Tracheid anatomical responses to climate in a forest-steppe in Southern Siberia

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ABSTRACT

Tree-rings are precious natural archives to assess ecosystem variability over time. Xylem anatomy in woody tissue is a promising source of information in tree-rings since it is closely linked to tree hydraulics and carbon fixation. However, despite the rising interest for cell anatomy in dendrochronology, still little is known about the interpretation of the variability of cell anatomical responses observed across different environments and species.

Here we analyze cell anatomical responses to increasing summer drought on 18 trees from 3 conifer species (*Picea obovata*, *Pinus sylvestris*, and *Larix sibirica*) growing in the transition zone between forest and steppe in the Republic of Khakassia (Russia). Analyses include the comparison of tracheid size distributions along climatic gradients and contrasting micro-topography from 1986 to 2008.

Results indicate an overall decrease of earlywood tracheid lumen and cell wall thickness to high temperature and drought regardless of species and site conditions. In particular an increase of one degree Celsius during the summer caused up to 5% reduction of earlywood cell lumen and wall thickness. These anatomical shifts suggest that a downscaling of hydraulic efficiency is not paralleled by increased hydraulic safety, presumably due to carbon limitation.

Based on the results of this case study, we suggest that increasing drought stress might hamper the formation of a functional xylem structure, thus being a possible trigger for a miss-acclimation causing long-term decline and higher exposure to hydraulic failures. Despite the promising study approach, more studies including more data (trees, years) and broader climatic gradients would be needed to further improve our mechanistic understanding.

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1. Introduction

Annually dated tree-rings are a precious natural archive for assessing past and current ecosystem adaptability. Tree growth is frequently documented to be sensitive to environmental changes (St. George, 2014) and thus the widths and properties of the annual layers are recording precious information of changes in growing conditions between and within years (Fritts, 1976). The newly formed tree-ring represents both a relative measure of the annual growth performance, as well as the means through which the xylem has been adjusted to contribute to the future tree functioning. Thus, not only the total number of cells, but also the structure and the material from which they are made contribute to define the functional xylem properties (Lachenbruch and McCulloh, 2014).

In this perspective, the anatomy of xylem cells (as tracheids and vessels) is a promising tree-ring proxy to reconstruct past tree responses to environment because of its high intra-annual resolution and its direct link to important functional and physiological processes as sap transport and carbon fixation (Fonti et al., 2010; Fonti and Jansen, 2012). Thanks to these characteristics, the study of cell anatomical changes over time can help understanding the causes and the impact of xylem in relation to changing environmental conditions, especially in drought-prone areas, where drought-induced tree mortality is increasing (van Mantgem et al., 2009; Allen et al., 2010; Phillips et al., 2010; Peng et al., 2011). There is in fact an increasing body of evidence suggesting that warming and increasing drought are important triggers for forest decline and dieback in several ecosystems across the globe (e.g.; Allen et al., 2010; Rigling et al., 2013; Sangüesa-Barreda et al., 2015). The mechanism for mortality in this environment, although still under debate, seems to be mainly related to unbalances in the availability of carbon assimilates and/or the hydraulic functionalities

(McDowell et al., 2008; McDowell and Sevanto, 2010; Sala et al., 2010; McDowell et al., 2011; Sevanto et al., 2014). In particular, it is proposed that these more stressful environmental conditions reduce net carbon assimilation and storage and/or alters the newly forming wood structure through which water moves from the soil up to the leaves, as observed for several species and environments (e.g.; Eilmann et al., 2009; DeSoto et al., 2011; Gea-Izquierdo et al., 2012; Martin-Benito et al., 2013; Hetzer et al., 2014; Olano et al., 2014; Venegas-Gonzalez et al., 2015). Yet, both characteristics are related to the process of wood formation and their results is permanently stored in the anatomical structure of the tree rings. The first determine the amount of resources available to build up the structure of an efficient water-transporting xylem, while the second determine the safety level against hydraulic failures when drought occurs. Thus, changed average environmental conditions or increased frequency and magnitude of extremes, will not only limit the trees' growth capacity, but, also deteriorate the hydraulic and mechanical properties of the xylem tissue over multiple sapwood layers, which can have an detrimental legacy on trees' future performance and survival (Anderegg et al., 2013; Heres et al., 2014).

