976; Schweingruber, 1983].

[4] Indications for such biases for oxygen isotopes were first reported by Treydte *et al.* [2006] in their reconstruction of long-term precipitation changes in Central Asia using an annually resolved 1000 year $\delta^{18}\text{O}$ chronology. This study included a comparison of chronologies composed of younger and older tree rings over a >300 year preindustrial segment (A.D. 1264–1599). This “split-pool” approach revealed that the chronology composed of juvenile rings contained a long-term declining trend that was not observed in the chronology composed of old rings over the same period. For $\delta^{13}\text{C}$, a tendency toward increasing values over the first decades, the so-called juvenile effect, has been reported in a number of studies [e.g., Francey and Farquhar, 1982; Bert *et al.*, 1997]. The trend was also assessed in a recent study based upon a number of annually resolved $\delta^{13}\text{C}$ time series from individual (nonpooled) trees from northern Scandinavia [Gagen *et al.*, 2008]. A comparison among tree clusters revealed age-related biases over the first 50 years after germination, but no trend up to age classes of 250 years [Gagen *et al.*, 2007]. Indications of a shorter-term age trend are further complicated since about 1800 A.D. by the increasingly depleted carbon isotopes in the atmosphere due to fossil fuel emissions and possible plant physiological reactions to increasing CO_2 concentrations [Treydte *et al.*, 2009]. To our knowledge, no study exists that compares stable isotope measurements among individual living and relict trees over the past 500 to 1000 years – a study design that may permit assessment of long-term age-related noise that is not limited by tree age, modern changes in the atmosphere, or climatic trends common to living tree cohorts.

[5] An assessment of existing data¹ indicates that ~43 long-term (>300 years) tree ring stable isotope records have been developed so far. The majority of these time series originate from Europe where two EC funded projects helped to substantially increase the density of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ data. Most (34) of these records are derived from whole ring analysis, 83% of the studies include samples from conifers, and only two of the conifer records are derived from latewood material. This pattern might appear surprising as several studies indicated clearer climate signals in latewood than in whole ring samples [e.g., McCarroll and Loader, 2004; Schleser *et al.*, 1999]. However, the lack of latewood only long-term records seems to be related to (1) studies indicating insignificant whole ring/latewood differences in conifers [Kress *et al.*, 2009], (2) the difficulty to determine earlywood/latewood boundaries in conifers [Schweingruber, 1983], and

(3) the typically narrow tree rings in old-growth conifer species making it virtually impossible to cut representative latewood samples throughout several centuries. Given the numbers and types of long-term isotopic records currently available, it appeared useful to assess whole ring signals in conifer species.

[6] We here address potential age-related biases in stable isotopes by analyzing a data set of 25 individual $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ measurement series from living and relict *Pinus uncinata* trees that germinated throughout the past millennium. Development of this large data set was only feasible at the expense of decreased resolution, i.e., all time series are decadal resolved as stable isotopes were measured after milling 10 year wood segments. This experimental design of (1) decadal resolved (2) single tree measurements from (3) living trees and relict wood (4) spanning the full millennium, enables an analysis of long-term tree age-related noise largely independent of climatic influences. Analysis of these data includes an assessment of the variance and trends in stable isotope data, decomposition of data into tree age classes, alignment of measurement series by biological age, and approximation and removal of trends using classical dendrochronological techniques. We also address – by comparing pre and post A.D. 1500 $\delta^{18}\text{O}$ (and partly also $\delta^{13}\text{C}$) data – the assumption that no underlying millennial-scale climate trend is biasing the age trend assessment. This work is part of an effort to optimally reconstruct low-frequency climate variations over the past 1000 years within the European Community project “Millennium,” in which a number of millennium-length stable isotope chronologies are being developed. Our analysis presents a basic assessment of long-term noises in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ associated with trees age that could be relevant to other studies using stable isotopes to assess long-term environmental changes.

2. Material and Methods

2.1. Tree Ring Material

[7] Tree ring samples originate from a *Pinus uncinata* tree line site at about 2300 MASL in the Spanish central Pyrenees. The site is located at 42°38'N/1°06'E near the northern border of the Sant Maurici National Park. *Pinus uncinata* is a shade-intolerant species dominating this open forest ecotone across the Pyrenees, where soils are shallow, skeletal leptosols, tree roots do not access groundwater, and snowmelt is the main water supply. At our site, between-tree distance ranges from 3 to 8 m and understory coverage is with 5–10% quite low. Mean annual temperature is ~4.0°C with a maximum in July (~13°C) and a minimum in January (~-3°C). Annual precipitation total is ~1200 mm with only minor seasonal variations.

[8] Twenty-five *Pinus uncinata* trees were selected for stable isotope measurement using a subset of core samples from a larger compilation of wood material collected during several field campaigns over the last years, for which TRW and MXD data were already available [Büntgen *et al.*, 2008, 2010] (Figure S1). Samples were selected based upon long segment lengths and to obtain an even distribution of series throughout the past millennium. The first criterion permits

¹Auxiliary materials are available in the HTML. doi:10.1029/2010GB003772.

investigation of age trends over several hundred years as segment lengths range from 186 to 644 years centered on a mean typical for old-growth *Pinus uncinata* in the central Pyrenees (330 years). The second criterion minimizes the contamination from climate in the assessment of age trends as climatic trends from various parts of the past millennium cancel each other out when the series are aligned by biological age. Both criteria are helpful to maintain a relatively high and constant sample replication, but replication declines below 5 trees before A.D. 1298. This unique collection of wood material is sufficient to derive decadal resolved stable isotope measurements reaching back to A.D. 924.

