Received: 11 March 2019 | Revised: 22 September 2019 | Accepted: 4 October 2019

DOI: 10.1002/joc.6349

RESEARCH ARTICLE

International Journal of Climatology RMetS

Climate signals in carbon and oxygen isotope ratios of *Pinus cembra* tree-ring cellulose from the Călimani Mountains, Romania

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Funding information
EEA Grants, Grant/Award Number: CLIMFOR18SEE; Helmholtz Climate Initiative, Grant/Award Number: REKLIM; Unitatea Executiva pentru Finantarea Invatamantului Superior, a Cercetarii, Dezvoltarii si Inovarii, Grant/Award Number: PN-III-P4-ID-PCE-2016-0253

Abstract

We analyse annually resolved tree-ring stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopic chronologies from Swiss stone pine (*Pinus cembra* L.) in Romania. The chronologies cover the period between 1876 and 2012 and integrate data from four individual trees from the Calimani Mts in the eastern Carpathians where climatic records are scarce and starts only from 1961. Calibration trials show that the $\delta^{13}C$ values correlate with local April–May relative humidity and with regional to larger scale (European) summer precipitation. $\delta^{18}O$ correlates significantly with local relative humidity, cloud cover, maximum temperature, as well as European scale drought conditions. In all cases, the climate effects on $\delta^{13}C$ values are weaker than those recorded in the $\delta^{18}O$ data, with the latter revealing a tendency toward higher (lower) values of $\delta^{18}O$ during extremely dry (wet) years. The most striking signal, however, is the strong link between the interannual $\delta^{18}O$ variability recorded in the Calimani Mts and large-scale circulation patterns associated with North Atlantic and Mediteraneean Sea sea surface temperatures. High (low) values of $\delta^{18}O$ occur in association with a high (low) pressure system over the central and eastern part of Europe and with a significantly warmer (colder) Mediterranean Sea surface temperature. These results demonstrate the possibility of using tree ring oxygen isotopes from the eastern Carpathians to reconstruct regional drought conditions in eastern Europe on long-term time scales and larger scale circulation dynamics over the preinstrumental periods.

KEYWORDS

atmospheric circulation, climate response, dendrochronology, Swiss stone pine, $\delta^{13}C$, $\delta^{18}O$

1 | INTRODUCTION

In terms of recent climate change, when heat waves and summer droughts become more frequent and more intense, the environment and property risks have increased and became more dangerous (IPCC, 2014; Spinoni *et al*., 2015). As such, the necessity for high precision climate predictions for better adaptation and mitigation has arisen. However, the complex characteristics of present and expected future climate changes can be better understood in the context of past climate variability (IPCC, 2014), due to fact that the trends based on short records are very sensitive to the beginning and end dates and do not, in general, reflect long-term climate trends (IPCC, 2014). In this respect, natural archives have become an important tool to supplement the short available instrumental records. Tree rings are widely used in paleoclimatology because of their annual resolution, precise dating, widespread availability on different parts of the globe, and they have the possibility to create chronologies of thousands of years and to explore climate through different proxies such as tree-ring width, maximum density, and/or stable isotopes (Gagen *et al*., 2004; Brugnoli *et al*., 2010; Hughes, Swetnam, and Diaz, 2011). Overall, the international tree-ring data bank (ITRDB) contain more than 4,000 records, however, most of them are based on the tree-ring width, and only few chronologies are based on the maximum density and even less are based on the variations of stable carbon or oxygen isotopes in tree-ring cellulose (https://www.ncdc.noaa.gov/data-access/paleoclimatology-data/datasets/tree-ring).

The carbon and oxygen stable isotope ratios in tree rings incorporate unique information since they record, through isotopic discrimination, plant-specific physiological processes that include climatic effects. The climatic signals registered by stable isotopes are less dependent on the ecoclimatic settings of the sampled trees when compared with tree-ring width and density proxies (Esper *et al*., 2018). Stable carbon isotope ratios depend on leaf internal concentrations of CO_2, which are influenced by the balance between stomatal conductance and the rate of carboxylation during photosynthesis (Farquhar *et al*., 1989). In dry environments, the $^{13}C/^{12}C$ ratio tends to be dominated by stomatal conductance, which is mainly controlled by differences in vapour pressure of the ambient air and the intercellular air spaces within the leaves (Young *et al*., 2015). In regions without strong moisture stress, the dominant signal recorded by the $\delta^{13}C$ values is the fluctuation of photosynthetic rate, which is mainly influenced by solar radiation and the production rate of the photosynthetic enzyme RuBisCo (Hafner *et al*., 2014). Tree-ring $\delta^{18}O$ is primarily affected by the isotopic ratio of source water and, secondly, by evaporation of leaf water via the stomata, which leads to increasing $\delta^{18}O$ values (Gessler *et al*., 2014). The isotopic composition of the source water usually mirrors the isotopic composition of precipitation infiltrated into the soil and taken up by the roots (Roden *et al*., 2000) and depends on the atmospheric circulation patterns and local climate. The evaporation intensity of leaf water depends on the stomatal conductance and the vapour pressure deficit, both of which are directly related to relative humidity (McCarroll and Loader, 2004). Stable isotopes in tree-ring cellulose have proven to be a good proxy in areas where tree-ring width (TRW) and maximum latewood density (MXD) are not strongly controlled by a single climate parameter (Hartl-Meier *et al*., 2015; Young *et al*., 2015; Nagavciuc *et al*., 2019). Oxygen isotopic ratios, in general, lack substantial tree-age effects, rendering statistical de-trending unnecessary, thereby preserving the low frequency inherent to the raw data (Rinne *et al*., 2013; Duffy *et al*., 2017). In addition, climate signals can be reliably detected in carbon and oxygen isotope chronologies comprising lower numbers of replicates (Gagen *et al*., 2008; Leavitt, 2010) and slight brown-rot decay wood has only a limited influence on the isotopic composition (Nagavciuc *et al*., 2018). Stable isotopes in tree rings can thus provide representative, accurate, and precise information on past climate variability, where other tree rings proxies fail (Kress *et al*., 2010; Konter *et al*., 2014; Cernusak and English, 2015; Hartl-Meier *et al*., 2015).

Tree-ring carbon and oxygen isotopes records have already been used to reconstruct various climate parameters including temperature (Treydte *et al*., 2009; Esper *et al*., 2015), precipitation (Danis *et al*., 2006; Rinne *et al*., 2013; Young *et al*., 2015), drought (Kress *et al*., 2010; Xu *et al*., 2014; Labuhn *et al*., 2016), relative humidity (Haupt *et al*., 2011), solar radiation (Young *et al*., 2010), and cloud cover (Gagen *et al*., 2011), in different parts of the world. There is still a strong contrast between the eastern part of Europe and other regions of the continent because most of the paleoclimatic reconstructions based on tree ring isotopes are distributed from Fennoscandia through western Europe to the Mediterranean region (Treydte *et al*., 2007; Konter *et al*., 2014; Young *et al*., 2015; Labuhn *et al*., 2016). Thus, by filling the eastern-European gap, we would provide a better understanding of past climate variability at the continental scale.

From a climatological point of view, Romania is located in a strategic position, in the eastern part of Europe where the climatic patterns that strongly influence the Atlantic, Mediterranean, and Scandinavian regions have convergent influences. Old-growth forests preserved in the Carpathian mountains (Popa, 2016) would allow the construction of very long stable carbon and oxygen isotope chronologies. The high altitude natural forests of the Călimani Mountains, in north-eastern Romania, retain an impressive collection of very old living trees and important deposits of relict wood in excellent states of preservation, covering at least past Millennium (Popa and Kern, 2009).

The aim of this study is to explore the climate signal registered by interannual variability of stable carbon and oxygen isotope ratios in the cellulose of Swiss stone pine tree rings (*Pinus cembra L.*) from Călimani Mountains, Romania. We statistically analyse the relationship between $\delta^{13}C$ and $\delta^{18}O$ and monthly local climate variables over AD1961-2012, gridded climatic data over a longer period (AD1901-2012), and also compare with large-scale circulation patterns. We discuss the skill of the potential climate reconstructions using established statistical calibration and verification tests in order to highlight, which climate parameter is most reliably registered by each isotope.

2 | METHODS AND MATERIALS

2.1 | Study site

The study area is located in the Călimani Mountains, in the eastern Carpathian Arc (Romania) (Figure 1). A detailed site description can be found in the Popa and Kern *et al*. (2009), as both studies are done at the same site. The forest 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*) toward higher elevations. The study site is characterized by a mountain temperate-continental climate, with severe cold winters and cool summers. The mean temperature ranges from −6.5°C in January to 13.3°C in July, while the mean annual precipitation amount is 889 mm, with a summer peak in June to July, for 1961–2012 period (Figure 1). The geological substrate is composed of the "andesitic arch," represented by the volcanic chain resting on a foundation of shale and Triassic sedimentary formations (Mutihac, 2004). The samples were collected around the timberline situated at ~1,700 m a.s.l., while the current treeline of Swiss stone pine is at ~1850 m a.s.l. in the study area (Kern and Popa, 2008). Human influence in the study area was limited after 1975 due to the establishment of a Natural Reserve with a high degree of protection. However, a sulfur extraction occurred near to the study site between 1965 and 1992, with most intense activity during the 1974–1986 period (Brându and Cristea, 2004) leaving clear signals also in the sulfur concentrations of the wood (Kern *et al*., 2009).

