How does climate influence xylem morphogenesis over the growing season? Insights from long-term intra-ring anatomy in *Picea abies*

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### Background and Aims
During the growing season, the cambium of conifer trees produces successive rows of xylem cells, the tracheids, that sequentially pass through the phases of enlargement and secondary wall thickening before dying and becoming functional. Climate variability can strongly influence the kinetics of morphogenetic processes, eventually affecting tracheid shape and size. This study investigates xylem anatomical structure in the stem of *Picea abies* to retrospectively infer how, in the long term, climate affects the processes of cell enlargement and wall thickening.

### Methods
Tracheid anatomical traits related to the phases of enlargement (diameter) and wall thickening (wall thickness) were innovatively inspected at the intra-ring level on 87-year-long tree-ring series in *Picea abies* trees along a 900 m elevation gradient in the Italian Alps. Anatomical traits in ten successive tree-ring sectors were related to daily temperature and precipitation data using running correlations.

### Key Results
Close to the altitudinal tree limit, low early-summer temperature negatively affected cell enlargement. At lower elevation, water availability in early summer was positively related to cell diameter. The timing of these relationships shifted forward by about 20 (high elevation) to 40 (low elevation) d from the first to the last tracheids in the ring. Cell wall thickening was affected by climate in a different period in the season. In particular, wall thickness of late-formed tracheids was strongly positively related to August–September temperature at high elevation.

### Conclusions
Morphogenesis of tracheids sequentially formed in the growing season is influenced by climate conditions in successive periods. The distinct climate impacts on cell enlargement and wall thickening indicate that different morphogenetic mechanisms are responsible for different tracheid traits. Our approach of long-term and high-resolution analysis of xylem anatomy can support and extend short-term xylogenesis observations, and increase our understanding of climate control of tree growth and functioning under different environmental conditions.

**Key words:** Cell size, cell-wall thickness, climate change, Norway spruce, quantitative wood anatomy, secondary growth, tracheid, tree ring, xylogenesis.

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**INTRODUCTION**

The anatomy of xylem determines the ability of a tree to transport water from soil to leaves and to support its own structure. Recent studies linking tree physiology with wood anatomy have demonstrated that the morphology of the xylem cells affects functional properties such as hydraulic safety and efficiency (Lachenbruch and McCulloh, 2014; Schuldt et al., 2016). Therefore, modifications in xylem anatomy strongly determine the performance and survival of trees, and consequently forests’ vulnerability to climate change and their capacity to fix carbon (Anderegg, 2015; Sperry and Love, 2015; Pellizzari et al., 2016).

Secondary growth, i.e. the formation of xylem cells through cambial activity, has been extensively investigated since the last century, providing detailed insights into the physiology that regulates plant cell development (Heyn, 1940; Růžička et al., 2015). Experimental manipulations have additionally shown how internal factors, e.g. hormones (Aloni, 1980), and external factors, e.g. temperature (Denne, 1971) and water availability (Hsiao, 1973), govern this process. However, the information gained was mostly limited to observations in controlled settings (such as in a greenhouse) performed on non-woody species or very young trees, being only partially indicative of growth mechanisms occurring in adult trees in complex and variable natural systems (Rathgeber et al., 2011; Wolkovich et al., 2012).

Wood formation (xylogenesis) is known to be influenced by environmental conditions that vary in both space and time (Cuny et al., 2015). Restricted water availability generally limits tree growth in warm and dry areas, while low temperatures control growth at high latitudes and elevations (Rossi et al., 2013). However, the mechanisms behind the environmental influence on meristematic processes are still rather poorly understood. This knowledge is critical for a mechanistic understanding of the climate control of tree growth [e.g. carbon source-limitation versus sink-limitation hypotheses (Körner, 2013)] and for assessing legacy effects on future xylem functioning under changing climatic conditions (Sass-Klaassen et al., 2016).

