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Multi-proxy crossdating extends the longest high-elevation tree-ring chronology from the Mediterranean

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ABSTRACT

Annually precise dating is the cornerstone of dendrochronology. The accurate crossdating of relict wood is, however, frequently challenged during early chronology periods when sample replication is typically low. Here we present a multi-proxy approach in which stable carbon ($\delta^{13}C$) and radiocarbon (^{14}C) isotope data are used to evaluate and correct dating errors in the early period of the longest high-elevation tree-ring chronology from the Mediterranean Basin. The record was initially developed using 878 tree-ring width (TRW) and 192 maximum latewood density (MXD) series from living and relict Bosnian pines (*Pinus heldreichii*) from Mt. Smolikas in Greece to reconstruct hydroclimate and temperature variability back to the 8th century. New annually resolved and non-pooled $\delta^{13}C$ series now suggest a re-dating of first millennium relict pine samples during a period when sample replication was too low for proper TRW and/or MXD crossdating. The associated correction shifts the start of the Mt. Smolikas chronology from 575 back to 468 CE, a change independently confirmed by wiggle-matching annual ^{14}C data along the 774/775 CE cosmic event. Our study demonstrates the importance of independent age validation for robust chronology development and shows how multi-proxy crossdating can improve dating success during periods of low sample replication.

1. Introduction

The fundamental principle of dendrochronology is crossdating, a method used to accurately assign tree rings to their exact year of formation, routinely accomplished by determining coherent patterns of inter-annual ring-width (TRW) variability (Stokes and Smiley, 1996). This matching technique allows the dating of relict samples, such as subfossil, archeological or historical construction timber, an indispensable step in developing multi-centennial to millennial-long tree-ring records (Esper et al., 2016; Ljungqvist et al., 2020). Absolute calendric dating is advantageous compared to other proxy archives and enables the development of high-resolution environmental reconstructions that provide unique information to study, for example, the climate response to volcanic forcing (Sigl et al., 2015; Büntgen et al., 2020a) or the history of natural forest disturbances (Esper et al., 2007) at annual resolution.

Crossdating is most effective when tree growth is constrained by a single limiting environmental factor (Fritts, 1976). Dating success based on TRW can be hampered either by favorable conditions resulting in invariant (complacent) rings or structural anomalies such as false, missing, and wedging rings or intra-annual density fluctuations (Schweingruber et al., 2008). Compared to TRW, maximum latewood density (MXD) measurements typically contain less non-climatic noise, such as biological memory effects (Esper et al., 2015) and spectral biases (Franke et al., 2013), and are less susceptible to large-scale disturbances (Rydval et al., 2018), improving crossdating success. Despite various methodological advances in recent decades (Björklund et al., 2019), dating relict wood of unknown age using well-established TRW/MXD records as a reference, however, remains challenging. This is

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particularly noticeable in the early period of many tree-ring chronologies, where sample replication and overlap usually decrease substantially. Incorrectly dated series can lead to critical errors in composite chronologies, propagating back through time and eliminating crucial high-frequency signals (Black et al., 2016). Hence, only samples with high dating certainty are routinely included in final records. As a result, a large number of undated relict wood is neglected, often limiting the further extension of absolutely dated tree-ring chronologies into the past.

Besides TRW and MXD, stable carbon (δ^{13}C) and oxygen (δ^{18}O) isotopes have proven valuable tools for dating living (Roden, 2008) and historic construction timber (Loader et al., 2019; Loader et al., 2021). As δ^{13}C reflects the balance between stomatal conductivity and photosynthetic CO$_2$ assimilation, and δ^{18}O is determined by source water and leaf transpiration (McCarroll and Loader, 2004), both tree-ring stable isotopes (TRSI) contain crucial information on past climatic conditions and show strong coherences across species and sites (Treydte et al., 2007; Saurer et al., 2008; Hartl-Meier et al., 2014). Climate signals in TRSI are less dependent on ecological conditions and thus increasingly used for paleoclimate reconstructions, particularly in lowland regions where traditional parameters are less sensitive to climate (e.g., Labuhn et al., 2016; Büntgen et al., 2021). Furthermore, despite juvenile effects