2.2. Stable Isotope Measurements

[9] Stable isotopes were measured for each tree separately using decadal wood pieces (A.D. 2000–1991, 1990–1981, ...) so that the resulting figures represent average isotope ratios of 10 consecutive tree rings approximately weighted by their width. Splitting of the decadal chunks was performed with a razor blade under a stereomicroscope. All wood samples were crossdated prior to splitting to ensure accurate dating. The decadal wood pieces were milled to a fine powder using a Retsch centrifugal mill with a mesh size of 0.5 mm to ensure sample homogeneity [Borella et al., 1998]. Cellulose was extracted in a two-step procedure [Battipaglia et al., 2008; Boettger et al., 2007; Rinne et al., 2005]. Resin, fatty acids, ethereal oils and hemicellulose were removed using a solution of 5% NaOH, twice for 2 h at 60°C. Lignin was then extracted with a NaClO₂ solution, prepared with 80% and 3–4 ml acetic acid per liter solution to reach pH4, for 36 h at 60°C. Samples were washed 3–4 times using boiling distilled water and dried overnight at 50°C. Resulting cellulose was then weighted into tin cups for $\delta^{13}\text{C}$ and silver cups for $\delta^{18}\text{O}$ measurements.

[10] Oxygen stable isotopes were measured using a continuous flow stable-isotope ratio mass spectrometer (Delta Plus XP, Thermo Finnigan) after decomposing 0.5–0.7 mg cellulose by thermal pyrolysis at 1080°C into CO. For carbon, 0.5–0.7 mg cellulose samples were forwarded to a mass spectrometer after combustion to CO₂ in an elemental analyzer. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ are expressed in units ‰ relative to V-SMOW and V-PDB, respectively, calibrated against α -cellulose, vegetal, and graphite laboratory standards (see Saurer et al. [1998] for details). The precision of these analyses is $\pm 0.1\text{‰}$ for carbon and $\pm 0.2\text{‰}$ for oxygen. We corrected the $\delta^{13}\text{C}$ data for changes in the isotopic composition of the atmosphere due to fossil carbon combustion (Figure S2), and then inverted these measurements (multiplied by -1) for age trend illustration and approximation using widely applied growth curve functions [Cook and Kairiukstis, 1990].

2.3. Time Series Assessment and Detrending

[11] To assess the variability of isotopic data, we calculated ranges for each parameter data set, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, MXD, and TRW, and for the individual measurement time series in these categories. Ratios of the standard deviation of mean chronologies to the mean standard deviation of individual time series, the normalized spread, were calculated to ease

comparison among the different measurement units. Coherence within and between parameters was assessed by calculating interseries correlations among individual trees and between mean chronologies. We also calculated correlations among the four parameters for each tree to add detail to the associations within the data, and used the interseries correlation to benchmark the Pyrenees data against widely recognized records from Scandinavia [Grudd et al., 2002] and North Africa [Esper et al., 2007a].

[12] To assess age trend in the various tree ring parameters, individual measurement series were aligned by biological age. This alignment considered the number of missing innermost rings on core samples, the so-called pith offset (PO), which we estimated from the curvature and width of central rings, and in some cases from the ratio between core sample length and stem circumference at sampling height. For age trend estimation, a number of straight lines were regressed to the arithmetic means of the age-aligned data. We also decomposed the data into age classes of 10, 20, ... 300 years, fitted gap-bridging splines to the resulting value clouds, and analyzed the variance between age classes for each parameter using the software SPOTTY [Esper et al., 2009]. Age trends were approximated considering a variety of functions, including Negative Exponential (NegExp) and Hugerhoff (Hug) using the software ARSTAN [Cook, 1985], and index chronologies assessed to appoint a ‘best fit’ detrending model for each parameter. We applied Regional Curve Standardization (RCS) to preserve long-term variability in mean chronologies and to assess low-frequency coherence between the tree ring parameters [Esper et al., 2003].

3. Results

[13] Examination of decadal resolved isotope time series revealed substantial variance with minimum-to-maximum parameter ranges of 8.35‰ and 4.33‰ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, respectively, over the past millennium (Figure 1). The 25 individual pine series have ranges between 1.9 and 6.9‰ for $\delta^{18}\text{O}$ and 1.2–3.0‰ for $\delta^{13}\text{C}$ (Table 1). Comparison with MXD and TRW data from the same trees indicates that the between- and within-tree variability of the isotopic data is in the order of the traditional parameters as quantified by the normalized spread (this metric typically changes to 1 after detrending). Visual inspection of the decadal measurements supports this conclusion, at least for the isotope and MXD data. The raw TRW measurements contain higher autocorrelations (0.68 at lag 1) and show more noticeable age trends than the other parameters. All data, however, indicate some association between measurement value and biological age (Figure 1, middle): TRW, but also MXD, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are larger when the mean biological age of the samples included in the chronology is low, such as the 13th and 18th centuries.

