

High-resolution climatic analysis of wood anatomical features in Corsican pine from Corsica (France) using latewood tracheid profiles

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Abstract

Key message We propose a new methodology to identify intra-annual density fluctuations in latewood using cell features and relative radial position within the latewood of pine trees growing on Corsica, France. Climatic forcing of latewood wood anatomical anomalies was analyzed.

Abstract We analyzed latewood anatomical features from Corsican pine (*Pinus nigra* ssp. *laricio*) of high-elevation sites in Corsica (France) derived from digital images of the wood surface. Latewood of each ring during the period 1950–2008 was partitioned into ten equal parts P1–P10. Mean values of the cell parameters cell lumen area (CLA), radial cell width (RCW), radial cell wall thickness (CWT), and modeled latewood density (MLD) were calculated for P1–P10. The cellular profiles for each cell parameter were subjected to principal component analyses. It was possible to quantify macroscopically visible variations of wood anatomy like intra-annual density fluctuations (IADFs) by latewood profiles of different cell parameters. A combination of cell parameter characteristics including their relative radial position within latewood provides a quantification of the cell anatomical variations in an IADF. Individual cell parameter chronologies and principal

components of cell parameter profiles were correlated with climate data to determine the climatic forcing on latewood formation. Average cell parameter profiles and deviations from the long-term means are able to describe “normal” and “anomalous” environmental conditions during latewood formation. Cell feature anomalies throughout the latewood during individual years allow the reconstruction of past weather conditions with a high temporal resolution.

Keywords Wood anatomy · Mediterranean climate · *Pinus nigra* · Dendroecology · Wood cell parameters · France

Introduction

Cellular characteristics of the vascular system of trees can be modified by environmental factors and thus show inter-annual variability. Wood anatomical properties of hardwood trees show modifications caused by flooding (St. George et al. 2002), temperature changes (Fonti and García-González 2004; Fonti et al. 2007), and precipitation variability (Fonti and García-González 2008; Pourtahmasi et al. 2011). Maximum latewood density is an indirect measure of wood anatomical modifications within coniferous tree species (e.g., Yasue et al. 2000; Fonti et al. 2010). In northern high latitudes, temperature variability influences wood density as well as cell characteristics (Panyushkina et al. 2003). Furthermore, climatic extremes like drought events can cause significant changes in the wood anatomy of hardwood (Cherubini et al. 2003; Battipaglia et al. 2010, 2014) and coniferous tree species (Eilmann et al. 2006). Cell parameter variations (density fluctuations) in conifer species are macroscopically visible (e.g., Campelo et al. 2007, 2013; Vieira et al. 2009;

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Bräuning 1999), however, quantitative measurements of cell sizes and cell wall widths require the application of image analysis systems (e.g., De Micco et al. 2012). Physical cell integrity is regarded as the most probable cause for cell adaptations to climate forcing since the risk of cavitation and a breakdown of the vascular system increase with cell diameter (Fonti et al. 2010).

Measurable cell dimensions and characteristics include tangential cell width, radial cell width, radial cell wall thickness, cell lumen area, and proportions of several of these parameters (e.g., Decoux et al. 2004; Rathgeber et al. 2006). The determination of the final cell dimensions is split into two main phases (e.g., Olano et al. 2012). First, cell enlargement determines the radial dimensions of the tracheids. In the phase of cell maturation, cell wall thickness is determined. Thus, cell size and cell wall thickness are potentially able to record different environmental forcing factors (e.g., Olano et al. 2012). Temporally, high-resolution climate reconstructions are based on a number of potential proxies (e.g., Moberg et al. 2005). Thus, cell parameters may be a valuable source of paleoenvironmental signals during different parts of the vegetation season (Fonti et al. 2010).

Maximum latewood density has been successfully used for temperature reconstructions in subtropical and Mediterranean high mountain regions (e.g., Bräuning and Mantwill 2004; Büntgen et al. 2008, 2010). Yet, it is less clear which processes are most strongly influencing maximum density values in different species and environments. On the one hand, water shortage can hamper cell enlargement, which is connected with the risk of xylem dysfunction (Fonti et al. 2010). On the other hand, carbohydrate allocation into cell walls leads to cell wall thickening (e.g., Panyushkina et al. 2003). High-resolution dendrometer data and microcoring studies of several species were able to shed light on the intra-annual xylogenesis and latewood formation (e.g., Rossi et al. 2008). However, long-term monitoring of trees in remote study areas with such methods is logistically difficult. In such cases, time series of modeled wood density derived from cell wall proportions can provide reliable estimates of X-ray wood density measurements (e.g., Decoux et al. 2004).

Intra-annual analysis of wood anatomical features is often rather qualitative in nature, heretofore usually only comparing rings of few years (e.g., Eilmann et al. 2011; DeSoto et al. 2011; De Micco et al. 2012). To reliably mirror the total spectrum of intra-annual wood anatomical characteristics, a higher number of analyzed years with varying climatic conditions are necessary. Cell characteristics of intra-annual density fluctuations (IADFs) are of special interest, since environmental triggers are often well known (Campelo et al. 2007). In comparison to studies on the imprint of climatic extremes on wood anatomy, less