Studies monitoring xylogenesis (i.e. weekly cytological observations of tree-ring formation) can provide important insights into environmental control of wood formation in adult trees. They have evidenced site-specific adaptation of xylogenesis processes (Grčar et al., 2015) and latitude and elevation...
influences on the timing and duration of cambial activity (e.g. Moser et al., 2010; Rossi et al., 2014). However, despite the strong inter-annual variability of cambial phenology (Treml et al., 2015), this process has usually been quantified with just a few years (typically 1–5 in most studies) of continuous observations. In addition, studies monitoring xylogenesis have provided clues on how climate before and during the growing season affects the onset of cambial activity (Rossi et al., 2013) and impacts the kinetics of cell formation, influencing xylem cell morphological traits (Cuny et al., 2014). However, these observations lack the necessary long-term perspective to thoroughly assess the responses of wood formation and structure to inter-annual climate variability (Boulouf Lugo et al., 2012). A long-term perspective can be obtained by the retrospective quantitative analysis of xylem anatomy in tree-ring series. Past investigations have shown that it is possible to link cell anatomical features with inter-annual climate variability (Fonti et al., 2010). Methodological advances (von Arx and Carrer, 2014; von Arx et al., 2016) now allow significantly longer (several centuries) and better replicated (two to three orders of magnitude more cells) time series of xylem anatomical features than in the recent past to be processed and analysed efficiently, thus opening new investigation prospects.

Based on the assumption that tracheids record the weather conditions influencing their formation, we investigated the long-term tracheid anatomical variation in the stem of mature Picea abies (Norway spruce) trees along a 900-m elevation gradient in the Italian Alps. The most conspicuous effect of increasing elevation is an adiabatic decline of temperature, which further reduces the mean photoperiod, and seasonality in precipitation and temperature) and genetic variability. In the European Alps, this is generally coupled to increased mean precipitation (Körner, 2007). Elevation gradients are thus valuable settings to assess responses to changing climate (Körner, 2007). Previous analyses at the same location had already shown a climate influence on tree-ring width and potential hydraulic conductivity associated with the 900-m gradient (Castagneri et al., 2015). Here we make use of this setting to evaluate how climatic variability controls the morphogenesis of two functionally important anatomical traits: tracheid size, resulting from the process of cell enlargement, and wall thickness, which results from the successive deposition of the cell wall. Tracheid anatomy was innovatively assessed at intra-ring level, with the aim at evaluating how and when climate variability affects tracheid morphogenesis within the growing season. Given that (1) tracheid enlargement and wall thickening are two distinct processes (Vaganov et al., 2006), (2) tracheid enlargement is more sensitive to water availability than other xylogenesis phases (Hsiao, 1973; Abe et al., 2003), and (3) the carbon-demanding latewood wall thickening is more limited at higher elevation by the temperature-sensitive process of carbon fixation (Petit et al., 2011; Rossi et al., 2013; Körner, 2015), we expect that these morphogenetic processes in P. abies are differently influenced by climate across elevation. Here we used an elevation gradient to evaluate the role of climate in shaping tracheid diameter and wall thickness, and to verify whether temporal shifts and duration of the influence of climate on morphogenesis change according to the length of the growing season, which is shorter at higher elevation (Körner, 2007).

FIG. 1. Annual course of maximum temperature and precipitation in the study area. In red, daily maximum temperature mean over 31-d moving windows with a daily step; in blue, precipitation sum over 31-d moving windows with a daily step. Weather data were recorded from 1926 to 2012 at the meteorological station of Cortina d’Ampezzo, 1230 m a.s.l., located ~3 km from the study sites.

MATERIALS AND METHODS

Study area

Samples were collected in the Eastern Italian Alps along a northeast-facing slope at Croda da Lago (46°30’ N, 12°07’ E). The slope, ranging from 1200 m a.s.l. up to the tree limit at 2150 m a.s.l., is covered by open, uneven-aged multi-layered forest stands composed of Picea abies occurring exclusively or mixed with other conifers (Larix decidua, Pinus cembra, Pinus sylvestris and Abies alba). The stands have not been managed for decades or affected by major disturbances. The soils are shallow and calcareous and the climate of the region is relatively moist and cool. The average daily maximum (minimum) temperature is 20.8 (8.9) °C in July and 3.1 (~5.8) °C in January (meteorological station of Cortina d’Ampezzo, 1230 m a.s.l., 1926–2012, ~3 km from the area). Mean annual precipitation, occurring as snow during winter, is 1080 mm, and is most abundant from May to November (Fig. 1).