Quantitative wood anatomy is a viable method commonly used to detect cell anatomical changes along variation in climatic conditions such as increasing warmth and drought. Moreover, thanks to recent developments in wood preparation (e.g.; Gartner et al., 2014) and computational image analysis (e.g.; Silkin, 2010; Wegner et al., 2013; von Arx and Carrer, 2014), it is now possible to quantify changes of different tissues and parameters at intra-annual scale with an acceptable effort. In particular, it has already been evidenced that environmental variability affects the size and structure of the water conducting xylem (see Fonti et al., 2010 for a review). Recent studies are also demonstrating that the xylem anatomical properties can have a possible impact on the hydraulic properties of the wood (e.g.; Schuldt et al., 2013; Chenlemuge et al., 2014; Hajek et al., 2014), and only few studies have demonstrated that on the long-term this affects tree vitality and performance (Heres et al., 2014). In addition to the measurement of the conduit size (e.g.; Abrantes et al., 2013; González-González et al., 2015), these developments have made possible for example to also analyze the conduit grouping (e.g.; von Arx et al., 2013), and the amount of ray parenchyma (e.g.; Olano et al., 2013; Fonti et al., 2015), making the link between anatomical changes and their functional meaning more quantifiable. However, despite the rising interest of cell anatomy in dendrochronology and its potential contribution in supplying a long-term perspective beyond the processes of mortality, the use of tree-ring anatomy in environmental science is still hampered by a limited mechanistic understanding. This is mainly due to complex interactions between short- and long-term environmental change on xylem formation and structure; and by difficulties in assessing how these changes in structure can long-term impact the functioning of trees, forests and ecosystems (Lachenbruch and McCulloh, 2014). To improve our mechanistic understanding of the environmental impact on xylem properties, we thus need to collect information of responses in a broader range of climatic gradient and contrasting environmental conditions.

In this study we analyzed intra-annual tracheid anatomical characteristics along series of tree-rings in order to assess how xylem structure of tree from the forest-steppe in the Republic of Khakassia (Siberia, Russia) are responding to increasing summer temperature and drought. In particular, based on a study case including mature trees from three conifer species (*Picea obovata*, *Pinus sylvestris*, and *Larix sibirica*) growing at two sites with differing soil water availability, we aim at assessing at what rate increasing temperature and/or water-limiting conditions affect the anatomical characteristics of the water conducting cells of the xylem.

Table 1
Sampling size and main tree characteristics (mean ± standard deviation).

Group	Number of trees[a]	DBH [cm]	Age [years]	Ring width[b] [mm]
Larix Dry	14 (5)	13.9 ± 3.6	138 ± 46.0	1.01 ± 0.78
Larix Wet	10 (5)	14.3 ± 3.0	156 ± 7.7	0.92 ± 0.61
Picea Wet	10 (5)	10.5 ± 2.5	110 ± 21.8	0.96 ± 0.67
Pinus Dry	14 (3)	15.7 ± 3.0	186 ± 46.9	0.85 ± 0.59

Dry = trees located on a south facing slope; Wet = trees located at the bottom of the slope (see Fig. 1).
[a] Numbers in brackets refer to the numbers of trees used for the anatomical measurements.
[b] Ring width data refers to the period 1986–2008.

2. Materials and methods

2.1. Study site, site characteristics, and sampled wood cores

The study site is situated in the Chulym–Yenisei Hollow (54°24′ N, 89°57′ E) in the Altai–Sayan region of the Republic of Khakassia in Russia (Fig. 1a). The region belongs to the forest-steppe belt in the Southern Siberia and is characterized by a moderately cold and dry continental climate. According to records from the Shira meteorological station (54°30′ N, 89°56′ E, 450 m asl, from 1966 to 2012, located 32 km northeast from the study site; Fig. 1b), the average annual temperature is 0.8 °C and the annual precipitation sum is 294 mm. The period with temperatures above 10 °C lasts 110–120 days, and the growing season usually onsets in April when daily temperatures rise above 5 °C. The majority of precipitation (90%) falls during the warm season (April–October), but summer droughts may occur in particularly warm and dry years. These events (defined as when total summer precipitation is at least one standard deviation less than the mean) occurred six times over the period 1969–2008.

The trees considered for this study have been selected in contrasting soil water conditions, i.e., on a steep south-facing slope and at its valley bottom, in the vicinity of the floodplain of an intermittent stream with a relatively high groundwater level (Fig. 1c). The distance between the sites is less than 500 m and the difference in elevation is 150 m. The slope site (Dry site) is characterized by higher amount of solar radiation, thin and well-drained soil and is covered by a mixture of *P. sylvestris* and *L. sibirica* with few *Betula pendula*. The floodplain site (Wet site) has podzolic chernozemic soil and is covered by an open woodland area dominated by *P. obovata* with sporadic *L. sibirica*.

A total of 48 wood cores have been collected at stem breast height from 4 group of dominant, healthy, damage-free, and mature trees, i.e.; from 14 *L. sibirica* (*Larix* Dry) and 14 *P. sylvestris* (*Pinus* Dry) at the dry site, and from 10 *L. sibirica* (*Larix* Wet) and 10 *P. obovata* (*Picea* Wet) at the wet site (Table 1 and Fig. 1e).

2.2. Tree-ring width and anatomical measurements and analyses

All 48 collected cores were sanded for tree-ring width (TRW) measurement (using a LINTAB measuring table connected to a TSAP Win software; RINNTECH, Heidelberg, Germany) and visually cross-dated. Cross-dating accuracy was assessed using COFECHA (Holmes, 1983). To perform climate-growth relationships, individual time-series were standardized to remove age-related and other trends using a flexible 32-yrs spline and averaged using a biweight mean into group chronologies (Cook and Kairiukstis, 1990). The strength of the common signal was quantified as the mean correlation between the detrended individual time-series (rbt).