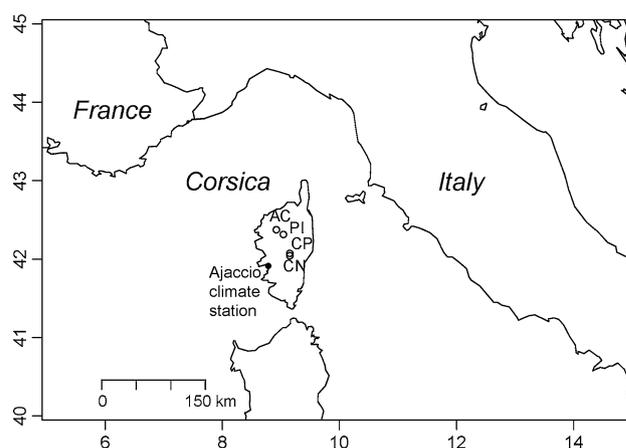


Fig. 1 Map of the Island of Corsica (France) indicating the locations of study sites Asco (AC), Cannereccia (CN), Capannelle (CP), and Pinerole (PN) and the climate station Ajaccio

work has been done in categorizing cell parameter profiles to search for similarities and differences between individual years and thereafter search for the triggering climatic factors. This approach may help to categorize years with similar intra-annual cell variability patterns that may provide linkages to the underlying climatic forcing.

As study species we selected *Pinus nigra* ssp. *laricio*, a long-living conifer species widely distributed in the mountain areas of the Mediterranean island of Corsica. The species has already successfully been used for the reconstruction of local climate conditions (Szymczak et al. 2012a). The aims of this paper are to (a) visualize, quantify, and categorize intra-annual cell dimension variability, (b) evaluate the climatic background of specific intra-annual cell parameter profiles, and (c) shed some light on the processes causing *Pinus nigra* intra-annual density variations in the mountain areas of Corsica Island.

Methods

During several field campaigns in the years 2006–2009, more than 1,000 increment cores were extracted from *P. nigra* ssp. *laricio* trees in Corsica, France. The sites are located in the higher mountain ranges at 1,300–1,500 m a.s.l. elevation. Chronologies of ring width and stable carbon and oxygen isotope variations were measured (Szymczak et al. 2012; Hetzer 2013) and a strong regional climate signal was found for all these wood parameters. The ring-width chronologies of the sites were highly correlated (Hetzer 2013), thus trees from four studied sites: Asco (two trees), Cannereccia (two trees), Capannelle (one tree), and Pinerole (four trees, Fig. 1) were combined for wood anatomical analyses to strengthen the regional climate signal. Since old-growth trees reveal low average ring

width during recent decades and hence a lower proportion of latewood (Griffin et al. 2011), we selected nine younger trees from our data pool showing significant correlations of their ring-width patterns to the overall chronology and forming wider rings to additionally investigate the climatic influence on anatomical cell parameters. The selected trees were collected at an elevation of around 1,500 m a.s.l. and had cambial ages ranging between 60 and 160 (mean age 115 years, standard deviation 30 years). The trees grew on rocky soils of slopes of varying aspect and inclination. To avoid any possible influence of reaction wood, the cores were taken perpendicular to the slope.

The samples were glued on sample holders and the wood surface was smoothed using a microtome. The sample surface was cleaned from resin and other extractives by acetone and ethanol. To enhance the cell wall–cell lumen contrast, viscous wall paint was rubbed into the cell lumina with a finger; water-soluble paint was removed from the sample surface by a slightly moist tissue. Thus, the optical contrast between earlywood and latewood was sharp and precisely detectable within one row of cells, thus minimizing subjectivity of the analysis (Hetzler 2013). A Keyence VHX-500-F digital microscope (Keyence Corporation, Japan) was utilized to capture digital images of the latewood of each ring. The microscope includes fast image-acquiring routines. The images were further processed with Adobe Photoshop CS4 (Adobe Systems Inc.) to produce black and white images of the individual cells.

Analysis of the tracheids was carried out with the image-analyzing software WinCell Pro 2010 (Regent Instruments Inc., Canada). Four cell parameters were selected for intra-annual analysis of the tree rings: cell lumen area (CLA), radial cell width (RCW), radial cell wall thickness (CWT), and a modeled latewood density (MLD). The latter was calculated by first measuring the cell lumina with the WinCell program. The outer boundaries of the individual cells were calculated by radial and tangential cell width measurements and assuming a rectangular cell shape. Cell wall area is the difference between the whole cell area (CA) minus CLA. The cell wall proportion (cwp) was then calculated as the ratio of CA.

$$\text{cwp} = (\text{CA} - \text{CLA})/\text{CA}. \quad (1)$$

Wood density was determined with a linear function and using a value of 1.530 kg/m^3 for the density of cell wall material (Stamm 1929):

$$\text{MLD} = \text{cwp} \times 1.53 (\text{kg m}^{-3}). \quad (2)$$

The obtained data were further processed in the statistical software package R (www.r-project.org). A modified tracheidogram method (Vaganov 1990) was applied to standardize all cell data. The latewood of a ring was divided into 10 equally spaced parts. As illustrated for