[14] Age-dependent characteristics are further detailed by decomposing the data into particular age classes and illustration of data points throughout the past millennium (Figure S3). We fitted gap-bridging splines to these age classes to emphasize level differences between juvenile

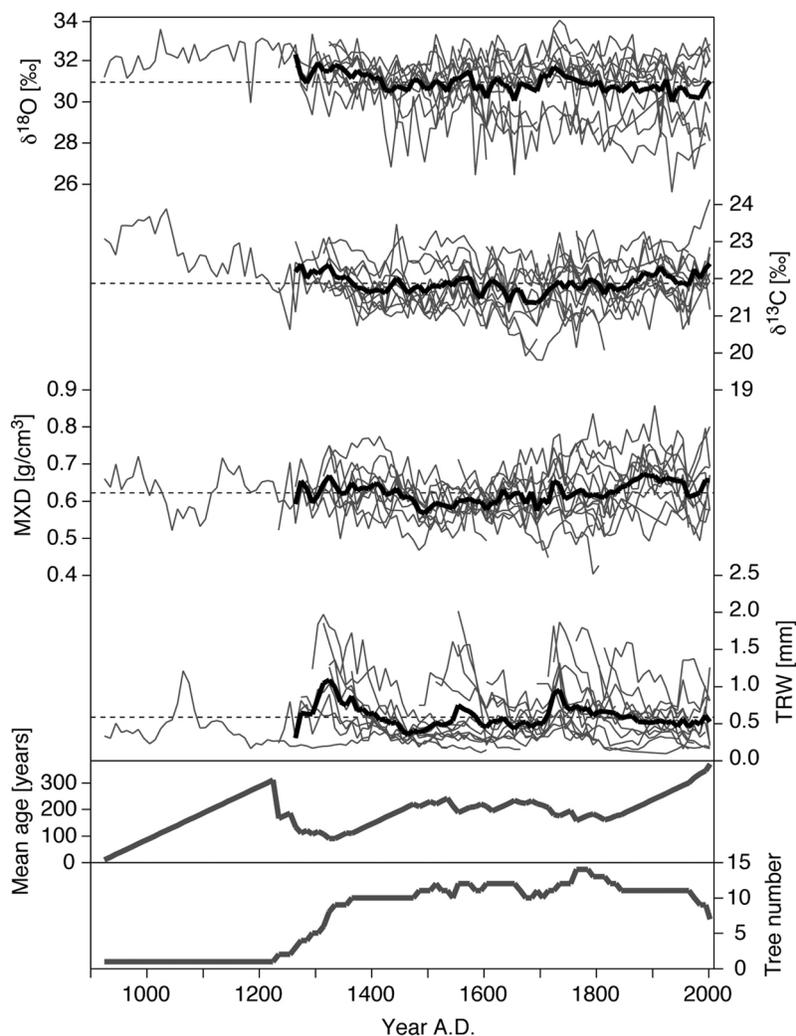


Figure 1. Decadally resolved long-term tree ring data from the Spanish Pyrenees. (top) The raw measurement time series of $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ (y axis inverted), MXD, and TRW, and bold curves indicate the means of these data. Dashed lines indicate arithmetic means: 30.95‰ $\delta^{18}\text{O}$, 21.88‰ $\delta^{13}\text{C}$, 0.621 g/cm³ MXD, and 0.586 mm TRW. (middle) The mean tree age. (bottom) The replication of the data.

(<100 years), middle (100–200 years), and old (>200 years) tree rings (Figure 2). This assessment demonstrated that the juvenile $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values were systematically larger than the middle and old values throughout most of the past 700 years – except for the 14th century ($\delta^{18}\text{O}$) and around 1800 ($\delta^{13}\text{C}$). The order of splines, from higher to lower ages, is also fairly systematic for TRW, but less clear for MXD. Overall, data decomposition into age classes reveals ranges of 3.3‰ $\delta^{18}\text{O}$, 1.5‰ $\delta^{13}\text{C}$, 0.25 g/cm³ MXD, and 1.1 mm TRW throughout the past 700 years (Figure 2). Whereas these ranges demonstrate how mean chronologies (of nondetrended data) are biased by tree age changes, the distribution of splines and age class data clouds illustrates how this problem is clustered in certain periods of the past millennium.

[15] The association between tree age and measurement value is additionally assessed after aligning the data by biological age (Figure S4). Linear regressions fit to arithmetic means of these data demonstrate systematic changes over the first 100–400 years of tree growth, and reveal age trend inherent to all tree ring parameters (Figure 3). Age trend is initially stronger in $\delta^{13}\text{C}$ and TRW, but appears more persistent in $\delta^{18}\text{O}$ and MXD. The density parameter shows a positive trend over the first 60–100 years after germination and a negative trend thereafter. This is the classical age-related shape that led to the utilization of Hugeshoff detrending curves with MXD data [Esper *et al.*, 2010]. Least square regressions fit to the normalized data translate into trends of -0.09% and -0.06% per decade over the first 100 years, for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, respectively

Table 1. *Pinus uncinata* Site and Tree Ring Parameter Characteristics^a

	Parameters			
	$\delta^{18}\text{O}$ (‰)	$\delta^{13}\text{C}$ (‰)	MXD (g/cm^3)	TRW (mm)
Mean	30.95	-21.88	0.62	0.59
Minimum	25.60	-24.13	0.40	0.09
Maximum	33.95	-19.80	0.86	2.02
Range	8.35	4.33	0.46	1.93
Individual ranges ^b	1.87–6.85	1.17–3.00	0.10–0.29	0.53–1.72
Normalized spread ^c	1.53	1.25	1.20	1.13
Mean lag 1 autocorrelation (raw data)	0.41	0.54	0.58	0.68
Mean lag 1 autocorrelation (detrended) ^d	0.11	0.37	0.30	0.04
Interseries correlation (raw data)	0.16	0.15	0.15	0.34
Interseries correlation (detrended) ^d	0.08	0.18	0.09	0.09
Interseries correlation (detrended, annual) ^d			0.22	0.15
Number of linear fits	10	17	17	11

^aSampling site, Gerber/Pyrenees; elevation (MASL), 2200–2350; number of trees, 25; period A.D., 924–2003; period A.D. with replication >5 trees, 1297–2004; mean segment length (years), 330.

