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Evaluation of the non-climatic (age-related) trends of stable oxygen and carbon isotopes in Swiss stone pine (*Pinus cembra* L.) tree rings from the Eastern Carpathians, Romania

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ABSTRACT

The presence, magnitude, and duration of age and/or size-related trends in the stable isotopes in tree-ring cellulose time series has been a subject of scientific debate. Where present, their evaluation and removal are key to the development of robust climate calibrations and reconstructions, especially in the low-frequency domain and where sample replication levels are low. Where reported, results suggest that the age/size/height-related trend of stable oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotope compositions in tree-ring cellulose may vary according to the tree species and the individual tree location. For this reason, it is important when developing long palaeoclimate reconstructions for a new species, geographical region or ecological setting to perform studies to investigate non-climatic (age-related) trends. This study evaluates the ontogenetic pattern in the $\delta^{18}O$ and $\delta^{13}C$ data in Swiss stone pine trees from the Eastern Carpathians, Romania. For this, we used a new multi-centennial dataset consisting of 5 living and 10 relict Swiss stone pine samples collected from the Eastern Carpathians, which were annually resolved for $\delta^{18}O$ and $\delta^{13}C$ composition. Age-related trends were evaluated using the conventional "slope of the mean" approach as well as the "mean of the slope" method. The results suggest that neither the traditional "slope-of-the mean" nor the "mean-of-the-slope" approach indicates a persistent linear trend in the Swiss stone pine $\delta^{18}O$ data, while for the $\delta^{13}C$ data a systematic enrichment in ^{13}C was observed over a $<$ 40 year-long period after germination. Despite the limited sample size of this developing dataset these findings help to inform more detailed analyses and future sampling strategies in the Eastern Carpathian Swiss stone pine stands.

1. Introduction

The annual woody increments formed by trees provide a temporally ordered archive of past variations of growing conditions (Schweingruber, 1996). Tree-ring derived stable isotope time series are becoming increasingly important tools for the investigation of past environmental changes (Badea et al., 2021; Gagen et al., 2011a; Siegwolf et al., 2022). In contrast to the growth-based proxies (e.g., ringwidth and relative X-ray density) that perform best when sampled close to their climatic ecotones, stable isotopes in tree ring cellulose offer potential for reconstructing environmental or physiological variability for non-marginal environments across their distribution (Cernusak and English, 2015; Hartl-Meier et al., 2015; Loader et al., 2020).

The stable oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopes in tree ring cellulose have been used in different parts of the world, from Boreal (Gagen et al., 2011b, 2008; Naulier et al., 2015) to tropical regions (Cullen and Grierson, 2007; van der Sleen et al., 2017; Vargas et al., 2022) as well as on broadleaf (Danis et al., 2006; Nagavciuc et al., 2019;

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Young et al., 2015) and needle species (Esper et al., 2010; Nagavciuc et al., 2020; Sidorova et al., 2008). Tree ring isotopes have been used to reconstruct different climatic variables such as precipitation amount (Danis et al., 2006; Loader et al., 2020; Rinne et al., 2013), sunshine duration/cloudiness (Gagen et al., 2011b; Hafner et al., 2014; Loader et al., 2013; Young et al., 2019), air humidity (Haupt et al., 2011), air temperature (Naulier et al., 2015; Sidorova et al., 2008), or drought (Kress et al., 2010; Nagavciuc et al., 2022). Despite the differences from one study to another, all need to consider the non-climatic factors that can affect the oxygen and carbon isotope values in tree ring cellulose, if a reliable and robust climate reconstruction is to be attained.

Age and/or size-related trends are the most common non-climatic factors that can affect or attenuate the potential climate-related signal (Klesse et al., 2018). In the case of annual ring widths, the age-related trend-bias is well-known and specific methods have been developed in order to remove them (Cook et al., 1990). In the case of the stable isotopes however, whilst there is growing convergence in the field, the presence/significance of non-climatic/age-related trend-bias remains a topic of investigation. At present there is no single unified approach to detect and correct for any such bias (Arosio et al., 2020; Esper et al., 2015, 2010; Gagen et al., 2007; Helama et al., 2015; Helama and Matskovsky, 2020; McCarroll et al., 2020a; b; Torbenson et al., 2022).