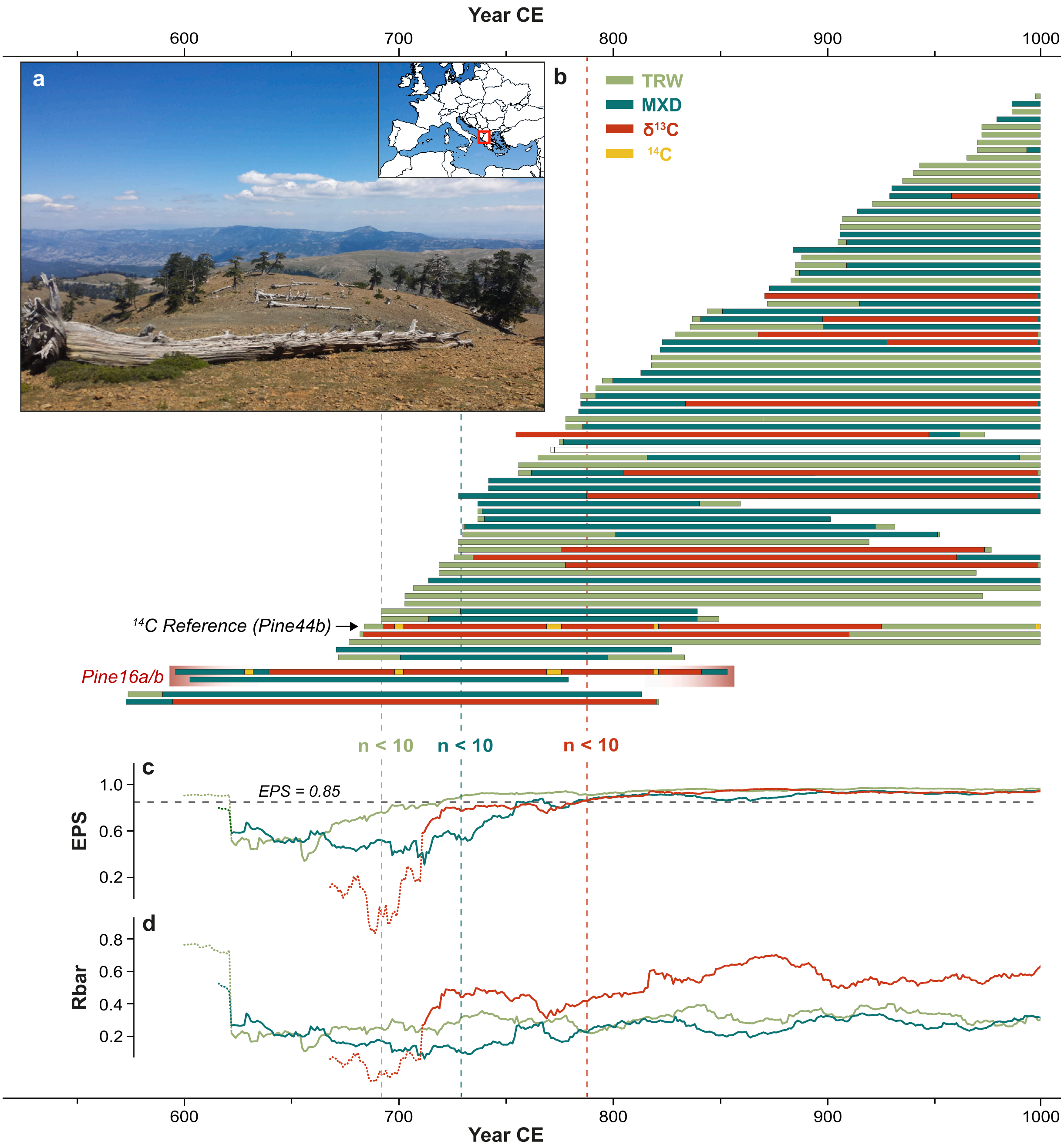

Fig. 1. Mt. Smolikas tree-ring data (as published in Esper et al., 2020a, 2021). (a) Photograph of relict *Pinus heldreichii* trunks and map showing the geographical location of Mt. Smolikas in Europe. (b) Segment length plot indicating the sample replication of the tree-ring width (TRW), maximum latewood density (MXD), stable carbon (δ^{13}C) and radiocarbon isotope (^{14}C) data in the first millennium. Each horizontal bar represents one measurement series. Vertical dashed lines mark the years when sample replication falls below 10 series/year. (c) Expressed population signal (EPS) and (d) mean inter-series correlation (Rbar) for each proxy calculated over 50-year windows, lagged by 49 years. Dotted curves highlight periods when Rbar/EPS-calculations are based on two series only. Note the sharp decrease in Rbar/EPS of δ^{13}C at the early chronology end caused by the misdating of Pine16a.

(Gagen et al., 2008), TRSI often lack long-term biological age trends (Gagen et al., 2007; Young et al., 2011; Büntgen et al., 2020b) and require comparatively small sample sizes (4–6 trees) to produce reliable site-representative chronologies (Leavitt, 2010; Belmecheri et al., 2022).

Independent of regional growth and climate, high-precision radiocarbon (^{14}C) measurements calibrated against the International Northern Hemisphere Radiocarbon Age Calibration Curve (IntCal20; Reimer et al., 2020) allow the determination of sample ages. Recent methodological advances have made annually resolved Accelerator Mass Spectrometer (AMS) measurements more readily available and reasonably affordable (Wacker et al., 2014; Sookdeo et al., 2016), opening new opportunities for independent crossdating confirmation. To minimize ^{14}C dating uncertainties resulting from the non-monotonicity of IntCal20, so-called ^{14}C "wiggle-matching" is performed where multiple rings along a single wood sample are measured and systematically compared to the calibration curve (Bronk Ramsey et al., 2001). Furthermore, abrupt, singular, and globally coherent ^{14}C enrichment events, likely due to short-term enhanced cosmic radiation, represent objective time markers allowing for absolute dating beyond the methodological dating uncertainty of ^{14}C (Miyake et al., 2012; Büntgen et al., 2018).

Here, we apply state-of-the-art multi-proxy crossdating, including TRW, MXD, δ^{13}C, and ^{14}C, to overcome dating issues of relict pine logs from Mt. Smolikas in Greece. We show that annually resolved and non-pooled δ^{13}C measurements of α-cellulose from whole wood samples enable the correction of TRW-based dating errors in the earliest period of the chronology despite weak sample replication. Verified by high-precision ^{14}C wiggle-matching, our findings extend the longest Mediterranean tree-ring record more than a century back in time.

