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ORIGINAL PAPER

Age effects and climate response in trees: a multi-proxy tree-ring test in old-growth life stages

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Abstract Aging in trees implies a progressive reduction in the growth rate, related to a shortening of the growing period and changes in the photosynthetic capability and efficiency. These changes may continue during the old-growth life stages following the juvenile phase and are reflected in tree-ring properties such as growth increment, density or stable isotopes. We studied possible climate age effects in time series of several tree-ring parameters (ring width, wood density and stable carbon and oxygen isotopes) of mature individuals from two age groups of *Pinus uncinata* and *P. nigra* at two locations in Spain. The aim was to test whether age differences in trees in the old-growth life stages could lead to diverging climate responses. The results show some differences in response to climate between age groups at a monthly level, but most of these divergences are not significant for seasonal climate variables. Regardless of the age group, the main limiting climate factors constrained tree growth equally. Although our findings do not support the idea of an age-dependent response to climate that may lead to inaccurate climate reconstructions, further studies using tree-ring density and stable isotope series are urgently needed to verify the current results.

Keywords Tree growth · Aging · Dendrochronology · Stable isotopes · Dendroclimatology

Introduction

The relationship between tree-ring proxies and climatic and ecological variables can be described using simple linear models. On the basis of such models, tree-ring variables, e.g., tree-ring width and maximum density have been used extensively for high-resolution reconstructions of climate of the past millennium. Tree rings have become the dominant data sources in most large-scale hemispheric temperature reconstructions (e.g., Mann et al. 1999; Briffa et al. 2004; Esper et al. 2002; D'Arrigo et al. 2006).

Additionally, new tree-ring proxies such as stable carbon and oxygen isotope ratios in tree rings (δ^{13}C and δ^{18}O, respectively) have emerged as new sources of information, not only for palaeoclimate research (McCarroll and Loader 2004; Gagen et al. 2006; Treydte et al. 2006; Treydte et al. 2007) but also to investigate long-term ecophysiological patterns, e.g., the water-use efficiency (WUE) (Saurer et al. 2004; Seibt et al. 2008; Andreu-Hayles et al. 2011).

Dendroclimatology has advanced considerably during the last decades but there are still some limitations and weaknesses lowering the quality of climate reconstructions based on tree-ring proxy records. Among these weaknesses is the question whether climate–growth relationships are

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controlled by tree-age and how changes in physiological processes associated with tree aging can alter these relationships.

Every tree-ring proxy seems to be affected to some extent by aging, e.g., is it well established that photosynthesis rates and related physiological attributes differ between juvenile (pre-reproductive plants) and full reproductive individuals (mature plants) (Yoder et al. 1994; Bond 2000). The existence of a "juvenile effect" for the first 50–100 years in tree-ring width, density and stable isotope series is well known (e.g., Lerman and Long 1979; Schleser 1992; Buchmann and Ehleringer 1998). The changes are the result of morphological and physiological trends characterizing the transition from a juvenile to a mature growth phase. However, such changes may continue as trees progress to even older life stages (Bond 2000; Day et al. 2002) but it is unclear how this aging into older life stages may affect the climate–growth responses.

Post-juvenile changes (usually after the first 50–100 years of tree life) during the aging process in trees imply a shortening of the growing period (Rossi et al. 2008), reduction in growth rates and thus, in the amount of wood produced (Yoder et al. 1994; Ryan and Yoder 1997; Bond 2000; Day et al. 2002; Peñuelas 2005), which eventually results in narrower tree rings. Tree-ring width series display a declining age-related trend interpreted as the geometrical consequence of the increasing trunk diameter, rather than a reduction in wood production associated with aging. This well-known and noticeable age trend in tree-ring width series is statistically removed by the so-called detrending process, assuming that after this, the climate–growth relationship becomes linear and stable over time (Fritts 1976; Cook 1985; Cook and Kairiukstis 1990). The same procedure is often applied in maximum density series. More controversy exists in regard to stable isotope series, for which the existence of a juvenile effect on δ^{13}C and δ^{18}O series is known but which can be avoided by deleting the first few decades of the individual series (Gagen et al. 2007). However, whether δ^{13}C and δ^{18}O series contain a long-term age signal is still debated (e.g., Esper et al. 2010).

The assumption that the climate–growth relationships are independent of age after the biological trend has been removed statistically from the tree-ring series has been questioned in a number of studies (Szeicz and MacDonald 1994, 1995; Carrer and Urbinati 2004; Rozas et al. 2009). But investigations in whether the climate–growth response is stable across different age groups are scarce and inconclusive due to the wide variety of results reported. Moreover, existing literature has only focused on tree-ring width series (e.g., Carrer and Urbinati 2004; Esper et al. 2008; Rozas et al. 2009), and comparable information on tree-ring density and stable isotope series is not available.

Hence, investigations studying whether increasing tree age after leaving the juvenile phase of growth behind promotes functional constraints which may modify the climate–growth responses are urgently needed. Furthermore, it is important to evaluate how the increasing tree age may affect not only tree-ring width series but also other tree-ring-related proxies such as maximum density and stable isotopes.

Therefore, in this study, we test at two old forest locations on the Iberian Peninsula whether the tree responses to climate are age related. The approach focuses on trees in old-growth phases, avoiding juvenility, and involves the evaluation of different annually resolved tree-ring variables (i.e., width, density, δ^{13}C and δ^{18}O) derived from the same samples in order to assess whether aging affects the separate tree-ring proxies differently.