Sample collection and processing

Increment cores were collected from three stands located at 2100 (EL21), 1600 (EL16) and 1200 (EL12) m a.s.l. at a distance of 1–2 km from each other. According to the standard sampling protocol for the analysis of tree-ring responses to climate (Schweingruber et al., 1990), we selected 15–20 dominant and undamaged adult trees in each stand. For each tree, two cores (5.15 mm in diameter) were collected with an increment borer (Haylöf, Längsele, Sweden) perpendicularly to the slope on opposite sides of the stem. To assign the annual rings to the corresponding calendar year, the ring widths were measured to the nearest 0.01 mm using TsapWin (Rinttech, Heidelberg, Germany) for visual cross-dating, and cross-dating quality was checked using COFECHA (Holmes, 1983). Subsequent cell anatomical measurements were then conducted for each site on a selection of eight cores from eight trees (Table 1), avoiding samples with visible defects such as nodes, reaction wood or rotten and missing parts. The selected cores were divided into pieces 4–5 cm long to prepare transverse sections (15–18 μm thick) for anatomical measurements using standard protocols.
Traits were built by calculating the bi-weight robust mean from the calculated detrended index as the ratio between the observed and fitted values for each annual ring (Cook and Kairiukstis, 1990). To assess the climate influence on xylem traits, we sought to emphasize high-frequency variation, related to inter-annual climate variability, through the removal of low-frequency variation not related to climate (Fritts, 1976). Indeed, anatomical time series usually show long-term trends, due to tree height growth during ontogenesis (Fig. 3A) (Carrer et al., 2015). We therefore fitted a cubic smoothing spline with 50% frequency cut-off of 30 years to each individual anatomical series, and calculated the detrended index as the ratio between the observed and fitted values for each annual ring (Cook and Kairiukstis, 1990) (Fig. 3B). Chronologies of the anatomical features (2 traits × 3 sites × 10 sectors = 60 sector chronologies) were built by calculating the bi-weight robust mean from the detrended series, using the R package dplR (Bunn, 2008).

**Statistical analyses and assessment of climate–anatomy relationships**

To evaluate the influence of elevation on xylem anatomical traits, one-way analysis of variance (ANOVA) was applied to test differences in CD and CWT in each sector among the three sites. When elevation showed a significant effect, Tukey’s pairwise comparisons were used to determine which group was significantly different from the others. Besides, to assess anatomical trait variations along the ring, one-way ANOVA was applied between the ten sectors. Tukey’s pairwise comparisons were used to identify significantly different groups.

The mean correlation coefficients between all possible combinations of the eight series [r between trees (rbt)] was adopted to assess the shared pattern across individual series building each sector chronology (Fritts, 1976) with the R package dplR (Bunn, 2008). Furthermore, the (in)dependence of information provided by ring sectors separated by different distances was evaluated for each anatomical feature and site by calculating the shared variance (coefficient of determination, coefficient of determination, $R^2 \times 100$) among sector chronologies. All statistical analyses were performed on anatomical parameters from 1926 to 2012, corresponding to the period covered by the weather records from Cortina d’Ampezzo meteorological station (Fig. 1).