2. Material and methods

2.1. TRW and MXD measurements

A total of 878 TRW and 192 MXD measurements on living (5-mm increment cores) and relict (discs) Bosnian pines (*Pinus heldreichii*) from Mt. Smolikas (≥ 2000 m a.s.l.) in northwestern Greece (Fig. 1a) enabled the development of tree-ring records dating back to early medieval times (Esper et al., 2020a; Esper et al., 2021). The distinct climate seasonality (warm-dry summers, cold-snowy winters) of the Mediterranean mountain massif permitted the preservation of relict logs for centuries. Core and disc samples were processed according to standard dendrochronological techniques (Stokes and Smiley, 1996). TRW was measured along two stem radii per tree (labeled as a- and b-core; e.g., Pine16a and Pine16b) using the high-precision Lintab (Rinntech GmbH, Heidelberg, Germany) and Velmex (Velmex Inc., Bloomfield, USA) devices, while MXD data were produced using the Dendro-2003 X-ray densitometer (Walesch Electronic GmbH, Effretikon, Switzerland). Visual and statistical crossdating was performed using the TSAP-Win (Rinn, 2012) and COFECHA (Holmes, 1983) software, respectively.

2.2. δ^{13}C measurements

A total of 78 TRW series (~17 % of the entire relict wood material) date to the first millennium. From this collection, 17 well-preserved discs were selected to develop a continuous, annually resolved δ^{13}C chronology including 4151 individual measurements. In contrast to TRW and MXD, δ^{13}C was measured along a single radius per tree. The disc samples were sawn into ~7-mm-wide radial sections to allow the precise separation of individual rings using a scalpel under a stereomicroscope. To avoid potential juvenile effects (Gagen et al., 2008), the 50 innermost rings of each sample (considering pith-offset) were omitted as a conservative precaution. After carefully cutting and dividing each tree ring into similarly sized fiber flakes, the α-cellulose was extracted from the whole wood via the modified Jayme-Wise isolation method (Boettger et al., 2007) at the Global Change Research Institute (CzechGlobe) in the Czech Republic. For this, the shredded wood fibers of each ring were packed in Teflon filter bags and washed successively with 5 % NaOH (twice for 2 h at 60 °C) and 7 % NaClO$_2$ solution (30 h at 60 °C) to remove hemicellulose, resin, fatty acids, ethereal oils, and lignin. Acetic acid (99.8 %) was added to the latter solution to set a pH of 4–5. The remaining α-cellulose was rinsed three times with 90 °C distilled water, dried at 50 °C for 24 h, locked in Eppendorf microtubes, and stored in a dark room at constant room temperature (21 °C).

For δ^{13}C measurements, 0.2–1.0 mg of α-cellulose was placed in tin capsules and combusted to CO$_2$ at 960 °C using the varioPYRO cube elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). From the CO$_2$ gas, the ratio of heavy (^{13}C) to light (^{12}C) carbon isotopes was determined using the ISOPRIME100 mass spectrometer (Isoprime Ltd., Manchester, UK) operating in a continuous flow mode. The ion source of the mass spectrometer was centered, tuned, and tested for stability ($\sigma \leq 0.04$‰ on ten pulses over three runs) and linearity ($\sigma \leq 0.03$‰/nA) before each measurement. ^{13}C/^{12}C ratios were referenced to caffeine (IAEA-600) and graphite (USGS24) and expressed in the conventional δ notation (in ‰), relative to the Vienna Pee Dee Belemnite standard according to the formula (Eq. 1):

$$\delta^{13}\mathrm{C} = \left(R_{sample}/R_{standard} - 1\right)x1000, \tag{1}$$

where R is the ratio between heavy and light carbon isotopes (^{13}C/^{12}C). Six consecutive measurements of the same α-cellulose sample were made to determine the precision of δ^{13}C ratios. The range between minimum and maximum values and standard deviation (σ) were 0.12‰ and 0.042‰, respectively.

2.3. Chronology development

To normalize the TRW, MXD, and δ^{13}C data, the individual tree-ring series were power-transformed and high-pass filtered by calculating residuals from cubic smoothing splines with a 50 % frequency-response cutoff at 30 years (Cook and Peters, 1981; Wigley et al., 1987), removing large portions of low-frequency variability, positive autocorrelation, and skewness. Master chronologies were developed using ARSTAN (Cook and Krusic, 2016) by calculating the bi-weight robust mean (Cook, 1985) and performing variance stabilization to account for altering sample replication and covariance over time (Frank et al., 2007). Coherences among the individual series were assessed using the inter-series correlation (Rbar) and expressed population signal (EPS; Wigley et al., 1984) computed over 50-year segments with a 49-year overlap. Although the new δ^{13}C series were dated according to their respective TRW/MXD measurements (Esper et al., 2020a; Esper et al., 2021), their degree of internal crossdating was evaluated using COFECHA (Holmes, 1983). Moving window correlations between the high-pass filtered δ^{13}C series and their COFECHA master chronology thereby cast doubt on the age of the second oldest relict pine (Pine16) by proposing a putative redating of the a-core (Pine16a) from its original TRW-derived placement at 597–852 CE (Opt1) to 468–723 CE (Opt2).

2.4. Radiocarbon dating

For independent age validation, annually resolved ^{14}C measurements were performed on two samples, Pine16a with a total ring number of 256 and Pine44b with 325 rings. The latter provided a high dendrochronological dating confidence due to significant ($p < 0.001$) strong correlations with the TRW ($r = 0.57$) and δ^{13}C ($r = 0.87$) master chronologies over the well-replicated period 695–925 CE ($n \geq 10$ series/year). From each sample, 13 rings from the initial, middle, and end section, ideally from identical calendric dates derived from TRW, were precisely cut and fragmented into equally sized fiber flakes using a scalpel. Material believed to cover the 772–777 CE period was selected to use the 774/775 CE cosmic event as an independent time marker.