^bMinimum to maximum value derived from individual measurement series.

^cStandard deviation of the mean divided by the average standard deviation of the measurement series.

^dNegExp detrending for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and TRW. Hug detrending for MXD.

(carbon data inverted). Over the first 300 years $\delta^{18}\text{O}$ declines by 0.06‰ per decade, but $\delta^{13}\text{C}$ by only 0.01‰. This reduced change in the carbon data is due to a lack of trend at tree ages >100 years, as is indicated by the 0.00‰ change in $\delta^{13}\text{C}$ over the 100–390 year period.

[16] To approximate and remove the identified age trends, a number of deterministic functions were applied to the data to search for a best fit detrending model for each parameter. These tests indicate that NegExp applied to $\delta^{18}\text{O}$, (inverted) $\delta^{13}\text{C}$, and TRW, and Hug applied to MXD approximate the measurements quite well and produce chronologies characterized by minimum squared sum residuals (Figure 4). Of the 25 pines considered in this analysis, the time series analysis program ARSTAN [Cook, 1985] also selected between 10 and 17 straight lines depending on parameter (Table 1), indicating that a number of measurement series were not optimally approximated by NegExp or Hug functions, or contain overall positive trends. The individual functions, however, nicely emphasize the trends of the single measurement series, as well as the level offsets inherent to the various parameters (Figure S5). Removal of these trends and offsets by either the best fit or RCS detrending methods, as expected, does not significantly change the high-frequency, interdecadal variations, but influence the low-frequency behavior of chronologies (Figure 5). For example, best fit and RCS detrending result in increasing $\delta^{18}\text{O}$ and TRW trends over the past 100–200 years, a feature also retained by RCS in the MXD data. For $\delta^{13}\text{C}$, RCS increases chronology variance after ~1650, and both detrending techniques yield maximum values in the 20th century.

[17] Detrending also increases the coherence among tree ring parameters, as is indicated by the RCS chronologies over the past 700 years (Figure 6). Cross-parameter coherence is strongest between $\delta^{18}\text{O}$ and TRW, and TRW and MXD. RCS and prewhitened (autocorrelation removed) chronologies correlate up to 0.65 and 0.58 over the past 700 years (effective samples size ~70; see the insert table

in Figure 6). Coherence is particularly obvious between ~1300–1500, ~1700–1850, and over the late 20th century, and is at least partly driven by overall positive trends in all parameters. Prewhitening the chronologies reduces most cross-parameter correlations (e.g., $\delta^{18}\text{O}$ versus TRW from 0.65 to 0.29), but increases coherence between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (from 0.34 to 0.44). These results are supported by an assessment of coherence among parameters for each tree that revealed measurements are positively correlated in 21 trees between MXD and TRW and in all 25 trees between $\delta^{18}\text{O}$ and TRW (Figure S6).

4. Discussion and Conclusions

[18] Detrending is a regular procedure to remove age-related, “biological” noise from classical tree ring parameters TRW and MXD. However, with the exception of juvenile and industrialization effects in $\delta^{13}\text{C}$, it is usually assumed that stem wood stable isotope measurements do not need to be statistically treated before being considered as a climate proxy [McCarroll and Loader, 2004]. This assumption requires that stable isotope data are not influenced by tree age, a hypothesis herein tested using an exclusive collection of decadal resolved measurement series covering the past millennium. Our results show that $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ time series decline systematically over the first 100 to 400 years after germination, and contain substantial level differences in the order of several ‰ between individual trees. These two characteristics, age trend and level offset, observed in *Pinus uncinata* from the Spanish Pyrenees, suggest that detrending of tree ring stable isotope data is necessary for the reconstruction of long-term environmental changes. If not considered, some of the low-frequency variance in nondetrended $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ pine chronologies would be misinterpreted as climatic signals.

[19] Our sampling design and analysis of living and relict wood increases the likelihood that the long-term trends

