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Research paper

Climate signals derived from cell anatomy of Scots pine in NE Germany

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Tree-ring chronologies of *Pinus sylvestris* L. from latitudinal and altitudinal limits of the species distribution have been widely used for climate reconstructions, but there are many sites within the temperate climate zone, as is the case in northeastern Germany, at which there is little evidence of a clear climate signal in the chronologies. In this study, we developed long chronologies of several cell structure variables (e.g., average lumen area and cell wall thickness) from *P. sylvestris* growing in northeastern Germany and investigated the influence of climate on ring widths and cell structure variables. We found significant correlations between cell structure variables and temperature, and between tree-ring width and relative humidity and vapor pressure, respectively, enabling the development of robust reconstructions from temperate sites that have not yet been realized. Moreover, it has been shown that it may not be necessary to detrend chronologies of cell structure variables and thus low-frequency climate signals may be retrieved from longer cell structure chronologies. The relatively extensive resource of archaeological material of *P. sylvestris* covering approximately the last millennium may now be useful for climate reconstructions in northeastern Germany and other sites in the temperate climate zone.

Keywords: cell structure, dendroclimatology, *Pinus sylvestris*, quantitative wood anatomy, tree rings.

Introduction

It is well known that tree-ring width (TRW) can be used for reconstructing past climate variations, most of which have been achieved with samples from trees growing near their altitudinal or latitudinal limits (Fritts 1976). Typically, prominent regional and hemispherical temperature reconstructions are mainly or entirely based on tree-ring series from extreme sites of the species sampled (e.g., Esper et al. 2002, Grudd et al. 2002, Büntgen et al. 2005), whereas most people live in the temperate zone. Thus, past climate information from this zone would be a valuable asset. Since climate change occurs with different regional impacts (Schönwiese 2008, Heinrich et al. 2013), increased knowledge about the past climate derived from proxy data such as tree rings in the more temperate regions of the world is sought after.

Tree-ring series derived from extreme sites often have the advantage that one particular climate variable such as summer temperature can easily be identified as the principal factor limiting growth. The climate–growth relationship at temperate sites is often more diffuse and challenging to the analyst. However, armed with good knowledge of the species and study region, a proper site selection may lead to significant correlations between tree rings and a climate factor (Drew et al. 2013). A second advantage of sampling at study sites with more extreme environments often is the fact that trees at such sites can usually grow to very old ages, which facilitates the establishment of long chronologies. People preferably settled within the more temperate regions and thus a larger amount of old construction material can be derived from such locations. The abundance of this material, which is often

lacking in extreme environments, may be used to expand the relatively short chronologies derived from living trees further back in time (Büntgen et al. 2011).

An additional advantage of sample material from long-living trees is the minimization of the segment length curse (Cook et al. 1995), which means that a mean chronology comprised of individual series of relatively short segment length can only reconstruct climate on frequencies that are at maximum the length of the average segment lengths. Since TRW series need to be detrended, even more low-frequency signals are lost during this process. Therefore, utilizing raw value series would be beneficial for sustaining low-frequency climate signals.

A big challenge of dendroclimatology conducted with sample material derived from tree species growing away from their distributional limits is to find a clear climate signal, in other words, to find a significant correlation between one tree-ring variable and one particular climate variable which can then be used for a reliable long-term reconstruction (Fritts 1976). To tackle this challenge, one of the potential candidate tree species in Europe is *Pinus sylvestris* L., because it is widely distributed and its tree rings have been explored comprehensively at extreme sites. Tree-ring width chronologies of *P. sylvestris* have been extensively used in dendrochronology in the past decades, especially in the cold climate of Scandinavia (e.g., Grudd 2008, Helama et al. 2008) and hot and dry climate of the Mediterranean (e.g., Gutiérrez 1989, Bogino et al. 2009, Martin-Benito et al. 2013). The only dendroclimatological investigation of *P. sylvestris* in the northeastern German lowlands was conducted by von Lührte (1991), who analyzed TRWs of *P. sylvestris* growing in forests in and near Berlin. The author found that tree growth was limited mostly by cold winters and dry summers. Except for the work of von Lührte (1991) no more tree-ring studies focusing on *P. sylvestris* in northern Germany have been published, as far as we know.

Apart from pure TRW chronologies, *P. sylvestris* has been used for palaeoclimatological studies on wood density (e.g., Briffa et al. 2001, Grudd 2008), blue reflectance/intensity (McCarroll et al. 2002, Campbell et al. 2007) and stable isotopes (e.g., Hlasvuori et al. 2009, Loader et al. 2013). Recently, quantitative wood anatomical measurements have been developed to examine the climate response of *P. sylvestris* (Eilmann et al. 2009, Seo et al. 2012, Martin-Benito et al. 2013). While Eilmann et al. (2009) in the dry Valais of Switzerland and Martin-Benito et al. (2013) in Spain mainly found drought signals in the cell variables, it was not surprising that Seo et al. (2012) identified mainly temperature signals in the cell chronology from northern Finland (Figure 1). In all three studies the authors were able to identify stronger or different climate signals than had previously been reported for other variables. The previous studies have focused on drought or temperature-limited sites at the northwestern and southwestern distribution limits of the species, but dendroclimatological studies from

regions with less limiting growth conditions such as the north-eastern German lowlands are much less common. Our study aims to analyze the climate response of *P. sylvestris* cell variables in northern Germany, which is well within the latitudinal and altitudinal limits of the species' distribution.

Most quantitative wood anatomical studies have been conducted on angiosperm wood (e.g., Sass and Eckstein 1995, García-González and Eckstein 2003, Tardif and Conciatori 2006, Fonti and García-González 2008, Giantomasi et al. 2009, Campelo et al. 2010), but comparable studies on conifer wood are less common (e.g., Yasue et al. 2000, Wang et al. 2002, Panyushkina et al. 2003, Vaganov et al. 2006, DeSoto et al. 2011). The relative shortage of such studies has been ascribed to the difficulty of developing long series of cell structure measurements from conifers with standard methods of quantitative wood anatomical research (Liang et al. 2013). The above-mentioned studies of *P. sylvestris* developed cell chronologies for relatively short periods (e.g., 45 years (Eilmann et al. 2009), 47 years (Seo et al. 2012) and 46 years (Martin-Benito et al. 2013)). As one solution to this dilemma, Liang et al. (2013) have proposed a novel approach applying confocal laser scanning microscopy to increment core surfaces for efficient histometric analyses.