Cell anatomical measurements were performed for the annual rings from 1986 to 2008 on a sub-selection of trees where the TRW was most highly correlated to its corresponding group average and
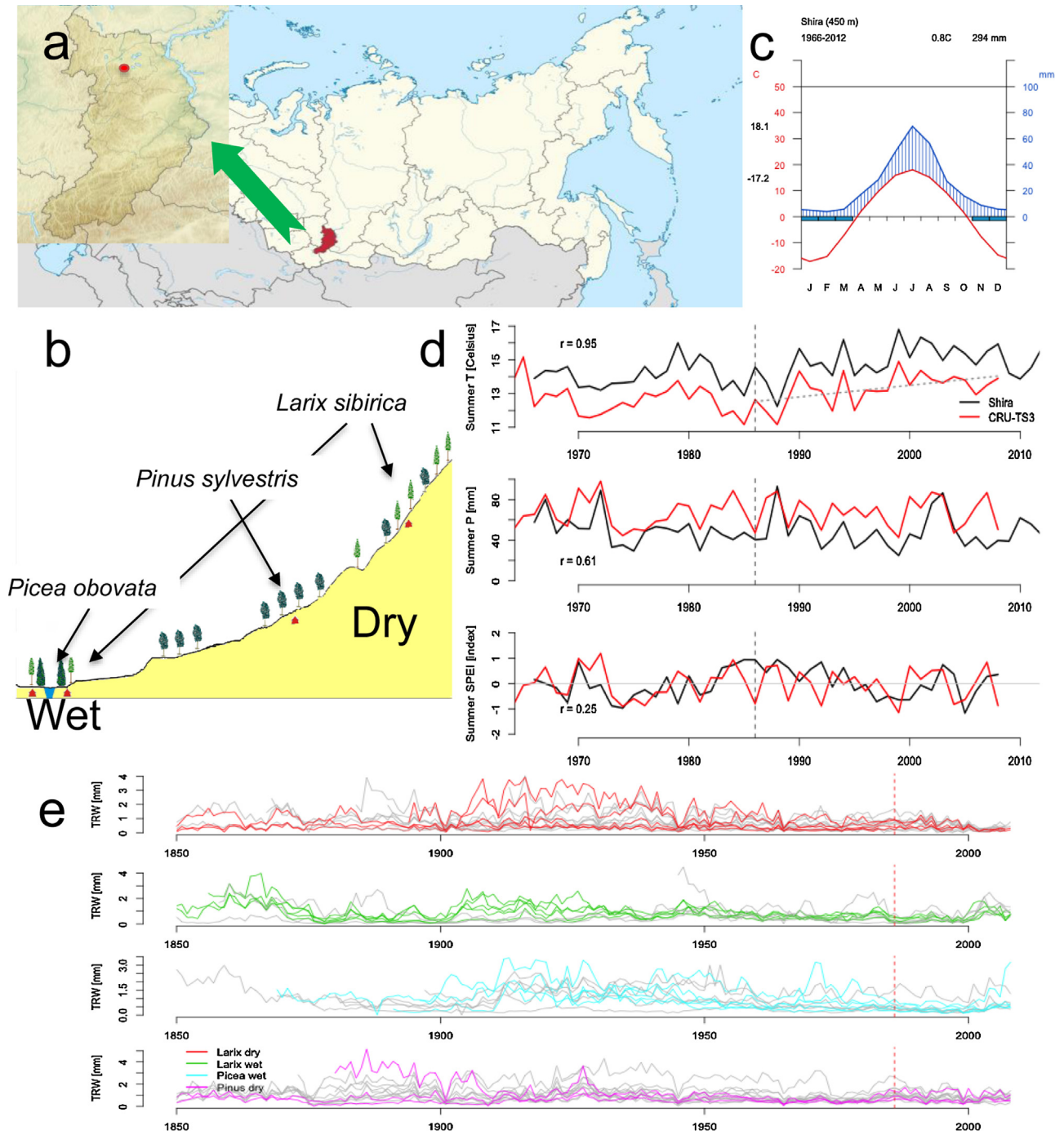
Fig. 1. (a) Location of the studied site, (b) schematic of the site microtopography, (c) climate diagram and (d) timeseries of summer (MJJ) temperature, precipitation and standardized drought index. Shira = meteorological station data for the period 1966–2012, CRU-TS3 = 0.5 degree grid data from Climate Research Unit for the period 1964–2008. (e) Raw tree-ring width time seris of the sampled trees (coloured lines refers to trees selected for anatomical meaurements). Vertical line indicates the beginning of the period covered by anatomical measurements.

that were free from reaction wood. A total of 18 cores (1 per selected tree) were chosen from anatomical analysis (5 *Larix* Dry, 3 *Pinus* Dry, 5 *Larix* Wet, and 5 *Picea* Wet). For the tracheid anatomical survey, 20 μm-thick transverse micro-sections were cut from the water-boiled wood cores using a sledge microtome and then stained with methylene blue.