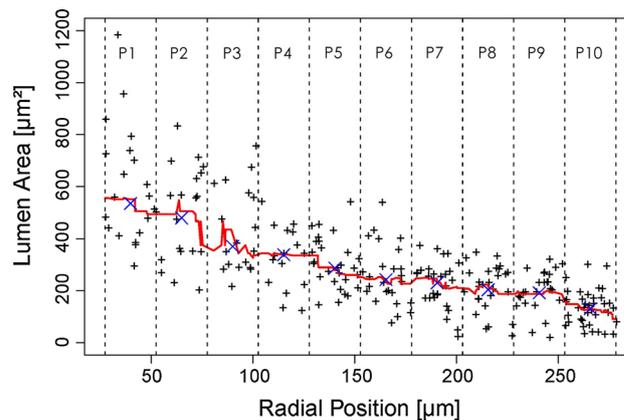


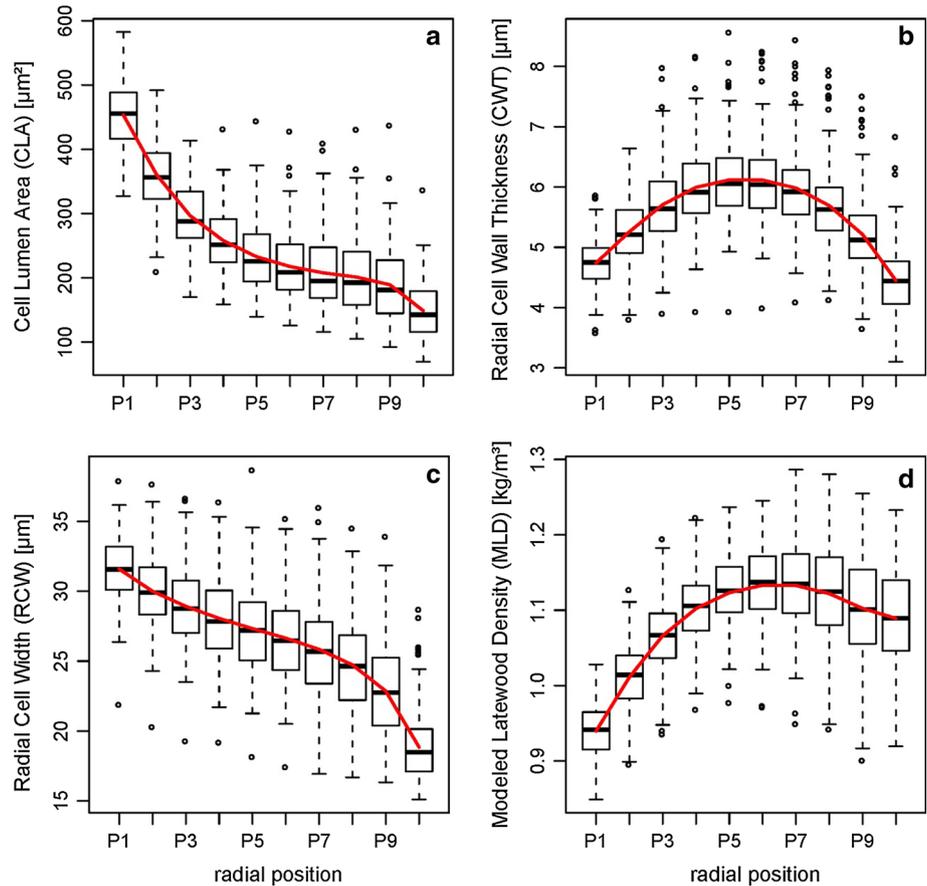
Fig. 2 Cell lumen area along a typical profile within the latewood of *Pinus nigra* showing a general decrease from the transition of earlywood to latewood towards the end of the ring. X indicates the mean values of the running median of the individual cells within 10 equal sections of the latewood, plus indicates single cell values, red solid line represents the running median

lumen area in Fig. 2, relative radial position of cells within latewood is used to divide tracheids into 10 equally spaced parts (P1–P10), with P1 beginning at the earlywood–latewood (E/L) transition (start of latewood) and P10 marking the section nearest to the tree-ring boundary (end of latewood). Running median curves of the single cells were calculated for each parameter (Härdle and Steiger 1995) and averaged within the ten parts. The variation of different cell parameters from E/L transition (P1) to the end of latewood (P10) is called a profile.

Chronologies were built from the individual samples by averaging the values of the same parameter of the same year for identical relative intra-ring positions. Thus, forty chronologies were produced ($10 \times$ cell lumen area: CLA-P1–CLA-P10, $10 \times$ cell wall thickness: CWT-P1–CWT-P10, $10 \times$ radial cell width: RCW-P1–RCW-P10 and $10 \times$ modeled latewood density: MLD-P1–MLD-P10), each parameter representing ten mean values at different relative positions within the latewood. The cell parameter time series show no severe bias by age-related trends, thus series of different samples were averaged by simple arithmetic means.

To detect deviations of average cell formation, the profiles were standardized to remove the overall trends (straight increase or decrease of a parameter/unimodal behavior) within each latewood by z -transformation. Principal component analysis (PCA) was applied to matrices of each radial position (P1–P10) and the mean values of CLA, CWT, RCW, and MLD separately to differentiate the intra-annual profiles (R-function prcomp). The calculation of the PCA was carried out by singular value decomposition. To identify years with a specific wood anatomical profile and define a threshold for low and high values of each cell parameter, a threshold for factor scores greater than three

Fig. 3 Mean intra-ring parameters (cell lumen area, radial cell wall thickness, radial cell width, modeled density) of *P. nigra* trees. *Boxplots* give the ranges and the median of the different cell parameters. *Red lines* represent the mean profile of a parameter within latewood



in absolute value was defined. All data and analyses results presented refer to the period 1990–2008, for which climate data for subsequent analyses were available. The analysis of climatic factors triggering the formation of different cell parameter profiles included monthly values of temperature means and precipitation sums from Ajaccio climate station (5 m a.s.l.; Fig. 1) for the years 1950–2008. Linear regression modeling was applied to assess the total climatic input potentially triggering the formation of exceptional cell parameter profiles using the *lm* function in R (www.r-project.org). The step function dismissed unnecessary climate variables by selecting only those variables explaining a significant proportion of variance and rejecting variables showing colinearity.