In this study, we utilized this new method to probe the potential of cell anatomical features of *P. sylvestris* from the north of Germany to encode climate-related information. Therefore, the objectives are (i) to build long chronologies of various cell features of *P. sylvestris*, (ii) to examine them for climatic signals and (iii) to compare the climate responses of TRW and cell structure measurements.

Materials and methods

Study area and sampling

The study site is located on the Telegrafenberg hill, Potsdam, Germany (52°23'N, 13°04'E, 94 m a.s.l.) (Figure 1). The Telegrafenberg hill is an end moraine hill situated ~60 m higher than the surrounding city of Potsdam (34 m a.s.l.). This study site was chosen because of the presence of an old mixed forest. Since Telegrafenberg was used by the Prussian army for long-distance message transmissions and later as a scientific observatory, the area including the forest was fenced in the early 19th century. This fence has been in place for >150 years and has resulted in undisturbed forest stands. Moreover, a high-quality meteorological station with several variables measured since 1893 is located only 200 m away from the study site. The long-term meteorological station Potsdam Telegrafenberg is one of the very few meteorological stations worldwide comprising such a comprehensive observation program going back without gaps over a period of >100 years in daily resolution (http://saekular.pik-potsdam.de/2007_en/index.html). The daily meteorological data mean, maximum and

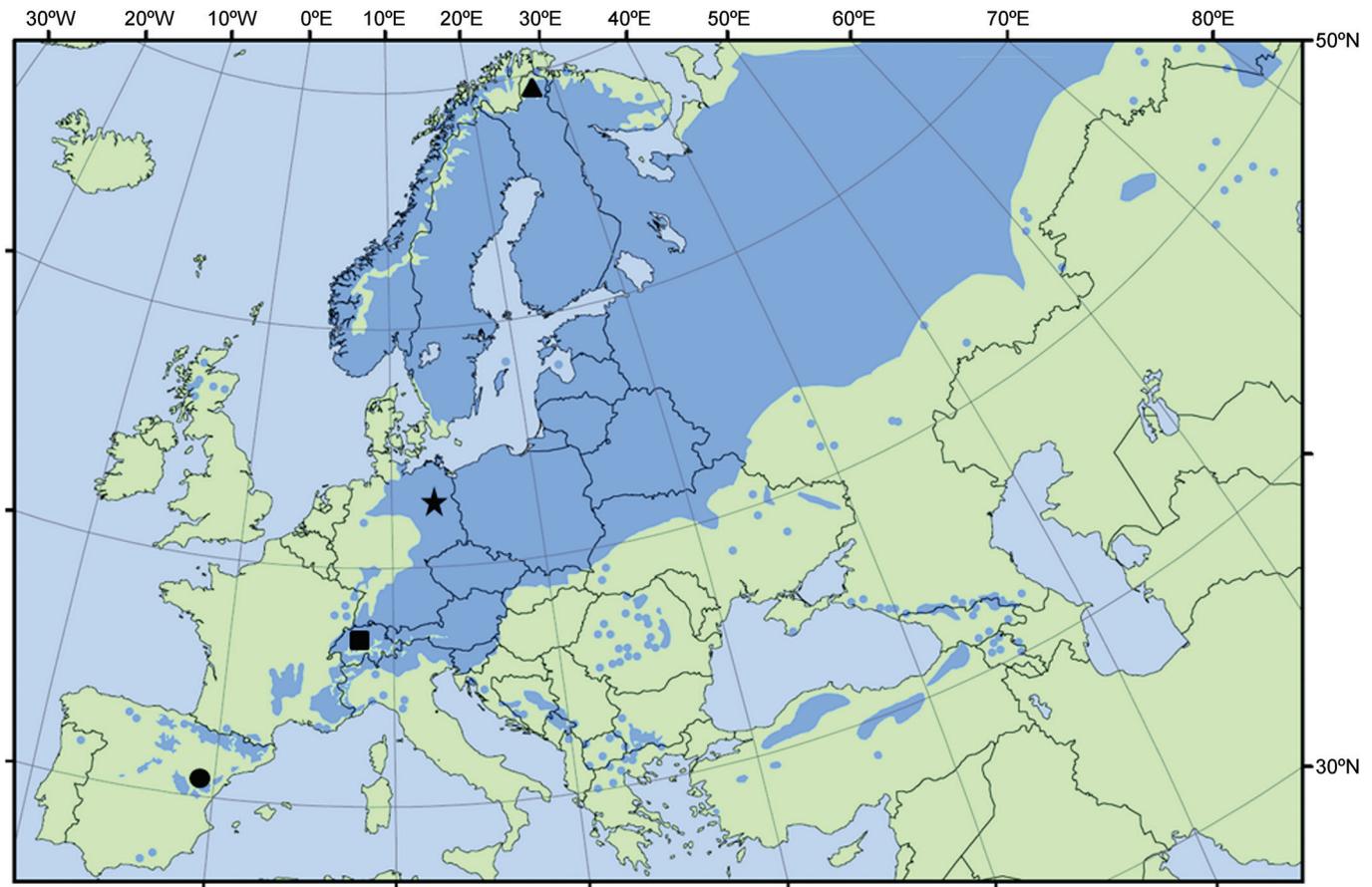


Figure 1. Natural distribution map of *P. sylvestris* (gray) and locations of the study sites. (Black star: our sample site in Germany, black square: site of Eilmann et al. (2009) in Switzerland, black circle: site of Martin-Benito et al. (2013) in Spain, black triangle: site of Seo et al. (2012) in Finland.) (Distribution map of Scots pine (*P. sylvestris*) EUFORGEN 2009, www.euforgen.org.)

minimum air temperature, precipitation sum, cloudiness, relative humidity, sun duration and vapor pressure available from the station were used for climate–growth comparisons. The local climate is characterized by a mean annual temperature of 8.8 °C and a total annual precipitation of 590.2 mm. The work was carried out on increment cores of 15 dominant, at least 140 years old, *P. sylvestris* growing in the forest of Telegrafenberg. Two 5-mm cores were collected at breast height from each tree parallel to the slope to avoid reaction wood.