Materials and methods

Sites and sampling

Two study sites are *Parque Natural Sierra de Cazorla, Segura y las Villas* (PN-S, *Pinus nigra*-Southern site) located in the southeast of Spain and *Parc Natural del Cadí-Moixeró* (PU-N, *P. uncinata*-Nothern site) located in the Pre-Pyrenees (see Table 1 for details). At the PN-S site (Fig. 1), *Pinus nigra* Arn. spp. *salzmannii* var. *salzmannii* (Dunal) was sampled. Here, it grows under oromediterranean dry/humid climatic conditions (Rivas-Martínez 1983), close to the climatic limit of its distribution. The Mediterranean climate is characterized by an annual recurrent summer drought followed by a cool–wet season with a precipitation maximum in autumn–winter. The scarcity of water combined with the limestone bedrock already points out water availability as the main limiting factor for plant growth.

At the PU-N site, in the transition from Mediterranean to Eurosiberian climatic regions (Rivas-Martínez 1983),

Table 1 Geographical characteristics of two sampling sites: Cazorla (PN-S) and Pedraforca (PU-N)

Site	Range	Latitude	Longitude	Altitude (m a.s.l.)	Aspect	Stand density
Cazorla	Baetic	37°48′N	02°57′W	1,800	SW	Open forest
Pedraforca	Pre-Pyrenees	42°14′N	01°42′E	2,100	E	Open forest

Fig. 1 Location of two studied sites: PU-N in the Pre-Pyrenees and PN-S in the Baetic Range

Pinus uncinata Ramond ex DC. in Lam. et DC. was sampled. This shade-intolerant and long-lived conifer species grows in an ecotonal forest ecosystem characterized by temperate subalpine climate conditions with a short growing period limited mainly by low temperatures.

In summer 2006, increment cores were taken at breast height with a 12 mm diameter increment borer. At PN-S, 52 cores were collected from 25 trees, and at PU-N, 42 cores were obtained from 20 trees. Trees were grouped into two different age groups: (1) adult trees ≤250 years old (youngest tree approximately 150 years old) and (2) old trees ≥500 years old (oldest tree approximately 950 years old). The aim was to obtain chronologies with consistent differences in age but avoiding the juvenile growth phase (first 50 years of growth). Relatively large intra-tree variations have been reported for density (Koga and Zhang 2004; Jyske 2008) and for the circumferential variability in $\delta^{13}C$ (Leavitt and Long 1984; Schleser 1999). In order to avoid increased noise because of such intra-tree variability, the same cores were used for tree-ring width and density measurements.

All cores were sanded and visually cross-dated following dendrochronological procedures described by Stokes and Smiley (1968). Ring widths were measured with an accuracy of 0.01 mm, using the linear table Lintab™ (Frank Rinn S.A., Heidelberg, Germany) and the TSAP-Win program (Rinn 2003). The accuracy of the visual cross-dating and measurements were verified using the program COFECHA (Holmes 1983).

From the set of tree-ring width series, only the cores with undamaged wood and the suitable age required for the present study were selected for the density measurements. Lathes perpendicular to the wood fibers were extracted from 34 cores from the PN-S and from 28 cores from PU-N and were analyzed following X-ray microdensitometric techniques developed by Polge (1965). Density profiles with a resolution of 0.01 mm in radial direction were obtained. Along this path, the parameters earlywood width (EWw), latewood width (LWw), total ring width (TRW) and maximum density (MXD) were measured.

Isotopic analyses, in general, require fewer samples than the more traditional tree-ring proxies to provide a representative average series for a site because the common signal strength among isotope series is higher (Leavitt and Long 1984; Gagen et al. 2004). From the collection of cores, five samples per age group and site were selected for isotopic analysis. Cores were analyzed individually and with annual resolution for ^{13}C and ^{18}O. Tree rings were split manually with a scalpel under a stereomicroscope and the α-cellulose extracted following the chemical method based on the use of sodium hydroxide and sodium chlorite (Loader et al. 1997).

The $^{13}C/^{12}C$ isotope ratios were measured as CO_2 by combusting the α-cellulose samples in an elemental analyzer (Fisons NA 1500NC) coupled via an open split to an isotope ratio mass spectrometer (Micromass Optima) operating in continuous flow mode. The reproducibility was better than 0.1‰. Similarly, oxygen isotopes were measured from CO utilizing a TC/EA pyrolysis furnace coupled online to a Delta V Advantage mass spectrometer (Thermo Scientific, Bremen, D) with an external precision of less than 0.2‰. The isotope ratios are given in the conventional delta (δ) notation, relative to the standards VPDB ($\delta^{13}C$) and VSMOW ($\delta^{18}O$).

Ring width, density and stable isotope chronologies development

Tree-ring width and density series were processed according to standard dendrochronological techniques (Cook and Kairiukstis 1990). Detrending procedures were applied to remove non-climatic information (noise) from tree-ring data to maximize the climate signal. Individual series were standardized using ARSTAN (Holmes 1983) by fitting a negative exponential function or a cubic smoothing spline of 67% of the series length with 50% frequency response cutoff to the raw series in order to remove age trends (Cook 1985). This relatively stiff standardization emphasizes inter-annual variations but also keeps multi-decadal scale wavelengths in the final chronology (Cook et al. 1995). Since the series need to be homogeneous in variance, the dependency between mean and variance was checked and dimensionless tree-ring indices were calculated as ratios or differences depending on the existence of trends in the variance. If trends in the variance were found, indices were calculated as ratios, dividing the real by the fitted value. If no trend was present, indices were calculated as the difference between the real and the expected values. Finally, for each site and variable, master chronologies based on two different age groups were built using a bi-weight robust mean, which reduces bias caused by extreme values.

Stable isotope chronologies traditionally do not undergo standardization since it is assumed that they are not affected by age-related trends. Independently of age trends, raw $\delta^{13}C$ series display a negative trend caused by the depletion in atmospheric $^{13}CO_2$ due to fossil fuel burning and deforestation since industrialization (ca. AD1850). This trend needs to be removed from the raw $\delta^{13}C$ series. The most common way is to subtract annual changes in $\delta^{13}C$ of atmospheric CO_2, obtained from ice cores and direct measurements. We applied this atmospheric correction to our series (see details at McCarroll and Loader 2004).