Results

Principal component differentiation of cell parameter profiles

Mean intra-annual cell characteristics in Corsican pines

CLA of Corsican pine latewood decreases from ca. $500 \mu\text{m}^2$ in E/L transition wood to $150 \mu\text{m}^2$ at the end of

latewood (Fig. 3a). RCW is characterized by a similar behavior, with maximum values of $32 \mu\text{m}$ located in the E/L transition wood and minimum values of $18 \mu\text{m}$ at the end of latewood (Fig. 3c). Cell wall thickness increases until P5–P6 and decreases afterwards (Fig. 3b). At the beginning of latewood (P1), mean CWT is $4.8 \mu\text{m}$, maximum CWT is $6.1 \mu\text{m}$, and mean CWT of the terminal rows is $4.4 \mu\text{m}$. The profile of MLD also shows a unimodal distribution of values, with a maximum at the P6 relative radial position within latewood (Fig. 3d).

Raw profiles of CLA show a huge variance, with a general decrease in size towards the end of the ring (Fig. 3a), whereas all CWT profiles are characterized by a unimodal behavior. RCW profiles show generally decreasing trends, with a higher variance in the P7–P9 sections of the latewood, and a lower variance in the smallest cell rows (P10), which is probably caused by their strongly reduced cell size. Average MLD increases until P4–P7, showing increasing variance in radial direction of the tree ring.

PCA separates CLA profiles into five groups. The first principal component (PC#1) explains 70 % of the total variance and PC#2 21 % (Table 1). Profiles with high CLA values throughout the latewood show high positive scores on PC#1 (Fig. 4a, b). Highest scores were found for the

Table 1 Linear regression modeling of cell lumen area PCA scores using climate data from the Ajaccio climate station

			MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
CLA PC#1 (70%) adj. $r^2 = 0.46$	TEMP					***					
	PRECIP						**	*	***		
CLA PC#2 (21%) adj. $r^2 = 0.27$	TEMP						*	*			**
	PRECIP										
CWT PC#1 (80%) adj. $r^2 = 0.17$	TEMP			**							
	PRECIP										
CWT PC#1 (13%) adj. $r^2 = 0.43$	TEMP				**				**	**	*
	PRECIP			**					*		
RCW PC#1 (80%) adj. $r^2 = 0.35$	TEMP				*						
	PRECIP					*	***		**		
RCW PC#2 (14%) adj. $r^2 = 0.25$	TEMP			*			*				**
	PRECIP							*			
MLD PC#1 (65%) adj. $r^2 = 0.60$	TEMP				***				*	**	
	PRECIP							**	*		
MLD PC#2 (22%) adj. $r^2 = 0.22$	TEMP							**			**
	PRECIP										

* $P < 0.05$, ** $P < 0.01$,
*** $P < 0.001$

Variables were selected using the step function in R

Black negative estimate, white positive estimate, grey variable dismissed by the step function

years 1995 and 1976. In total, 11 years are characterized by high CLA values. In contrast, CLA profiles with low values at each position have negative scores (7 years, e.g., 2003). Positive scores on PC#2 are years with high CLA values at the beginning of the latewood and low values at the end of the latewood (1 year: 1986). Negative scores are found for years typically showing density fluctuations with a first minimum of CLA at cell position P3 and a second minimum at the end of the latewood (3 years: e.g., 1950). The last group consists of years without significant positive or negative loadings on both axes.

CWT profiles share a high common variability with PC#1 (80 %); a minor part is included in PC#2 (13 %) (Fig. 4c, d, Table 1). PC#1 separates profiles with continuously thick cell walls from profiles that show only small cell walls throughout the latewood. Scores above 3 and below 3 are only found on the first axis. Seven years share high scores on PC#1 (e.g., 1994 and 1997), 6 years show low scores (e.g., 1962). High scores include years in which thick cell walls were formed, strong negative loadings are found for years with thin walls. All other years are within the two extremes. No year is detected with very high or very low scores on PC#2.

The PCA separates the profiles of RCW into three groups. PC#1 and PC#2 explain 80 and 14 % of the total variance, respectively (Fig. 4e, f, Table 1). Profiles with high scores on PC#1 (e.g., 1994, 1997) are radially enlarged; whereas negative scores (e.g., 1982) correspond to radial small cells. PC#2 is associated with a RCW fluctuation with negative scores (e.g., 1950). Many years with radially wide cells correspond to years with large cell lumen areas (e.g., 1963, 1973, and 1992). Some profiles

with small cell widths correspond to years with intra-annual low lumen area (e.g., 1982, 2003–2004). One profile (the year 1950) is representative for PC#2, with a clear intra-latewood cell size fluctuation (Table 2).

PC#1 of MLD represents 65 % of the common variance (Fig. 4g, h, Table 1). Low scores on axis #1 mirror high density rings and high scores characterize low density profiles. Axis #2 represents density fluctuations that are characterized by a fast increase of density and a subsequent decrease until the end of the tree ring (years 1991 and 1998). PC#2 accounts for 24 % of the common variance.

Wood anatomical profiles and climate variability

Linear modeling including June temperature and May to September precipitation is able to explain about 46 % of the variance of CLA PC#1. The climatic forcing on cell lumen size is less clear, with an adjusted r^2 of 0.27. Temperature variability seems the only factor triggering the formation of lumen area fluctuations or a sharp lumen area decline within the latewood profile (PC#2 in Table 1).