At the Curt-Engelhorn-Center of Archaeometry (Mannheim, Germany), holocellulose was extracted from each ring using the BABA bleaching method (Brock et al., 2010) by washing the wood fibers sequentially at 60 °C in 4 % NaOH (overnight), 4 % HCl (30 min), 4 % NaOH (30 min), 4 % HCl (30 min), and repeatedly 5 % $NaClO_2$ (60 min) until cellulose turned white. HCl was added to the $NaClO_2$ solution to set a pH of 3. The material was rinsed 1–3 times between each step with Milli-Q water. After chemical extraction, samples were dried overnight at 60 °C. Holocellulose yield ($m_{holocellulose}/m_{wood}$) ranged from 48 % to 62 %. Holocellulose was combusted using the varioMICRO cube elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany), and the resulting CO_2 was catalytically graphitized using either a custom build or the commercially available graphitization system AGE3 (IonPlus Inc., Dietikon, Switzerland; Němec et al., 2010; Wacker et al., 2010a). The cellulose-based graphite was subsequently pressed into aluminum targets and analyzed for its ^{14}C concentration using the high-precision MICADAS-type (IonPlus Inc., Dietikon, Switzerland) AMS (Synal et al., 2007; Wacker et al., 2010b; Kromer et al., 2013). To determine absolute calendrical dates (CE), the ^{14}C measurements from Pine16a and Pine44b were wiggle-matched against the IntCal20 curve (Reimer et al., 2020) using the D_Sequence function in OxCal 4.4 (Bronk Ramsey et al., 2001; Bronk, 2009).

3. Results and discussion

3.1. TRW, MXD, and $\delta^{13}C$ chronology statistics

Although the statistical properties of raw $\delta^{13}C$ were less problematic for correlation analyses than those of TRW and MXD, a first-order autocorrelation of 0.41 and pronounced skewness highlighted the need for detrending. High-pass filters effectively removed multi-decadal scale variability while emphasizing high-frequency variability and moving the data closer to a normal distribution (Fig. A1). In the first millennium, the chronology replication decreased continuously toward the past, falling below a threshold of $n < 10$ series/year before 693 CE for TRW, 730 CE for MXD, and 790 CE for $\delta^{13}C$ (Fig. 1b). Over the well-replicated 790–1000 CE period, the detrended records (excluding Pine16) correlated < 0.5, indicating high independence between the tree-ring proxies, with more than 75 % of the variance unexplained. Mean segment length of the $\delta^{13}C$ data (244 years) was 60–70 years shorter than those of TRW and MXD (Table 1), while mean tree age decreased almost equally for all proxies, from an average of 154 years in 1000 CE to 61 years in 790 CE.

In periods of sufficient sample replication ($n \geq 10$ series/year), EPS values of all detrended proxy chronologies persistently exceeded 0.85 (Fig. 1c), indicating an adequate signal strength for robust chronology development (Wigley et al., 1984). Rbar values, however, were substantially higher in $\delta^{13}C$ than in TRW and MXD throughout the first millennium (Fig. 1d), despite a constantly lower $\delta^{13}C$ replication (n_{avg} ~25% of TRW). Since the isotope data included only one radial measurement per tree – unlike TRW and MXD – the high Rbar values in $\delta^{13}C$ reflected strong correlations among the Bosnian pines. These results were in line with previous studies from the Mediterranean reporting comparatively high Rbar values for $\delta^{13}C$ from several pine species, e.g., *Pinus heldreichii* from the Dinarides in Montenegro (Lukač et al., 2021), *Pinus nigra* from Corsica (Szymczak et al., 2012), *Pinus uncinata* from the Spanish Pyrenees (Konter et al., 2014) and Cazorla Natural Park in southern Spain (Dorado Liñán et al., 2011). The observed strong correlations among the individual trees outlined $\delta^{13}C$ as a valuable crossdating proxy, especially in periods where the Mt. Smolikas record is based on less than ten replicates.

3.2. Dendrochronological dating of Pine16a

While 16 of the 17 $\delta^{13}C$ series displayed highly consistent year-to-year variations, a strikingly low correlation with the isotopic master chronology ($r_{(643-840)} = 0.15$) was found for Pine16a at its TRW-based dating position (Opt1, 597–852 CE), causing a sharp decline in Rbar/EPS values. At an alternative $\delta^{13}C$-derived position (Opt2, 468–723 CE) proposed by COFECHA and supported by visual inspection, Pine16a showed higher agreement in all proxies (Fig. 2). However, only the $\delta^{13}C$ ($r_{(597-711)} = 0.62$) and TRW ($r_{(575-723)} = 0.26$) correlations were significant at $p < 0.001$, while MXD correlations were not. The lack of MXD coherence is striking, especially since its annual-to-decadal scale variability is highly temperature-sensitive (Klippel et al., 2019; Esper et al., 2020a). However, this is likely related to the insufficient sample replication after 850 CE, which is up to 50 % lower than for TRW. Similar replication-related inconsistencies were found in *Larix decidua* MXD from the Tatra Mountains in Slovakia, hindering the further extension of living tree chronologies utilizing historical construction wood (Klippel et al., 2020).

While sole TRW and MXD crossdating yielded inconclusive results, the synergistic combination with non-pooled $\delta^{13}C$ measurements provided convincing evidence for the actual age of Pine16. Although the $\delta^{13}C$ chronology is only based on a single tree between 597 and 683 CE, the agreement with Pine16a at Opt2 was remarkably high after detrending ($r_{det} = 0.57$, $p < 0.001$) and still significant in the raw $\delta^{13}C$ data ($r_{raw} = 0.27$, $p < 0.05$). Visual inspection outlined distinct marker years (e.g., 660–661 CE) and periods (654–681 CE) in both time series (Fig. A2) with only a brief period of high-frequency discrepancies between 613 and 625 CE. Minor level offsets in absolute $\delta^{13}C$ values were likely related to a mixture of varying microsite conditions (Hartl-Meier et al., 2014) and tree-internal variability (Esper et al., 2020b). However, unlike the observed TRW results (Klippel et al., 2017), the high-frequency variability in $\delta^{13}C$ appeared to be less or not at all affected, reinforcing its high suitability for crossdating.