Therefore, the age and/or size-related trends in tree ring isotope series remains an important element in the study of past climatic variations (Klesse et al., 2018). The results reported until now show a range of different outcomes for different sites and species. This variability confirms that a single age/size/height-related trend in the δ^{13}C and δ^{18}O values of tree-ring cellulose is unlikely and instead may be dependent on the species and the individual tree location (Daux et al., 2011; Gagen et al., 2008; Leavitt, 2010; Xu et al., 2017). For example, several recent studies show that there is very little to no trend in the δ^{13}C and δ^{18}O values in the native oak species, such as *Quercus robur* L. and *Q. petraea* Liebl., in NW and Central Europe (Büntgen et al., 2020; Duffy et al., 2019; McCarroll et al., 2020a); while for pine species the findings are somewhat contradictory. The δ^{13}C and δ^{18}O data of *Pinus uncinata* from the Spanish Pyrenees showed a systematic decline in the first 100–400 years of growth (Esper et al., 2010). No significant trend was reported for δ^{13}C and δ^{18}O data in Scots pines (*Pinus sylvestris* L.) from NW Norway (Young et al., 2011) and northern Fennoscandia (Loader et al., 2013) beyond a short juvenile period, but a significant age-related trend of 0.04 ‰ per 100 years (δ^{13}C) was reported for *Pinus sylvestris* from northern Finnish Lapland (Helama et al., 2015). Living and relict Scots pines from northern Fennoscandia suggested a steeper trend (~0.035 ‰ per decade) for δ^{13}C and no significant trend in δ^{18}O data (Torbenson et al., 2022), however the dataset inhomogeneity (i.e., whole-wood and cellulose) might bias these conclusions. The δ^{13}C values in *Pinus cembra L.,* and δ^{18}O values in *Larix decidua* Mill. and *Pinus cembra* L. from the European Alps have shown a statistically significant trends in the first 100 years of growth (Arosio et al., 2020). By contrast, a small-scale study conducted on *L. decidua* from the United Kingdom did not identify any strong or persistent trends in this species (Kilroy et al., 2016). Therefore, the age-related trend is still a controversial issue, with results varying with isotope, location, and tree species. The potential regional differences for the same species have also not been extensively studied and may require future considerations. Scrutiny of the age-related trends of the stable isotope series of the main chronology building species are necessary from different geographical origins in order for a better evaluation of the age-related trends of isotope ratios in the tree-ring cellulose, especially since a new approach was developed to examine those trends (Duffy et al., 2019).

Swiss stone pine (*Pinus cembra* L.) has prominent importance in European tree-ring studies, being the only species with continuous availability over the multi-millennial dataset of the Eastern Alpine Conifer Chronology (Nicolussi et al., 2009). Stone pine presents the greatest dendroclimatological potential also in the Eastern Carpathians (Popa and Bouriaud, 2013; Popa and Kern, 2009) and Southern Carpathians (Popa and Nechita, 2011; Știrbu et al., 2022) owing to the i) significant longevity of the species (Schweingruber and Wirth, 2009); ii) strictly constrained ecological preference (i.e. timberline habitat) and the related pronounced temperature regulated growth; and iii) abundance of well-preserved snags and relict material. The exploitation of the palaeoclimatological information of the stable isotope signals stored in the Swiss stone pine tree ring datasets has been initiated both in the Alps (Arosio et al., 2020; Haupt et al., 2014) and in the Carpathians (Nagavciuc et al., 2020, 2022), so a detailed assessment of the ontogenetic trends of the tree ring isotope series is necessary (Arosio et al., 2020). Whilst we recognise that these trends are not a direct effect of tree age per se, but instead likely reflect progressive development of roots, stand dynamics and canopy development (Klesse et al., 2018; McCarroll and Loader, 2004), it is nevertheless appropriate to consider them as age-related owing to their often reported progressive or trending nature as the tree matures.

Here, we present measurements on a new multi-centennial data set of annually resolved stable oxygen and carbon isotope compositions from 5 living and 10 relict Swiss stone pine samples collected at a timberline habitat in the Eastern Carpathians (Romania) to evaluate any potential systematic ontogenetic pattern on their δ^{18}O and δ^{13}C data. The hypothesis was that both isotopic compositions display a decreasing trend with increasing cambial age. Age-related trends were evaluated using the conventional "slope of the mean" approach as well as the more recently developed "mean of the slopes" method (McCarroll et al., 2020b).

2. Materials and methods

2.1. Sample description

Increment cores were collected from living Swiss stone pines (Fig. 1A, B) in autumn 2012 ($n = 3$) and 2015 ($n = 2$) from below the timberline of the Călimani Mts (Eastern Carpathians, Romania) (Fig. 1). The forest stand is dominated by Swiss stone pine (*Pinus cembra* L.) mixed with Norway spruce (*Picea abies* Karst., L.) which are replaced by mountain pine (*Pinus mugo*) towards higher elevations. Relict samples ($n = 10$) were selected from the archive of deadwood disks (Fig. 1 C,D) collected from an elevation range from 1450 m a.s.l. to 1850 m a.s.l between 2006 and 2008 (Popa and Kern, 2009). Relict samples were all crossdated during the construction of the Călimani site chronology (Popa and Kern, 2009) and wiggle-matched ^{14}C measurements on single and double tree-rings also assured the dendrochronological cross-matching of the relict samples (Sava et al., 2019).

Increment cores that did not reach the pith of the living trees or disks that lost their innermost rings through erosion or decay had the pith offset estimated by the concentric circles method (Villalba and Veblen, 1997) in this cases assuming constant initial radial growth. Cambial age was then determined considering any unanalysed rings and the pith offset estimates (Table 1). The cambial ages ranged from 125 to 292 years in the living trees, and from 90 to 380 years in the relict samples (Table 1). The subsets of living and relict samples cover the periods of 1739–2015 CE and 1251–1894 CE, respectively.

2.2. Analytical methods

The dated tree ring series were split with a scalpel to obtain annual resolution before cellulose preparation and isotopic measurement. Each ring was measured individually (i.e., not-pooled) since age-related trends are by definition intrinsic to individual tree-ring series and pooling of rings may prevent the detection of the trends.