Measuring TRW and wood anatomical variables

For the analysis of TRW and earlywood width (EWW), all 30 increment cores were prepared and analyzed following dendrochronological procedures described by Stokes and Smiley (1968), Schweingruber (1983) and Cook and Kairiukstis (1990). The earlywood and latewood classification were performed using Mork's formula (two double cell wall thicknesses (CWT) exceed the lumen diameter) (Denne 1988).

For cellular analyses, some cores were less suitable for preparation of cell structure measurements due to poorer quality of the ring structure visibility resulting from occasional

missing rings, light rings and resin ducts. Therefore, 10 cores of 8 trees were selected for further analysis. The sample preparation and image analysis followed the procedures described by Liang et al. (2013) for the period 1900–2009. This novel procedure combines readily available hardware and software equipment to conduct cell structure measurements on increment cores of conifers. The essential part of the procedure is the application of a confocal laser scanning microscope with the big advantage of capturing images directly from increment core surfaces. Cell wall and lumen are displayed with a strong contrast due to the monochrome black and green nature of the images. In the current study, the long cores were subdivided into pieces of 5-cm length because shorter wood samples were easier to handle during the following wood anatomical preparation. Then, the subdivided cores were surfaced carefully with the advanced core-microtome of the Swiss Federal Institute for Forest, Snow and Landscape Research (Gärtner and Nievergelt 2010). The evenness of the core surfaces facilitated images of relatively small magnification (×100) taken directly from the surface of the cores. The small magnification ensured that considerable parts of the sample surface could be fit into one image. After all individual successive images of

one core sample were taken they were precisely merged into one long image using Adobe Photoshop image editing software. Cell structure measurements were made on the merged micro-images using the WinCELL 2011a (Regent Instruments Inc., Québec, Canada) image analysis program.

All tracheid measurements were done on the earlywood part of the tree ring. We concentrated on earlywood because the earlywood cells are still large enough for analyses with relatively small magnifications such as 100 times. Wood structures such as resin ducts, ray cells and gaps were excluded manually from the analysis to avoid measurement mistakes. A special type of analysis procedure is provided in WinCELL 2011a to analyze the cell structures in individual tree rings. The process was conducted semi-automatically, that is, the ring boundaries were first identified visually, the boundaries traced and the years entered by the operator. Once all ring boundaries were identified, WinCELL created analysis regions in each tree ring by closing the regions delimited by the ring boundary paths. The result was one analyzed region per tree ring and the cell measurements were stored individually for each tree ring. Furthermore, potential measurement mistakes are automatically removed by setting reasonable filters (area, length, width, form and length-to-width ratio) for excluding large or small morphological features (Liang et al. 2013). Several earlywood variables were considered for this study, all derived from the initial WinCELL data files: total lumen area (TLA), average lumen area (ALA), average lumen diameter (ALD), cell number (CN) and CWT. In addition, following the procedures suggested by Campelo et al. (2010) for angiosperm wood, we selected only a subset of cell measurements from each tree ring: starting always with the first row of tracheids, we selected as many tracheid lumen as needed adding up to the first 30% lumen area. The individual values of the first 30% lumen area were then averaged into 30% ALA (30%ALA). Moreover, we selected the 30 largest tracheid lumen from each tree ring and then averaged them into 30 maximum ALA (30MAX). The measurements of TLA, ALA, ALD, CN, CWT, 30%ALA and 30MAX taken for individual series were then averaged into site chronologies

Data analysis

The statistical properties of the time series were evaluated by means of five coefficients commonly used in dendrochronology: mean sensitivity (MS), the expressed population signal (EPS), the mean correlation between trees (MC), the first-order autocorrelation coefficient (AutoR) and the signal-to-noise ratio (SNR). Mean sensitivity is an expression of the year-to-year variation and is calculated for individual series by the formula:

$$MS = \frac{2}{N-1} \times \sum_{i=1}^{i=N} \frac{|C_{i+1} - C_i|}{C_{i+1} + C_i}$$

where i is the ring number, C_i is the value associated with a ring number i and N is the number of measured rings per core. The individual values of MS are then averaged into the overall MS.

Expressed population signal indicates the extent to which the sample size is representative of a theoretical population with an infinite number of individuals, according to the following equation:

$$EPS(t) = \frac{\bar{r}_{bt}}{\bar{r}_{bt} + (1 - \bar{r}_{bt}) / t} = \frac{t\bar{r}_{bt}}{t\bar{r}_{bt} + (1 - \bar{r}_{bt})}$$

where t is the number of tree-ring series averaged (one core per tree) and \bar{r}_{bt} is the mean between-tree correlation.

Mean correlation is an average of the correlations calculated pairwise between the individual samples and thus a measure of the strength of the common signal; AutoR, calculated for individual series and then averaged into one mean AutoR, is a measure of persistence of the time series and expresses the influence of previous years upon growth; and SNR is an expression of the strength of the observed common signal among the trees in a chronology and thus used to identify the reliable portion of the mean (Cook and Kairiukstis 1990). The SNR is defined as:

$$SNR = \frac{N\bar{r}}{1 - \bar{r}}$$

where \bar{r} is the average correlation between samples and N is the number of samples in the ensemble of standardized tree-ring indexes.

The time series were examined visually for age trends. If present, the series were first power transformed and then detrended applying a 66-year cubic smoothing spline function with a 50% cutoff in conjunction with the residuals method, i.e., 50% of the variance in each series at the period of 66 years was removed, using the program ARSTAN (Cook et al. 1990). Cross-correlations between the chronologies of all tree-ring variables were compared to examine whether they vary similarly and thus are likely to contain the same climatic and environmental information for the common period 1900–2009. Cross-correlations were also calculated with the detrended data of TRW, EWW, TLA and CN.