2.2 | Sample collection, preparation, and stable isotope measurements

Four living trees (labelled Trees 1 to 4) were cored in autumn 2012, using an 11 mm Pressler increment borer. Tree ring

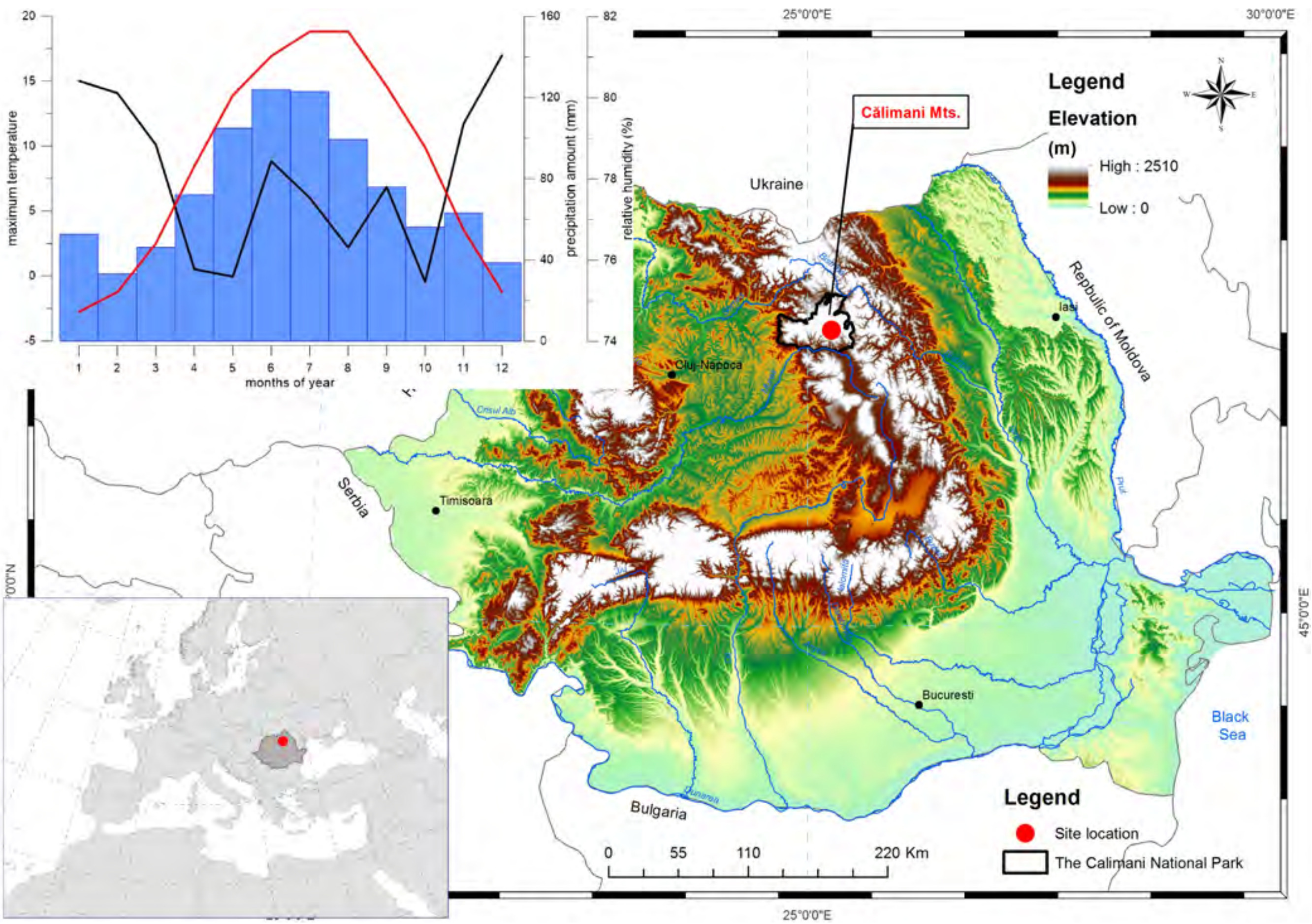

FIGURE 1 Location of the investigation area in Europe (inset bottom left) and a topographic map of Romania showing the sampling site. Insert in the top left represents the annual variation of the max temperature (red), precipitation (blue), and relative humidity (black) over the 1961–2013 period form the ROCADA gridded data (Dumitrescu and Birsan, 2015) for the nearest grid point of study site

width (TRW) was measured using LINTAB equipment and TSAP 0.53 software, with 0.001 mm accuracy. TRW was cross-dated against the local master chronology (Popa and Kern, 2009) and checked for missing rings with COFECHA software (Holmes, 1983). The stable isotope analyses were performed for the 1876–2012 period (Table 1). The tree rings were separated with a scalpel ring by ring and were not pooled prior to the measurements. After that, the α-cellulose was extracted using the modified Jayme-Wise method (Loader *et al*., 1997; Boettger *et al*., 2007), homogenized by a standard ultrasonic protocol (Laumer *et al*., 2009) using VCX130 (Sonics & Materials Inc) device and dried at 70°C for 24 hr.

After being encapsulated in silver, 0.2 mg (±10%) of α-cellulose was pyrolized over glassy carbon at 1450°C and simultaneous measurements of oxygen and carbon isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) were performed (Leuenberger and Filot, 2007; Loader and Waterhouse, 2014) using a ThermoQuest TCEA interfaced with a Thermo Delta V Advantage IRMS. The isotopic ratios are reported in per mil (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW) for oxygen, and Vienna Pee Dee Belemnite (VPDB) for carbon (Coplen, 1994), respectively, using the traditional δ (delta) notation. The analytical precision of the measurements was better than 0.2 ‰ for both oxygen and carbon. All samples were measured in triplicates; if their *SD* exceeded 0.2‰, two additional measurements were performed. If one of the five delta vaules was further from the mean of the other four values than their 2 *SD* that value was considered as outlier and was omitted. The final $\delta^{13}C$ and $\delta^{18}O$ values were calculated as the arithmetic mean of the multiple measurements.

2.3 | Correction for nonclimatic factors and construction of the dendroisotope chronologies

The raw carbon isotopic ratios need to be corrected for changes in carbon isotope composition and concentration of atmospheric CO_2 due to anthropogenic coal and hydrocarbon combustion from the start of the industrial revolution because these anthtropogenic effects can heavily overprint the climate signals (McCarroll and Loader, 2004; Treydte *et al*., 2009). To remove the long-term depletion in ^{13}C of the atmospheric CO_2, the so-called Suess effect (Keeling, 1979), we applied the correction scheme established for the northern hemispheric variations in atmospheric CO_2 isotopic composition based on a compilation of the $\delta^{13}C$ values of CO_2 ($\delta^{13}C_{atm}$) derived from air inclusions in ice cores (Leuenberger, 2007). Even after the $\delta^{13}C_{atm}$ correction, the resulting chronology is still influenced by the increasing values of pCO_2 above the pre-industrial level, causing the amount of carbon isotopic fractionation per unit ppm to decrease. This effect was removed following the procedure described by Schubert and Jahren, (Schubert and Jahren, 2012) (Figure S1).

Several studies have shown that the increase of $\delta^{13}C$ and $\delta^{18}O$ values in the juvenile years of tree growth is dependent on the species and the individual tree location (Gagen *et al*., 2008; Leavitt, 2010; Daux *et al*., 2011; Xu *et al*., 2017). The duration of these juvenile trends vary from short periods or non-existent trends (Daux *et al*., 2011; Kilroy *et al*., 2016; Duffy *et al*., 2017) up to 50 years or more than 80 years (Leavitt, 2010). In case of $\delta^{13}C$, for instance, it can be explained by the fact that young trees, growing close to forest floor or below the canopy, reuse the respired air from the old surrounding trees, which is already depleted in ^{13}C (Treydte *et al*., 2009). In order to examine the juvenile trends of $\delta^{13}C$ and $\delta^{18}O$ ratios in tree-ring cellulose, Trees 1, 2, and 3 were aligned by cambial age and the corresponding anomalies were calculated (Figures S2 and S3). Tree 4 was not taken into account because the first 198 years from the pith were not analysed yet, and the remaining data falls outside the juvenile lifespan.

The negative exponential curve (neg) detrending method was applied using the ARSTAN software (Cook and Peters, 1981; Cook, 1985). The final $\delta^{13}C_{res}$ chronology was developed by (a) inverting the $\delta^{13}C$ (corrected for changes in carbon isotope composition and concentration of atmospheric CO_2) by multiplying the individual series with (−1), in order to have positive values and (b) by removing the $\delta^{13}C$ age trend by calculation of residual values from a negative exponential function (Esper *et al*., 2015); and (c) calculation of the robust means of the detrended $\delta^{13}C$ series, (d) inverting the resulting $\delta^{13}C$ chronology by multiplying with (−1) in order to have the original trend of chronology, which was used further for climate correlations.