Alpha-cellulose, extracted using the modified Jayme-Wise method (Loader et al., 1997), was homogenized using a standard ultrasonic protocol (Laumer et al., 2009) and a VCX130 (Sonics & Materials Inc/USA) device. Samples were dried at 70 °C for 24 h before analysis. For the simultaneous measurements of carbon and oxygen isotope ratios

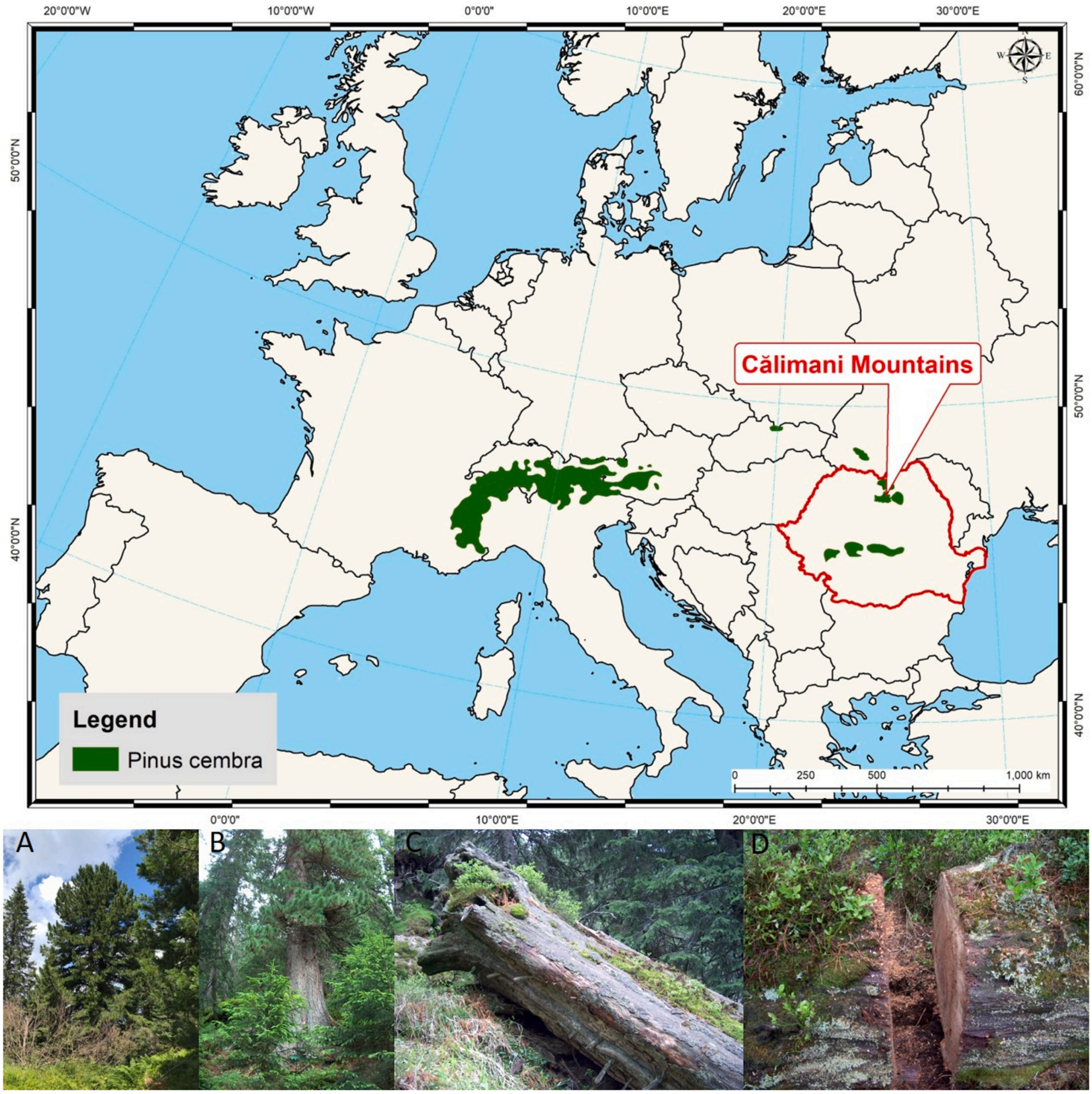

Fig. 1. Location of the study area with green shading the distribution of *Pinus cembra* in Europe (Ulber et al., 2004). Photos show old solitary Swiss stone pines from the treeline-timberline ecotone (A, B) and relict samples (C, D).

(Loader et al., 2015), 0.2 mg (± 10%) of α-cellulose was packed in silver foil and pyrolyzed over glassy carbon at 1450 °C (Leuenberger and Filot, 2007) using a ThermoQuest TCEA interfaced with a Thermo Delta V Advantage isotope ratio mass spectrometer. Carbon and oxygen isotope values are reported in per mille (‰) relative to the Vienna PeeDee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW), respectively, using the traditional δ (delta) notation (Coplen, 1994). IAEA-CH-3 and IAEA-CH-6 were used as laboratory standard reference material and commercial Merck cellulose was used as an internal standard. The analytical reproducibility (2σ) based on repeated measurements of the internal standards was better than 0.16‰ and 0.20‰, for $\delta^{13}C$ and $\delta^{18}O$, respectively (Kern et al., 2023). All samples were measured in triplicates, but if the standard deviation exceeded 0.2‰ either for $\delta^{18}O$ or $\delta^{13}C$ two additional measurements were performed to improve replication for outlier detection and reduce uncertainty. For further details, including the isotopic range of calibration standards and outlier detection, see (Kern et al., 2023).