After the correction of the stable carbon isotope measurements, individual series of $\delta^{13}C$ and $\delta^{18}O$ were z-transformed and averaged to build the final chronologies. The Expressed Population Signal (EPS, Wigley et al. 1984) was computed to assess the common signal representativeness of the final chronologies.

Chronologies comparison and climate signals

A common overlap period from 1900 to 2006 was used for comparison among chronologies. All single series and the master chronologies underwent 10-year low- and high-pass filter with a centered moving average to enhance either inter-decadal or inter-annual variations and thus to allow more comprehensive comparisons at different wavelengths. The first principal component (PC1) and Pearson's correlation coefficients (r) were computed to estimate common variance between individual series and chronologies. Correlation coefficients were also calculated for high- (r_{hf}) and low-pass (r_{lf}) filtered series and chronologies.

Homogenized mean monthly temperatures and precipitation were available from nearby stations of the *Instituto Nacional de Meteorologiá* (INM) network. For the PN-S site, temperature data were available from Pontones (38°01′N; 2°52′W) and precipitation data from Nava de San Pedro (37°52′N; 2°53′W) with distances from the sampling site of 48.9 km and 16 km, respectively. For the PU-N site, temperature data were available from Fígols station (42°17′N; 1°51′E) and precipitation from Lillet (42°24′N; 1°57′E) located from the sampling site 14 km and 23 km, respectively. The influence of climate on each tree-ring variable was investigated by computing simple linear correlations (r) between monthly climate variables using a period from July of the previous year ($t - 1$) to October of the current year (t) and all individual tree-ring series of each age group. For variables displaying significant correlations ($P \leq 0.05$) for consecutive months, the correlations with the seasonal variables were also calculated, even if correlations with some months were not significant. Tree-ring variables lacking significant correlations with the climate parameters are not shown.

The Mann–Whitney test was used to assess the significance of the difference in response to climate between groups of adult and old trees. A non-parametric approach was chosen since the existing replication in the group of adult trees does not ensure a robust normal distribution of the data. The test was performed between proxy and climate and for months and seasons when correlations were statistically significant for the mean of both age groups.

Results

Inter-tree common signals

Pearson's mean inter-series correlation coefficients suggest a generally higher agreement among adult trees than among old trees in both study areas (see Table 2). The strength of the relation, based mainly on the coherence in the year-to-year variations (r_{hf}) and shared variance (PC1), is consistently higher for adult trees in all proxies.

The tree-ring width records (TRW, EWw and LWw) and $\delta^{13}C$ seem to be the more sensitive proxies at the PN-S site indicated by the higher common variances than the other records from the same site. It is remarkable that $\delta^{13}C$ shows very similar inter-tree correlation values in the high frequency for both adult and old trees. In contrast, TRW,

Table 2 Tree-ring statistics at the sites Cazorla (PN-S) and Pedraforca (PU-N) for the chronologies of two age groups (Adult and Old) of every tree-ring variable

Chronology ID		Cazorla (PN-S)						Pedraforca (PU-N)					
		Number of trees/cores	Mean inter-series correlation	HF mean inter-tree correlation	LF mean inter-tree correlation	PC1 (%)	EPS	Number of trees/cores	Mean inter-series correlation	HF mean inter-tree correlation	LF mean inter-tree correlation	PC1 (%)	EPS
TRW	Adult	8/8	0.42**	0.45**	0.14	0.50	0.85	8/8	0.29*	0.31**	0.14	0.39	0.76
	Old	12/18	0.38**	0.38**	0.26	0.46	0.88	11/18	0.20*	0.19*	0.07	0.29	0.73
EWw	Adult	8/8	0.50**	0.55**	0.16	0.56	0.89	8/8	0.28*	0.29**	0.21	0.38	0.76
	Old	12/18	0.33**	0.34**	0.24	0.44	0.85	11/18	0.18*	0.18*	0.07	0.27	0.71
LWw	Adult	8/8	0.51**	0.57**	0.11	0.57	0.89	8/8	0.31**	0.41**	0.17	0.41	0.78
	Old	12/18	0.35**	0.37**	0.19	0.43	0.86	11/18	0.15	0.18*	0.07	0.29	0.66
MXD	Adult	8/8	0.34**	0.38**	0.00	0.41	0.80	8/8	0.25*	0.28**	0.08	0.36	0.73
	Old	12/18	0.27*	0.29**	0.07	0.35	0.81	11/18	0.26*	0.18*	0.07	0.38	0.79
δ^{13}C	Adult	5/5	0.46**	0.54**	0.14	0.57	0.81	5/5	0.37**	0.55**	0.23	0.51	0.75
	Old	5/5	0.36**	0.47**	0.15	0.46	0.74	5/5	0.25*	0.43**	0.05	0.42	0.63
δ^{18}O	Adult	5/5	0.28*	0.34**	0.15	0.43	0.66	5/5	0.50**	0.61**	0.27	0.60	0.83
	Old	5/5	0.12	0.20*	0.00	0.36	0.41	5/5	0.45**	0.50**	0.24	0.54	0.81

Total ring width (*TRW*); early-wood width (*EWw*); late-wood width (*LWw*); maximum density (*MXD*); stable carbon isotope ratios ($\delta^{13}C$); and stable oxygen isotope ratios ($\delta^{18}O$). Chronology statistics were calculated for the period 1900–2006. *LF* low frequency, *HF* high-frequency inter-tree correlation calculated after low- and high-pass filtering with a 10-year centered moving average. *PC1* represents the first principal component in percentage of explained variance. *EPS* is the express population signal

Significance of correlation coefficients is shown: * $P \leq 0.05$; ** $P \leq 0.001$

EWw and LWw at the PU-N site display poor agreements, but stable carbon and oxygen isotope series exhibit the highest inter-tree coherence for both adult ($r = 0.37$, $P < 0.001$ and $r = 0.50$, $P < 0.001$ for δ^{13}C and δ^{18}O, respectively) and old trees ($r = 0.25$, $P = 0.005$ and $r = 0.45$, $P < 0.001$, respectively). In contrast, correlations among δ^{18}O series are always higher at the PU-N site than at the PN-S site, and among density and ring-width series, correlations are higher at the PN-S site (Table 2).