The robustness of the obtained mean chronology was assessed by Expressed Population Signal (EPS) and the interseries correlation (Rbar). EPS is a measure of how well the available finite sample of tree-ring data represents an infinite population chronology (Wigley *et al*., 1984; Buras, 2017).

TABLE 1 The length of the tree ring width chronology

Tree name	TRW chronology length	Tree age	Analysed period	Analysed years
Tree 1	1871–2012	141	1876–2012	136
Tree 2	1888–2012	124	1893–2012	119
Tree 3	1878–2012	134	1883–2012	129
Tree 4	1,680–2012	332	1876–2012	136

EPS and Rbar values were calculated for detrended $\delta^{13}C$ series and for raw $\delta^{18}O$ series for the 1876–2012 period, with a running window of 50 years with an overlap of 25 years.

2.4 | Climate data and statistical methods

The linear relationship between the tree-ring $\delta^{13}C_{res}$ and $\delta^{18}O$ records and cloud cover (CLD), precipitation amount (PP), relative humidity (RH), mean, maximum, and minimum temperature (Tm, Tx, and Tn) were analysed using ROCADA gridded data for the nearest grid point to the study site (Dumitrescu and Birsan, 2015) with a resolution of 0.1° × 0.1° for the period 1961–2012. Given that the plant physiological processes regulating the isotopic fractionation are sensitive to precipitation and temperature, we also tested the relationship with the drought index as it integrates these two parameters (Bégin *et al*., 2015). For this, we analysed the relationship with a climate index (CI) as well as with the Standardized Precipitation-Evapotranspiration Index (SPEI). The CI index is computed by subtracting the standardized temperature from the standardized precipitation. Thus, negative values of CI indicate dry and/or warm conditions, whereas positive values of CI indicate wet and/or cold conditions. To calculate the SPEI, we used monthly precipitation totals, 2 m surface air temperature means, and potential evapotranspiration. Since the study site is not known to exhibit long-term dry spells, we focus on short-term drought and wetness variability, by calculating SPEI for 3 months of accumulation period (SPEI3 from now on) (Beguería *et al*., 2014).

To have a longer term perspective of the relationship between the tree-ring parameters and climate variables, the PP and CLD over the closest grid points near the study site were obtained from the monthly CRU T.S. 4.01 data set for the 1901–2012 CE period (Harris *et al*., 2014), with a spatial resolution of 0.5° × 0.5°. Also, CI and SPEI3 indices were calculated basis on data from the CRU T.S. 4.01 climate data set.

To investigate the link with the large-scale atmospheric circulation patterns, we used the seasonal means of Geopotential Height at 500 milibar (mb) (Z500), zonal wind (U500), and meridional wind (V500) at 500 mb from the Twentieth Century Reanalysis (V2) data set (Whitaker *et al*., 2004; Compo *et al*., 2006, 2011) on a 2° × 2° grid, over the 1876–2012 CE period. For sea surface temperature (SST), we used the 1° × 1° Hadley Centre Sea Ice and SST data set—HadISST (Rayner *et al*., 2003). These data sets have a global coverage.

Linear correlations between $\delta^{13}C_{res}$ and $\delta^{18}O$ values and monthly or seasonal climate parameters along with their associated 95% bootstrap confidence intervals were calculated using the treeclim package (Zang and Biondi, 2015) in the R environment (R Development Core Team, 2014). To identify connections with the large-scale atmospheric circulation and the North Atlantic Ocean SST, we constructed the composite maps of Z500 and SST standardized anomalies for the summer season by selecting the years when the value of the normalized dendroisotope time series was >1 *SD* (High) and < −1 *SD* (Low), respectively. This threshold was chosen as a compromise between the strength of the climate anomalies associated with $\delta^{13}C_{res}$ ($\delta^{18}O$, respectively) anomalies and the number of maps that satisfy this criterion. Further analysis has shown that the results are not sensitive to the exact threshold value used for the composite analysis (not shown). The significance of the composite maps is based on a standard *t*-test (confidence level 95%).

The calibration and verification model was analysed using the R packages dplR (Bunn, 2008) and treeclim (Zang and Biondi, 2015). Three statistical tests were performed to evaluate the strength of the calibration model: the Reduction of Error (RE), the Coefficient of Efficiency (CE), and the Durbin-Watson Test (DW), in the split window approach (Cook *et al*., 1994). The calibration/verification model with meteorological data was performed by splitting in forward and reverse periods. For the short local climate data, a calibration length of 75% of the chronology was used and for the longer gridded data 50%.

2.5 | Stability maps

To test the stability of the relationship between the dendroisotope records and climate variables, we make use of stability maps, a methodology successfully used in the seasonal forecast of the European rivers and Antarctic sea ice to examine the stationarity of the long-term relationship between our proxies and the gridded climate data (Ionita *et al*., 2008, 2014, 2018). In order to detect stable predictors, the variability of the correlation between the tree-ring parameters and the gridded data is investigated within a 31-year moving window over the 1901–2012 period. The correlation is considered stable for those regions where the tree-ring parameter and the gridded data are significantly correlated at the 90 or 80% level for more than 80% of the moving window. A detailed description of the methodology is given by Ionita (Ionita, 2017). The basic idea of this methodology is to identify regions with stable correlations (meaning the correlation does not change over time) between $\delta^{13}C_{res}$, $\delta^{18}O$ and gridded data (e.g., PP, CI, and SPEI3) with different time lags.

3 | RESULTS AND DISCUSSION

3.1 | Characteristics of the carbon and oxygen isotope chronologies

The mean of the combined chronology of $\delta^{13}C$ raw values is −22.3‰ and the data vary between −23.5 ‰

and − 20.9 ‰. Lags 1 and 2 autocorrelations of the $\delta^{13}C$ data are high (r = 0.78 and r = 0.68). Such a high autocorrelation can be explained by the accentuated trend of the chronology. A high autocorrelation can be expected as the trees store the glucose assimilated in the late autumn and winter, and use it in the next spring when a new ring starts growing (Pallardy, 2008; Kimak and Leuenberger, 2015).

The detrended data of Trees 1, 2, and 3, which have similar ages (around 130 years), show a negative slope of approximately 1.5–2 ‰, while Tree 4 (representing >300 years cambial age) does not show any trend for the analysed period, indirectly supporting the absence of age-related trends in $\delta^{13}C$ data with a cambial age of 100 years and older (Gagen *et al.*, 2008). The inter-series correlation (Rbar = 0.45) and Expressed Population Signal (EPS = 0.75) reveal an acceptable internal coherence but low confidence of the site chronology.

The $\delta^{18}O$ values of the combined chronology vary around the mean of 29.3‰, ranging from 27.6‰ to 31.5‰. The $\delta^{18}O$ chronology is characterized by low autocorrelation (r = 0.28 and r = 0.05 on the Lag 1 and Lag 2, respectively), which indicates that previous year conditions do not have a strong effect on oxygen isotopic variability in tree-ring cellulose, indicating that the source water originates mainly from the current season rainfall (McCarroll and Loader, 2004). The $\delta^{18}O$ series show no juvenile effects or common increasing or decreasing trends in the first 140 years of tree age (Figure 2b and Figure S3). Therefore, we conclude that $\delta^{18}O$ values from Swiss Stone pine tree-ring cellulose from Calimani Mts can be used for dendroclimatological studies without any detrending procedure. The mean inter-series