2.3. Statistical methods

2.3.1. Carbon isotope correction

Raw measured $\delta^{13}C$ values have been corrected for changes in the atmospheric CO_2 regarding both its stable isotope signature (Leuenberger, 2007) and mixing ratio (Schubert and Jahren, 2012). Anthropogenic activities (e.g., coal and hydrocarbon combustion) have led to an increase of atmospheric CO_2 concentration and an associated change in the carbon isotopic ratio of the atmosphere. This directly influences the carbon isotopic composition of tree-ring cellulose and needs to be corrected for prior to evaluation of the age-trends or the to reconstruction of past climate (McCarroll and Loader, 2004). In order to remove the long-term depletion in ^{13}C of the atmospheric CO_2 (Keeling, 1979), the $\delta^{13}C$ time-series were corrected by applying the compilation of the $\delta^{13}C$ values of CO_2 derived from air inclusions in ice cores (Leuenberger, 2007). This version is marked as $\delta^{13}C_{\text{atm-only}}$. Secondly, in order to account for past changes in carbon isotopic fractionation of C3 vascular land plants to changing atmospheric CO_2 levels, we applied the

Table 1
Time span of the analysed samples and their mean isotopic composition. The sections of the living trees 1–4 covering the period from 1876 to 2012 CE were analysed in Nagavciuc et al. (2020). Estimated growth periods take into account pith offset and the presence of rings counted but not measured isotopically due to poor preservation. StDev abbreviates standard deviation.

Sample code	Sample type	Estimated growth period	Period analysed	$\delta^{18}O_{VSMOW}$ [‰] (± 1 StDev)	$\delta^{13}C_{VPDB}$ [‰] (± 1 StDev)
Tree 1	living	1871 – 2012	1876 – 2012	29.3 (± 0.8)	-23.9 (± 0.8)
Tree 2		1888 – 2012	1893 – 2012	29.4 (± 0.9)	-24.1 (± 0.6)
Tree 3		1878 – 2012	1883 – 2012	29.4 (± 0.9)	-23.4 (± 0.7)
Tree 4		1724 – 2015	1739 – 2015	29.0 (± 1.0)	-23.2 (± 1.0)
Tree 5		1770 – 2015	1843 – 2015	29.3 (± 1.0)	-22.5 (± 0.9)
Tree 6	relict	1766 – 1894	1779 – 1894	28.9 (± 0.9)	-22.4 (± 0.6)
Tree 7		1621 – 1869	1645 – 1869	28.5 (± 0.7)	-21.6 (± 0.7)
Tree 8		1485 – 1864	1495 – 1864	28.2 (± 0.8)	-21.9 (± 0.6)
Tree 9		1620 – 1759	1622 – 1759	28.4 (± 0.8)	-22.5 (± 1.1)
Tree 10		1513 – 1790	1513 – 1757	28.6 (± 0.8)	-22.2 (± 0.8)
Tree 11		1373 – 1534	1373 – 1534	29.1 (± 0.8)	-22.2 (± 0.4)
Tree 12		1270 – 1695	1331 – 1522	28.9 (± 0.9)	-21.6 (± 0.5)
Tree 13		1410 – 1499	1410 – 1499	29.1 (± 0.8)	-22.3 (± 0.6)
Tree 14		1204 – 1789	1251 – 1436	28.5 (± 0.8)	-21.0 (± 0.5)
Tree 15		1441 – 1775	1459 – 1670	29.0 (± 0.8)	-21.2 (± 0.7)

correction based on the averaged hyperbolic relationships quantified for above-ground tissues of C3 species (Schubert and Jahren, 2012). Historical annual northern hemispheric mean atmospheric CO_2 concentrations were retrieved from the compilation of the Institute for Atmospheric and Climate Science at ETH Zürich, Switzerland (Accessed on 03.08.2020 at https://www.co2.earth/historical-co2-datasets). This version is marked as $\delta^{13}C_{full\text{-}corr}$.

2.3.2. *Age trend analysis*

The age-trend was tested by considering both the conventional *slope-of-the-mean* (Helama et al., 2015) and the more recent *mean-of-the-slope* (Duffy et al., 2019) approaches. The mean of the longest common ontogenetic period (from the 35th to the 115th cambial year) was calculated for each time series and was subtracted from all data of the corresponding time series before the mean of the joint dataset was computed. The trend analysis was limited for both approaches to a 161-yr threshold of the cambial age providing the longest period with modest fluctuation in replication (Fig. 2) to minimize the risk of trend bias due to level-offsets when shorter records drop out (McCarroll et al., 2020a). The trend analysis was performed on both $\delta^{13}C_{atm\text{-}only}$ and $\delta^{13}C_{full\text{-}corr}$ versions of the carbon-isotope chronologies.

Inspired by recent observations on the same species from an Alpine dataset (Arosio et al., 2020) linear trends were evaluated in moving windows to explore the potential age-dependent patterns of the isotope mean series at the multidecadal scale. To avoid a window-specific bias, this evaluation was repeated in 11, 15, 19, and 23-yr windows. The slope and significance of the linear trends were assessed together. The calculations were done in R (R Core Team, 2019) using the lipdR (McKay and Heiser, 2015) package and the regressEns() and other functions of the geoChronR package (McKay et al., 2021) and the authors own scripts.