Maximum latewood density (MXD) of PN-S and PU-N shows smaller correlations between individual series and thus, a lower common variance. This lack of coherence for year-to-year and decadal variations is more evident in the older tree group. In general, tree-ring proxies at the PN-S site display higher inter-tree correlations and *PC1*, denoting more similarities among individual series.

Coherence of chronologies

Chronologies of each variable derived from adult and old trees are plotted to visually examine coherence of the chronologies (Fig. 2). At the drier site (PN-S), TRW, EWw and LWw chronologies of adult and old trees display consistently strong inter-series correlations ($r = 0.82$, $r = 0.80$ and $r = 0.81$, respectively; $P < 0.001$), which is mostly due to the high coherences of their inter-annual variations ($r_{\mathrm{hf}} = 0.83$, $P = 0.001$). Similarly, δ^{13}C also shows strong agreement between the adult and old chronologies ($r = 0.80$, $P < 0.001$), in the high-frequency domain ($r_{\mathrm{hf}} = 0.82$, $P < 0.001$) as well as for decadal oscillations ($r_{\mathrm{lf}} = 0.80$, $P = 0.011$). For MXD and δ^{18}O, the correlation coefficients decrease due to a smaller agreement of the inter-annual variations ($r_{\mathrm{hf}} = 0.78$, $P = 0.003$ and $r_{\mathrm{hf}} = 0.72$, $P = 0.007$, respectively) and of the low-frequency variations.

The correlations between the two different age group chronologies are generally lower at PU-N than at PN-S. The stable isotopes δ^{13}C and δ^{18}O display high year-to-year synchrony ($r_{\mathrm{hf}} = 0.79$, $P = 0.003$ and $r_{\mathrm{hf}} = 0.83$, $P = 0.001$, respectively) but small coherences inter-decadally ($r_{\mathrm{lf}} = 0.28$, $P = 0.216$ and $r_{\mathrm{lf}} = 0.06$, $P = 0.43$, respectively). The correlations of EWw and MXD ($r_{\mathrm{lf}} = 0.73$, $P = 0.007$ for both) are higher in the low-frequency domain than in the high-frequency suggesting more differences in the year-to-year variability, while TRW and LWw show less agreement between age groups in the low-frequency domain.

Climate–growth relationship

Tree growth at the PU-N and PN-S sites, especially variables based on ring width, correlates well with the previous summer to autumn temperatures. However, it generally correlates less with precipitation of the current year, which only has a significant influence on the isotope records. At the PN-S site, the previous summer to autumn temperatures