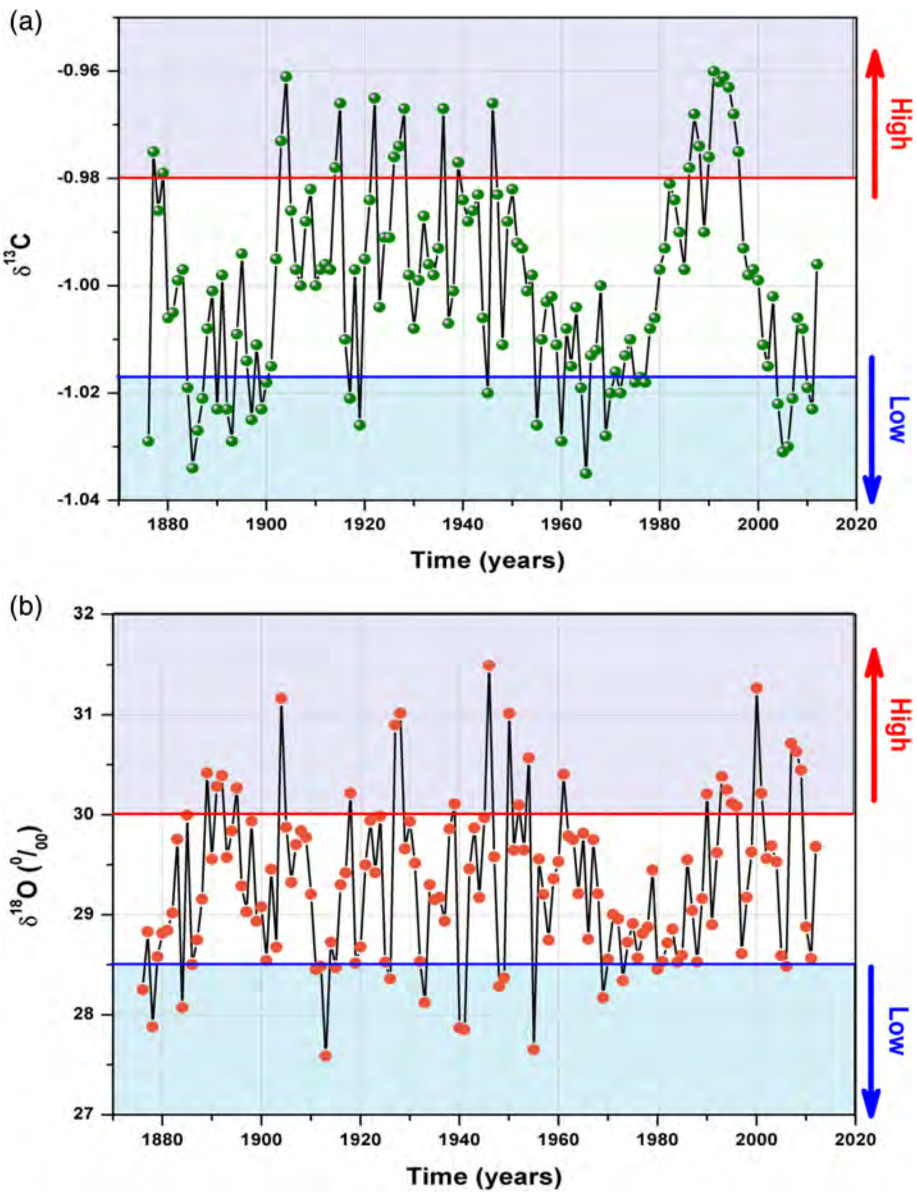

FIGURE 2 The temporal evolution of the (a) detrended $\delta^{13}C$ chronology and (b) $\delta^{18}O$ chronology over the period 1876–2012. The shaded areas indicate the years used for the composite maps in Figures 6 and 7

correlation (Rbar = 0.77) and Expressed Population Signal (EPS = 0.92) demonstrate the robustness of the $\delta^{18}O$ chronology and indicate that the $\delta^{18}O$ values display a high amount of shared variance originating from a common controlling factor related to climatic conditions.

3.2 | $\delta^{13}C$ and $\delta^{18}O$ climate response on local scale

Detrended carbon isotopic data from Călimani Mts correlate significantly with June to August (JJA) precipitation ($r = -0.49$, $p < .05$), with March and April RH ($r = 0.43$ and $r = 0.45$, respectively, $p < .05$) and with July and August SPEI3 ($r = -0.45$ and $r = -0.52$, respectively, $p < .05$) (Table 2). No significant correlation was found between $\delta^{13}C_{res}$ and temperature (Table 2). The sampled trees are located at high elevation, where a thick layer of snow cover accumulates during the winter. The resulting water from snowmelt, starting in March–April, infiltrates in the highly permeable soil, which allows the retention of soil water. Thus, the derived spring soil moisture content together with summer precipitation amounts become the most important factors which controlling the carbon isotopic composition in tree-ring cellulose (McCarroll and Loader, 2004). The RH in spring is related to high precipitation and snowmelt, which contribute to supplement the soil moisture and water aquifers. When tree rings start to grow, water availability in soil, and high RH leads to high stomatal conductance, leading to low $\delta^{13}C$ values because of strong ^{13}C discrimination (McCarroll and Loader, 2004; Loader *et al.*, 2008).

However, the split-period calibration model shows very poor statistical reconstruction skills (Table S1 and S2). This, as well as the relatively low signal-strength statistics of the mean chronology (see section 3.1) underlines that a robust and reliable reconstruction is still not achieveable based on carbon isotopes in Călimani Mountains. These results might be hindered by the low replication (four trees). Nevertheless, by using more replicates a robust and reliable reconstruction might be achievable.

The stable oxygen isotope chronology shows a high correlation with the local climate parameters during the summer months (June, July, and August) (Table 3), which is also in agreement with the commonly reported signal in European tree sites (Treydte *et al.*, 2007; Saurer *et al.*, 2008; Hartl-Meier *et al.*, 2015). The $\delta^{18}O$ values are negatively correlated with the precipitation ($r = -0.55$, $p < .05$), CLD ($r = -0.67$, $p < .05$), RH ($r = -0.64$, $p < .05$), and CI ($r = -0.69$, $p < .05$); and positively correlated with the Tx ($r = 0.60$) in JJA (Table 3). The high correlation with CLD and RH can be related to the high correlation between these two climatic parameters ($r = 0.75$, $p < .05$). Relative humidity has a direct effect on leaf transpiration rate and stomatal conductance through the ratio of vapour pressure inside to

TABLE 2 Correlation coefficients of $\delta^{13}C$ with local monthly climate data: Cloud cover (CLD), precipitation (PP), relative humidity (RH), maximum temperature (Tx), mean temperature (Tm), minimum temperature (Tn), climate index (CI), and SPEI3

	CLD	PP	RH	Tx	Tm	Tn	CI	SPEI3
SEP	−0.12	−0.08	0.15	0.01	−0.02	−0.06	−0.03	**−0.42**
OCT	0.01	−0.07	0.14	0.04	0.04	−0.01	−0.08	−0.30
NOV	−0.09	−0.21	0.17	−0.33	−0.33	−0.30	0.08	−0.17
DEC	−0.08	0.15	0.32	−0.06	−0.04	−0.02	0.13	−0.12
Jan	0.10	−0.05	**0.43**	0.06	0.09	0.13	−0.10	−0.05
Feb	−0.29	−0.22	0.09	0.00	−0.02	−0.04	−0.14	−0.11
Mar	−0.07	−0.11	**0.43**	−0.05	0.00	0.02	−0.08	−0.16
Apr	0.12	0.00	**0.45**	−0.10	−0.07	−0.04	0.05	−0.16
May	−0.08	−0.16	0.18	−0.04	−0.04	−0.04	−0.08	−0.13
Jun	−0.07	−0.33	0.25	0.02	0.01	−0.05	−0.21	−0.24
Jul	−0.17	−0.31	0.07	0.07	0.06	−0.01	−0.25	**−0.45**
Aug	−0.20	−0.28	−0.03	0.11	0.08	−0.03	−0.24	**−0.52**
Sep	0.01	0.00	0.18	−0.02	0.00	0.02	0.00	**−0.37**
Oct	0.11	−0.02	0.20	0.02	0.08	0.08	−0.07	−0.24
Nov	−0.16	−0.31	0.09	−0.20	−0.21	−0.20	−0.07	−0.15
Dec	−0.12	0.23	0.28	−0.10	−0.08	−0.07	0.22	−0.13
JJA	−0.25	**−0.49**	0.13	0.09	0.07	−0.04	**−0.38**	**−0.45**
JA	−0.26	**−0.41**	0.03	0.11	0.09	−0.03	−0.34	**−0.52**

Note: Correlations coefficients at 95% significance level ($p < .05$) are bolded, red colour indicates positive correlation and green colour indicates negative correlation.

	Cloud	PP	RH	Tx	Tm	Tn	CI	SPEI3
SEP	0.05	0.08	0.01	−0.04	−0.01	0.03	0.05	−0.13
OCT	0.03	0.01	0.06	0.29	0.35	0.32	−0.22	−0.05
NOV	−0.12	−0.18	−0.10	−0.05	−0.09	−0.11	−0.06	0.01
DEC	−0.09	0.10	0.03	0.10	0.08	0.09	0.01	−0.08
Jan	0.19	0.01	0.17	0.11	0.15	0.18	−0.10	−0.06
Feb	0.00	−0.03	0.07	0.07	0.11	0.12	−0.10	0.01
Mar	0.08	0.23	0.11	0.13	0.19	0.20	0.03	0.19
Apr	−0.30	−0.20	−0.26	**0.36**	0.34	0.27	−0.38	−0.04
May	−0.34	−0.33	−0.30	0.25	0.20	0.10	−0.33	−0.25
Jun	**−0.50**	**−0.50**	**−0.52**	**0.45**	**0.41**	0.21	**−0.57**	**−0.51**
Jul	**−0.40**	−0.25	**−0.49**	0.34	0.27	0.08	−0.34	**−0.55**
Aug	−0.30	−0.27	**−0.45**	**0.42**	**0.40**	0.28	**−0.45**	**−0.57**
Sep	0.11	0.17	−0.10	−0.03	−0.03	−0.03	0.12	−0.21
Oct	−0.08	−0.08	−0.05	0.32	0.31	0.22	−0.26	−0.14
Nov	0.01	0.00	0.02	0.22	0.22	0.22	−0.15	−0.01
Dec	−0.13	0.08	−0.16	0.03	−0.02	−0.01	0.07	−0.08
JJA	**−0.66**	**−0.55**	**−0.64**	**0.55**	**0.47**	0.23	**−0.69**	**−0.61**
JA	**−0.49**	**−0.36**	**−0.55**	**0.46**	**0.40**	0.20	**−0.51**	**−0.60**

Note: Correlations coefficients at 95% significance level ($p < .05$) are bolded, red colour indicates positive correlation and green colour indicates negative correlation.