3. Results

The stable isotopic composition of the analysed Swiss stone pine trees from Călimani Mts displayed a range of 5.86 ‰ (from 26.47 ‰ to 32.34 ‰) for $\delta^{18}O$ and 6.48 ‰ (from −26.97 ‰ to −20.49 ‰) for $\delta^{13}C$ for the living subset, while the same measures were 6.22% (from 26.18 ‰ to 32.40 ‰) for $\delta^{18}O$ and 5.55 ‰ (from −25.40 ‰ to −19.85 ‰) for $\delta^{13}C$ for the relict subset (Fig. 2 A,B). The mean of individual series ranged from 28.99 ‰ to 29.44 ‰ for $\delta^{18}O$ and from − 23.89 to − 22.48 ‰ for $\delta^{13}C$ in the living subset and from 28.16 ‰ to 29.12 ‰ for $\delta^{18}O$ and from − 22.55 to − 20.97 ‰ for $\delta^{13}C$ in the relict subset (Table 1). Neither of the isotopic parameters investigated showed any age-related variance bias, suggesting a homoscedastic character (Büntgen et al., 2020).

3.1. *The slope-of-the-mean and mean-of-the-slope*

The conventional average trend approach provided an insignificant increasing slope (0.0793 ‰ per 100 yr, $p = 0.113$) for the mean of the $\delta^{18}O$ data of the joint dataset terminated at 161-yr cambial age. Of the 15 individual $\delta^{18}O$ records, eight showed an increase with increasing ring number of which one was statistically significant ($p < 0.05$). Seven series showed a declining trend but none of these were statistically significant (Fig. 3A).

Regarding the corrected carbon isotope records, in the first 161-yr cambial age significant increasing slopes were found both for the mean $\delta^{13}C_{atm\text{-}only}$ (0.274 ‰ per 100 yr, $p < 0.001$) and the mean $\delta^{13}C_{full\text{-}corr}$ (0.204 ‰ per 100 yr, $p < 0.001$) series. Of the $\delta^{13}C_{atm\text{-}only}$ records 11 showed an increase with increasing ring number of which 10 were statistically significant ($p < 0.01$), while four records showed a decline of which three were statistically significant ($p < 0.01$) (Fig. 3B). The trend analysis for considering similarly the first 161-yr cambial age of the $\delta^{13}C_{full\text{-}corr}$ version showed 11 increasing trends of which nine were significant ($n = 7$ at $p < 0.01$ $n = 2$ at $p < 0.05$), while four records showed a declining trend of which three were significant ($p < 0.01$) (Fig. 3C).

3.2. *Linear trend in moving windows and juvenile effect*

The visual inspection of the mean isotope series does not reveal any obvious multidecadal pattern for oxygen (Fig. 4A), while for carbon depleted compositions can be clearly seen for the cellulose before the ~40th cambial year (Fig. 4B). The pattern seen for the first decade of growth must be treated with caution since the replication is lower ($n < 9$) compared to the rest of the analysed period. So, despite the larger amplitude of the decrease seen in the average $\Delta^{13}C_{full\text{-}corr}$ data peaking at the 8th increment it is safer to state that the juvenile-to-mature enrichment observed in the earliest years of this Stone pine dataset corresponds to > 0.8 ‰ in $\delta^{13}C$ data.

Decadal-scale trend analysis revealed finer structures and helped to quantify the trends. The longest ontogenetic period with significant trend was found for both isotopes up to the ~40th cambial year (Fig. 4C to F). The slope changed from positive to negative within this period for $\delta^{18}O$ data (Fig. 4C) but continuously remained positive (Fig. 4D) although losing significance over a subperiod for the $\delta^{13}C$ data (Fig. 4F).

4. Discussion

Considering the cost of development of large tree ring isotope datasets for palaeoclimatological and palaeoecological analysis and the scientific and socio-economic consequences of incorrect reconstructions of past climatic change, testing for the presence of non-climatic (age-related) trends represents an important step in the development and understanding of this proxy. It requires isotope data from many trees,