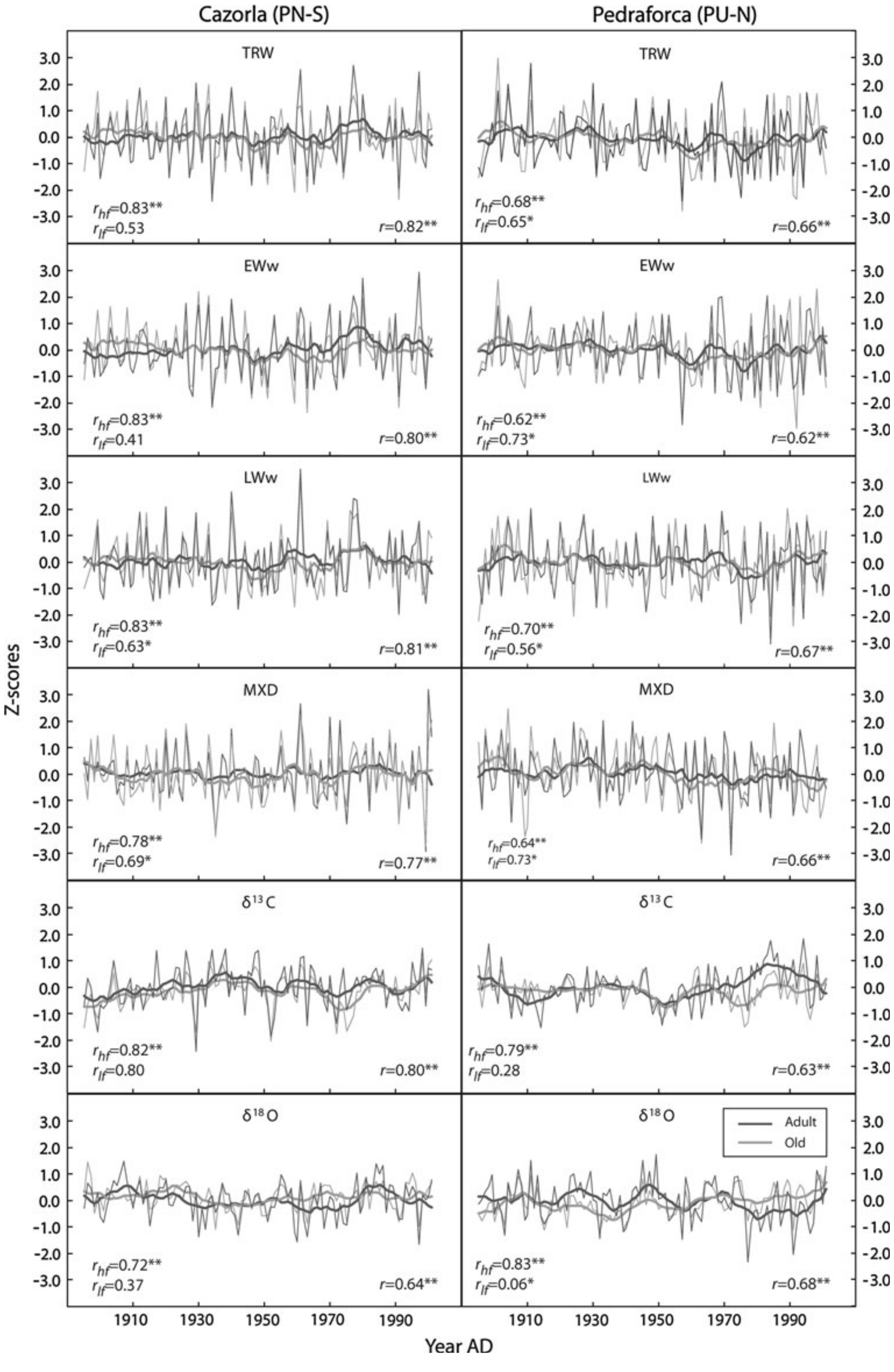

Fig. 2 Comparison of adult and old trees master chronologies for every proxy at two study sites: Cazorla (PN-S) and Pedraforca (PU-N). Tree-ring variables are abbreviated as in Table 2. Medium-to-low frequencies are highlighted by a 10-year moving average and correlations between adult and old chronologies in different time domains listed by *plot*: Pearson's correlation coefficients for unfiltered chronologies (*r*); correlation coefficient for 10-year high-pass (r_{hf}) and for low-pass (r_{lf}) filtered chronologies. Significance of correlation coefficients is shown: * $P \leq 0.05$; ** $P \leq 0.001$

correlate negatively with tree growth (Fig. 3). It affects TRW and EWw significantly but has smaller effects on LWw and no clear influence on MXD or δ^{13}C. Precipitation sums of the previous July–September affect TRW and EWw positively, and late autumn precipitation (November) exerts a positive influence on δ^{13}C variations although it is not always significant.

The PN-S correlation profiles reveal almost identical correlation patterns between current year temperatures and TRW, EWw, LWw and MXD, that is, positive and negative correlations from February to May and June to July, respectively. Precipitation and δ^{13}C correlate negatively from July to September but significant only in July. Similarly, ring-width and density variables correlate negatively