TABLE 3 Correlation coefficients of $\delta^{18}O$ with local monthly climate data: Cloud cover, precipitation (PP), relative humidity (RH), maximum temperature (Tx), mean temperature (Tm), climate index (CI), and SPEI3

that outside of the leaf (McCarroll and Loader, 2004). The low RH determines an increase of leaf transpiration rates, causing a high intensity of stomatal conductance. Thereby, evaporation through the open stomata enriches the isotopic composition of leaf water, which is transferred to photosynthate (sucrose) and effectively transferred to tree-ring cellulose (Gessler *et al.*, 2014). A similar direct link between cloud cover and stable isotopes in plant water is not known so, despite the slightly lower correlation coefficient, RH is considered as the actually most influential environmental parameter regulating interannual $\delta^{18}O$ variability of cellulose in stone pine tree rings in the Calimani Mts.

3.3 | Asymmetric signal in high- and low-extreme proxy years

The comparison between extreme values of $\delta^{13}C_{res}$ and $\delta^{18}O$ and the seasonal cycle for precipitation and relative humidity for the instrumental period 1961–2012 shows interesting patterns. Years recording the highest (> 1 *SD*) and lowest (< −1 *SD*) values for $\delta^{13}C_{res}$ (Figure 3a) and $\delta^{18}O$ (Figure 3b) were selected and used to calculate the seasonal cycle in precipitation (Figure 3c,f) and relative humidity (Figure 3e,f). Extreme low values of $\delta^{13}C$ and $\delta^{18}O$ are connected to wetter conditions than average from May until August (Figure 3c,f). In contrast, drier conditions are detected in JuneAugust in association with high $\delta^{13}C$ and in MayAugust in association with high $\delta^{18}O$ values. In the case of RH, the differences in the seasonal cycle are not so obvious for extreme $\delta^{13}C_{res}$, whereas for extreme $\delta^{18}O$ years, there is a clear and distinct seasonal cycle (Figure 3e,f). More humid conditions than average are detected from June-September associated with extreme low values of $\delta^{18}O$, while less humid conditions than average are detected from AprilSeptember accompanied by high values of $\delta^{18}O$. The differences in the seasonal cycle of precipitation are captured more clearly by $\delta^{13}C_{res}$ extreme years compared to $\delta^{18}O$. Based on these relationships, we can argue that the seasonal distribution of precipitation amount acts as a limiting factor for $\delta^{13}C_{res}$ extreme values, whereas the seasonal cycle of RH acts to strongly influence the $\delta^{18}O$ extreme values, especially in the case of high $\delta^{18}O$. The seasonal cycle analysis indicates that months with deviations from average climatic conditions differ between the years characterized by low or high values in the proxy data, especially in the case of RH for extreme $\delta^{18}O$ years.

3.4 | Spatial correlation with climate data

Several studies have detected a loss of climate sensitivity in ring-width (Jacoby *et al.*, 2000; Esper *et al.*, 2010) and density (Briffa *et al.*, 2004) chronologies, especially at high

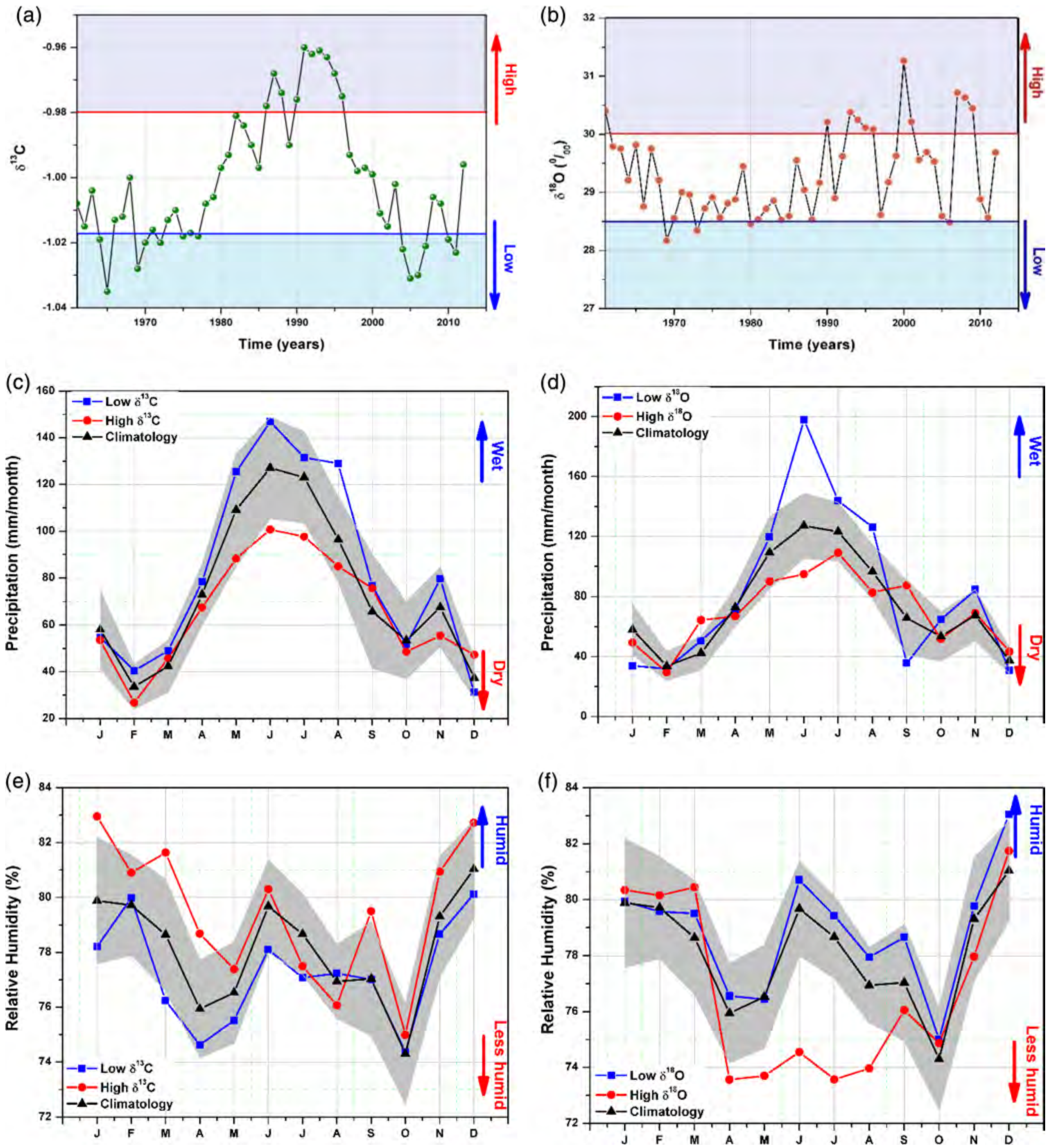

FIGURE 3 The temporal evolution of the (a) detrended $\delta^{13}C$ chronology; (b) $\delta^{18}O$ chronology over the period 1961–2013; (c) the seasonal cycle for precipitation during years with extreme $\delta^{13}C$ values (shaded area in a); (d) the seasonal cycle for precipitation during years with extreme $\delta^{18}O$ values (shaded area in b); (e) as in (c) but for relative humidity and (f) as in (d) but for relative humidity. In (c-f), the grey lines indicate the seasonal cycle over the whole analysed period (1961–2013), the red lines indicate the seasonal cycle for low values of $\delta^{13}C$ and $\delta^{18}O$ and the blue lines indicate the seasonal cycle for high values of $\delta^{13}C$ and $\delta^{18}O$. Where the coloured lines lie outside the grey shading, deviations higher/smaller than 1 *SD* from average conditions occur

latitudes, as well as for several species at the upper treeline in the European Alps (Carrer and Urbinati, 2006; Leonelli *et al.*, 2009). Ocassionally, unstable correlations have been found also for stable isotopes (Treydte *et al.*, 2007; Bale *et al.*, 2010). In this respect, a stable relationship over time between tree-ring proxies and climate variables is crucial for climate reconstructions. In order to test the strength of the relationship between our proxy data and gridded data, at European level, we have applied a methodology, the so-called stability maps, successfully used for the monthly and seasonal prediction of streamflow for central European Rivers (Ionita *et al*., 2012, 2014).