3.3. Radiocarbon verification

The ^{14}C measurements on Pine16a and Pine44b, calibrated against the IntCal20 record, provided unambiguous dating results. Wiggle-matching and calibrated ^{14}C ages of Pine44b were in line with the dendrochronological derived position. The innermost ring, dated to 699 CE by both TRW and $\delta^{13}C$, returned a ^{14}C age of 1307 ± 19 BP that was calibrated to 688–711 CE at 99.7 % (3σ), 689–706 CE at 95.4 % (2σ) and 691–700 CE at 68.3 % probability (1σ). The dating accuracy of the twelve remaining rings along Pine44b was similarly accurate (Table A1). Furthermore, the sharp decline in uncalibrated ^{14}C ages of rings dated to 773–776 CE, ranging from 1318 ± 19 BP to 1159 ± 19 BP, coincided with the 774/775 CE cosmic event (Fig. 3) and thus confirmed the annual dating precision of the Mt. Smolikas tree-ring

Table 1
TRW, MXD, and $\delta^{13}C$ statistics of the Mt. Smolikas tree-ring chronology.

	n	Period	MSL	Mean ± σ	Rbar raw | det	AC1 raw | det	Reference
TRW	449 | 878	468–2019	314	0.82 ± 0.38	0.30 | 0.31	0.77 | 0.25	Esper et al. (2021)
MXD	103 | 192	468–2017	303	0.69 ± 0.04	0.21 | 0.30	0.51 | 0.08	Esper et al. (2020a)
$\delta^{13}C$	17 | 17	514–1235	244	-21.09 ± 0.55	0.47 | 0.60	0.41 | 0.08	This study

n: total number of trees | radii, Period: first and last year (CE) of the chronologies, MSL: mean segment length [years], Mean: average raw TRW [mm], MXD [g/cm^3] and $\delta^{13}C$ [‰] values ± 1σ, Rbar: mean inter-series correlation, AC1: first-order autocorrelation. The latter two statistics are provided for the raw and detrended (det) data. Chronologies are Pine16 corrected.

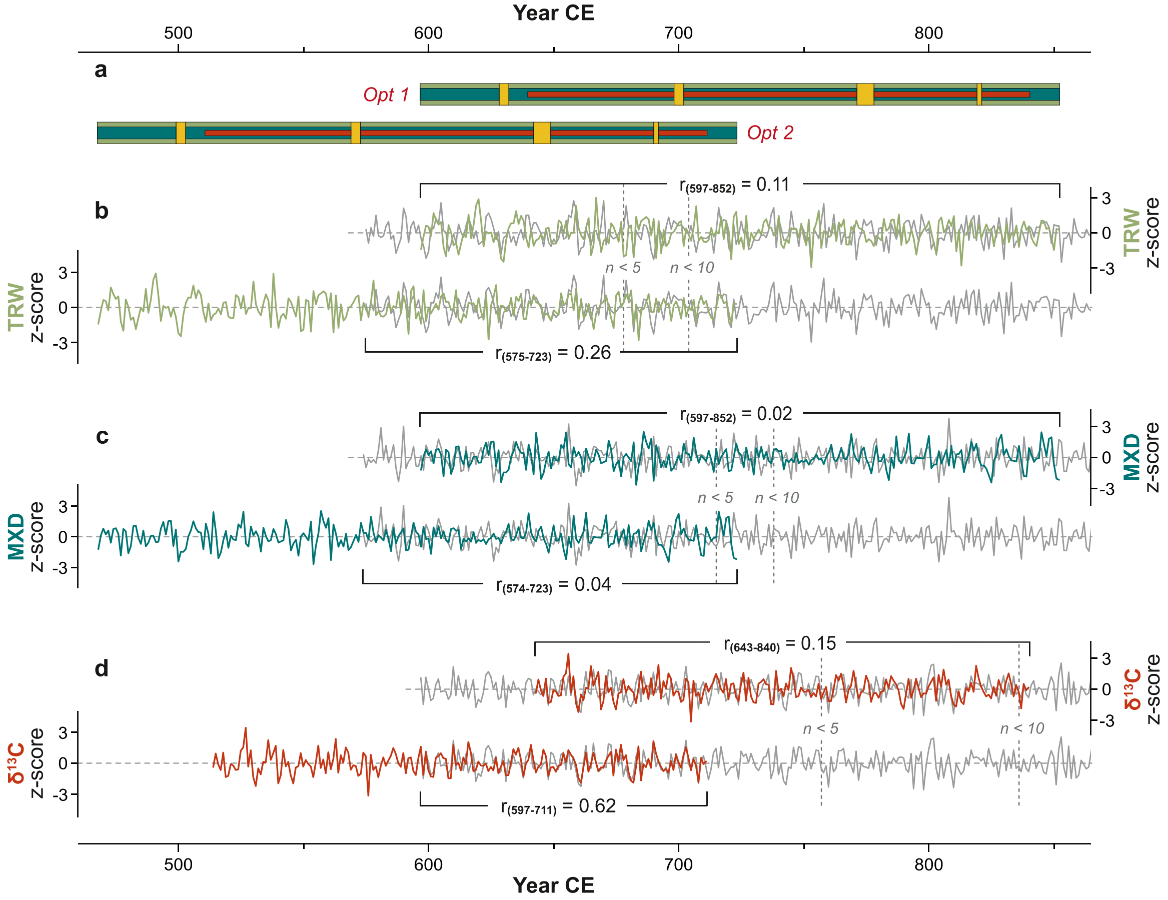

Fig. 2. Crossdating of Pine16a. (a) Bars indicating the two potential dating positions: Opt1 (597–852 CE) and Opt2 (468–723 CE) with color code as in Fig. 1. High-pass filtered (b) TRW, (c) MXD and (d) $\delta^{13}C$ time series of Pine16a (color) and the master chronologies (gray). All series are z-transformed for better comparison. Brackets highlight the maximum overlap and corresponding Pearson correlations.