Climate influence on cell anatomical features was quantified by correlating each sector chronology with daily maximum temperature and precipitation sum with Pearson’s correlations using 31-d windows slid 1-d steps from 1 July of the previous year to 31 October of the current year. Climate time series were obtained using daily-resolved weather records from the Cortina d’Ampezzo meteorological station (Fig. 1). Besides this conventional approach with climatic data aligned to the calendar date [day of year (DOY) approach], we also calculated the correlations aligning the climatic data to the date when a specific degree-day temperature sum is reached (DD approach). The DD temperature sums of each year (McMaster and Wilhelm, 1997) were calculated as:

$$\sum_{j=1}^{m} \left( \frac{T_{max} + T_{min}}{2} \right) - 5$$

where $j = 1, 2, \ldots, m$ are days with a mean temperature $> 5^\circ C$, which is widely used as the lower limit for plant growth (Grigorieva et al., 2010), and $T_{max}$ and $T_{min}$ are the daily maximum and minimum air temperatures (°C), respectively. As EL16 and EL21 are located at higher elevations than the meteorological station, for these two sites temperature was adjusted considering a lapse rate of 0.6°C/100 m of elevation (Auer et al., 2005; Körner, 2007). For the alignment of the climatic data, we considered seven DD thresholds (0, 20, 40, 60, 80, 100

### Table 1. Characteristics of trees selected for ring anatomical measurements

<table>
<thead>
<tr>
<th>Plot</th>
<th>No. of samples</th>
<th>Height (m)</th>
<th>Diameter (cm)</th>
<th>Ring width (mm)</th>
<th>No. of rings</th>
<th>Estimated age (years)</th>
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<tbody>
<tr>
<td>EL21</td>
<td>8</td>
<td>19.8±3.5</td>
<td>55.3±14.2</td>
<td>0.9±0.26</td>
<td>236±46</td>
<td>263±54</td>
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<tr>
<td>EL16</td>
<td>8</td>
<td>29.1±3.1</td>
<td>57.6±7.9</td>
<td>0.89±0.36</td>
<td>289±53</td>
<td>301±42</td>
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<tr>
<td>EL12</td>
<td>8</td>
<td>36.6±1.6</td>
<td>70.3±8.9</td>
<td>1.86±0.36</td>
<td>141±12</td>
<td>149±12</td>
</tr>
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Values are mean ± standard deviation of eight trees at site EL21 (2100 m a.s.l.), EL16 (1600 m a.s.l.) and EL12 (1200 m a.s.l.). Stem diameter was measured 1.3 m above the ground. Ring width is averaged over the period 1926–2012. No. of rings is the total number of rings in the core. Estimated age includes estimation of rings missing to the stem pith.

(von Arx et al., 2016). In short, micro-sections were cut with a rotary microtome (Leica, Heidelberg, Germany), stained with safranin (1% in distilled water) and fixed on permanent slides with Eukitt (BiOptica, Milan, Italy). Overlapping digital images were captured with a light microscope at 40× magnification (Nikon Eclipse 80 with distortion-free lenses) with a resolution of 0.833 pixels μm⁻¹, and stitched together with PTGui software (New House Internet Service B.V., Rotterdam, The Netherlands) to obtain a single image of 2–3 mm width from each section. The images were then processed with the image analysis software ROXAS v2.0 (von Arx and Carrer, 2014), which provided the lumen size, wall thickness and relative position within the dated annual ring for each of the ~4 million measured tracheids. Analysis was restricted to the period covered by weather records, i.e. 1926–2012.

**Intra-ring anatomical series and chronologies**

Diameter and wall thickness were assessed for each tracheid in the ring (Fig. 2). To analyse xylem anatomy at the sub-annual time scale, each annual ring was divided into ten tangential sectors of equal width (Fig. 2). For each sector, we assessed the 90th percentile of the size distribution of cell diameter (CD) and cell-wall thickness (CWT). For each core, we then built ten time series for both CD and CWT, which represented the inter-annual variations of tracheid anatomical features at intra-ring level.

To assess the climate influence on xylem traits, we sought to emphasize high-frequency variation, related to inter-annual climate variability, through the removal of low-frequency variation not related to climate (Fritts, 1976). Indeed, anatomical time series usually show long-term trends, due to tree height growth during ontogenesis (Fig. 3A) (Carrer et al., 2015). We therefore fitted a cubic smoothing spline with 50% frequency cut-off of 30 years to each individual anatomical series, and calculated the detrended index as the ratio between the observed and fitted values for each annual ring (Cook and Kairiukstis, 1990) (Fig. 3B). Chronologies of the anatomical features (2 traits × 3 sites × 10 sectors = 60 sector chronologies) were built by calculating the bi-weight robust mean from the detrended series, using the R package dplR (Bunn, 2008).