The micro-sections were used to capture magnified digital images of the wood cross-section with the distortion free microscope (100 × magnification, Karl Zeiss, Germany) connected to a digital camera (756 × 581 image resolution, Karl Zeiss, Germany).

Measurements of radial lumen diameter (LD) and radial double cell wall thickness (CWT) were performed along 5 radial files per annual ring using the image analysis software package consisting of the programs Lineyka, SuperMoment and ProcessorKR (Fig. 2, Silkin, 2010). In each ring, tracheids were assigned to earlywood (EW), transition wood (TW), or latewood (LW) according to the Mork's index (i.e.; 2CWT/LD; Denne, 1989). Tracheids belonging to the ring sector with RTSR < 0.3 were assigned to EW, those with RTSR > 0.7 to LW, while the cells in between (i.e.; when 0.7 < RTSR > 0.3) where assigned to TW.
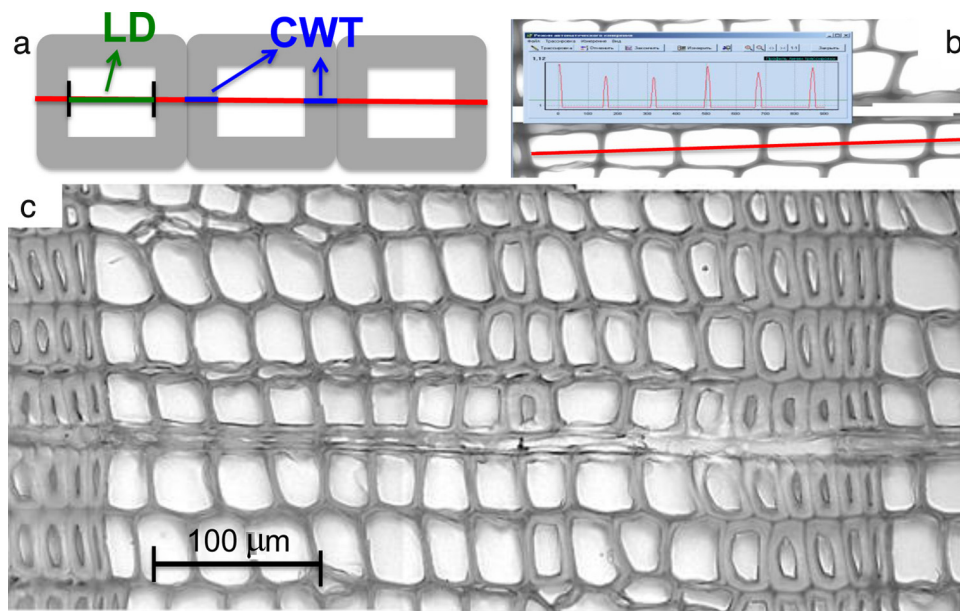
Fig. 2. Example of tracheid anatomical measurement as performed on the wood cross-section. (a) Indicates the parameters measured (LD = radial lumen diameter; CWT = radial double cell wall thickness). (b) Shows how the measurement is perfomed along a radial files. (c) Shows an example of measured image from an annual ring of *Pinus sylvestris*.

2.3. Climatic data

The influence of climate on the tree growth and on the anatomical tracheid characteristics have been performed using monthly temperature and precipitation data from the gridded CRU-TS3 (for the period 1901–2008; grid cell 54°N–84.5°E; http://climexp.knmi.nl, Mitchell and Jones, 2005) and from the meteorological station closest to the site (Shira, from 1966 to 2012), respectively. Additionally, based on the monthly temperature and precipitation data, we also quantified the standardized precipitation-evapotranspiration index (SPEI) (Vicente-Serrano et al., 2010) using the R package SPEI (v 1.6). A resolution of only one month was selected for covering short drought episodes, since short-time drought might already affect drought-sensitive tree species on shallow soils. The agreement between gridded data with the records of the Shira meteorological station is shown in Fig. 1d.

3. Results

3.1. Growth responses to climate

The studied trees were mature and relatively similar in age (species average at each site ranges from 110 to 186 years), size (DBH from 10.5 to 15.7 cm), and growth rate (ring width from 0.85 to 1.01 mm, Table 1), with decreasing TRW trend from 1966 to 2008 for *Larix* Dry, stable for *Pinus* Dry, and increasing for the trees on the Wet site (Fig. 1e). The analyses of the inter-annual growth pattern indicated that the trees at the selected sites have a good common signal and respond to annual climatic variability (Table 2), except for the trees from the *Larix* Wet group which does not significantly correlate ($p < 0.05$) with the other TRW chronologies ($r < 0.11$ over the period 1901–2008). The common signal within the species ranged from a minimum of 0.26 to a maximum of 0.55. Climate-growth correlations between the ring-width chronologies and the monthly climate parameters (i.e. with the monthly temperature, precipitation and SPEI data from 1901 to 2008) indicate that tree growth at the dry site (i.e. for *Larix* Dry and *Pinus* Dry) mainly responded to early summer drought (e.g. with the average for May, June and July: MJJ) with both significant ($P < 0.05$) responses to temperature (negative) and to precipitation (positive), while the