The stability map between $\delta^{13}C_{res}$ and PP and SPEI3 (Figures S4 and S5) shows no stable and significant correlations are found over the period 1902–2012, at European level, although for the period 1961–2012 significant correlations with local data have been found between $\delta^{13}C_{res}$ and PP and SPEI3 for summer months (Table 2). This indicates that the relationship between the available $\delta^{13}C_{res}$ data and the climate drivers is nonstationary in time. A higher replication might help to improve the potential climate signal and make the stable carbon isotope data set of Calimani Stone pine tree rings suitable for paleoclimatological purposes.

For $\delta^{18}O$, we have computed the stability maps with the gridded data for CI and SPEI3. The stability map between $\delta^{18}O$ and SPEI3 (Figure 4), with different time lags, indicates that stable and significant correlations persist from June until September. Significant, stable, and negative correlations are observed over the eastern part of Europe (e.g., Romania, Serbia, Bulgaria and Ukraine), while significant, stable and positive correlations are observed over Fennoscandia (e.g., Norway, Sweden and the western part of Finland). This dipole-like structure (opposite correlations over Fennoscandia and the eastern part of Europe) in the spatial correlation with SPEI3 gridded data is a common feature of drought occurrence at European level (Ionita, 2015). The spatial extent of the correlation is largest for August SPEI3. The stability maps between $\delta^{18}O$ and CI (Figure 5) shows that stable and significant correlations are observed throughout the summer months (JuneAugust), and the spatial extent of the correlations is also characterized by a dipole-like structure, similar to the one obtained for SPEI3. The largest spatial extent of the stable correlations is found when CI is averaged throughout the summer months (JJA). Based on the results obtained from the stability maps, we can argue that $\delta^{18}O$ values of the Calimani stone pine isotope record reveal both local as well as European scale climate variability. Overall, during summer, high $\delta^{18}O$ values are associated with dry conditions and positive temperature anomalies over the central, southeast, and eastern Europe, while low $\delta^{18}O$ values are associated with negative temperature anomalies and wet summers over the eastern part of Europe (Figures 4 and 5). The dry and warm climatic conditions determine the increase of ambient/intercellular vapour pressure gradient, causing enrichment of ^{18}O in the leaf water, which is them transferred to the tree ring cellulose (Roden *et al*., 2000; McCarroll and Loader, 2004).

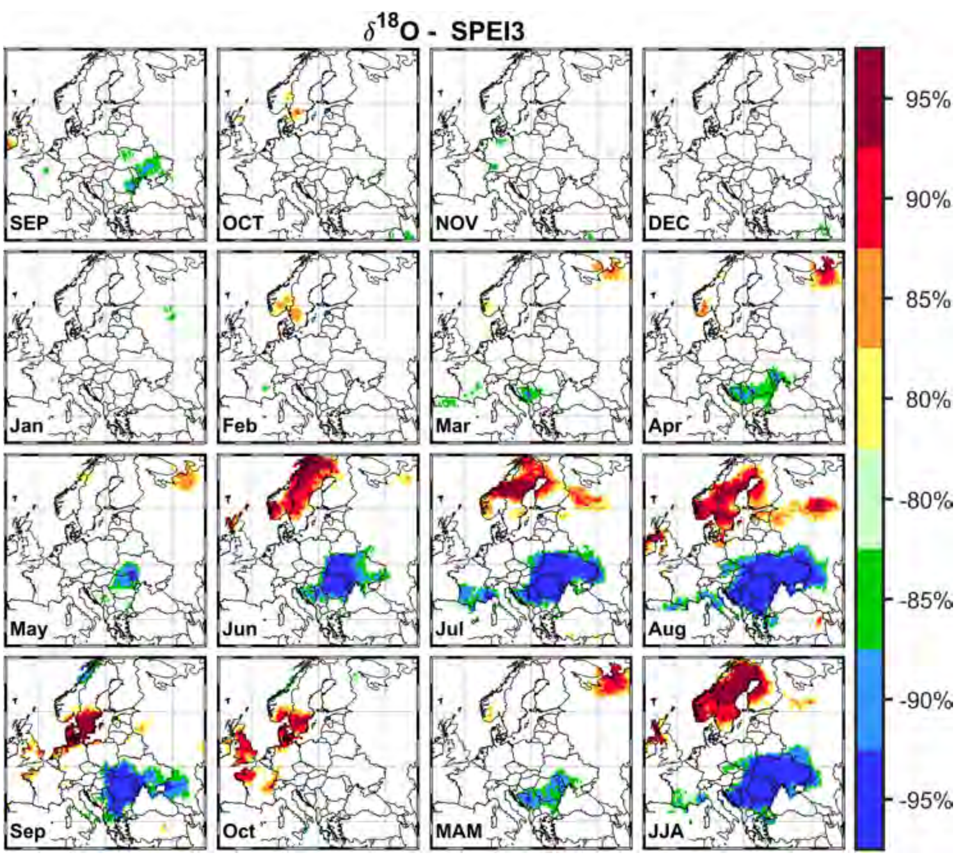

FIGURE 4 Stability map of the correlation between $\delta^{18}O$ and SPEI3 from previous year September until current year October. Regions where the correlation is stable, positive, and significant for at least 80% windows are shaded with dark red (95%), red (90%), orange (85%), and yellow (80%). The corresponding regions where the correlation is stable, but negative, are shaded with dark blue (95%), blue (90%), green (85%), and light green (80%). SEP – September previous year, OCT - October previous year, NOV – November previous year, DEC – December previous year, Jan – January, Feb – February, mar – March, Apr – April, may – May, Jun – June, Jul – July, Aug – August, Sep – September, Oct – October, MAM – March/April/may and JJA – June/July/ august. Analysed period: 1902–2012

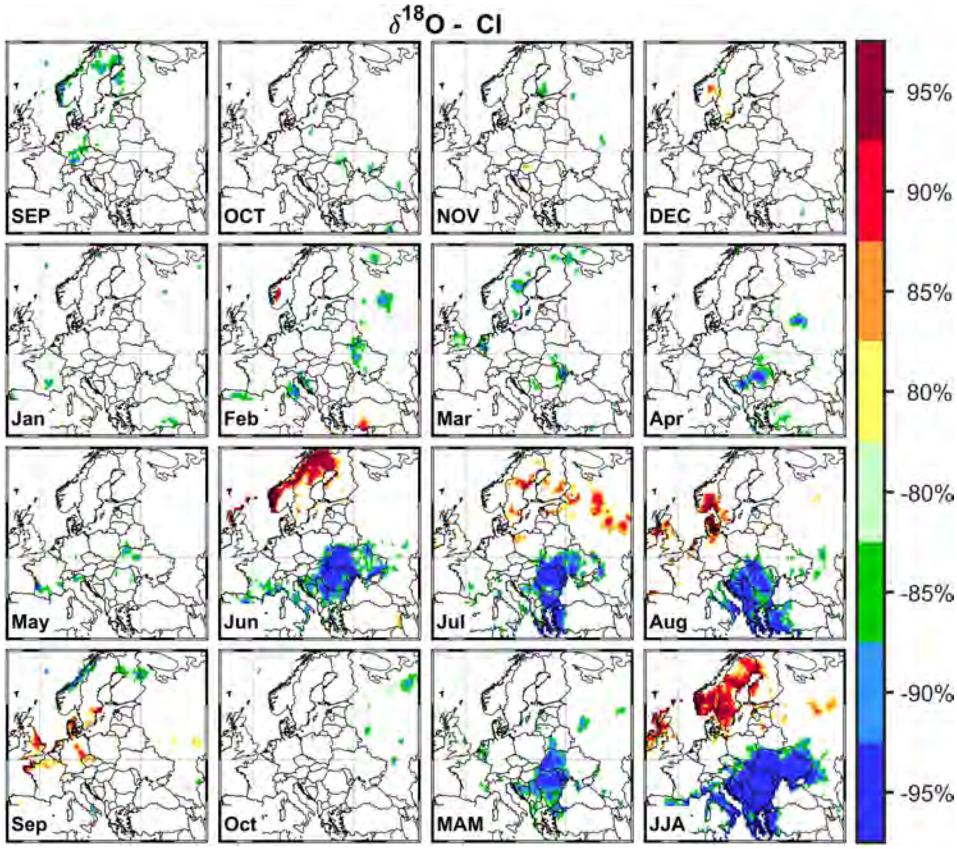

FIGURE 5 Stability map of the correlation between δ^{18}O and CI from previous year September until current year October. Regions where the correlation is stable, positive and significant for at least 80% windows are shaded with dark red (95%), red (90%), orange (85%) and yellow (80%). The corresponding regions where the correlation is stable, but negative, are shaded with dark blue (95%), blue (90%), green (85%) and light green (80%). SEP, September previous year; OCT, October previous year; NOV, November previous year; DEC, December previous year; Jan, January; Feb, February, Mar, March; Apr, April; May, May; Jun, June; Jul, July; Aug, August; Sep, September; Oct, October; MAM, March/April/May; JJA, June/July/August. Analysed period: 1902–2012

Based on the results from the stability maps (Figures 4 and 5), we have defined two indices, one for August SPEI3 and one for JJA CI, by averaging the gridded data sets over the region (22°E30°E, 45°N50°N) for August SPEI3 and over the region (23°E30°E, 43.5°N50°N) for JJA CI. We choose these particular regions, because significant and stable correlations are found over these two areas, and they are located in the vicinity of our studied forest. In order to verify the reconstruction skill for these two indices, the calibration-verification model was performed for both SPEI3 and CI (Figure 6a,b). The positive and significant values for SPEI3 (CI) of RE = 0.58 (0.59), CE = 0.54 (0.58), and the significant correlation coefficients indicate that the regression model provides predictive skill for reconstruction (Cook *et al*., 1994) while the Durbin-Watson statistics (2.28 for SPEI3, 1.80 for CI) do not suggest any linear trend in the model residuals (Table S3SPEI3 and Table S4CI). This temporally stable relationship between δ^{18}O and SPEI3 (CI) was also tested by applying the stability maps methodology (Figures 4 and 5). Overall, the best verification result was obtained for CI (Table S4), indicating the robustness of the regression model. This shows that the CI model based on δ^{18}O is significantly related to the actual variation of dry-warm/wetcold climatic conditions over the eastern part of Europe, including the study area.