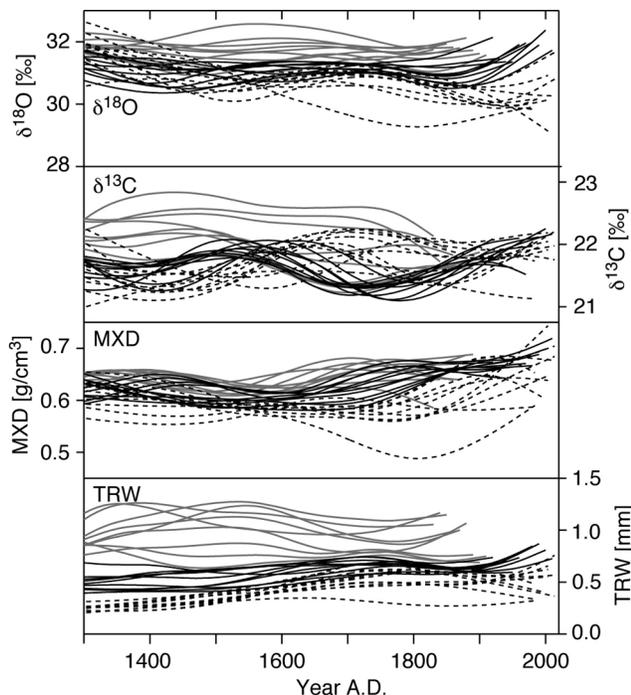


Figure 2. Long-term age trend changes. 30 year splines fit to differently old $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, MXD, and TRW data points over the past 700 years (earlier data are too sparse to fit reasonable splines; see Figure S3). Grey curves are splines fit to data points of 20, 30, ..., 100 years, black curves to 110, 120, ..., 200 years, and dashed black curves to 210, 220, ..., 300 years. For each of these age classes (e.g., tree age 20) one spline was fit. No splines were fit to data <20 and >300 years, as replication in 3 classes drops below 10 data points.

observed in the age-aligned data are indeed due to plant age, but they alone do not ensure that this is the case. Long-term trends observed in both the carbon, and particularly the oxygen isotope data could be due to millennial length changes in climate and/or lowest-frequency changes in atmospheric circulation patterns [Trouet *et al.*, 2009], and the RCS analysis of the full data set as carried out here would be challenged to detect this difference. The additional tests, whereby data for the earlier and latter past millennium for carbon (Figure S2) and oxygen (Figure S7) show no offset (and additionally comparable trends), demonstrate that the long-term changes are in fact age-related and strengthen the conclusions from this study.

[20] High-resolution studies on shorter time series revealed evidence for systematic differences between stable isotope values derived from earlywood and latewood samples, and steep intra-annual gradients [Helle and Schleser, 2004; Schulze *et al.*, 2004]. Such changes within single tree rings could also influence the herein identified $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ age trends, as the ratios of earlywood-to-latewood widths [Fritts, 1976] and their densities [Schweingruber *et al.*, 1978] often

change slightly over the trees lifespan. Other work, however, indicated that differences between latewood and whole ring samples are restricted to deciduous trees, where earlywood formation is dominated by the mobilization of reserves [Barbour *et al.*, 2002; Hill *et al.*, 1995], while conifers show a high degree of coherence between early and latewood isotope values [Kress *et al.*, 2009]. It seems relevant in this context to recall that the majority of long-term records are derived from conifers and that only two of these are composed of latewood material (Table S1), which again reinforces the use of whole ring samples as considered in this current study. Our results are independent of measurement resolution as the identified age trends are much longer (>100 years) than the decadal blocks used for isotope measurements, i.e., results would be the same if annually resolved time series would have been used.

[21] For the carbon data, there are three basic sources for nonclimatic trends that need to be considered. These include (1) changes in the source signal, i.e., the isotopic ratio of the atmosphere, (2) influences of increasing CO_2 on plant physiology including isotope discrimination, and (3) tree age-related effects. Whereas the first two factors can be attributed and removed to some extent (as for example done in Figure S2, but see also Treydte *et al.* [2009]), age-related effects have so far only been associated with young trees exposed to increased CO_2 levels from soil respiration or reduced direct light influences, which appear particularly important in dense forests [Francey and Farquhar, 1982]. However, longer-term $\delta^{13}\text{C}$ changes as detected here may as well be associated with increasing hydraulic resistances of taller trees and subsequent lower stomatal conductance [McDowell *et al.*, 2002], a link also found in branch length and isotope discrimination analyses [Warren and Adams, 2000]. For the results presented here, it seems most likely that this association between $\delta^{13}\text{C}$ and hydraulic resistance changes lasted longer than some decades resulting in carbon trends over the first 100 years in Spanish pine trees.

[22] Even if changes in stomatal conductance and hydraulic conductivity with plant age are widely known in tree physiology, nonclimatic long-term changes in $\delta^{18}\text{O}$ are rarely addressed so far. Generally, a decreasing trend in conductance with age would lead to higher $\delta^{18}\text{O}$ due to a reduced Peclet effect in the needles [Barbour *et al.*, 2004] and therefore cannot explain the decreasing trend revealed in our study. It has been shown though that young conifers start growing earlier in the season [Rossi *et al.*, 2008], which could translate into some temporal shift in environmental signals associated with tree age. Longer growing seasons and more immediate responses to climate conditions [Villalba and Veblen, 1994; see also Esper *et al.*, 2008] have been related with a greater sensitivity of young trees to drought [Copenheaver *et al.*, 2006; Vieira *et al.*, 2009]. Such age-dependent growth responses could particularly be important in Mediterranean environments, where growing seasons are long (6–8 months) and water stress might influence growth even in high-elevation environments – an argument that also accounts for $\delta^{13}\text{C}$. Moreover, Marshall and Monserud [2006] found differing long-term $\delta^{18}\text{O}$ trends in cooccurring species and explained this with the varying ability of root