The climate variables are more strongly correlated to the second axis of CWT-PCA, which corresponds to years with marginally higher cell wall thickness at the end of the ring (rotation #2 of PCA; not shown). The overall differentiation of the rings with globally low and high wall thickness is consequently barely explained by a linear regression model (adjusted $r^2 = 0.17$; Table 2). About 43 % of PC#2 variability can be explained by temperature in the months of June, September–November, and by precipitation in May, August, and September.

Fig. 4 Mean profiles of **a** cell lumen area, **c** cell wall thickness, **e** radial cell width, and **g** modeled density (1950–2008). PCA of **b** cell lumen area, **d** cell wall thickness, **f** radial cell width, and **h** modeled density profiles showing individual years and relative intra-ring positions within the latewood (CLA-P1 to CLA-P10, CWT-P1 to CWT-P10, RCW-P1 to RCW-P10, and MLD-P1 to MLD-P10)

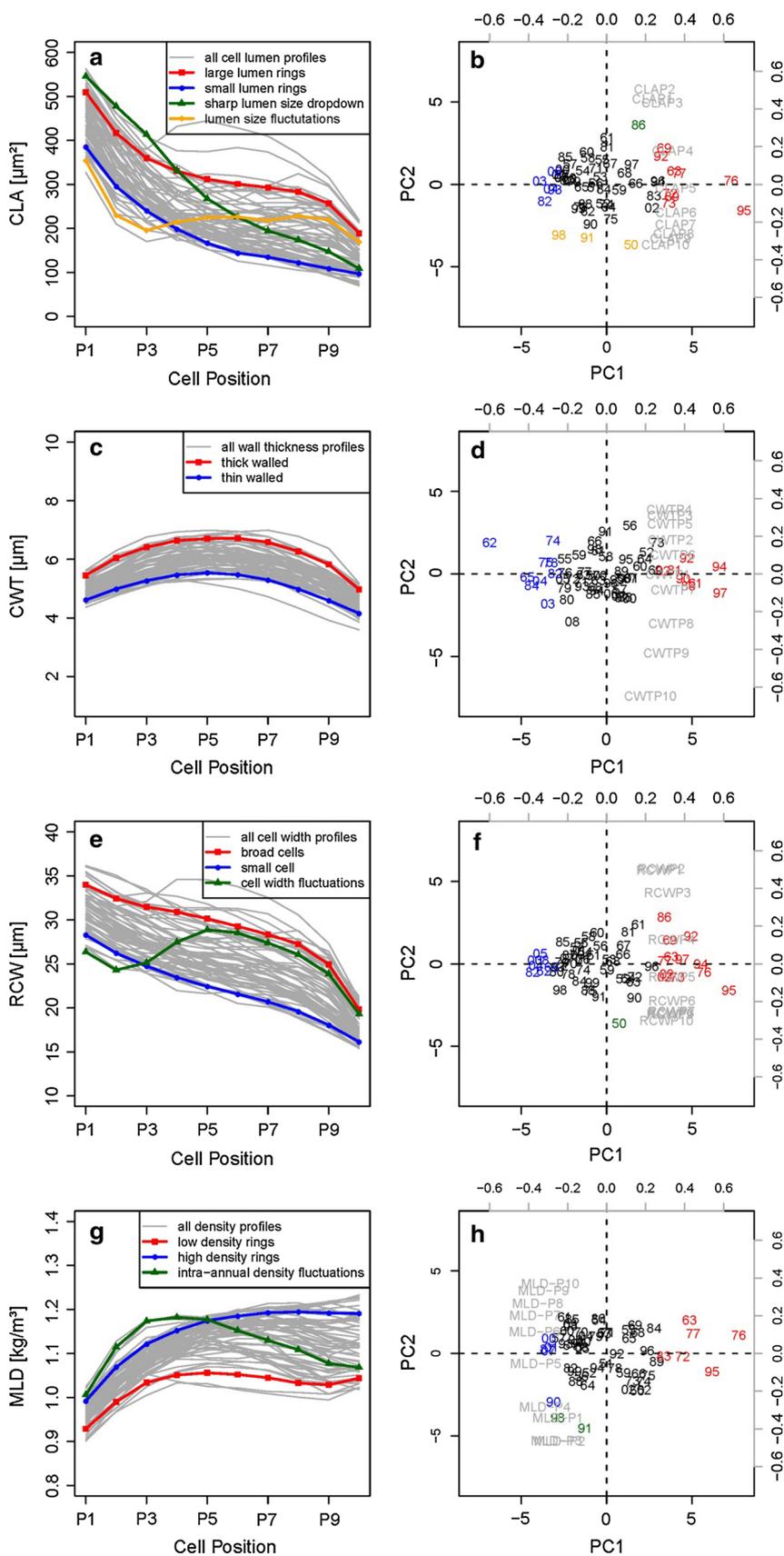


Table 2 Comparison of the different wood anatomical features detected by PCA (Fig. 4a–h)