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and 120 DD). For each of these cases with newly aligned climatic data, moving correlations were calculated with the 31-d windows as for the DOY approach. For both DOY and DD approaches, correlation $P$ values were adjusted for multiple comparisons using the false discovery rate correction (Benjamini and Hochberg, 1995). Analyses were implemented in R (version 3.1.0, R Development Core Team, 2014).

RESULTS

Sub-annual anatomical variability and common pattern

Variations in cell anatomical features along the ring sectors were consistent among elevations. Within the ring, CD steadily decreased from sector 1 to 10, with a pronounced reduction in the last three sectors (Figs 2 and 3, Table 2). Cell-wall thickness showed the opposite trend, with a sharp increase in the last sectors. In addition, the negative relationship between CD and CWT along the sectors was consistent at the three sites (EL21, $r = -0.97$, $P < 0.001$; EL16, $r = -0.98$, $P < 0.001$; EL12, $r = -0.98$, $P < 0.001$; Fig. 4). For all sectors, both CD and CWT were larger at EL12 than at the other two sites (Table 2).

The detrended anatomical series had a fairly strong common pattern (rpt), revealing good synchronization of the inter-annual variations in anatomical features within the same sector on different trees. In general, for both CD and CWT, rpt increased across the annual ring (Table 2). The highest value (rpt = 0.56) occurred for CWT in the last sector at EL21.

Fig. 2. Example of a tree ring split into ten sectors (A) and representation of the anatomical features measured on each single tracheid (B). In (A), the overlay curves show the intra-ring variation of the mean 90th percentile of cell diameter (CD) and cell-wall thickness (CWT) as obtained for the ten tree-ring sectors with equal width. The coloured cells illustrate on a narrow strip how individual tracheids were assigned to the sectors.

Fig. 3. Cell diameter (CD) time series. (A) Variability of CD along a single core, as grouped by ring sector. The shaded area indicates the investigated period (1926–2012). (B, C) Thirty-year spline-detrended CD series for sectors 1 (B) and 10 (C) for the eight trees measured at the 2100 m site over the period 1926–2012. Thick lines indicate the mean ring-sector chronologies.
TABLE 2. Mean 90th percentile of cell diameter (CD) and cell-wall thickness (CWT), and mean correlation between the detrended time series (Rbt) of eight trees for tree-ring sector averaged over 1926–2012, at EL21 (at 2100 m a.s.l.), EL16 (1600 m a.s.l.) and EL12 (1200 m a.s.l.)

<table>
<thead>
<tr>
<th>Sector</th>
<th>CD</th>
<th>CWT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EL21 Mean (µm)</td>
<td>EL16 Mean (µm)</td>
</tr>
<tr>
<td>1</td>
<td>A 50.6c</td>
<td>A 52.5b</td>
</tr>
<tr>
<td>2</td>
<td>A 50.4c</td>
<td>AB 51.7b</td>
</tr>
<tr>
<td>3</td>
<td>A 49.8c</td>
<td>B 50.9b</td>
</tr>
<tr>
<td>4</td>
<td>B 48.4c</td>
<td>C 49.2b</td>
</tr>
<tr>
<td>5</td>
<td>C 46.9c</td>
<td>D 47.5b</td>
</tr>
<tr>
<td>6</td>
<td>D 45.3b</td>
<td>E 45.8b</td>
</tr>
<tr>
<td>7</td>
<td>E 43.4b</td>
<td>F 44.0b</td>
</tr>
<tr>
<td>8</td>
<td>F 40.1b</td>
<td>G 40.7b</td>
</tr>
<tr>
<td>9</td>
<td>G 33.2b</td>
<td>H 33.5b</td>
</tr>
<tr>
<td>10</td>
<td>H 22.8b</td>
<td>I 22.5b</td>
</tr>
</tbody>
</table>

Different upper-case letters on the left of the value indicate significant differences between sectors for the same site (column), according to ANOVA with Tukey’s pairwise comparisons. Different lower-case letters on the right indicate significant differences between the three sites EL12, EL16 and EL21, for the same sector (row).