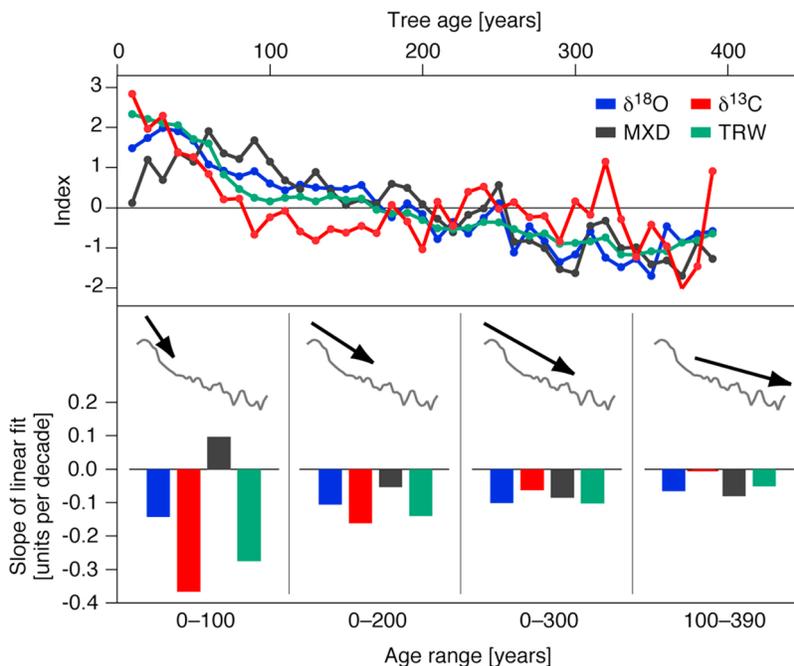


Figure 3. Age trend in tree ring parameters. (top) Arithmetic means of 25 $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, MXD, and TRW time series after aligning the data by biological age. (bottom) Histogram shows the slopes of linear least square regressions fit to the various parameters over the first 100 years, 200 years, 300 years, and the 100–390 year periods. These periods are also highlighted by the arrows. All age-aligned mean curves are shown after normalization over the 0–390 year period that is replicated by 5 or more trees. $\delta^{13}\text{C}$ data have been inverted.

systems to reach deeper soils. As top layers are usually isotopically enriched, due to evaporation, these gradients in soils and root systems could as well explain age-related trends in $\delta^{18}\text{O}$.

[23] In this study, age-related noise in tree ring stable isotopes has been assessed using a collection of 25 individual measurement series covering the past millennium. This particular replication and temporal distribution of samples appeared sufficient to estimate age trend and to assign best fit detrending models based upon a minimum squared residual criterion. It has been shown how NegExp (and linear) models remove the association between tree age and isotopic value, and thereby changes the long-term behavior of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ chronologies. Detrending also removes much of the lag 1 autocorrelation from the tree ring parameters (particularly TRW), substantially shrinks the range of data – from originally 25.6–33.95‰ $\delta^{18}\text{O}$ and 19.8–24.13‰ $\delta^{13}\text{C}$ – and reduces the interseries correlation in $\delta^{18}\text{O}$, MXD, and TRW (Table 1). The herein identified best fit detrending options, however, also remove low frequency variance from the chronologies, as the maximum wavelength of retainable variance is fundamentally associated with the individual lengths of the underlying data (the ‘segment length curse’) [Cook *et al.*, 1995]. Mean segment length of the Pyrenees pine data is quite large (330 years), which to some degree eases the segment length curse in the given example. Removal of low frequency variance would be

more substantial though, if the data set were composed of shorter segments, as is typically the case in a European context.

[24] While data replication appeared sufficient to estimate age trend, it seems premature to conclude on the nature and origin of level offset between individual pine isotope time series. Trees growing at the same site have been shown to display differences up to several per mil, which could be caused by small-scale soil patchiness (e.g., mounds and troughs resulting in varying water supplies), microclimatic differences due to canopy structure and forest density, and/or genetic differences governing tree properties including water use efficiency [Leavitt and Long, 1984; Brendel *et al.*, 2002]. Sample replication in our study appears too low though to estimate whether these substantial offsets are also driven by changes in climate, and as such should be retained in long-term chronologies. In classical dendroclimatology, level differences in TRW [e.g., Esper *et al.*, 2007b] and MXD [e.g., Büntgen *et al.*, 2006] data are regarded as both climatic signals and noise. The climatic component of the offset may be preserved via the application of RCS, a technique that allows deviations from the mean of all age-aligned data to be retained in the detrended chronology [Esper *et al.*, 2002]. The noise component of the offset is mitigated by the large sample sizes that are required when performing RCS [Esper *et al.*, 2003], a finding that may be troublesome to the common accep-

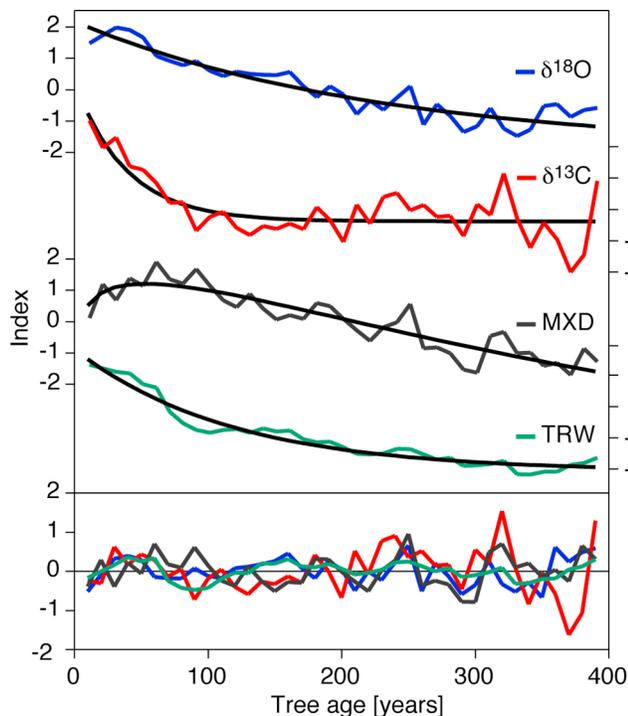


Figure 4. Age trend approximation. (top) Deterministic functions fit to the mean of the age-aligned data; NegExp for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and TRW, and Hug for MXD. (bottom) Residual time series from these functions.

tance of a low sample replication in most isotope studies (e.g., $n = 5$) justified by measures of interseries correlation [Wigley *et al.*, 1984].