Climate–growth relationships were investigated with the CLIMTREG V.4 program (Beck 2007). CLIMTREG allows investigation of the relation between the measured wood variables and climate data with a high temporal resolution rather than using the traditional monthly correlations. In the program, moving Pearson correlations between climate and tree-ring data are calculated. Daily meteorological data are summarized by the program for a minimum period of 21 days and a maximum of 121 days. Correlations are first calculated between 21-day

averages of the climate data and the selected tree-ring variables. For the next round of analysis, 1 day is added until the maximum window length of 121 days is reached. The moving correlation process starts on 1 July of the previous year and stops at 31 October of the current year, moving day by day along the calendar overall resulting in 42,218 correlations per analysis. The CLIMTREG software was used for the first time to investigate correlations between wood anatomical variables and daily sets of climate data for a period of 109 years (Beck 2007). Cross-correlations between eight daily climate factors (mean, maximum and minimum air temperature, precipitation sum, cloudiness, relative humidity, sunshine duration and vapor pressure) and six variables derived from tree rings and cell measurements (tree-ring index (TRI), ALA, CWT, ALD, 30%ALA and 30MAX), respectively, were calculated.

Results and discussion

Comparing the chronologies

We found very high correlations between TRW, EWW, CN and TLA ($r > 0.85$). Earlywood tracheids are large empty conduits with thin cell walls serving the purpose of water transport from the roots up to the canopy (Vaganov et al. 2006). In our study, EWW composed 70–80% of the total TRW. Moreover, all chronologies of the above-mentioned variables displayed an age trend and a peak in the late 1920s (Table 1, Figure 2). Since the age trend in TRW and thus in EWW, CN and TLA variables are related to the increasing circumference of the tree stem, they needed to be accounted for and were detrended.

High correlations between TRW and EWW, as in our study ($r = 0.98$), were also found in *P. sylvestris* located at its south-western limit of distribution by Martin-Benito et al. (2013) and Seo et al. (2012), although the latter observed lower correlations in comparison ($r = 0.74$). High positive correlations between TRW and CN were also observed by Martin-Benito et al. (2013) for the same species, but also for *Pinus uncinata* Ramond ex DC. in Lam. et DC. and *Larix cajanderi* Mayr. (Camarero et al. 1998, Panyushkina et al. 2003). The results of Olano et al. (2012) concerning *Juniperus thurifera* L. also point

in the same direction with similar climatic signals encoded in the CN and ring-width chronologies. Giantomasi et al. (2009) also noticed positive and strong correlations between the total vessel area and TRW of *Prosopis flexuosa* DC.

As opposed to the tree-ring variables discussed above, ALA, CWT, ALD, 30%ALA and 30MAX chronologies presented a distinct positive trend that could not be explained with increasing age. Moreover, chronologies of TRW, EWW, CN and TLA no longer exhibited significant correlations with ALA, CWT, ALD, 30%ALA and 30MAX after detrending by fitting a cubic smoothing spline function with 66-year stiffness and 50% cut-off, using the program ARSTAN (Cook et al. 1990) (results not shown). Since the correlations were significantly negative before detrending (see Table 1), it may be derived that the significant correlations were mainly due to the long-term age trend in the first group of variables. It is interesting that ALA differed so much from the TLA but further investigations are needed to clarify why this is the case in *P. sylvestris*. The correlations between ALA and the other wood anatomical variables (CWT, ALD, 30%ALA and 30MAX) are significantly positive, with the highest correlation found with ALD ($r = 0.83$), which suggests that both variables might respond to similar environmental variables and thus, might contain comparable climate information.

Apart from the detrended TRW (further referred to as TRI for tree-ring index), raw values of ALA, CWT, ALD, 30%ALA and 30MAX were selected for further investigations of their chronology qualities and climate–growth relationships. The variables EWW, TLA and CN were discarded from further analyses because of their similarities with TRI.

Quality of chronologies

Before correlation analyses with climate data could be conducted, the statistical quality of the selected chronologies was tested. Several statistical parameters were calculated to evaluate chronology qualities (Table 2).

In general, TRI exhibited a stronger common signal than those of the cell measurement variables. Similar results were also found by Seo et al. (2012) and Martin-Benito et al. (2013)

Table 1. Correlations among raw measurement chronologies of all tree-ring variables (common period 1900–2009).

	TRW	EWW	TLA	CN	ALA	ALD	CWT	30%ALA
EWW	0.98							
TLA	0.95	0.98						
CN	0.86	0.88	0.89					
ALA	−0.60	−0.59	−0.58	−0.51				
ALD	−0.46	−0.42	−0.42	−0.30	0.83			
CWT	−0.51	−0.51	−0.54	−0.52	0.65	0.56		
30%ALA	−0.54	−0.53	−0.51	−0.39	0.81	0.77	0.51	
30max	0.16	0.19	0.22	0.22	0.47	0.48	0.17	0.53

TRW, tree-ring width; EWW, earlywood width; TLA, total lumen area; CN, cell number; ALA, average lumen area; ALD, average lumen diameter; CWT, cell wall thickness; 30%ALA, the first 30% average lumen area; 30MAX, 30 maximum average lumen area.