Among the ten sectors, anatomical chronologies were relatively independent of each other. For both CD and CWT, the proportion of shared variance in neighbouring sectors was high (inter-sector distance = 1, Fig. 5). However, it sharply decreased when considering more distant sectors (generally <25% at inter-sector distance >3), indicating that anatomical features of cells formed in different periods of the season (not consecutive sectors) were quite independent of each other.

Climate influence on xylem anatomy

Correlation analyses between inter-annual climate variability and sector chronologies revealed that different anatomical features showed distinct relationships with climate factors, mostly during spring and summer. Moreover, we observed temporal shifts in climatic responses along the ring, which reflect the seasonal changes in the timing of environmental constraints on cell formation.

At EL21, a positive correlation between CD and maximum temperature occurred from mid-June in the first ring sectors to early July in the last ones (P < 0.05 in sectors 1, 2, 3 and 5; P < 0.01 in sectors 6 and 10; P < 0.001 in sectors 7–9; Fig. 6A). This correlation increased markedly when considering the DD temperature sums. Specifically, when considering the 40-DD threshold, which over the study period occurred on average on 19 June (corresponding to DOY 170), correlations improved for all the sectors (P < 0.001 in all sectors; Fig. 7). During August, there was a negative correlation with temperature (P < 0.05 in nine sectors; Fig. 6A). At EL16, the negative correlation with temperature was observed ~15–30 d earlier than at EL21, specifically around DOY 170 (late June, sectors 3–7, P < 0.05 to P < 0.01; Fig. 6C) to 215 (end of July, last sectors, P < 0.05; Fig. 6C). Positive correlations with precipitation occurred a few days before, specifically around DOY 160 (mid-June, sectors 3 and 4, P < 0.05; Fig. 6D) to 215 (July, last sectors, P < 0.05; Fig. 6D). At EL12, the negative correlation of CD with temperature (P < 0.05 in sectors 4–0; P < 0.001 in sectors 6 and 7; Fig. 6E) and positive correlations with precipitation (significant in all sectors; P < 0.001 in sectors 2, 6 and 7; Fig. 6F) were slightly stronger than at EL16, and occurred...
10–15 d earlier. At this elevation, positive correlation with precipitation emerged in the first sectors and remained quite stable across the whole ring, with a time lag of 40–50 d from the first to the last sectors. At all three sites, CD was weakly connected to climatic variability before the growing season, with generally unclear patterns except for a positive relationship with late June temperature at EL12 (\( P < 0.05 \)) in sectors 8, 9 and 10, and February precipitation at EL21 (\( P < 0.05 \)) in sectors 1, 2, 3, 8 and 10 (Supplementary Data Fig. S1).

Cell-wall thickness showed completely different relationships with climate compared with CD (Fig. 8). Temperatures in late spring and/or summer were positively related to this feature, while relationships with precipitation were weak at all elevations. May temperature was positively correlated with CWT for the last three sectors at EL21 (\( P < 0.05 \) in sectors 8, 9 and 10; Fig. 8A) and the central sectors at EL12 (\( P < 0.05 \) in sectors 4 and 5; Fig. 8E). Furthermore, a positive association with late-summer temperature emerged for the last four sectors, being stronger at EL21, particularly for the last three sectors (\( r > 0.5, P < 0.0001 \); Fig. 8A), weaker at EL16 (\( r > 0.4, P < 0.05 \); Fig. 8C), and not significant at EL12 (Fig. 8E). Similarly to CD, this association increased at EL21 using the DD approach (\( r > 0.6 \); Fig. 7 compared with Fig. 8). Climatic conditions of the previous growing season and winter were slightly related to CWT (Supplementary Data Fig. S1).