[25] We here applied RCS to four tree ring parameters and found similar low frequency variance in the detrended chronologies. While this finding may suggest that some of the level differences in stable isotope measurements could be related to a common forcing, more samples are needed to test this hypothesis and evaluate the applicability of stable isotope data for RCS or similar composite detrending approaches [Briffa *et al.*, 2001; Esper *et al.*, 2009]. This conclusion stems from the variance of age-aligned isotope data as shown in Figure S4, as well as the number of MXD measurements (>200 series) considered in an RCS approach to estimate long-term temperature changes in the Spanish Pyrenees [Büntgen *et al.*, 2007, 2008]. The differences in low frequency characteristics between best fit and RCS detrended chronologies, as revealed here, indicates a methodological uncertainty that appears to now be linked with isotope measurements, and not just TRW or MXD [Esper *et al.*, 2007a; Frank *et al.*, 2007].

[26] The reduced, decadal resolution of our time series prohibited an analysis of the climatic response and robust calibration of these data against instrumental measurements [Frank *et al.*, 2010]. However, the overall positive trends retained in the RCS chronologies, the low-frequency coherence between tree ring parameters, as well as the reasonable

calibration of annually resolved MXD data against temperature [Büntgen *et al.*, 2008], together suggest that the various parameters contain information on long-term climatic changes in the region. An assessment of regional climate data (not shown) indicated that precipitation time series show fewer trends, suggesting that temperature changes might be the prime driver of low frequency variance in RCS detrended chronologies. It is planned to develop a data set of annually resolved $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ measurements over the last 100–150 years to test this hypothesis.

[27] Sample replication of the *Pinus uncinata* data set presented here (~ 10 trees back to A.D. 1300) is in the order of a widely recognized temperature reconstruction from the Tornetraesk region in Sweden (10–19 series) [Grudd *et al.*, 2002], but lower than a drought reconstruction from the Atlas Mountains in Morocco (14–85 series) [Esper *et al.*, 2007a], for example (Figure S8). An assessment of the internal coherence of these reconstructions shows that Swedish MXD and Moroccan TRW data are characterized by higher common variances than the Spanish isotope series (Figure 7), suggesting that fewer data are required in the Northern European and Northern African sites to robustly estimate a population mean and reconstruct climate variations [Wigley *et al.*, 1984]. A comparison of the annually versus decadal resolved time series also reveals a reduction in cross coherence when aggregating data over 10 year chunks – particularly for the Tornetraesk MXD measurements. Similar changes were found in stable isotopes from the European Alps [Kress *et al.*, 2010], suggesting that climate signals in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, as well as MXD, are somewhat weighted toward the higher-frequency domain [Frank and Esper, 2005; Frank *et al.*, 2005].

[28] The significance of biological biases depends on the size and shape of age trends, as well as the length and distribution of measurement series throughout time. The latter is particularly relevant in data sets composed of even-aged samples, where most trees germinated around the same calendar period such as the European isotope network [Treydte *et al.*, 2007], or in data sets from only living trees spanning the past millennium such as the cedar collection from Northern Africa [Esper *et al.*, 2007a]. For data sets composed of living and relict samples, as is the case for the Pyrenees pine data, the mixture of old and young data throughout time naturally mitigates age-related biases. However, mean tree age of the Pyrenees chronology still changes notably from >300 years in the early 13th to <100 years in the early 14th century (see Figure 1, middle), for example, which induces age-related biases when left untreated.

[29] Our results validate findings by Treydte *et al.* [2006] suggesting that $\delta^{18}\text{O}$ data from Central Asian juniper trees contain age trend. The more systematic analysis of living and relict pine material presented here, now indicates that assumptions for the nonexistence of age-related trends in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ time series are no longer tenable. If this pattern is supported by similar tests elsewhere and for other species, a general reevaluation of methods and procedures will be required before robust reconstructions can be derived from isotopic data. Whereas 20th century $\delta^{18}\text{O}$ trends that have been reported and interpreted in a climatic