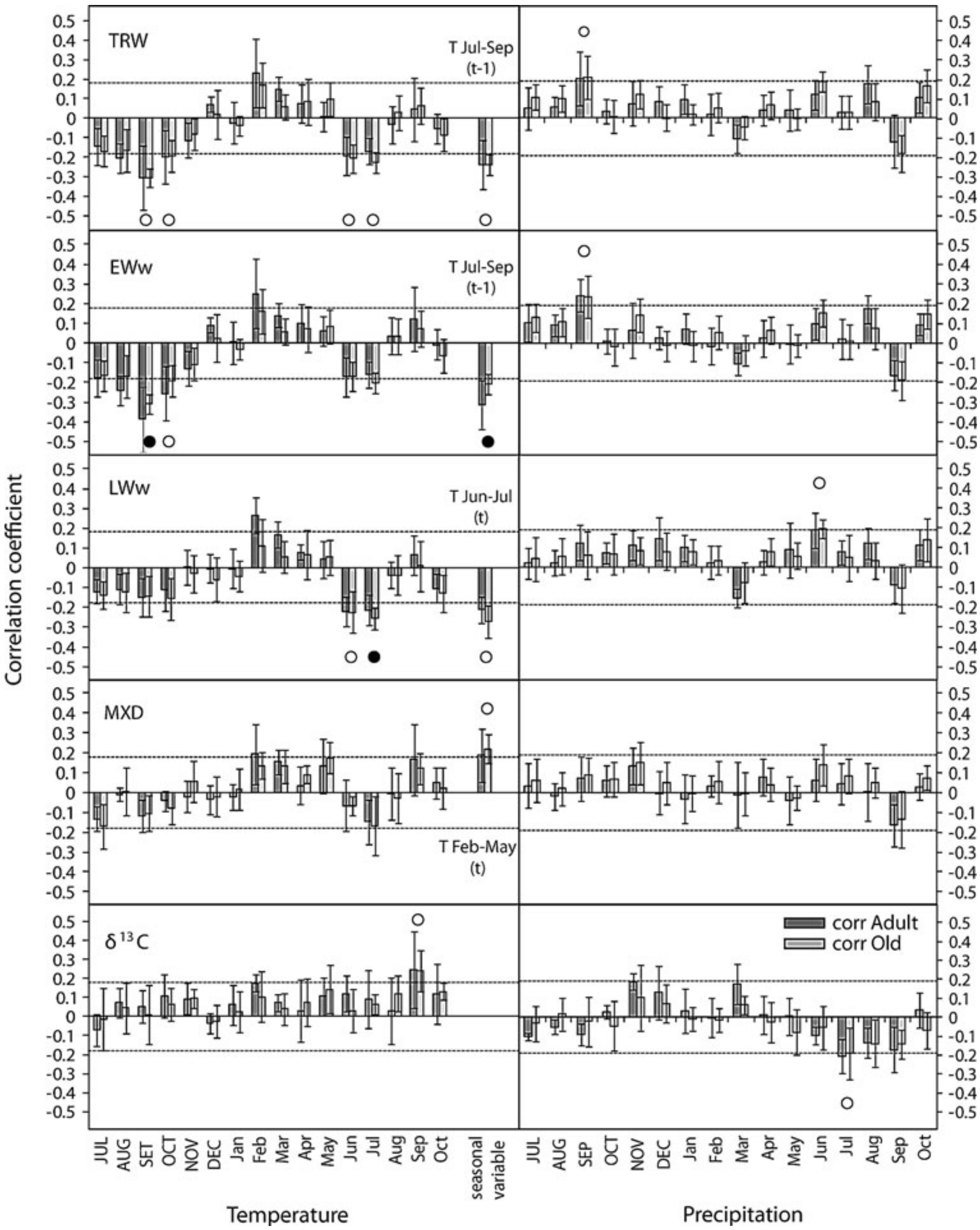

Fig. 3 Cazorla (PN-S) climate and tree-ring proxy mean correlation coefficients and standard deviation from July of the previous year ($t-1$) to October of the current year (t). Only variables with significant correlations with climate are shown, and tree-ring variables are abbreviated as in Table 2. Correlation with the most representative seasonal variable is also shown when significant. *Dashed lines* indicate 95% significance level. The Mann–Whitney test significance is shown: *Open circles* denote non-significant differences and *closed circles* indicate significant differences ($P < 0.05$)

with September precipitation and for TRW and LWw only, positively with summer rainfall.

At the PU-N site (Fig. 4), all tree-ring width parameters are affected positively by the previous summer (July–August) and autumn (October–November) temperatures only separated by a negative influence in September, but no clear effect of previous year precipitation is evident. δ^{13}C at the PU-N site correlates negatively with July–August and October temperatures and September precipitation of the previous year. The δ^{18}O series do not display consistent correlations with climatic factors of the previous year.

At the PU-N site, δ^{13}C and δ^{18}O display the highest correlations with temperatures (positive for late spring to summer) and precipitation (negative for early summer) of the current year. Among ring width parameters, only LWw correlates with temperature (positive in spring and negative in summer) and rainfall (positive in June to July).

MXD does not correlate significantly with climate neither in the previous nor current year of growth. However, summer temperatures (May–September) exert some influence which is, however, inconsistent among age groups. MXD also contains a clear but non-significant summer precipitation signal (May–September) (results not shown).

Age effect on sensitivity to climate

A total amount of 12 (PU-N) and 17 (PN-S) Mann–Whitney tests were performed in order to reveal possible differences between correlation mean values. The test results indicate four significant differences between the two age