	CLA: cells in latewood with large lumen	CLA: cells in latewood with small lumen	CLA: sharp lumen size dropdown in latewood	CLA: lumen size fluctuation in latewood	CWT: Thick walled cells in latewood	CWT: Thin walled cells in latewood	RCW: broad cells	RCW: small cells	RCW: radial cell width fluctuation	MLD: high density rings	MLD: low density rings	MLD: density fluctuations	possible climatic factor
1950				■					■				hot Jul/Aug
1951													
1952													
1953													
1954													
1955													
1956													
1957													
1958													
1959													
1960													
1961					■								
1962					■	■		■					
1963	■					■	■				■		dry summer wet summer
1964													
1965						■		■					dry summer
1966													
1967													
1968													
1969	■						■						
1970													
1971													
1972	■										■		
1973	■						■						
1974						■	■						cold Oct
1975						■							cold Oct
1976	■						■				■		wet summer
1977	■						■				■		wet summer
1978						■							
1979													
1980													
1981					■								
1982		■				■		■					hot summer
1983													
1984						■							
1985													
1986			■		■		■						dry-warm Oct/Nov
1987										■			
1988													
1989	■						■						
1990					■	■				■			
1991	■			■	■		■				■		dry summer/wet autumn
1992	■				■		■						
1993		■											
1994	■				■		■						dry-hot summer
1995	■						■				■		
1996													
1997					■	■	■						
1998				■								■	dry summer/wet autumn
1999													
2000										■			
2001										■			
2002					■		■						
2003		■				■		■					dry-hot summer
2004		■				■		■					dry Jun/Jul
2005													
2006													
2007										■			
2008	■						■						dry Jul/Aug

RCW variability can be explained mainly by temperature in July for PC#1 (large and small cells in the total profile) and by precipitation in May, June, July. PC#2 is correlated with May, July, August, and November temperature (cell size fluctuations; Fig. 3e). Models of PC#1 and PC#2 explain 80 and 14 % of the total RCW variance, respectively (Table 1). Besides precipitation variables, PC#1 includes temperature in June and November as significant influential factors.

Modeled density variability is influenced by numerous climatic variables on both axes of the PCA (Table 1). Precipitation in May, July–September as well as temperature in September and October has a significant influence. The linear regression model of PC#1 explains 65 % of the variance, still including eight significant variables after using the step function in R to exclude non-significant vectors. The model of PC#2 (density fluctuations) uses temperatures in August, October and November, as well as precipitation in August (Table 1).

All years were tabulated according to their attributes to summarize the categorization of CLA, CWT, RCW and MLD profiles (Table 2). Interestingly, the year 1950 with a RCW and CLA fluctuation (1950) did not correspond with density fluctuations (1991 and 1998). Rings with generally low density, large cells, and large lumens can be found in 4 years. Five years can be recognized as years with small thin-walled cells and 5 years show large thick-walled cells.

Discussion

Water conductivity and xylem variability

Our results showed that individual cell parameters of Corsican pine respond differently to different climatic forcing factors: latewood cells are significantly enlarged if precipitation is above average, whereas cell walls are generally thicker in years with higher temperatures throughout the vegetation season. This seems to contradict an irrigation experiment that showed significant differences between irrigated Scots pine (*Pinus sylvestris*) trees and trees exposed to drought conditions: non-irrigated trees formed fewer radial cells; cells were significantly enlarged and had thinner cell walls (Eilmann et al. 2011). Therefore, it can be hypothesized that xylogenesis of Mediterranean high-elevation *P. nigra* trees differs from that of *P. sylvestris* in the inner Alps. In *Pinus halepensis*, IADFs in latewood at coastal sites in Portugal are formed if precipitation in September and October is high (Vieira et al. 2009), whereas at moderately high elevations (844 m a.s.l.) in southeastern Spain, latewood IADFs occur if August precipitation is above average (de Luis et al. 2011). At our high-elevation Mediterranean sites, soil water availability

is hardly limiting during the early and late growing season. Relief rainfall supplies water except during the summer months that are characterized by high temperatures and occasional rain events. These rain events are probably triggering the production of large cells by increasing the turgor pressure of young tracheids in their enlargement phase. Thus, the hypothesis that cell size is strongly dependent on water availability can be confirmed for *P. nigra* latewood. Large cells are not formed in periods of high water deficits to minimize the risk of xylem dysfunction (Fonti et al. 2010). This is in accordance with other studies in the Mediterranean region, where drought events leave measurable imprints on cell dimensions of different species like *Juniperus thurifera*, *Arbutus unedo*, and *Erica arborea* in the form of smaller cells with thicker cell walls (DeSoto et al. 2011; Battipaglia et al. 2010, 2014). An investigation of cell parameters using consecutive years of *J. Thurifera* trees (Olano et al. 2012) found radial cell width and cell wall width influenced by summer temperatures.

Tracheidogram standardization

Ten equal parts were used for standardizing intra-latewood cell variability to allow a statistically robust investigation of climatic triggers and common between-tree variability. Mediterranean tree species are known to show many wood density variations (e.g., Campelo et al. 2007), thus only high radial resolution of the latewood cell data is able to analyze climatic triggers of latewood cell variability in detail. Olano et al. (2012) stated that the tracheid number after standardization using the tracheidogram method (Vaganov 1990) should be kept as low as possible to get a high intra-annual resolution as well as temporal homogeneity due to non-synchronous cambial activity of individual trees (e.g., Bouriaud et al. 2005; Rossi et al. 2008; Seo et al. 2011). The question of how many tracheids are most suitable must be tested for each tree species individually. In some cases, less than six consecutive units in latewood can be chosen to represent intra-latewood variability of cell parameters adequately (Olano et al. 2012). However, intra-annual density fluctuations are not detectable if the number of cells is very low.

Is there information in earlywood cells?