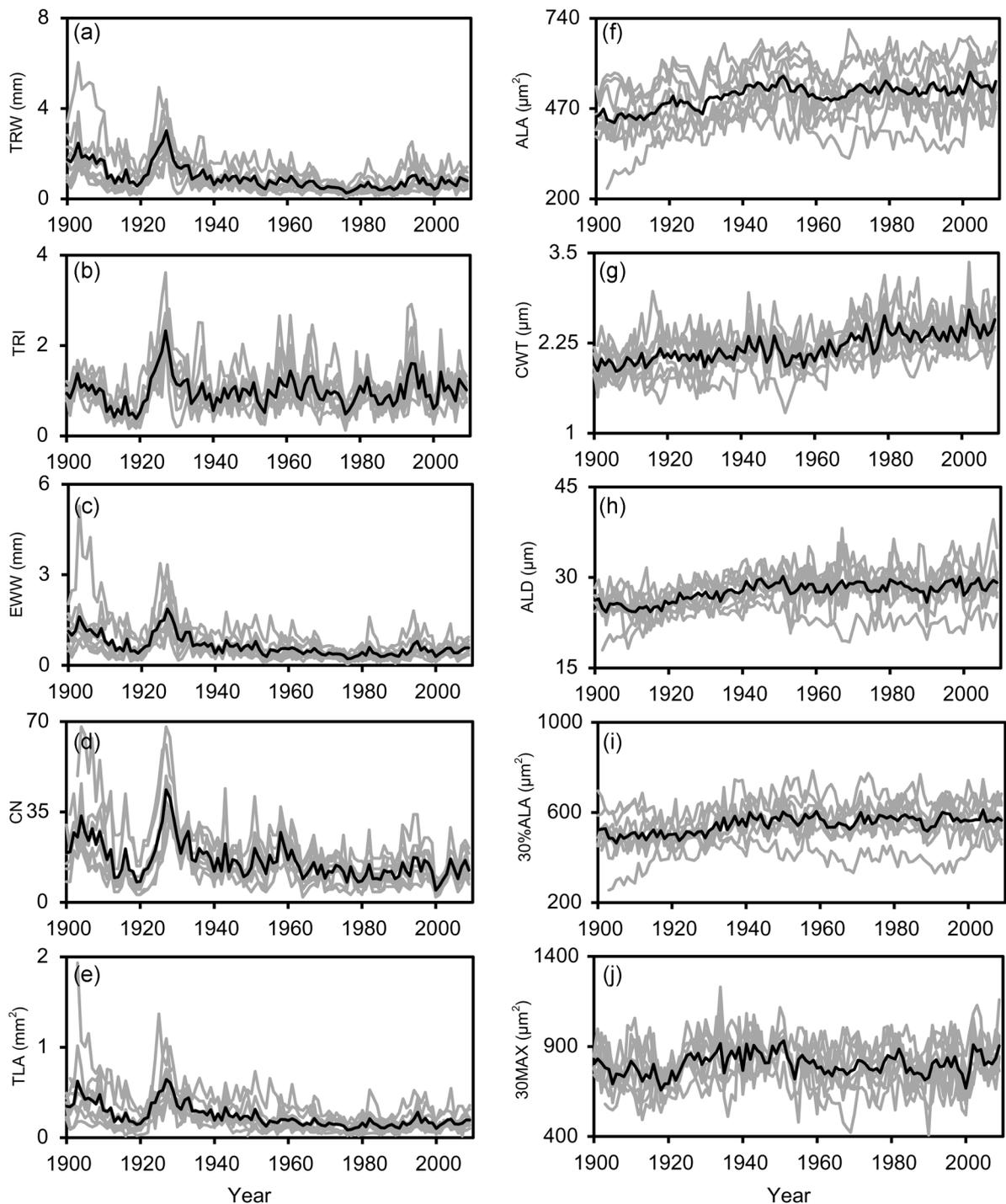


Figure 2. Time series of (a) tree-ring width (TRW), (b) tree-ring index (TRI), (c) earlywood width (EWW), (d) cell number (CN), (e) total lumen area (TLA), (f) average lumen area (ALA), (g) cell wall thickness (CWT), (h) average lumen diameter (ALD), (i) the first 30% ALA (30%ALA), (j) the 30 maximum ALA (30MAX) for the period 1900–2009. Gray lines refer to individual and thick bold lines to the chronology.

for *P. sylvestris* and by Olano et al. (2012) for *J. thurifera*. They observed that cell lumen and cell wall chronologies showed in most cases lower common signals than ring-width series. This was also reported in several studies on ring-porous species (e.g., Fonti and García-González 2004, Tardif and Conciatori 2006, Fonti and García-González 2008). Fonti and

García-González (2008) demonstrated that a higher chronology quality does not necessarily guarantee a better climatic signal in cell measurement variables. A common signal indicates the statistical quality of each variable, but does not describe the climatic information they record (Fonti and García-González 2004). It is therefore beneficial to explore the relation

Table 2. Statistical characteristics of TRW and cell measurement variables: TRI, tree-ring index; ALA, average lumen area; ALD, average lumen diameter; CWT, cell wall thickness; 30%ALA, the first 30% average lumen area; 30MAX, 30 maximum average lumen area. MS, mean sensitivity; AutoR, first-order autocorrelation coefficient; MC, mean correlation; EPS, expressed population signal; and SNR, signal-to-noise ratio.

	TRI	ALA	CWT	ALD	30%ALA	30MAX
MS	0.32	0.07	0.09	0.08	0.07	0.09
AutoR	0.82	0.19	0.16	0.13	0.13	0.15
MC	0.52	0.29	0.27	0.15	0.20	0.22
EPS	0.89	0.78	0.87	0.81	0.80	0.65
SNR	8.05	3.56	6.92	4.20	4.01	1.88