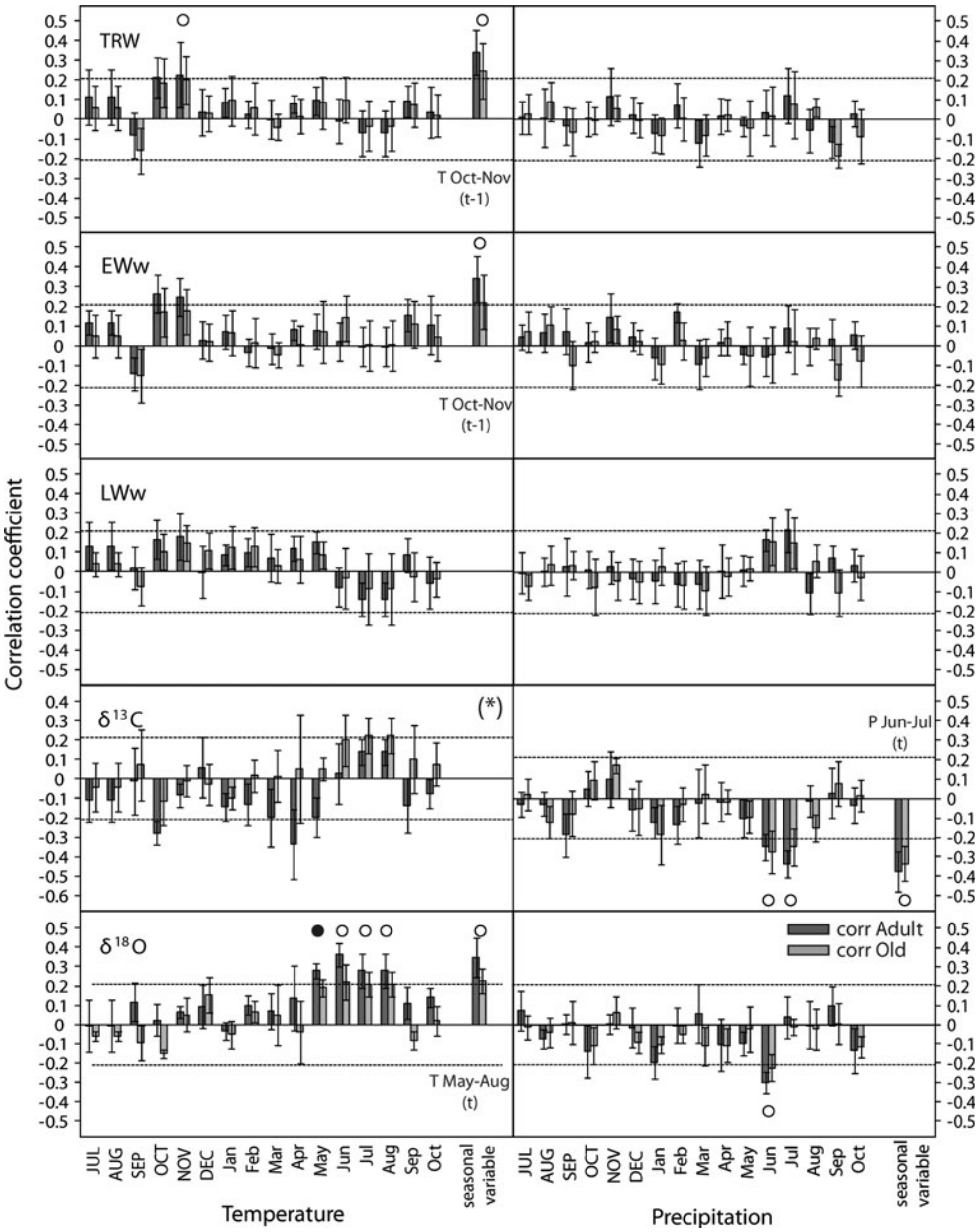

Fig. 4 Pedraforca (PU-N) climate and tree-ring proxy mean correlation coefficients and standard deviation from July of the previous year ($t-1$) to October of the current year (t). Only variables with significant correlations with climate are shown, and tree-ring variables are abbreviated as in Table 2. Correlation with the most representative seasonal variable is also shown when significant. *Dashed lines* indicate 95% significance level. The Mann–Whitney test significance is shown: *Open circles* denote non-significant differences and *closed circles* indicate significant differences ($P < 0.05$). (*) Notice *different scales*

groups. Most of them are related to correlations between proxies and climate for individual months but the differences are insignificant when testing on a seasonal level. For instance, at the PU-N site, significant differences in correlations exist for $\delta^{18}O$ between two age groups and May temperature of the current year ($P = 0.037$) but not for the seasonal temperature variable May to August (t) ($P = 0.060$).

Furthermore, at the PN-S site, significant differences in correlations between LWw and July temperature (t) ($P = 0.043$) have been indicated by the tests. However, these differences are insignificant for the seasonal variable June–July temperature (t) ($P = 0.133$).

At the PN-S site, the strength of the seasonal climate signal expressed as correlations between EWw and July–September temperatures ($t-1$) ($P = 0.019$) is statistically different between adult and old trees (at the 95% level of significance), which suggests that the sensitivity to summer temperatures of the previous year decreases significantly with age.

Discussion

Climate signals in tree ring

The climate–growth relationships displayed by our adult and old tree chronologies are consistent with the reports of previous studies concerned with TRW, EWw and LWw at the Cazorla Range (Martin-Benito et al. 2008; Andreu et al.

2008), TRW at the Pyrenees (Tardif et al. 2003; Büntgen et al. 2007; Andreu et al. 2008) and with regional climate signals found by Andreu et al. (2007).

Tree growth of *P. nigra* individuals at PN-S is correlated negatively with late summer to autumn temperatures. In addition, September precipitation of the previous year exerts a positive influence on TRW and EWw. This suggests a dependency of the metabolic reserves on the previous growing season because warm late summers prolong the growing season and limit the amount of photosynthates stored for the following period of growth (Rozas et al. 2010).

The δ^{13}C series displayed significant negative correlations with July, August and September rainfall denoting a certain degree of summer water stress as may be expected in a Mediterranean climate. Drought conditions are known to result in lower stomata conductance, lower isotope discrimination and therefore in higher tree-ring δ^{13}C (Farquhar et al. 1982; Leavitt and Long 1989; Saurer et al. 2008), but high temperatures promote high evapotranspiration rates which also increase with more rainfall. Negative correlations between δ^{13}C and summer precipitations have also been reported for different pine species at the Iberian Peninsula (Andreu et al. 2008; Voltas et al. 2008) and also across different locations in Europe (Treydte et al. 2007).

The δ^{18}O chronologies at PN-S do not display significant correlations with any of the climatic parameters, and the climate signal is inconsistent among trees. Since the oxygen isotope composition of plant tissues often reflects the variations in δ^{18}O of the source water, differences among individuals may be due to a difference in the water uptake, that is, a variable contribution of surface water versus water from deeper layers may obscure the signal (Marshall and Monserud 2006; Saurer et al. 2008). Ferrio and Voltas (2005) also found that in Mediterranean species such as *Pinus halepensis* Mill. the source water signal is lost due to a strong stomatal regulation and thus a great variability in evaporative enrichment at the leaf level.

At the Pyrenees site (PU-N), late autumn temperatures of the previous year promoting tree-ring growth were reported by Tardif et al. (2003) (November), Büntgen et al. (2007) (October) and Andreu et al. 2008 (October and November). In environments mainly controlled by temperature as at the PU-N site, higher temperatures in autumn favor net photosynthesis and increased food storage that will be available tree growth in the following season (Fritts 1976). We could not identify any significant correlations between the current year temperatures and ring width parameters and MXD series, respectively, at the Pyrenees as has been reported by Tardif et al. (2003) and Büntgen et al. (2007, 2008, 2010). These differences concerning the climate sensitivities are also indicated by the low values for the inter-tree correlations, the common variance and the EPS falling below 0.80 for both adult and old chronologies.

Nevertheless, we found significant May to September temperature signals in the stable oxygen isotope ratios, which suggest an increased leaf water enrichment caused by a combination of low relative humidity and high transpiration rates (Ferrio and Voltas 2005). The correlations between δ^{18}O and climate found in the current study are consistent with earlier reports from the Swiss Alps (Saurer et al. 2008; Battipaglia et al. 2009), and they are also in accordance with regional patterns described by Treydte et al. (2007) for a European network of δ^{18}O.

Age-dependent sensitivity to climate

Previous research on age effects on the tree growth–climate relationship produced mixed results and conclusions. While some studies reported no consistent differences in response to climate across age groups (Linderholm and Linderholm 2004; Carrer and Urbinati 2004; Esper et al. 2008), others pointed out how aging affects tree sensitivity to climate (Szeicz and MacDonald 1994, 1995; Rozas et al. 2009).

In this study, the pattern of significant correlations with precipitation and temperature exhibited by adult and old trees is generally consistent among individuals and across age groups. The correlation patterns between climate and individual trees suggest a homogeneous climatic control by those parameters that affect tree growth significantly and equally to the individual trees. However, the relationship between tree-ring variables and climate is generally stronger in the group of adult trees at both sites (PN-S and PU-N), and the common signal among individuals is also higher in the group of adult trees.