Unfortunately, earlywood cell parameters were not extractable from surfaced wood specimen as carried out in this study. Thus, IADFs occurring in earlywood (type ‘E’ sensu Campelo et al. 2007) were not analyzed and probably still require the preparation of microscopic thin sections (e.g., de Luis et al. 2011) for quantitative cell parameter analysis. The investigation of Olano et al. (2012) revealed

that earlywood cell parameters respond to environmental triggers, mainly occurring during the early growing season. Bouriaud et al. (2005) concluded from their analysis that climate, but not wood density, affects the growth rate in early parts of a ring. A preliminary analysis of *P. nigra* cell dimensions from a microsection showed no statistically significant differences of earlywood cell parameters between individual years (Hetzer 2013). Nevertheless, a detailed analysis of *P. nigra* earlywood cell characteristics should be conducted to verify the impact of climate on earlywood cell characteristics.

The advantage of cell parameter profiles

In contrast to single wood parameters like maximum latewood density or ring width, the analysis of cell parameter profiles throughout the latewood is suitable to identify specific climate patterns and episodic climatic extremes. A cell profile over an entire annual ring or the latewood integrates temperature and precipitation signals over most of the vegetation season. Contrasting effects on different cell parameters can be identified. For example, density fluctuations are mainly constituted by cell lumen size fluctuations, whereas cell wall thickness variability plays a minor role. Climatic triggers can be identified as high temperatures in August and low temperatures in October and November, associated with more intensive drought stress during summer and more humid conditions during autumn.

Conclusions

This study evaluated the variability of tracheid dimension profiles of *P. nigra* trees after careful surfacing of wood samples to enhance microanatomy. Previously, cell analyses based on digital images had mainly been performed from stained microscopic thin sections, requiring major effort in sample preparation and subsequent image analysis of individual tree rings. New surface preparation and imaging techniques like confocal laser scanning (e.g., Kitin et al. 2003; Knebel and Schnepf 1991; Liang et al. 2013) will facilitate future research of numerical cell analysis, although adequate surface preparation will always be the prerequisite for high image quality.

Our study in a Mediterranean mountain climate showed that macroscopically detectable variations in wood density like intra-annual density fluctuations (IADFs) can be quantified by the analysis of cell parameter profiles, and a combination of cell parameter characteristics (e.g., cell wall thickness and/or cell lumen area), including their relative radial position within latewood, provides a better description of IADFs and can improve understanding of the variability in IADFs. The average cell parameter profiles

and the deviations from the long-term means are able to describe “normal” environmental conditions during latewood formation and deviations from these conditions in individual years. The correlation with specific climatic elements during years showing wood anatomical anomalies allows reconstruction of former climatic anomalies with a high temporal resolution, possibly enabling the reconstruction of underlying atmospheric circulation conditions responsible for seasonal variations of climate elements in the Mediterranean region (Luterbacher et al. 2012).

Author contribution statement TH made all wood measurements, statistical calculation and graphics, participated in the interpretation of the results and wrote parts of the text. AB is PI of the project, participated in the interpretation of the results and wrote large parts of the text. H-HL is PI of the project, supervised the wood anatomical measurements, participated in the interpretation of the results and wrote parts of the text.

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References

- Battipaglia G, De Micco V, Brand WA, Linke P, Aronne G, Saurer M, Cherubini P (2010) Variations of vessel diameter and $\delta^{13}\text{C}$ in false rings of *Arbutus unedo* L. reflect different environmental conditions. *New Phytol* 188:1099–1112
- Battipaglia G, De Micco V, Brand WA, Saurer M, Aronne G, Linke P, Cherubini P (2014) Drought impact on water use efficiency and intra-annual density fluctuations in *Erica arborea* on Elba (Italy). *Plant Cell Environ* 37(2):382–391
- Bouriaud O, Leban JM, Bert D, Deleuze C (2005) Intra-annual variations in climate influence growth and wood density of Norway spruce. *Tree Physiol* 25:651–660
- Bräuning A (1999) Dendroclimatological potential of drought-sensitive tree stands in southern Tibet for the reconstruction of the monsoonal activity. *IAWA J* 20:325–338
- Bräuning A, Mantwill B (2004) Summer temperature and summer monsoon history on the Tibetan plateau during the last 400 years recorded by tree rings. *Geophys Res Lett* 31:L24205
- Büntgen U, Frank D, Grudd H, Esper J (2008) Long-term summer temperature variations in the Pyrenees. *Clim Dyn* 31:615–631
- Büntgen U, Frank D, Trouet V, Esper J (2010) Diverse climate sensitivity of Mediterranean tree-ring width and density. *Trees* 24:261–273
- Campelo F, Nabais C, Freitas H, Gutiérrez E (2007) Climatic significance of tree-ring width and intra-annual density fluctuations in *Pinus pinea* from a dry Mediterranean area in Portugal. *Ann For Sci* 64:229–238
- Campelo F, Vieira J, Nabais C (2013) Tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* responses to climate: does size matter? *Trees* 27:763–772
- Cherubini P, Gartner BL, Tognetti R, Bräker OU, Schoch W, Innes JL (2003) Identification, measurement and interpretation of tree