summer growth responses of the trees from the wet site (i.e. for *Larix* Wet and *Picea* Wet) were not clearly distinct (i.e., not significant at $P < 0.05$ for both the average for May, June, and July: MJJ; or June, July, and August: JJA) despite the occurrence of significant responses to single month parameters (e.g.; with a positive response to June temperature for *Larix* Wet).

3.2. Cell anatomical characteristics

The tracheids of *L. sibirica* had on average larger lumen diameter and thicker walls than the tracheids of *P. obovata* and *P. sylvestris* (Table 3). For example, the LD of the earlywood tracheid of *Larix* Wet (49.23 μm) were on average 59% and 50% larger, and latewood CWT (7.9 μm) 86% and 58% thicker than for *Picea* and *Pinus*. For *L. sibirica*, the tracheid lumen diameters were slightly larger and the wall thicker within the Wet site than at the Dry site. Over the considered time window for the anatomical measurements (from 1986 to 2008) the time-series of the cell anatomical parameters showed a rather stable and slight decreasing trend (e.g., showing a tracheid size reduction of *L. sibirica* trees from the Dry site) indicating that the inter-annual cell anatomical data variability was not mainly driven by the widening occurring as trees growth in height (*sensu* Anfodillo et al., 2013; Carrer et al., 2015; Fig. 3). The common signal of the cell anatomical characteristics (LD and CWT) grouped per tree-ring sectors (EW, TW and LW) was stronger at the Dry site (Rbt from 0.12 to 0.70, Table 3) than at the Wet site (from −0.01 to 0.40), where in some cases the signal was very weak or even absent. In general the common signal was observed to be stronger for *P. sylvestris* (although only based on 3 time-series) and weaker for *L. sibirica*.

3.3. Tracheid size variation and summer climatic variability

The variability of the summer weather conditions (i.e.; the June, July and August average) during the period considered for the anatomical analyses were characterized by a slight warming trend (rate of increase of 0.07 °C/year, significant at $p < 0.05$) and by relatively stable precipitation and drought occurrence (Fig. 1d). However, year-to-year weather conditions recorded at the Shira meteorological station were varying within a band of 4.6 °C for

Table 2

Overview of climatic characteristics of the tree-ring width chronologies. Values refer to the period from 1901 to 2008 and correlations with climate are calculated using the CRU-TS3 monthly data. Value in brackets refers to the same calculations but for the period from 1966 to 2008 and using the data from the Shira Meteorological station.

Correlation	*Larix* Wet	*Picea* Wet	*Larix* Dry	*Pinus* Dry
Inter-series (rbt)	0.48 (0.55)	0.26 (0.31)	0.47 (0.35)	0.55 (0.59)
Between chronologies				
*Larix*Wet	–	0.11 (0.27)	0.00 (−0.05)	0.04 (−0.05)
Picea Wet	–	–	0.26 (0.30)	0.22 (0.31)
*Larix*Dry	–	–	–	0.54 (0.38)
With temperature				
May	−0.02 (−0.11)	0.07 (−0.12)	−0.18 (−0.36)	−0.26 (−0.37)
June	0.23 (0.50)	0.03 (0.14)	−0.15 (−0.06)	−0.24 (−0.26)
July	−0.15 (−0.22)	0.02 (−0.16)	0.02 (0.24)	−0.18 (−0.19)
August	−0.11 (−0.24)	−0.22 (−0.03)	−0.02 (0.02)	−0.09 (−0.07)
MJJ	0.02 (0.10)	0.07 (−0.07)	−0.17 (−0.15)	−0.35 (−0.46)
JJA	−0.01 (0.11)	−0.07 (−0.01)	−0.09 (0.11)	−0.21 (−0.33)
Precipitation				
May	0.00 (0.00)	0.03 (0.05)	0.17 (0.02)	0.17 (0.12)
June	0.03 (0.06)	−0.09 (−0.38)	0.14 (−0.10)	0.19 (0.10)
July	0.09 (0.18)	0.19 (0.11)	0.10 (−0.20)	0.21 (0.20)
August	0.05 (−0.08)	0.01 (−0.18)	−0.10 (−0.35)	0.02 (−0.19)
MJJ	0.07 (0.17)	0.07 (−0.08)	0.21 (−0.19)	0.30 (0.23)
JJA	0.09 (0.11)	0.06 (−0.16)	0.07 (−0.31)	0.22 (0.10)
SPEI				
May	−0.02 (0.30)	0.04 (0.05)	0.17 (0.01)	0.19 (0.27)
June	0.04 (0.02)	0.09 (0.05)	0.14 (−0.08)	0.19 (0.10)
July	0.10 (−0.09)	0.19 (0.02)	0.09 (0.22)	0.19 (0.22)
August	0.06 (−0.05)	0.02 (0.30)	−0.09 (0.24)	0.03 (0.06)
MJA	0.06 (0.13)	0.07 (0.07)	0.22 (0.09)	0.30 (0.34)
JJA	0.10 (−0.07)	0.06 (0.22)	0.07 (0.22)	0.21 (0.23)