**DISCUSSION**

**Intra-annual variability in xylem anatomy**

This study demonstrated that the analysis of xylem anatomy along tree-ring series can be used to assess the long-term climate influence on xylem morphogenesis. Compared with previous analyses of anatomical features considering the whole ring or earlywood and latewood sections (Fonti et al., 2013), our approach aimed at improving the detail of results by increasing the resolution at intra-seasonal level. Across the entire elevation gradient, both CD and CWT showed a substantial synchronous
variability among trees (rpt; Table 2), although with differences among the ring sectors. This suggests that cell anatomy, and thus morphogenesis, is sensitive to environmental variability (Fritts, 1976), but this sensitivity can also vary over the course of the growing season (Sass and Eckstein, 1995). The different patterns of rbt, with generally higher values for CWT towards the last ring sectors and with increasing elevation, also indicate that not all anatomical traits are equally sensitive to climate. This is in line with previous observations showing different sensitivity to climate in different cell anatomical features, and between earlywood and latewood (Martin-Benito et al., 2013; Szymczak et al., 2014).

The decreasing similarities with increasing distance between ring sectors (Fig. 5) likely reflect changes in the environmental influences that impact cell formation during the growing season. This suggests that successive tracheid rows along the ring encode a distinct climate imprint throughout the growing season, demonstrating the potential of splitting the ring into sectors for investigating the associations between cell morphogenesis and intra-seasonal climate.

Temporal match of intra-ring climate correlations with cambial activity observations

Sector-based correlations showed that cell anatomical features were significantly affected by seasonal climate variability. The timing of cell enlargement and wall thickening, recorded in previous investigations on cambial activity, matches well with the timing of most of the observed significant correlations. Indeed, significant correlations between CD and temperature at high elevation started in early June for the first ring sectors, lasting to mid-July in the last sectors (Fig. 6). This timing matches the most intense phases of cambial activity and cell enlargement observed for P. abies at similar elevation, a few kilometres from the study site (Rossi et al., 2008). Information on cambial activity in the same species under environmental conditions similar to those at the low-elevation sites are unfortunately not available. However, the observed lapse rate of 10–15 d for both the temperature and precipitation associations with CD within the same sector from intermediate to low elevation, and of 20–40 d for temperature associations in CD from high to intermediate elevation, fits the lapse rate of ~3–4 d per 100 m in elevation reported for xylogenesis phases of Larix decidua in the Swiss Alps (Moser et al., 2010). These observations indicate that the proposed approach properly captures phenological shifts along elevation gradients.

Climate influence on CWT (Fig. 8) was evident in two distinct periods: a moderate positive temperature effect at the beginning of the season, and a second one at the end of the summer for the last three or four sectors, lasting for up to 2 months. The latter, much stronger at high elevation, overlaps with the wall-thickening phase in the last part of the ring observed in P. abies at high elevation (Gindl et al., 2001; Rossi et al., 2008), and its duration is in line with current knowledge on the duration of wall formation, which can last from 30 d (mild conditions) to 70 d (cold conditions) in latewood cells (Kirdyanov et al., 2003; Rossi et al., 2008, 2013; Cuny et al., 2013).

Intra-ring anatomy reflects climate impact on cell morphogenesis

Our retrospective analysis of the climate influence on cell features provided indirect indications on xylem morphogenesis that are in line with current knowledge, mainly derived from more direct approaches based on much shorter periods, such as observations of cambial activity (e.g. Cuny et al., 2014; Rossi et al., 2014) and diel stem radial variations (e.g. King et al., 2013; Steppe et al., 2015). In particular, our results indicate that climate drivers are diverse for different anatomical features, depending on the nature and timing of the underlying processes during the growing season, and on the limiting conditions prevailing at different elevations.