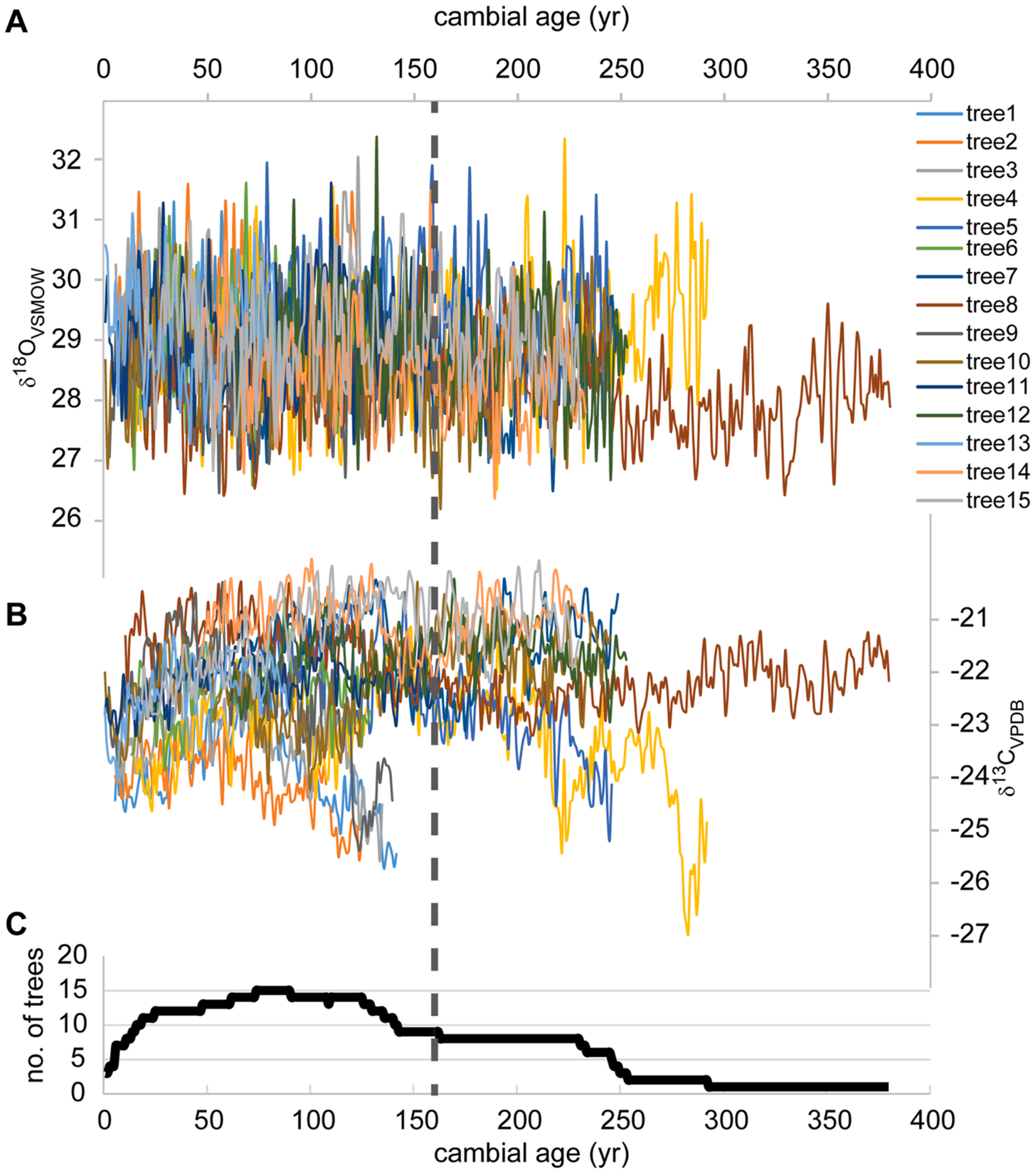

Fig. 2. Age-related behaviour of (A) raw δ^{18}O and (B) δ^{13}C series from 15 individuals (living trees: trees 1–5; relict wood: from 6 to 15) aligned by cambial age. (C) The number of age-aligned samples for each year. The vertical dashed line marks the termination of the period over which the age-related trends were calculated.

preferably covering non-overlapping and partially overlapping calendar periods, so avoid that trends in climate are not confused with parallel trends due to increasing age (Duffy et al., 2019). Suitable data sets are still sparse and new datasets are required to improve our knowledge about the statistical behaviour of the dendroisotopic parameters (Duffy et al., 2019). The presented dataset comprising δ^{18}O and δ^{13}C data from 5 living and 10 relict Stone pines are the first from the Carpathian region which fulfil these requirements with scope to develop this dataset in the future. Note here that the actual age of the individuals at the level that samples were collected is not known (i.e. pith date is very likely younger than germination date), it is reasonable to assume that the actual age of the individual is a few years more than the observed cambial age (Niklasson, 2002).

In the 81-yr-long common ontogenetic period (from the 35th to the 115th ontogenetic year) a very narrow range of mean values between trees (< 1 ‰) of offset was observed both for δ^{18}O (from 28.32 ‰ to 29.31 ‰) and for δ^{13}C (from −23.07 ‰ to −22.14 ‰) in the joint dataset (Fig. 2), although the level offset for overlapping growth periods often exceeded this (Nagavciuc et al., 2022). It may therefore be appropriate to scale overlapping records prior to combination to minimize the risk of trend bias, which can corrupt the low-frequency signal, as individual time-series enter or exit the average chronology (Hangartner et al., 2012; Nakatsuka et al., 2020).

Neither the traditional slope-of-the mean nor the more novel mean-of-the-slope approach indicates an overall or persistent linear trend in the Swiss stone pine δ^{18}O data. This is in agreement with many current results (Büntgen et al., 2020, 2011). The trend analysis in moving windows revealed a positive slope up to ~20th year and a negative slope up to ~40th year of growth. This pattern resembled the one described recently for a large Alpine stone pine dataset (Arosio et al., 2020), however the duration of the ontogenetic period they reported lasted longer (~100 yrs divided similarly to two equal subperiods), suggesting