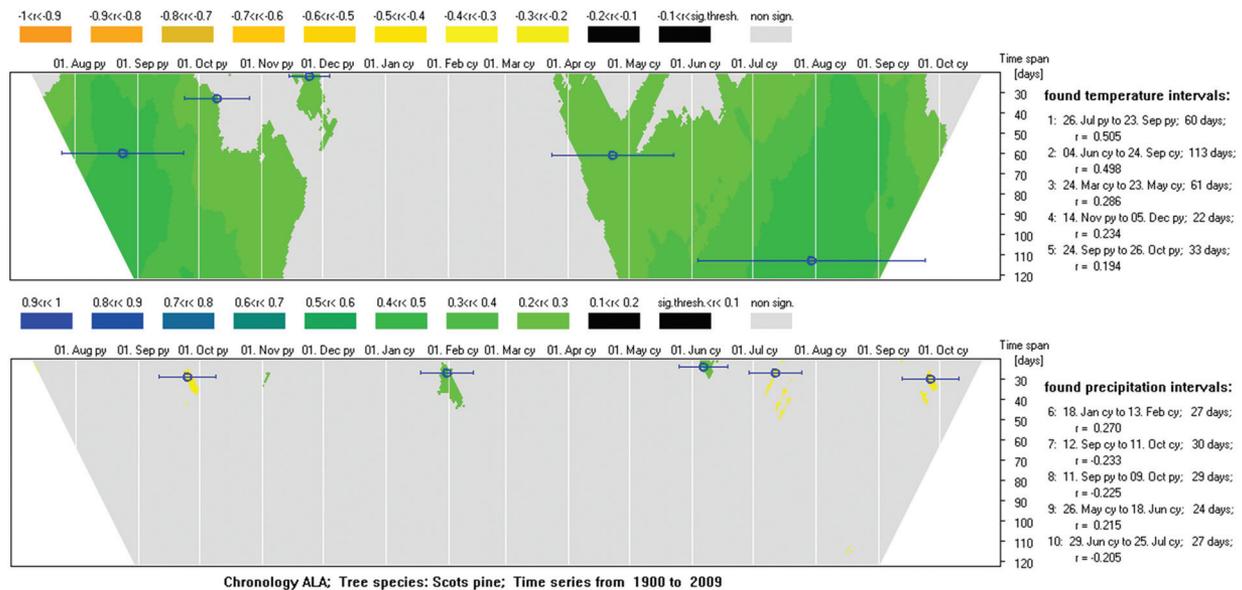


Figure 3. Example of a CLIMTREG analysis output calculated between ALA and daily minimum temperature (top) and daily precipitation (bottom): Grey bars indicate strength and direction of correlations.

between cell anatomical variables and climate data for a comparison with TRI, even though the statistical parameters for the cell measurement variables may indicate a smaller statistical quality.

Climate–growth relationships

The application of the CLIMTREG software was successful in identifying several significant climate–growth relationships (see Figure 3 for an example of a CLIMTREG analysis output). The most significant correlations were assembled and compared for further discussion (Table 3). In general, 48 positive and 12 negative significant correlations were identified. These associations between climate factors and tree-ring variable chronologies revealed important similarities but also different signals between TRI and cell measurement variables.

Correlations between TRI and May-to-June mean and maximum temperatures were significantly negative, while the correlations between TRI and July/August minimum temperature and precipitation, respectively, were significantly positive. Cloudiness and sunshine duration of March–April and May–June, respectively, negatively correlated with TRI. However, the

strongest correlations with TRI were displayed for May-to-July relative humidity and July-to-August vapor pressure ($r = 0.408$ and $r = 0.425$, respectively). The combination of positive correlations with relative humidity and vapor pressure on the one hand and negative correlations with mean and maximum temperatures and sunshine duration on the other strongly indicates high sensitivity to drought for *P. sylvestris* at Telegrafenberg during the summer months. Obviously, the climate signal encoded in TRI was more related to moisture stress variables of the current year. Similar results were presented by von Lürthe (1991) for *P. sylvestris* growing in southwestern Berlin, 5 km away from our site, and for *Pinus nigra* J.F. Arnold and *P. sylvestris* in Spain (Martin-Benito et al. 2013). Furthermore, von Lürthe (1991) found significant positive correlations between TRWs and winter temperatures, which could not be corroborated at our site.

Generally, the cell variables were more sensitive to temperature especially of the late summer conditions of the previous year (Table 3). The highest correlations for cell variables were found between ALA and minimum July-to-September temperatures and mean August-to-October temperatures of the

Table 3. Correlations between tree-ring variables and climate parameters for the period 1900–2009. TRI, tree-ring index; ALA, average lumen area; ALD, average lumen diameter; CWT, cell wall thickness; 30MAX, 30 maximum average lumen area; 30%ALA, the first 30% average lumen area. Cell measurement variables are raw values. Bold values indicate 10 most significant correlations (critical signal values: 95%: 0.1874, 99%: 0.2446 and 99.9%: 0.3095). Grey backgrounds reflect negative correlations. Months in capital letters indicate current year, small letters indicate previous year.

	Mean temperature	Maximum temperature	Minimum temperature	Precipitation	Cloudiness	Relative humidity	Sunshine hours	Vapor pressure
TRI	17 MAY to 27 JUN -0.272	09 MAY to 28 JUN -0.355	11 JUL to 10 AUG 0.275	03 jul to 25 jul 0.303	13 MAR to 08 APR -0.277	08 MAY to 07 JUL 0.408	09 MAY to 24 JUN -0.323	03 JUL to 12 AUG 0.425
ALA	27 jul to 16 oct 0.452	01 AUG to 27 AUG 0.401	26 july to 23 sep 0.505	18 JAN to 13 FEB 0.270	19 JAN to 16 FEB 0.228	08 MAR to 21 APR -0.299	20 jul to 27 oct 0.349	27 jul to 29 sep 0.375
ALD	30 jul to 22 aug 0.419	01 AUG to 27 AUG 0.341	28 jul to 12 sep 0.427	01 sep to 21 sep -0.293	26 APR to 31 MAY 0.291	27 APR to 27 MAY 0.293	19 jul to 05 sep 0.362	02 JUN to 10 AUG 0.356
CWT	27 jul to 26 aug 0.440	27 july to 26 aug 0.426	28 july to 25 aug 0.432	24 JUN to 16 JUL -0.301	23 JUL to 22 AUG -0.212	23 july to 26 aug -0.228	23 july to 26 aug 0.276	27 july to 24 nov 0.381
30MAX	02 aug to 31 aug 0.234	08 JUN to 28 JUN 0.231	25 jul to 28 aug 0.220	10 dec to 30 dec -0.299	26 JAN to 19 FEB 0.285	03 MAR to 26 APR -0.230	03 MAR to 22 APR 0.259	25 jul to 26 aug 0.297
30%ALA	01 APR to 14 JUL 0.266	01 APR to 14 JUL 0.266	27 APR to 16 JUN 0.226	19 JAN to 14 FEB 0.320	19 MAY to 16 JUN 0.281	30 jul to 27 aug -0.200	21 july to 19 oct 0.331	27 july to 27 sep 0.323

previous year ($r = 0.452$ and $r = 0.505$, respectively). Similarly high were the correlations between ALD and CWT and mean, maximum and minimum temperatures. Positive correlations with late-summer to early-autumn temperatures of the previous year suggest that tracheid formation and growth partly depend on the carbohydrate reserves formed during the preceding year. Although the photosynthetic capacity of evergreen tree species remains active during winter (Kozłowski 1992), the reserves may contribute to the formation of earlywood of *P. sylvestris* as was indicated in a previous study (McCarroll et al. 2003).