The seasonal cycle of August SPEI3 (CI) (Figure 6c,d) associated with extreme values of δ^{18}O (Table S5, Figure 3) over the period 1902–2012 show that wetter conditions than average are detected from March until September by extreme low values of δ^{18}O (Figu67re 6c), whereas drier conditions are recorded from JanuarySeptember for extreme high values of δ^{18}O (Figure 6c). The highest differences in the amplitude of the seasonal cycle of SPEI3 are recorded from JuneAugust. Combined wetter and colder conditions than average are observed from May until August for the years characterized by low values of δ^{18}O (Figure 6f), whereas drier and warmer conditions than average are observed from January until August for extreme high δ^{18}O values (Figure 6f). The seasonal cycle analysis indicates that there is a clear change in the absolute values of the analysed variables (SPEI3 and CI) through the year for extreme δ^{18}O values. For example, the values of CI in June and July for extreme low years is more than double compared to the ones recorded during years with extreme high δ^{18}O values. This verifies that the δ^{18}O in tree rings is able to properly capture the occurrence of extreme summers in terms of SPEI3 and CI.

3.5 | Large-scale atmospheric circulation

To investigate the relationship between the interannual variability of δ^{18}O values in tree rings from Calimani Mts. and large-scale atmospheric circulation composite maps of the

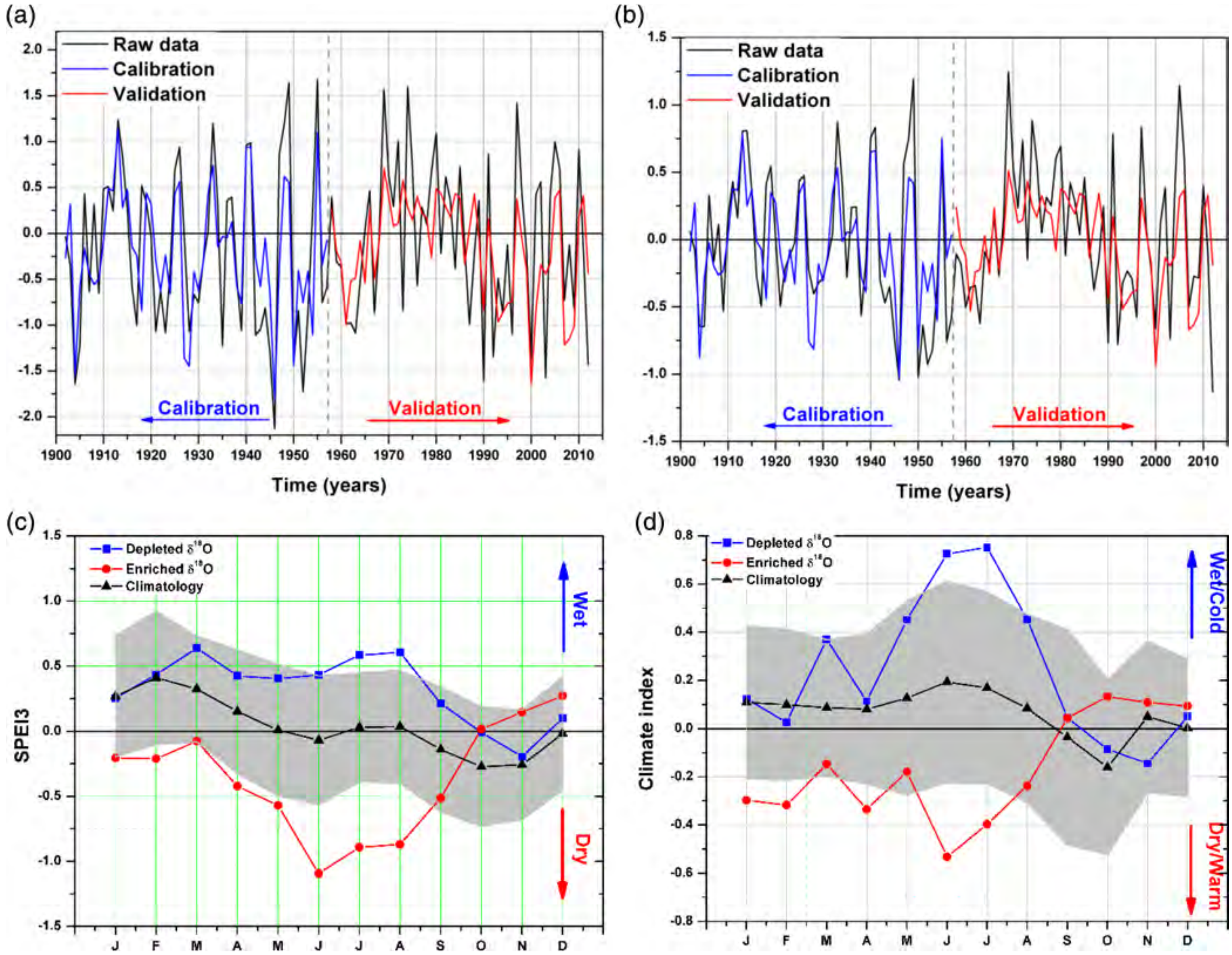

FIGURE 6 Calibration-verification model for (a) august SPEI3; (b) for CI JJA; (c) the seasonal cycle for august SPEI3 during years with extreme δ^{18}O values and (d) the seasonal cycle for JJA CI during years with extreme δ^{18}O. In (a) and (b) the black line indicates the observed data; the blue line indicates the reconstructed SPEI3 (CI) for the calibration period and the red line indicates the reconstructed SPEI3 (CI) for the verification period. Where the coloured lines lie outside the grey shading, deviations higher/smaller than 1 *SD* from average conditions occur. Analysed period: 1902–2012

geopotential height at 500mb (Z500) and SST for high (> 1 *SD*) and low (< −1 *SD*) δ^{18}O values for the summer months (JJA) were generated (Figure 7). Because, δ^{18}O records from Swiss stone pine reflects very well the drought conditions at European scale, it is fair to argue that δ^{18}O can partially reflect also the prevailing large-scale circulation (e.g., Rossby waves, atmospheric blocking) and the variability of the North Atlantic Ocean SST (Ionita *et al.*, 2012, 2017; Schubert *et al.*, 2014; Kingston *et al.*, 2015; Spinoni *et al.*, 2015). High values of δ^{18}O are associated with a high-pressure system over southern and central Europe and the Mediterranean Sea, and with a low-pressure over the northern Atlantic Ocean, northern Europe and Russia, which are linked to Rossby-wave oscillations (Ionita *et al.*, 2012, 2017; Van Lanen *et al.*, 2016) (Figure 7a). This pattern favours the advection of dry and warm air from the northern part of Africa toward the south-eastern part of Europe (including the study site). In contrast to this, low values of δ^{18}O are associated with a low-pressure center over central and eastern part of Europe and a high-pressure system over the northern Atlantic Ocean, Western, and northern Europe (Figure 7b). The negative Z500 anomalies centred over the central and eastern part of Europe are consistent with enhanced precipitation over this region and the advection of moist air from the Mediterranean region toward Romania (the wind vectors in Figure 7b). A similar large-scale pattern has been found to be associated with enhanced summer precipitation and high streamflow anolmalies over Romania, including our study region (Ionita, 2015). Throughout the summer period, the high-pressure centers are associated with