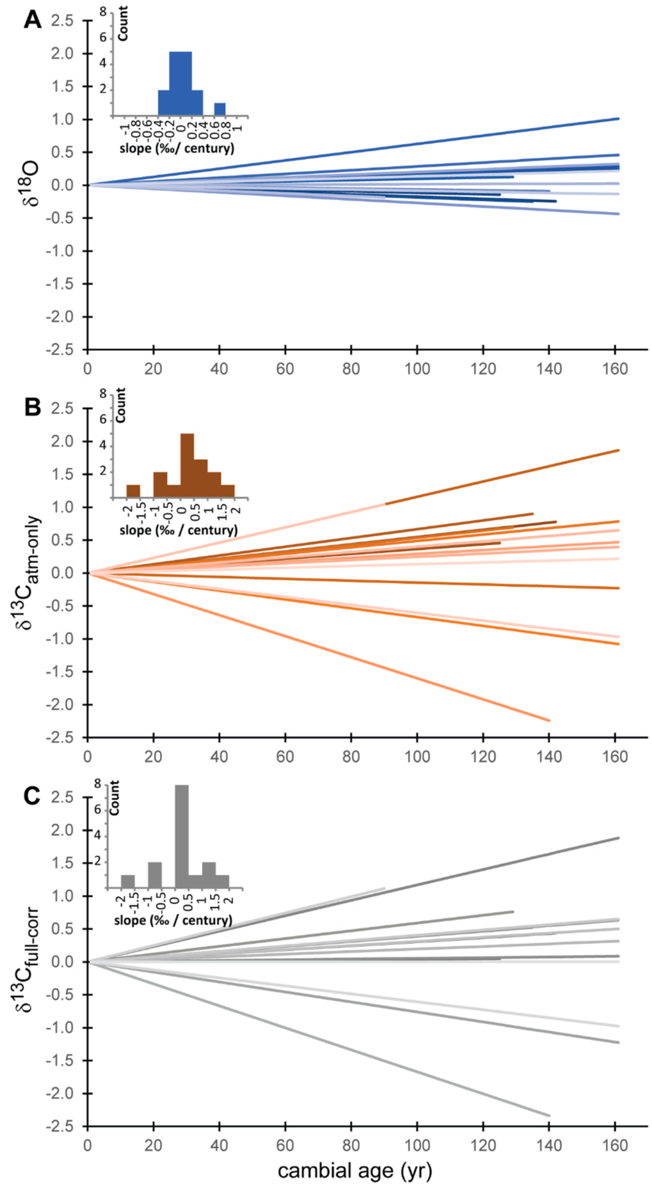

Fig. 3. Linear trends of stable oxygen and carbon isotope ratio of cellulose plotted against cambial age for 15 stone pines sampled from the timberline of the Călimani Mts (Romania). (A) δ^{18}O (B) δ^{13}C$_{\text{atm-only}}$ (C) δ^{13}C$_{\text{full-corr}}$. Inset histograms show the distribution of the centennial trend.

a potential difference among the Alpine and the Eastern Carpathian Swiss stone pine stands sampled. In addition, similar shifts can be observed for other (non-juvenile) periods in the Calimani dataset (Fig. 4). Such shifts may represent stand dynamics or disturbances and may provide insight into the controlling factors that underpin the juvenile trends observed. Increased replication might help to clarify this issue in the future, and if these events are random through a forest stand and over time, they may also be reduced to negligible levels through increased replication.

Alignment of the cellulose-derived δ^{13}C data by cambial age revealed a relatively short period of common behaviour manifested in a ≥ 0.8 ‰ enrichment in δ^{13}C values over a < 40 years-long period after germination and can be clearly seen in the anomaly curve for corrected carbon isotope ratios (Fig. 4. B). The duration and magnitude of this δ^{13}C shift agrees with the isotope "juvenile" effect reported from many species in previous tree ring isotope studies (Leavitt, 2010). The magnitude matches fairly well the pattern reported from the aforementioned Alpine Swiss stone pine dataset (Arosio et al., 2020) however the duration of the juvenile period in both isotopes seems to be significantly less in the Calimani data compared to the Alpine stands.

Moving window analyses suggest that a significant trend in the δ^{13}C record is present only for a < 40 year-long period after germination and so it seems likely that this juvenile section causes the significantly larger portion of the increasing trend of the individual records when extended (Fig. 3 B,C).

When developing isotope timeseries from trees a traditional recommendation is to exclude juvenile growth and to use only those rings for dendroclimatological analyses that formed after any 'juvenile effect' ceased (McCarroll and Loader, 2004, Gagen et al., 2008; Leavitt, 2010). This simple approach is both easy to apply and conservative, but it relies upon the prior characterisation of juvenile/non-climatic trends. Such truncation of the usable length of the given δ^{13}C records would adversely affect the replication of the mean δ^{13}C chronologies, but the incorporation of juvenile (trending) data could be equally damaging to any resulting palaeoclimate reconstruction, especially where levels of replication are low. Juvenile wood may contain useful ecological or micro-climatic information that may be worth conserving (McCarroll and Loader, 2004), so it deserves further investigation. When developing climate reconstructions from tree ring stable isotopes it is always preferable to have high-levels of sample replication as this reduces random error and provides a more robust mean. Efforts to test detrending techniques (Gagen et al., 2008; Esper et al., 2010) that compare the signals from the juvenile with older sections of the Swiss stone pine records, covering the same time, may help to better understand the nature of these early growth trends.

The difference in isotopic age-trends observed between *P. cembra* for an Alpine assemblage (Arosio et al., 2020) and an Eastern Carpathian stand (this study), or for populations of *P. sylvestris* in NW Scandinavia (Loader et al., 2013; Young et al., 2011) and northern Finnish Lapland (Helama et al., 2015), emphasises the need for a detailed understanding of isotopic variability when developing isotopic timeseries that may span or extend beyond the lifetime of an individual tree. This study contributes to the growing body of evidence and case studies, that could help to establish generalized protocols for working with juvenile trees.