Correlations between cell variables, precipitation and relative humidity, respectively, are mostly negative ($r = -0.20$ to -0.30), whereas TRI was positively correlated ($r = 0.30$, $r = 0.41$ for precipitation and relative humidity, respectively). Eilmann et al. (2009) demonstrated strong positive correlations between tree-ring chronologies of *P. sylvestris* and May precipitation of the current year, while Martin-Benito et al. (2013) identified positive correlations with September-to-October precipitation of the previous year. Olano et al. (2012) observed positive correlations with June precipitation of the current year. In our study, TRI correlated negatively with mean and maximum May-to-June temperatures, similar to the results of Martin-Benito et al. (2013), which detected positive and negative correlations in winter and summer, respectively, under Mediterranean climate conditions. However, *P. sylvestris* from the northern boreal forests (Seo et al. 2012) was found to be affected positively by July-to-August temperatures and July precipitation of the previous year.

In our study, CWT correlated positively with the previous summer temperatures and negatively with precipitation of the current summer. However, in Spain, Martin-Benito et al. (2013) identified positive/negative correlations with summer precipitation/temperature, respectively. While Eilmann et al. (2009) did not find such correlations, Seo et al. (2012) reported positive correlations between CWT and April-to-May temperatures of the current year. The variables of 30MAX and 30%ALA did not improve the correlations with climate, unlike the results reported by Campelo et al. (2010) for angiosperm wood.

Comparing the climate correlations of all six tree-ring variables (Table 3), it was noticed that TRI correlated mainly with climate variables of the current year, largely within the months May to August. The cell variables exhibited mixed climate responses toward climate variables of the current year, but also of the previous year. Since only earlywood cell variables were analyzed, this might have made the signal of the previous year more pronounced.

Although we did not examine the relationship between latewood cell variables and climatic factors, other studies have indicated that latewood cell parameters may encode important climate signals. In some instances the size of the latewood part may not be large enough for cell measurements as was found by Seo et al. (2012). Since the samples of Seo et al. (2012) came from the northern boreal zone, the latewood part was not considered because of the typically small numbers of tangential tracheid rows. Eilmann et al. (2009) only analyzed CNs in the latewood part. Negative correlations between numbers of latewood cells and temperatures during the previous August

and during the months before and during their formation (March–August) were demonstrated. In contrast, Harris (1955) reported that different types of latewood are directly related to rainfall during the times of latewood formation. Similarly, Martin-Benito et al. (2013) found a clear drought signal in the latewood cell parameters of *P. sylvestris*, that is, especially in the latewood CWT and lumen diameter. It seems that climate signals derived from latewood cell parameter series are depending on the particular cell parameter analyzed and on the specific site chosen. This means that our latewood samples may contain a significant climate signal, but images of higher magnification are needed to investigate the latewood cell parameters accurately.

The comparison with the results of the other three studies on quantitative cell measurements of *P. sylvestris* demonstrates how this species is more temperature limited at the northern site

and more reliant on precipitation at the southern Mediterranean site. Our site seems to contain various climate signals but the summer temperatures of the previous year have the strongest impact on the cell variables. The studies of Seo et al. (2012) and Martin-Benito et al. (2013) focused on *P. sylvestris* growing under harsh conditions near the latitudinal distribution limits of the species. Although our study trees grew under less extreme conditions, we found new climate signals in the cell variables in addition to those of the TRW. Various cell structure variables of *P. sylvestris* exhibited significant correlations with climate, which is in accordance with previous studies stating that cell variables from trees growing in less extreme habitats can provide additional information (Fonti et al. 2010). Therefore, the results indicate that there is valuable potential for applying cell structure variables for understanding historical climate at sites that would traditionally be ignored for dendroclimatic studies (Drew et al. 2013).