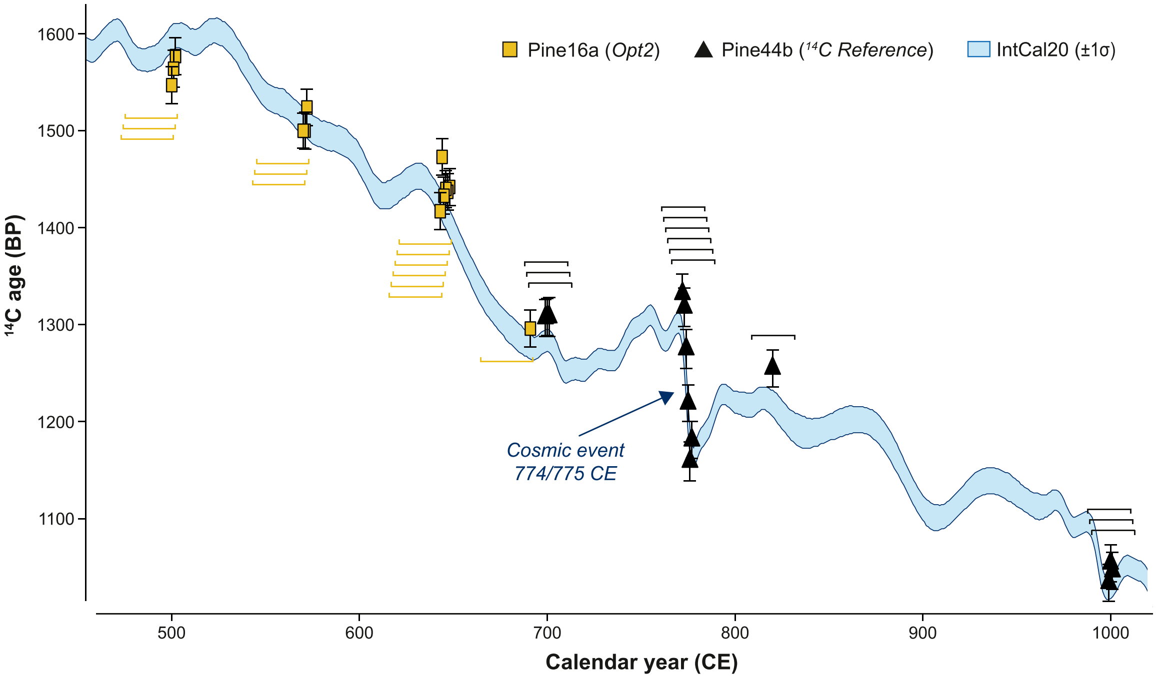

Fig. 3. Radiocarbon dating. Symbols represent ^{14}C ages (BP) ± 1σ (whiskers) of single rings and brackets the calibrated age ranges (CE) at 99.7 % probability (see Fig. A4 for details). The uncalibrated ^{14}C ages (BP) are aligned to their $\delta^{13}C$ dates (CE). The blue line is the IntCal20 calibration curve.

Table 2
^{14}C and δ^{13}C dating results of specimen Pine16a.

Ring	F^{14}C ± σ	^{14}C age ± σ	Unmodeled		Modeled		δ^{13}C	δ^{13}C date
			from	to	from	to		
629	0.8248 ± 0.0019	1547 ± 19	430	596	**473**	**501**	-	-
630	0.8231 ± 0.0019	1564 ± 19	421	579	**474**	**502**	-	-
631	0.8218 ± 0.0019	1577 ± 19	419	565	**475**	**503**	-	-
699	0.8296 ± 0.0019	1500 ± 18	540	641	**543**	**571**	-21.06	**570**
700	0.8296 ± 0.0019	1500 ± 19	538	643	**544**	**572**	-21.14	**571**
701	0.8272 ± 0.0020	1524 ± 19	435	634	**545**	**573**	-21.60	**572**
772	0.8383 ± 0.0020	1417 ± 19	591	663	**616**	**644**	-21.50	**643**
773	0.8325 ± 0.0020	1473 ± 19	551	648	**617**	**645**	-21.24	**644**
774	0.8366 ± 0.0020	1433 ± 19	578	658	**618**	**646**	-21.14	**645**
775	0.8359 ± 0.0019	1440 ± 19	572	655	**619**	**647**	-20.50	**646**
776	0.8362 ± 0.0020	1437 ± 19	575	656	**620**	**648**	-20.78	**647**
777	0.8357 ± 0.0020	1442 ± 19	570	655	**621**	**649**	-21.64	**648**
820	0.8510 ± 0.0020	1296 ± 19	659	778	**664**	**692**	-20.54	**691**

Ring: Original TRW-based dates CE (Opt1), F^{14}C: Normalized ^{14}C activity ratios (± 1σ), ^{14}C age: Uncalibrated ^{14}C ages BP (± 1σ), Unmodeled: Individually calibrated ^{14}C age ranges CE (3σ; 99.7 % probability), Modeled: Wiggle-matched ^{14}C age ranges CE (3σ; 99.7 % probability), δ^{13}C: Stable carbon isotope ratios [‰], δ^{13}C date: δ^{13}C-derived dates CE (Opt2). Note the discrepancies between the TRW and δ^{13}C dates CE (129-year offset).

record. In contrast, no similar ^{14}C anomaly was found in the corresponding tree rings of Pine16a. Wiggle-matching of Pine16a resulted in a calibrated ^{14}C age of 487–678 ± 28 CE (3σ) supporting Opt2 as the correct dating position by the distinct agreement between δ^{13}C and ^{14}C (Table 2, Fig. A3).