5. Conclusions

Understanding the non-climatic (age and/or size-related) trends in tree ring stable isotope times series is an important step in the development of long palaeoclimatic reconstructions as an ability to quantify the magnitude and duration of non-climatic signals helps to inform sampling strategy. Although this study was conducted in a relatively small dataset it comprised 2711 tree rings δ^{18}O and δ^{13}C data from 5 living and 10 relict Swiss stone pines from the Eastern Carpathians to provide an insight into non-climatic trends for trees growing across this region. The analysed samples are representative of the study area and allow the investigation of age-related trends in tree ring isotope data. Age-related trends in δ^{18}O and δ^{13}C data were evaluated considering the most used approaches and for the averaged and the individual isotopic data.

No consistent age-related trend was found among the individual δ^{18}O records from the Calimani Mts, Romania, consequently we conclude that δ^{18}O data from the compiled Swiss stone pine cellulose dataset can be used without detrending to build isotope chronologies without a serious risk of significant bias in the low-frequency variability in the parameter. Trend analysis based on moving windows revealed an δ^{18}O pattern similar in form to one from the Alpine Swiss stone pine dataset (Arosio et al., 2020), but much shorter in duration, suggesting a potential difference between the Alpine and the Eastern Carpathian Swiss stone pine stands. This supports the investigation of the age-trends as a routine element in isotope dendroclimatology as part of the development of a long isotopic record.

The results for the δ^{13}C data reveal a clear trend over a relatively

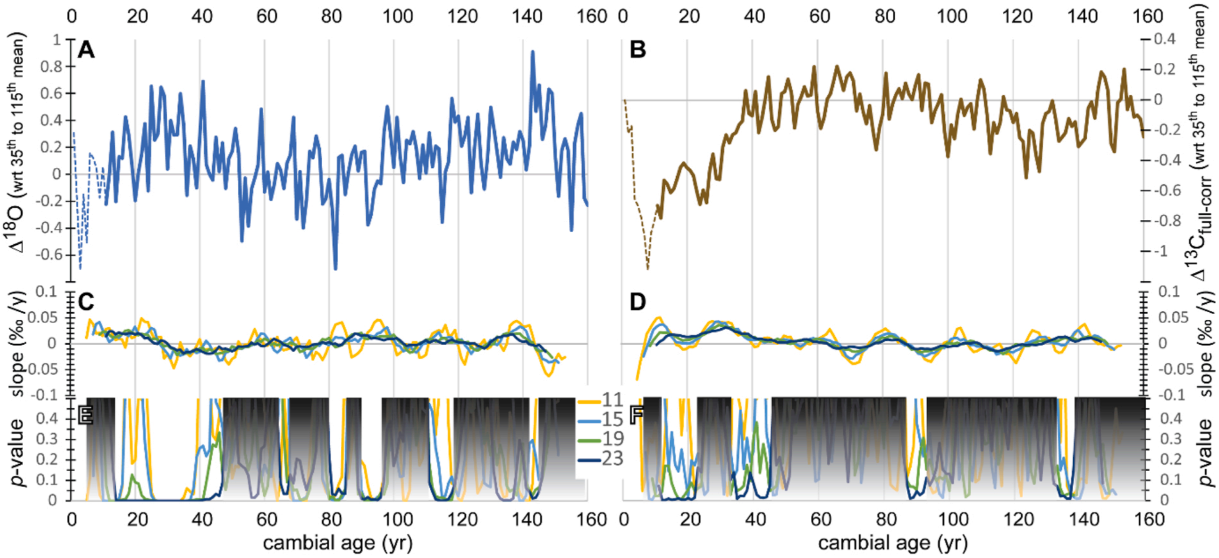

Fig. 4. Average fluctuation of stable oxygen and carbon isotope ratios of stone pines obtained after the individual series were centred on the mean of the longest common ontogenetic period (35th to 115th cambial years). The anomaly curve for oxygen ($\Delta^{18}O$) and corrected carbon isotope ratios ($\Delta^{13}C_{full\text{-}corr}$) are shown in panels A and B, respectively. The mean curve is plotted as a dotted line up to the 11th cambial year, to indicate the presumably higher uncertainty due to the relatively lower replication ($n < 9$) compared to the rest of the analysed period. The slope (C, D) and significance (E, F) of the linear trend calculated in moving windows are shown with uniform colour coding 11, 15, 19, and 23-yr windows are yellow, light blue, green, and dark blue, respectively. Intervals where the estimated significance of the linear trend calculated in 23-yr window is $p > 0.05$ are shaded. The selection of the 23-yr window is arbitrary but does not affect the conclusions.

short period (~40 years). Alignment of the $\delta^{13}C$ data by cambial age indicates a systematic enrichment in ^{13}C over a < 40 years-long period after germination. The magnitude of the effect is > 0.8 ‰. A persistent trend was not detected for carbon discrimination afterward.

At present, the simplest, approach to dealing with this non-climatic trend would be to exclude $\delta^{13}C$ data for the first < 40 years from any composite record however it risks insufficient replication for certain periods of the mean $\delta^{13}C$ chronology. To avoid this adverse situation detrending techniques must be tested comparing the signals from the juvenile with older sections of the Swiss stone pine records to find a capable method to retain the common signal also for the $\delta^{13}C$ data of the first ~40 years growth. Despite the very coherent level found for the studied stone pine individuals scaling of the overlapping records and/or thoughtful compositing with enhanced replication is highly recommended to minimize the risk of trend bias as individual time-series enter or exit the average chronology (Hangartner et al., 2012; Nakatsuka et al., 2020).

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.2023.126061.

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