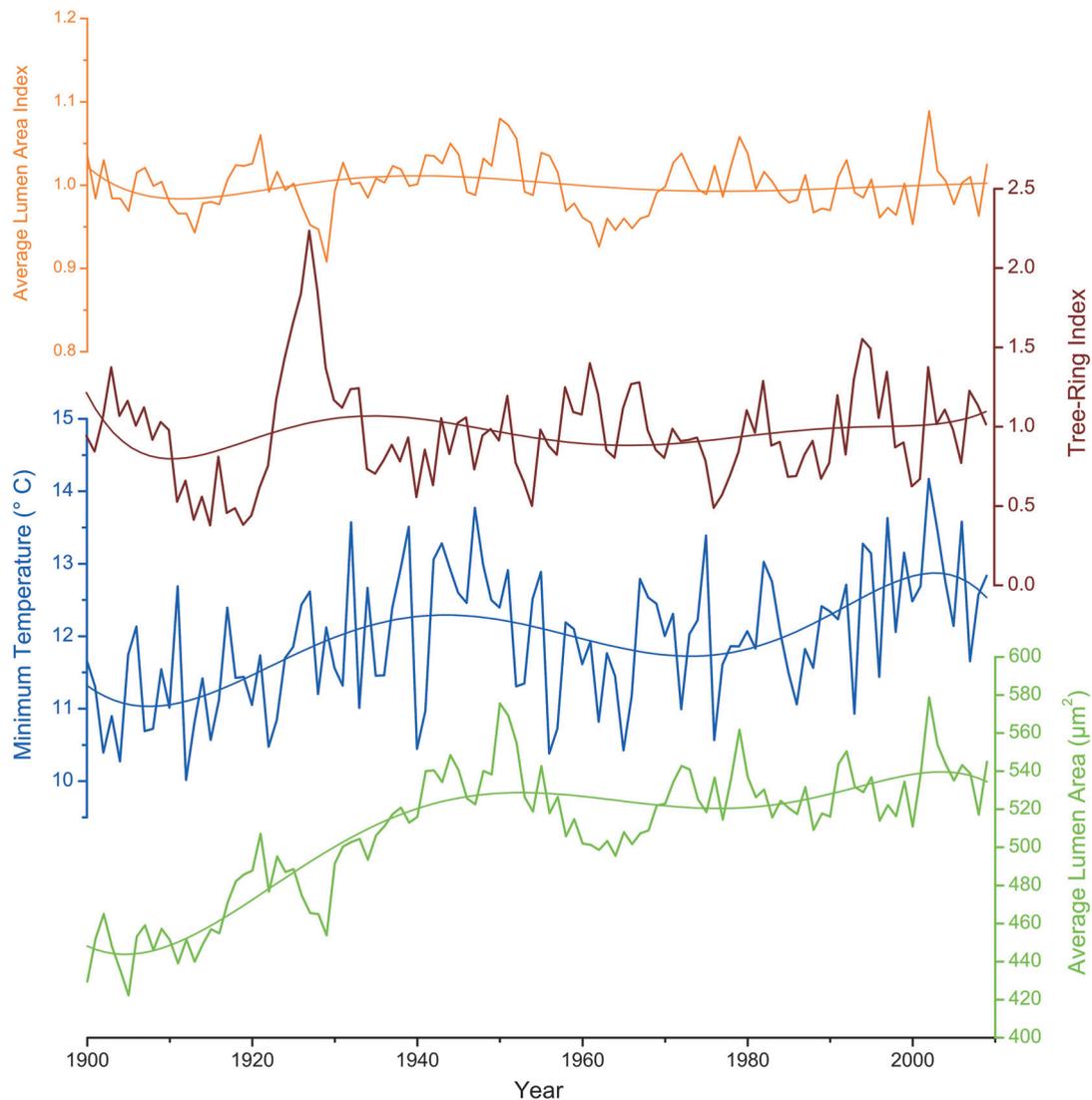


Figure 4. Comparison of ALA index (top graph), tree-ring index (second graph), 26 July to 23 September minimum temperature of previous year (third graph) and ALA (bottom graph). The smooth curves represent sixth-order polynomial fitting.

Raw versus detrended data

Raw TRW data contain a mixture of varying signals (i.e., biological age trends, climate forcing and ecological disturbance; Cook 1985), and therefore it is standard practice to remove the associated trends (Fritts 1976) from the raw tree-ring data. Correspondingly, in studies on the wood anatomy of conifer species, linear regression (Yasue et al. 2000), negative exponential curves (Panyushkina et al. 2003), straight lines (Wang et al. 2002), autoregressive modeling (Olano et al. 2012) or negative exponential functions (Martin-Benito et al. 2013) were applied to detrend chronologies of wood anatomical variables. However, detrending always bears the danger of losing climate information from the time series (Cook et al. 1995). Since we do not know of any study demonstrating long-term age trends in the cell measurement data series, we decided to use raw data for a comparison with climate data. Hence, we compared raw ALA and minimum temperature data which were annually averaged for the period from 26 July to 23 September. The low-frequency trends of raw ALA and minimum temperature are very similar with a correlation of $r=0.51$. If the low-frequency trend in ALA is removed, by fitting a cubic smoothing spline function with 66-year stiffness and 50% cutoff, using the program ARSTAN (Cook et al. 1990), the correlation between the ALA index and minimum temperature is quite low ($r=0.23$). Similarly, the low-frequency trends are also lost in TRI (Figure 4).

So far, possible non-climatic trends in cell structure measurements of conifers have only been studied sporadically (Lindström 1997, Mitchell and Denne 1997). The studies hint at an increasing trend within approximately the first 20 years in Norway (*Picea abies* (L.) H.Karst.) and in Wales (*Picea sitchensis* (Bong.) Carr.) when the trees are still young. First results of an ongoing research project indicate that this youth trend wears out within the first 10–30 years in *P. sylvestris* from Sweden (Pritzkow 2013). This is in line with the hypothesis set up by Aloni and Zimmermann (1983) in an attempt to explain the mechanism influencing vessel size and density. According to their theory, vessel sizes and densities are controlled by the plant hormone auxin at the location of cell growth. Low auxin levels result in slow cell differentiation, which permits more cell expansion before the secondary cell wall layer is produced, which marks the end of the cell expansion period. Since auxin is produced in and near the swelling buds at the beginning of the vegetation period, in young trees, the distance between the location of auxin production and cambium at the lower part of a tree stem is much shorter than in older trees. This changing distance and the decreasing impact of auxin might explain the youth trend of small cells becoming larger within the first 10–30 years. Once a tree has reached a certain size, the youth trend stops. Similarly, Gagen et al. (2007) and Young et al. (2011) demonstrated that there are no long-term trends in the

carbon isotope series that are related to tree age. Instead, they proposed that stable carbon isotope ratios from tree rings exhibit a youth trend within the first 20–40 years, but no age trends afterwards and thus may be used to reconstruct environmental variability without any detrending. Rather than applying any detrending it was recommended to leave out the sample parts containing the youth trends. In our study, we analyzed the samples from the period 1900–2009. Since the sampled trees are several decades older, our period of analysis was outside of this possible youth trend, and thus justified the non-detrending method. For future long-term reconstructions utilizing long series of cell measurement data, we recommend a similar procedure as followed by Gagen et al. (2007) and Young et al. (2011), thereby exorcising the segment length curse in long series not only of stable isotope data but also of cell measurement data.

Conclusions

In this study, we have developed chronologies of TRW and cell structure variables from *P. sylvestris* growing in northeastern Germany, and investigated the climate–growth relationships. Cell structures contain climate signals which were different and more significant than those found in TRWs as has been stated elsewhere (Wimmer 2002, Fonti et al. 2010). The strongest correlation between ALA and temperature and between TRW and vapor pressure will now be useful as part of a broader effort to reconstruct climate in the more temperate zones, away from the extreme environments. The results have shown that various tree-ring variables of *P. sylvestris* may be applied to a multi-proxy approach (Hughes et al. 1994, McCarroll et al. 2003). Since our chronologies of cell structure variables were >100 years long, we were able to compare the raw series with climate data throughout the 20th and into the 21st century. The comparison showed the parallel course of the series on a decadal to multi-decadal scale which indicates that longer chronologies of cell structure variables may be useful in reconstructing low-frequency climate signals. Overall, the results of this study suggest that, at least partly, the advantages of long chronologies from extreme sites may be outbalanced at sites in the temperate climate zone by analyzing other tree-ring variables such as cell structures. Cell structures may not need detrending and thus may overcome the segment length curse. Combining samples from living trees and archaeological material will lead to millennial-long robust chronologies.

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Conflict of interest

None declared.

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