Dry = trees located on a south facing slope; Wet = trees located at the bottom of the slope (see Fig. 1). Values in bold indicates a significant correlation ($P < 0.05$, i.e. $r > 0.15$ for $n = 43$; and $r > 0.25$ for $n = 108$, respectively). MJJ = average for May, June and July; JJA = average for June, July and August.

Table 3

Tracheid anatomical characteristics (mean ± standard deviation).

Characteristic	*Larix* Wet	*Picea* Wet	*Larix* Dry	*Pinus* Dry
Number of series (rings)	5 (135)	5 (135)	5 (135)	3 (135)
Number of tracheids				
Earlywood (EW)Transition wood (TW)Latewood (LW)	2030	2420	1540	1819
	1042	2426	863	2230
	1305	2492	1298	2593
Common signal LD				
Earlywood (EW)Transition wood (TW)Latewood (LW)	0.14	0.07	0.12	0.70
	0.01	0.18	0.41	0.40
	0.00	0.40	0.13	0.21
Common signal CWT				
Earlywood (EW)Transition wood (TW)Latewood (LW)	0.01	0.17	0.13	0.29
	-0.01	0.29	0.25	0.26
	0.12	0.17	0.27	0.25
Lumen diameter LD [μm]				
Earlywood (EW)Transition wood (TW)Latewood (LW)	49.23 ± 11.02	31.01 ± 6.09	47.29 ± 11.20	32.63 ± 6.88
	43.68 ± 11.05	27.76 ± 5.67	41.35 ± 10.50	30.77 ± 6.42
	21.47 ± 13.86	11.93 ± 7.17	19.74 ± 14.02	14.03 ± 8.51
Cell wall thickness CWT [μm]				
Earlywood (EW)Transition wood (TW)Latewood (LW)	2.86 ± 0.80	2.07 ± 0.53	2.91 ± 0.87	2.70 ± 0.70
	4.06 ± 1.35	2.72 ± 0.72	3.82 ± 1.29	3.13 ± 0.80
	7.90 ± 3.08	4.25 ± 1.22	5.98 ± 2.62	5.01 ± 1.80

Dry = trees located on a south facing slope; Wet = trees located at the bottom of the slope (see Fig. 1). Common signal refers to the correlation between the trees (rbt) for the period from 1986 to 2008 ($n = 27$).

temperature (ranging from 12.3 °C to 16.8 °C with a coefficient of variation = 0.07), and of 68 mm for precipitation (25–93 mm, coefficient of variation = 0.37). This climatic variability has, in some cases, affected the size distribution of the tracheid formed during the growing season (Fig. 4). When the distributions of the tracheid size grouped by classes of summer temperature and droughts are compared (e.g.; with tracheid grouped to the annual rings when average summer temperature was 14, 15 and 16 °C, respectively, see Fig. 5),