Specifically, we interpret the positive influence of temperature in June to early July on CD at the highest elevation as an indication that warm conditions promote the first phases of xylem formation (Lenz et al., 2013). In this context, temperature at the onset of the growing season affects the production and transport of hormones such as auxin and gibberellin (Schrader et al., 2003; Aloni et al., 2015) and the translocation of nutrients. Correspondingly, the important role of temperature in cold environments in the kinetics of cell differentiation and enlargement has already been observed both empirically (Gricar et al., 2006; Rossi et al., 2008; Petit et al., 2011; Lenz et al., 2013) and correlatively (Kirdyanov et al., 2003; Kalliokoski et al., 2012; Fonti et al., 2013; Rossi et al., 2014). Stronger and more
defined associations emerged when the climatic data were aligned according to the DD temperature thresholds at high elevation (especially for CD in the first ring sectors; Figs 6 and 7), but not at lower elevations (see Cuny et al., 2015). These add additional evidence and further confirm that late-spring temperature controls the onset of cambial activity and the kinetics of cell formation in cold environments (Kirdyanov et al., 2003; Seo et al., 2008; Thibeault-Martel et al., 2008; Moser et al., 2010; Olano et al., 2013). However, we also observed water-related constraints on tracheid morphogenesis. At high elevation, a negative temperature influence on CD emerged a few weeks after the positive temperature stimulus. At lower elevation, the negative effect of temperature on CD was stronger, better defined across the ring sectors, anticipated by a few weeks, and associated with a positive effect of precipitation. Apparently, at low elevation, early summer temperature does not limit cell enlargement, resulting in generally larger tracheids compared with higher elevation. However, during dry and warm years, low soil water content and high tree transpiration rates decrease water availability, reducing the cell turgor pressure necessary to stretch the primary cell wall of the forming cells (Hsiao, 1973; Taiz and Zeiger, 2006).

While our results support the hypothesis that the climate effects on CD are directly connected to the processes shaping cell size (i.e. cell enlargement), the responses encoded in CWT seem to be primarily related to the amount of carbon assimilates reaching the cambial zone (Wolf et al., 2012). Coherently with studies on maximum latewood density, we found correlations with climate for the last ring sectors in relation to the conditions at the beginning and towards the end of the growing season (Wimmer and Graber, 2000; Frank and Esper, 2005). The positive effect of April–May temperature on CWT might relate to the improved carbon assimilation during spring (source activity) corresponding to an earlier onset of the growing season and more intense assimilation activity. The late summer temperature association is instead probably linked to carbon mobilization and deposition rates at the time of wall thickening (sink activity) (Hoch et al., 2002; Körner, 2015), which were apparently strongly related to temperature at high elevation. Our observations support previous findings that carbon fixation and
carbohydrate mobilization are constrained by temperature at the altitudinal limit of a species (Hoch and Korner, 2003; Simard et al., 2013). Examples of this relationship are also given by observations that the long-lasting wall thickening process can be slowed or interrupted by reduced temperatures at the end of summer (Gindl, 1999; Vaganov et al., 2006; Xu et al., 2013), in extreme cases resulting in rings characterized by thin walls (known as light rings; Filion et al., 1986) or un lignified walls (known as blue rings; Piermattei et al., 2014) in the last cells.

Concluding remarks and future prospects

Our study shows that xylem anatomy assessed through tree-ring partitioning provides both high-resolution and long-term indications on how, when and for how long climate drivers influence the processes of xylem morphogenesis. Under different conditions along an elevation gradient, climate either affects cell enlargement through regulation of the kinetics of the process (influenced by temperature) or by influencing the cell tangential pressure necessary for cell enlargement (related to water availability). Similarly, we have shown that the deposition of the cell wall in the second part of the ring is sensitive to the temperature occurring both at the beginning of the growing season, which seemingly promotes carbon assimilation, and at the end of the season, when deposition in the cell wall occurs.

Tree-ring anatomy provides a long-term perspective on tree responses to climate, fundamental for soundly assessing forest responses to ongoing climatic change (Reyer et al., 2015). This can improve understanding of xylem formation, and help to assess changes over time in (1) growth phenology, (2) climatic sensitivity, (3) xylem functioning and (4) xylem plasticity of different species – all of them important topics for improving our mechanistic understanding of how climate impacts xylem hydraulic and structural properties and ultimately tree performance.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: correlations of ring-sector chronologies with temperature and precipitation over all year round.

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LITERATURE CITED