3.4. Chronology improvements

The revised age of Pine16 extends the existing tree-ring records from Mt. Smolikas by more than a century back in time. The TRW and MXD chronologies now start in 468 CE. Due to the exclusion of juvenile wood portions, the new δ^{13}C chronology begins 47 years later (514 CE). The corrected proxy records display a greater year-to-year variability, higher common signal strength, and reduced error range in the 6th and 7th century CE (Fig. 4). These findings are in line with error simulations by Black et al. (2016), who demonstrate the loss of distinct high-frequency variability by adding incorrectly dated tree-ring sequences. Due to higher inter-series correlations, the EPS values of the corrected records exceed the 0.85 threshold up to 65 years longer into the past, indicating stronger temporal robustness in the first millennium. However, using the EPS as an indicator of sufficient sample replication – to explain ≥ 85 % of the variance of a theoretical population (Wigley et al., 1984) – outlines notable differences between the proxies. While more than ten series are necessary to obtain a representative stand-level signal in TRW and MXD, only three δ^{13}C series are required to produce a robust isotope chronology. This result is consistent with previous TRSI studies that outline a sample replication of four to six trees sufficient for developing robust site-representative chronologies (Leavitt, 2010; Belmecheri et al., 2022). The application of high-pass filters and the calculation of coherence statistics that neglect inter-tree differences in absolute isotope ratios (up to ~3.5‰), however, limit the assessment of the entire δ^{13}C variance spectrum and restrict the explanatory power of this study to the high-frequency domain.

4. Applications, limitations, and conclusions

Inter-annual δ^{13}C variability of *Pinus heldreichii* α-cellulose holds great potential to overcome tentative crossdating of relict wood and improve robust chronology development. Several studies have previously shown that TRSI can support conventional ring-width dating of living trees (e.g., Leavitt et al., 1985; Roden, 2008) and historical construction wood (Loader et al., 2019; Loader et al., 2021) when isotopic reference chronologies are sufficiently replicated. In this study, we now demonstrate that combining TRW and non-pooled δ^{13}C measurements enables robust dating in the earliest chronology period, overcoming typical crossdating issues such as low sample replication and poor inter-series correlations. Although the δ^{13}C Mt. Smolikas record is only based on a single tree prior to 683 CE, the highly consistent year-to-year variations among the individual pines ensure a precise age determination that is independently confirmed by high-resolution ^{14}C dating.

Due to the exclusion of juvenile tree rings, it remains unclear whether these strong inter-series correlations in δ^{13}C are stable within the innermost stem sections. Even though juvenile effects are primarily described as low-frequency phenomena in the scientific literature (Bert et al., 1997; Duquesnay et al., 1998; Raffalli-Delerce et al., 2004; Gagen et al., 2008; Esper et al., 2010), δ^{13}C depletions in early growth rings may challenge an isotope-based dating of relict tree samples with highly decayed sapwood (Reinig et al., 2018). We, therefore, recommend further in-depth investigations on inter-annual δ^{13}C variability incorporating initial tree rings to assess whether juvenile effects exist in *Pinus heldreichii* and how they may affect the common variability among trees.

Despite many technological and methodological advances in recent decades (e.g., Schollaen et al., 2017; Andreu-Hayles et al., 2019), it is unlikely that TRSI will replace TRW for routine crossdating due to resource-intensive sample preparation and measurement. Traditional dendrochronological dating techniques, such as pointer year analysis, skeleton plotting and consideration of wood anatomical features, remain key for the development of long tree-ring chronologies (Schweingruber et al., 1990). However, the observed robust δ^{13}C covariance among individual trees provides a unique opportunity to date problematic samples with reasonable effort. Annually resolved and non-pooled δ^{13}C measurements thus offer a high-quality alternative to ^{14}C dating if suitable reference records exist. The strong high-frequency coherence is likely related to climatic drivers that require further in-depth investigations. It is important to note that crossdating success was only possible through annually performed δ^{13}C measurements across various trees containing common high-frequency variability. Although the pooling of material from different trees significantly reduces costs and analysis time, and is often the only way to develop multi-centennial TRSI records with sufficient sample depth, our results highlight the superior advantage of annual and tree-individual stable isotope measurements for quasi-independent age validation through multi-proxy crossdating. Furthermore, non-pooled TRSI provide the opportunity to enhance our knowledge of isotopic age trends, low-frequency variability, and underlying physiological processes, topics that are lively debated in the dendro-isotopic community (e.g., Esper et al., 2010; Helama et al., 2015; Büntgen et al., 2020b; Torbenson et al., 2022). Thus, our results stress the need for more high-resolution TRSI measurements on an individual tree level.