it emerges that both the distribution of LD and CWT have slightly shifted. Coherently for all the groups, in warmer summers the shift was toward tracheids with smaller lumina and thinner cell walls, especially in the earlywood and transition wood. Similarly, but less coherent among the groups, there was a slight distribution shift observed for the tracheid lumen diameter with increasing summer drought. For example, with increasing summer wetness (i.e. with SPEI values >0) we observe larger LD in the earlywood of *Pinus* and
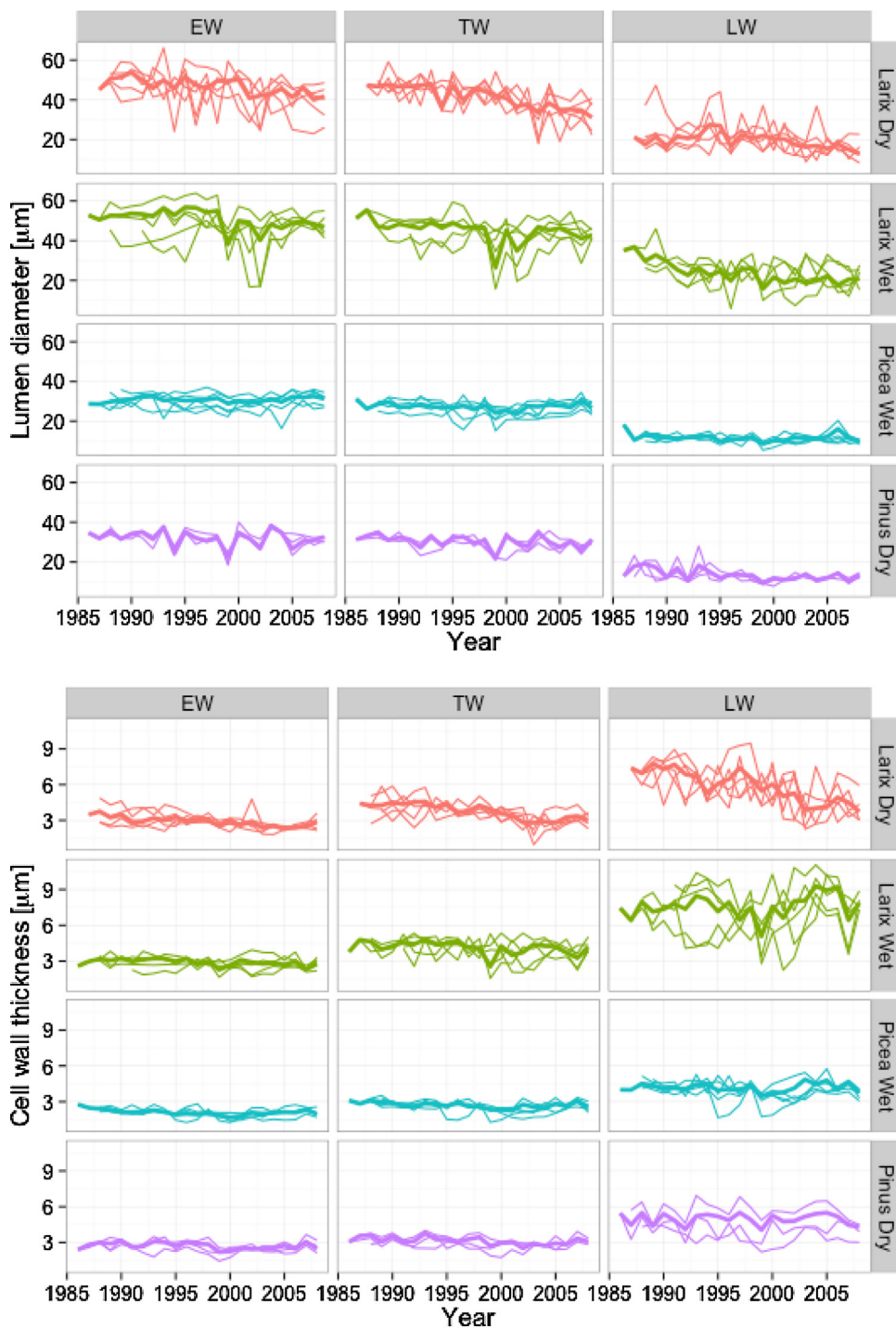
Fig. 3. Anatomical time-series of lumen diameter (upper panel) and cell wall thickness (lower panel) grouped by earlywood (EW), transition wood (TW), and latewood (LW), over the period 1986–2008. Thin lines show single individual time-series, thick lines indicate the average for the group.

Larix from the Dry site, and decreasing LD for *Larix* Wet and almost no changes for *Picea* Wet.

In order to quantify the magnitude of summer climate induced-shifts in the distribution of the tracheid anatomical parameters, we also compared the changes in the average values by grouping the tracheids in the ring sectors characterized by early- (EW), transition- (TW) and late-wood (LW) (Fig. 5). These comparisons confirmed that increased temperatures generally reduced the lumina and wall thickness of the EW and TW tracheids (with the