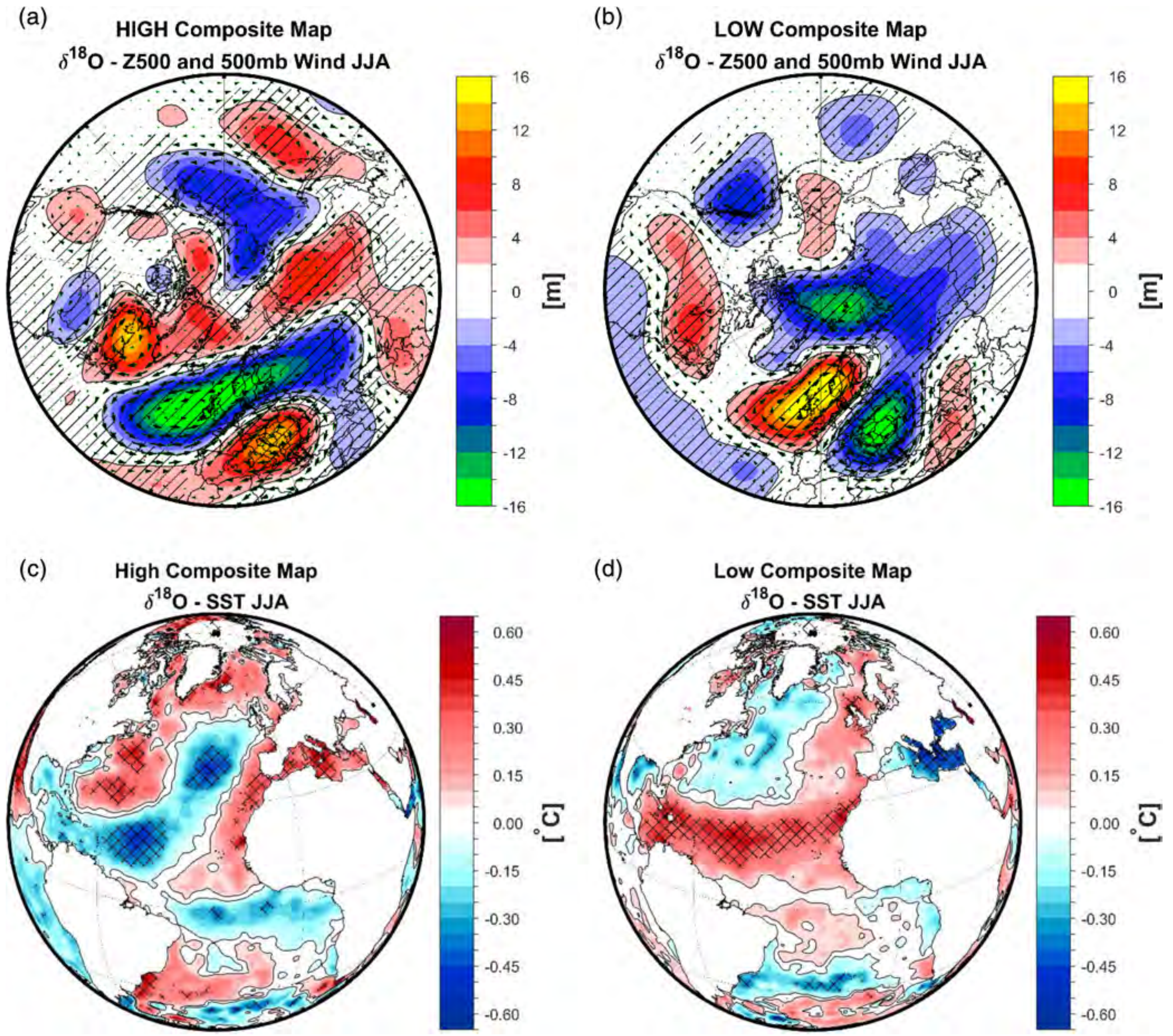

FIGURE 7 (a) the composite map between low $\delta^{18}O$ (< -1 *SD*) and summer Geopotential height at 500mb (Z500—Shaded coloured areas) and summer 500 mb wind vectors (black arrows); (b) the composite map between high $\delta^{18}O$ (> 1 *SD*) and summer geopotential height at 500 mb (Z500—Shaded areas) and summer 500 mb wind vectors; (c) as in (a) but for the summer sea surface temperature (SST) and (d) as in (b) but for the summer sea surface temperature (SST). The hatching highlights significant values at a confidence level of 95%. Analysed period: 1876–2012

anticyclonic circulation, which generates heat waves and droughts, while the low-pressure centers are associated with cyclonic circulation thus generates wet summers (Ionita *et al*., 2012).

Significant and stationary spatial correlations were found between $\delta^{18}O$ and CI and SPEI3 at European level (Figures 4 and 5). In Figure 7c,f, we further examined the relationship between $\delta^{18}O$ and oceanic conditions, because the occurrence of droughts and heat waves over the European region is significantly affected by adjacent oceanic condition on yearly to decadal time scales (Cassou *et al*., 2005; Della-Marta *et al*., 2007; Schubert *et al*., 2014; Ionita *et al*., 2017). The role of the North Atlantic Ocean and Mediterranean Sea SST in triggering extreme drought at European level has been demonstrated by previous studies (Feudale and Shukla, 2011; Ionita *et al*., 2012, 2017; Kingston *et al*., 2013; Ionita, 2015). Following this line, significant correlations between $\delta^{18}O$ values and North Atlantic Ocean SST indicate possible connections between the moisture availability over the eastern part of Europe and remote ocean areas. The high $\delta^{18}O$ values correspond to the positive SST anomalies over the Mediterranean Sea and the northern Atlantic Ocean and negative SST anomalies over the southern Atlantic Ocean (Figure 7c). In contrast, the low $\delta^{18}O$

values correspond to negative SST anomalies over the Mediterranean Sea and the Black Sea and positive SST anomalies over the Atlantic Ocean (Figure 7f). Overall, the structure of the SST anomalies in Figure 7 resembles the SST anomalies responsible for the occurrence of extreme drought events over the southern and eastern part of Europe (e.g., 2003, 2015) (Van Lanen *et al*., 2016; Ionita *et al*., 2017). In a recent paper, Ionita et al. (2017) shown that warm Mediterranean SSTs have preceded and occurred concurrently with dry summers over most of the central and eastern part of Europe. Moreover, the SST anomalies associated with high/low values of δ^{18}O over our analysed region are similar to the SST anomalies associated with δ^{18}O extreme values recorded by latewood cellulose of oak (*Quercus robur* L.) trees growing in the NW part of Romania (Nagavciuc *et al*., 2019).

4 | CONCLUSIONS AND PERSPECTIVES

Although tree-ring-based carbon and oxygen isotope records have been extensively used to reconstruct various climate paramaters at European scale (Treydte *et al*., 2009; Kress *et al*., 2010; Esper *et al*., 2015), currently there is a lack of such studies over the eastern part of Europe, including Romania. Thus, in this study, we have analysed the climate signal registered by stable carbon and oxygen isotopes ratios in the cellulose of Swiss stone pine tree rings from Calimani Mountains, Romania. Stable oxygen isotope ratio in Swiss stone pine tree ring cellulose from Calimani Mountains represents a better indicator for dendroclimatological application than the stable carbon isotope ratio. The correlation of all climatic parameters is higher and temporally more stable with δ^{18}O than with δ^{13}C. The poor statistical skill for carbon as a proxy for paleoclimate reconstructions as well as the relatively low signal-strength statistics of the mean carbon chronology underlines that reliable reconstruction is still not achievable based on carbon isotopes in Călimani Mountains. These results might be hindered by the low replication (four trees). Nevertheless, by using more replicates a robust and reliable reconstruction might be achievable.

For δ^{18}O values, the calibration and verification results demonstrate that δ^{18}O is correlated with local summer relative humidity, cloud cover, maximum temperature, as well as the drought conditions at a European scale. The highest correlation coefficients and best statistical skills were obtained for δ^{18}O values and relative humidity at local scale, and δ^{18}O and SPEI3 and CI at European scale. As such, this calibration could be used to provide a long record of summer drought conditions over the eastern part of Europe.

At interannual time-scales, the variability of δ^{18}O reflects changes in the large-scale atmospheric circulation and the SST from the North Atlantic Ocean and the Mediterranean Sea. High values of δ^{18}O are associated with an extended atmospheric blocking over the central and eastern part of Europe and a warm Mediterranean Sea and a cold central Atlantic Ocean. This kind of prevailing large-scale atmospheric circulation (e.g., anticyclone over the central and eastern part of Europe and cold central north Atlantic Ocean and warm Mediterranean Sea) is usually associated with extreme droughts and heatwaves over the central and eastern part of Europe (Van Lanen *et al*., 2016; Ionita *et al*., 2017).

Stable oxygen isotope composition of Swiss stone pine tree ring cellulose from northeastern Romania represents a great potential for long paleoclimatic reconstructions. Such records offer the opportunity to reconstruct both regional drought and large-scale circulation variability over southern and central Europe and allows us to fill the gap, over the eastern part of Europe, in order to be able to better understand past climatic variability at continental scale.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from EEA Financial Mechanism 20092014 under the project contract no CLIMFOR18SEE. PI was supported partial by the project number PN-III-P4-ID-PCE-2016-0253. VN was supported partial by German Federal Environmental Foundation (DBU). MI was funded by the Helmholtz Climate InitiativeREKLIM.

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How to cite this article: Nagavciuc V, Kern Z, Ionita M, *et al.* Climate signals in carbon and oxygen isotope ratios of *Pinus cembra* tree-ring cellulose from the Călimani Mountains, Romania. *Int J Climatol*. 2019;1–18. https://doi.org/10.1002/joc.6349