- rings in woody species from Mediterranean climates. *Biol Rev* 78:119–148
- De Luis M, Novak K, Raventós J, Gričar J, Prislán P, Čufar K (2011) Climate factors promoting intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) from semiarid sites. *Dendrochronologia* 29:163–169
- De Micco V, Battipaglia G, Brand W, Linke P, Saurer M, Aronne G, Cherubini P (2012) Discrete versus continuous analysis of anatomical and $\delta^{13}\text{C}$ variability in tree rings with intra-annual density fluctuations. *Trees* 26:513–524
- De Soto L, de La Cruz M, Fonti P (2011) Intra-annual patterns of tracheid size in the Mediterranean tree *Juniperus thurifera* as an indicator of seasonal water stress. *Can J For Res* 41:1280–1294
- Decoux V, Varcin É, Leban JM (2004) Relationships between the intra-ring wood density assessed by X-ray densitometry and optical anatomical measurements in conifers. Consequences for the cell wall apparent density determination. *Ann Forest Sci* 61:251–262
- Eilmann B, Weber P, Rigling A, Eckstein D (2006) Growth reactions of *Pinus sylvestris* L. and *Quercus pubescens* willd. to drought years at a xeric site in Valais Switzerland. *Dendrochronologia* 23:121–132
- Eilmann B, Zweifel R, Buchmann N, Graf Pannatier E, Rigling A (2011) Drought alters timing, quantity, and quality of wood formation in Scots pine. *J Exp Bot* 62:2763–2771
- Fonti P, García-González I (2004) Suitability of chestnut earlywood vessel chronologies for ecological studies. *New Phytol* 163:77–86
- Fonti P, García-González I (2008) Earlywood vessel size of oak as a potential proxy for spring precipitation in mesic sites. *J Biogeogr* 35:2249–2257
- Fonti P, Solomonoff N, García-González I (2007) Earlywood vessels of *Castanea sativa* record temperature before their formation. *New Phytol* 173:562–570
- Fonti P, von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H, Eckstein D (2010) Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytol* 185:42–53
- Griffin D, Meko DM, Touchan R, Leavitt SW, Woodhouse CA (2011) Latewood chronology development for summer-moisture reconstruction in the USA Southwest. *Tree Ring Res* 67:87–101
- Härdle W, Steiger W (1995) Algorithm AS 296: optimal median smoothing. *J r stat soc Ser C (Appl Stat)* 44:258–264
- Hetzer T (2013) Xylem Variability as a Proxy for Environmental and Climate Change in Corsica During the Past Millennium. PhD Thesis. University of Erlangen-Nürnberg, Germany. urn:nbn:de:bvb:29-opus4-36421
- Kitin P, Sano Y, Funada R (2003) Three-dimensional imaging and analysis of differentiating secondary xylem by confocal microscopy. *IAWA J* 24:211–222
- Knebel W, Schnepf E (1991) Confocal laser scanning microscopy of fluorescently stained wood cells: a new method for three-dimensional imaging of xylem elements. *Trees* 5:1–4
- Liang W, Heinrich I, Helle G, Dorado Liñán I, Heinken T (2013) Applying CLSM to increment core surfaces for histometric analyses: a novel advance in quantitative wood anatomy. *Dendrochronologia* 31:140–145
- Luterbacher J et al (2012) A review of 2000 years of paleoclimatic evidence in the Mediterranean. In: Lionello P (ed) *The climate of the Mediterranean region: from the past to the future*. Elsevier, The Netherlands, pp 87–185
- Moberg A, Sonechkin DM, Holmgren K, Datsenko NM, Karlén W (2005) Highly variable Northern Hemisphere temperatures reconstructed from low- and high-resolution proxy data. *Nature* 433:613–617
- Olano JM, Eugenio M, García-Cervigón AI, Folch M, Rozas V (2012) Quantitative tracheid anatomy reveals a complex environmental control of wood structure in continental Mediterranean climate. *Int J Plant Sci* 173:137–149
- Panyushkina IP, Hughes MK, Vaganov EA, Munro MAR (2003) Summer temperature in northeastern Siberia since 1642 reconstructed from tracheid dimensions and cell numbers of *Larix cajanderi*. *Can J For Res* 33:1905–1914
- Pourtahmasi K, Lotfiomran N, Bräuning A, Parsapajouh D (2011) Variations of tree growth and vessel characteristics of *Fagus orientalis* along an altitudinal gradient in the Caspian Forests, northern Iran. *IAWA J* 32(4):461–473
- Rathgeber CBK, Decoux V, Leban J-M (2006) Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). *Ann For Sci* 63:699–706
- Rossi S, Deslauriers A, Anfodillo T, Carrer M (2008) Age-dependent xylogenesis in timberline conifers. *New Phytol* 177:199–208
- Seo JW, Eckstein D, Jalkanen R, Schmitt U (2011) Climatic control of intra- and inter-annual wood-formation dynamics of Scots pine in northern Finland. *Environ Exp Bot* 72:422–431
- St. George S, Nielsen E, Conciatori F, Tardif J (2002) Trends in quercus macrocarpa vessel areas and their implications for tree-ring paleoflood studies. *Tree Ring Res* 58:3–10
- Stamm A (1929) Density of wood substance, absorption by wood, and permeability of wood. *J Phys Chem* 33:398–414
- Szymczak S, Joachimski MM, Bräuning A, Hetzer T, Kuhlemann J (2012) A 560 years summer temperature reconstruction for the Western Mediterranean basin based on stable carbon isotopes from *Pinus nigra* ssp. *laricio* (Corsica/France). *Clim Past Discuss* 8:2111–2143
- Vaganov EA (1990) The tracheidogram method in tree-ring analysis and its application. In: Cook ER, Kairiūkštis L (eds) *Methods of dendrochronology*. Kluwer Academic Publishers, Boston, pp 63–76
- Vieira J, Campelo F, Nabais C (2009) Age-dependent responses of tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* to Mediterranean climate. *Tree Struct Funct* 23:257–265
- Yasue K, Funada R, Kobayashi O, Ohtani J (2000) The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. *Trees Struct Funct* 14:223–229