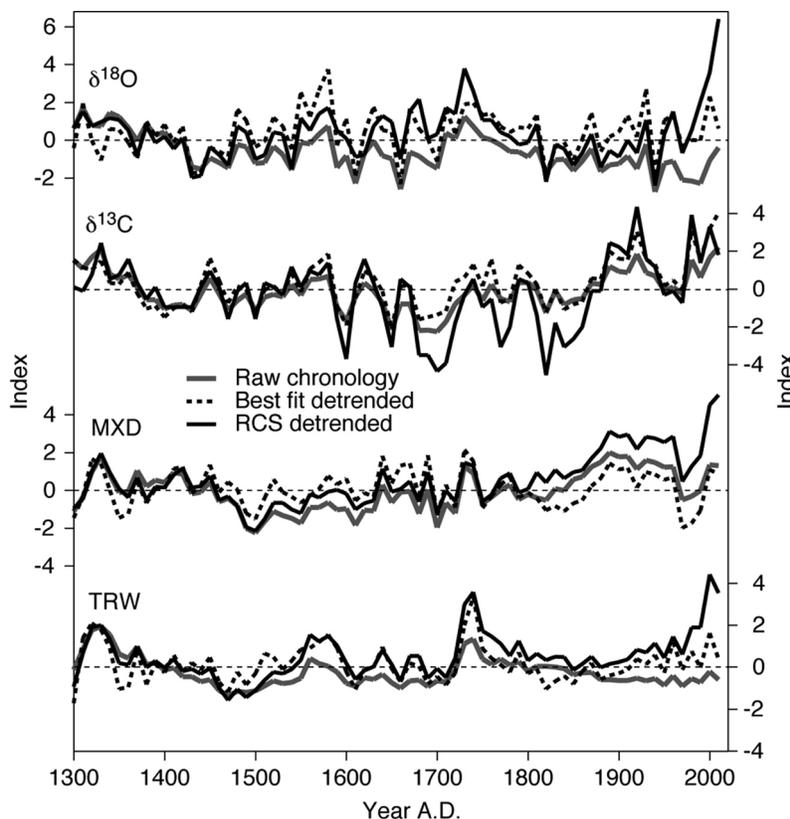


Figure 5. Influence of detrending. Raw chronologies (gray curves) are shown together with best fit (dashed curves; NegExp for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, TRW, and Hug for MXD) and RCS (black curves) detrended chronologies. All records are normalized over the 1300–1600 period.

context [e.g., Saurer et al., 2002; Hlasvuori et al., 2009] may need to be reassessed, the strong high-frequency coherence typical for tree ring stable isotopes suggests that these data can become important estimators of climate variability over the past millennia. Advances in measurement

speed are required though to increase replication and allow for an assessment of age-related biases, necessary for the development of skillful reconstructions of high-to-low frequency climate variations.

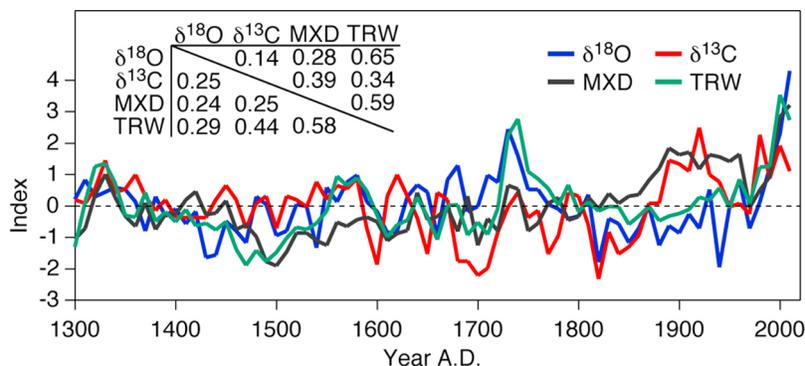


Figure 6. Long-term coherence between tree ring parameters. Colored curves are RCS detrended $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, MXD, and TRW chronologies. Data from only the well-replicated age classes 2 to 30 years are included in the chronologies. Time series have been normalized over the 1300 to 2010 period. Inserted table shows the correlations between (top right) the RCS chronologies and (bottom left) prewhitened versions of these time series over the 1300 to 2010 period.

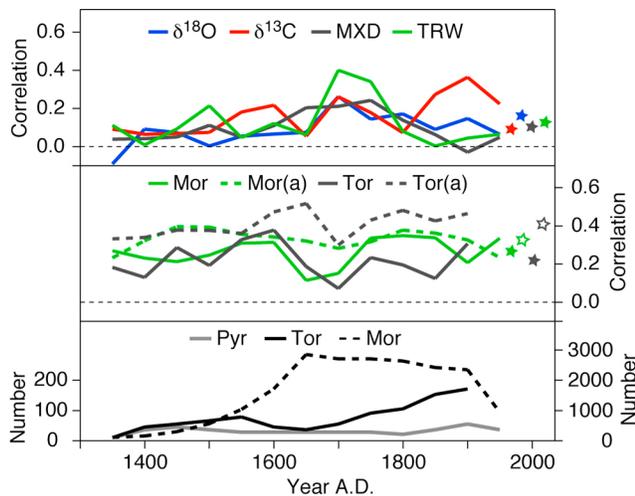


Figure 7. Coherence of the Pyrenees and other European data. (top and middle) Interseries correlations computed over 100 year periods (50 year offset) since 1300. Mor and Mor(a) are the decadal and annually resolved TRW data from the Atlas mountains in Morocco used for drought reconstruction [Esper et al., 2007a], and Tor and Tor(a) are the decadal and annually resolved MXD data from Torneträsk in Sweden used for temperature reconstruction [Grudd et al., 2002]. Asterisks indicate mean interseries correlations averaged over all 100 year segments; $r = 0.09$ for $\delta^{18}\text{O}$, 0.16 for $\delta^{13}\text{C}$, 0.10 for MXD, 0.12 for TRW, 0.26 for Mor, 0.33 for Mor(a), 0.22 for Tor, and 0.41 for Tor(a). (bottom) Number of correlations computed for each period (numbers for Mor are on the right hand axis).

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