The new Mt. Smolikas δ^{13}C chronology spanning from 514 to 1235 CE is the oldest, high-resolution TRSI record in the Mediterranean Basin. Our results call for renewed efforts to reassess the still significant amount of undated Mt. Smolikas relict wood and extend the existing tree-ring records further back in time. Moreover, the new δ^{13}C record can serve as a benchmark for the development of robust stable isotope records in neighboring Mediterranean regions. The unique synchronicity between the individual δ^{13}C measurements, hinting at a common climatic driver, underscores the need for further investigation of the

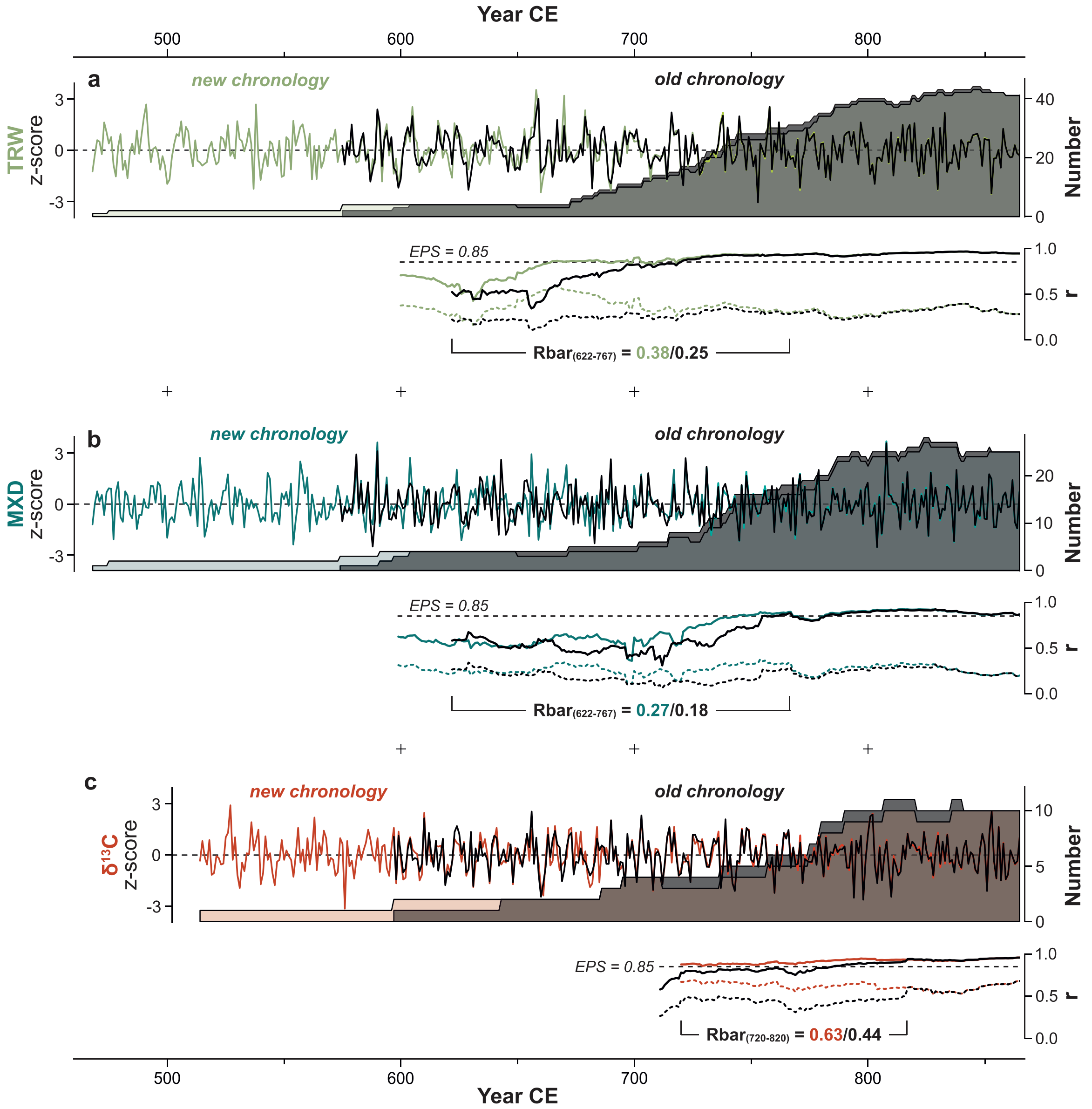

Fig. 4. Changes in the (a) TRW, (b) MXD and (c) δ^{13}C chronologies. Upper panels show the re-dated, new chronologies (color) compared to their old versions (black). Filled areas in the background indicate the underlying sample replication. Lower panels show the expressed population signal (EPS; solid lines) and mean inter-series correlation (Rbar; dashed lines) of the new (color) and old (black) chronologies calculated over 50-year windows with a lag of 49 years and truncated at N < 2 correlations. Horizontal, black-dashed lines mark the EPS threshold of 0.85. All chronologies are high-pass filtered and z-transformed.

paleoclimatic value of δ^{13}C, preferably by extending the isotope record into the 21st century. Several multi-centennial tree-ring records from the Mediterranean region exist (Luterbacher et al., 2012) and our results highlight the potential for supra-regional reconstruction based on δ^{13}C measurements.

CRediT authorship contribution statement

P.R., F.R., and J.E. conceived and planned the study. P.R. prepared samples for isotopic analyses; J.Č., N.P., and O.U. processed and measured the stable carbon isotopes; radiocarbon data were produced by R.F. P.R. performed the final analyses and drafted the manuscript with input from F.R., O.K., R.F., O.U., U.B., and J.E. All authors provided discussion and agreed to the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dendro.2023.126085.

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