Only a few significant differences in climate–growth correlations were found between age groups. The differences, however, appear in the first or last month of the key climatic periods influencing tree growth. Therefore, such differences may well be due to changes in the timing and duration of the tree-ring formation during the life span of the individual trees, which, in turn, may result in similar, but delayed annual dynamics (Rossi et al. 2008). This might explain the existence of significant differences between adult and old trees concerning the influence of the previous year July to September temperatures on EWw formation at PN-S, while no significant differences were found for TRW during the same period. Except for this particular case, differences in correlation with climate across age groups were statistically not significant on a seasonal basis for any of the tree-ring parameters.

Previous studies have considered different age group categorizations including trees in juvenile growth phases, which are known to differ significantly in structure and function from old trees (Ryan and Yoder 1997; Bond 2000). Thus, significant differences in their climate responses in comparison with mature trees can be expected. In contrast, in the current study, only older trees were considered for two age classes, and thus, our results are comparable only to non-juvenile age groups. In this context, our results are in agreement with those reported for older age groups in Linderholm and Linderholm (2004), Carrer and Urbinati (2004) and Esper et al. (2008). Likewise, Szeicz and MacDonald (1994, 1995) and Rozas et al. (2009) have shown increasing similarities in the climate response of older trees.

Aging and long-term trends

A screening of the standardization procedures applied in publications focusing on the relationship between age effects and climate signals in tree-ring series indicates no general agreement concerning the methods used: they vary from very conservative techniques that retain inter-annual to multi-decadal variations (Szeicz and MacDonald 1994; Linderholm and Linderholm 2004; Esper et al. 2008) to very flexible splines which remove low- to medium-frequency signals and enhance the year-to-year variations (Carrer and Urbinati 2004; Rozas et al. 2009). As a consequence, the total amount of climate information preserved in tree-ring series may vary substantially.

We applied a stiff detrending method in order to preserve medium- to low-frequency climate information. At both sites, the visual comparison of adult and old series suggests a strong synchrony in most instances, which implies a common sensitivity to annual and decadal climate variations independently of age which is in accordance with results reported by Esper et al. (2008).

In the context of low-frequency signal preservation, the needless to standardize has been proposed as one of the crucial advantages of isotope dendroclimatology, in contrast to the more traditional tree-ring proxies (e.g., TRW and MXD). This is based on the assumption that after passing through the "juvenile effect" observed in younger rings (Freyer 1979; Francey and Farquhar 1982), stable isotope series may not contain any long-term age-related trend (McCarroll and Loader 2004; Gagen et al. 2007, 2008). Our results show a general high synchrony in the high-frequency domain but, except for PN-S δ^{13}C, differences in the inter-decadal variations exist pointing to the existence of variations unrelated to climate, as reported recently by Treydte et al. (2009). Especially at PU-N, adult and old chronologies diverge consistently during the last 30 years. In general, δ^{18}O data display a poorer agreement in the low-frequency domain at both sites, in contrast to some studies reporting stable oxygen isotopes as good recorders of low-frequency climate variations (Treydte et al. 2006; Treydte et al. 2007; Saurer et al. 2008). Nevertheless, our study did not identify any significant differences concerning the climate response of stable isotopes across age groups. Therefore, it remains unclear whether this noise in the low-frequency domain is age dependent, related to individual phenotypical plasticity or due to microsite-condition changes, and thus, further studies are needed to clarify the origin of this trend.

In general terms, trees of different ages and sizes often experience substantially different external environments (Day et al. 2002). Older trees are described to experience more hydraulic limitations and lower photosynthetic capacities (Ryan and Yoder 1997). However, it is still unclear how individuals cope with physiological changes associated with aging and what is the ultimate cause of declining tree growth (Becker et al. 2000; Ryan et al. 2006). Tree-ring records are integral results of tree–water relations and carbon balance under the constraining influence of climate (Zweifel et al. 2006; Fonti et al. 2010). Tree growth can proceed only as fast as allowed by the primary limiting climate factor restricting growth (Fritts 1976). According to the rule of limiting factors, the differences between individuals are minimized under more restrictive external conditions because the range of possible responses is reduced. This may explain the consistency in response of every single tree to the same climate factor (limiting factor) as well as the absence of significant differences across age groups in most of the cases presented here.

Conclusions

Information concerning the influence of aging on the relationship of tree-ring proxies and climate is mostly restricted to tree-ring width, while data for density and stable isotope trends are scarce. To our knowledge, no literature seems to be available concerning age effects on density data, while insights from studies about age effects on isotope series were inconclusive.

Our findings do not support the idea of an age-dependent response to climate in trees in old-growth life stages that may lead to inaccurate climate reconstructions. No significant differences across age groups were found in response to the key climatic parameters and periods of different tree-ring proxies studied. Moreover, the responses of individual trees to climate are very similar, denoting that the same factor has constrained growth in all trees.

In general terms, many links between tree-physiological processes and wood production are still unclear, but functional and ecophysiological adjustments associated with tree aging do not seem to result in significant changes in sensitivity to climate factors limiting tree growth as has been evidenced by our study.

It has been emphasized previously that climate–growth responses in relation to age effects may vary with changing locations and tree species, but variations may also be due to the tree-ring proxy used, the age class categorization or even the standardization method applied.

More investigations focusing on crucial topics such as non-climate-related trends in stable isotopes are needed since such trends may obscure inter-decadal to multi-centennial climate signals. It would also be of interest to use long tree-ring proxy records in ecophysiological studies to clarify the link between ecophysiological processes and long tree-ring series; however, such records barely exist, and their potential is still unexplored.

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