exception of *Picea* Wet). Similar decreasing size changes occurred with increasing summer dryness (toward negative SPEI values) except for the latewood cells, which remain unchanged or slightly decreases. In most cases (e.g. for LD and CWT of earlywood tracheids), the rate of anatomical changes within the available band of year-to-year summer climatic variability has been quantified up to ~4–5% per degree Celsius. For example, the average annual cell lumina of all earlywood tracheids of *Larix* Dry was 49.22 μm during summers with average temperature of 14 °C and was 44.77 μm
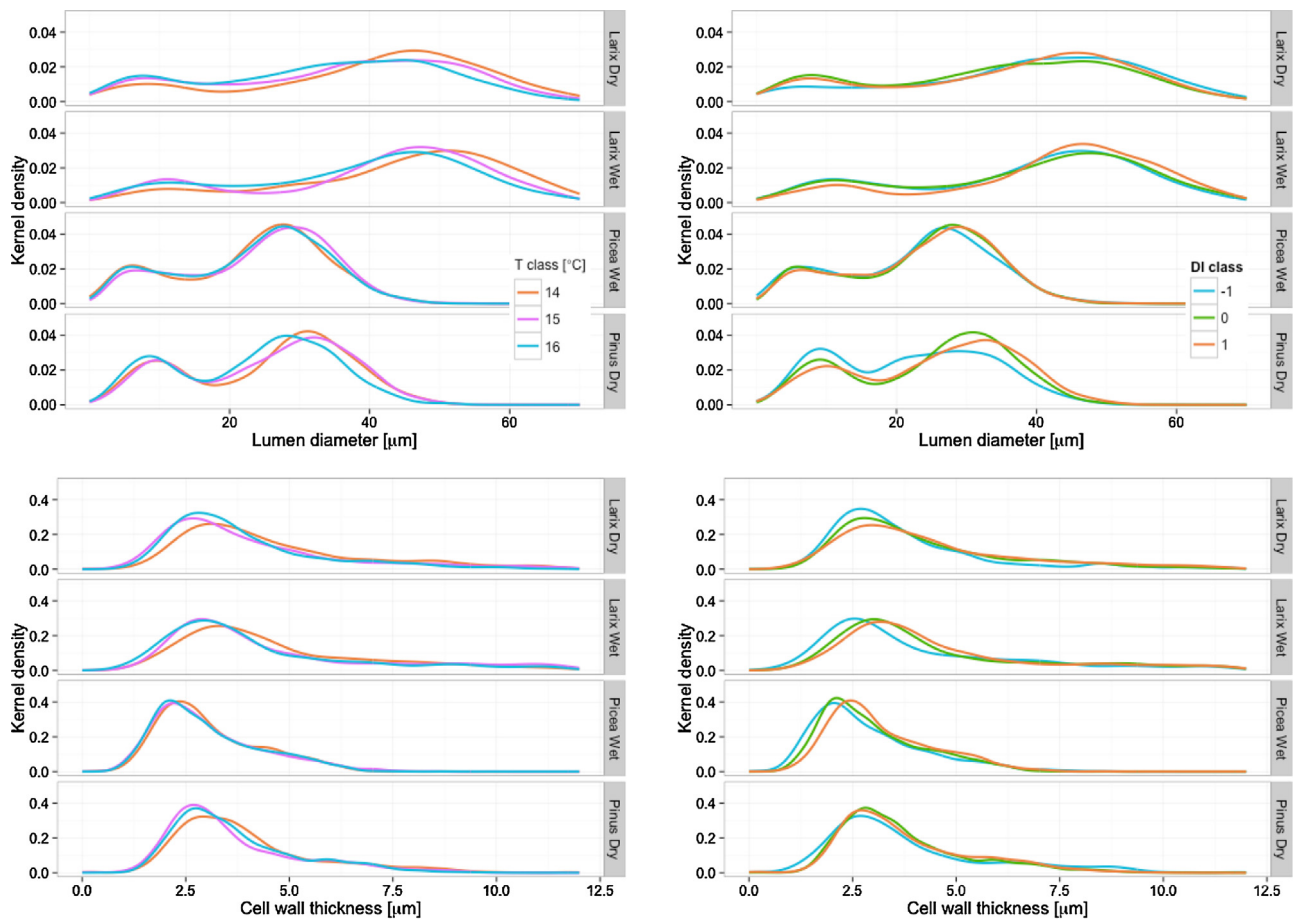
Fig. 4. Comparison of density distributions of all individual tracheid lumen diameters (LD: upper panels) and cell wall thickness (CWT: lower panels) grouped per species and along changes in summer climatic conditions (temperature: left panels; and SPEI drought index: right panels). Negative values of DI index (SPEI) indicate dryness, positive values indicate wetness. The density distributions have been calculated with a Gaussian smoothing and the "nrd0" bandwidth using the "density" function of R (R Development Core Team, 2014). Temperature data are from the Shira Meteorological station over the period 1986–2008. The lines for the T class include tracheid data from 5, 9, and 7 years for the classes of average summer temperature (JJA) of 14, 15, and 16 °C respectively; while the lines for the DI Class includes tracheid data from 4, 14, 5 years for the classes of DI of −1, 0, and 1. The T class 12, 13 and 17 are not shown since only represented by tracheid formed during only one year.

(−9.0%) in summer with 16 °C, while the cell wall thinned from 3.16 μm to 2.86 μm (−9.7%). For *Pinus*, these changes were quantified in −7.8% for LD and −5.6% for CWT. This assessed lapse rate corresponds to the average slope of the segments plotted in Fig. 5.

4. Discussion

4.1. Response to climate

As expected, since this study was performed in a relatively drought-sensitive environment (annual rainfall of 294 mm and mean temperature of 0.8 °C), the ring width from the trees relatively more exposed to drought (i.e. the trees from the south-facing hill slope) were more sensitive to summer water deficit, this despite that the average growth rate did not differ from the trees in the floodplain (see Table 2 and also previous study in the same setting of Babushkina and Belokopytova, 2014). Moreover, we observed that the *Larix* trees from the Dry site are showing a clear and constant decrease of CWT in the latewood tracheid in contrast to the Wet site (Fig. 3). These contrasting responses, i.e.; that the Wet site trees were less drought sensitive, are explained by the fact that the trees were still profiting from wet and deep soil and not yet negatively affected by the summer water deficit (Fig. 1d). Nevertheless, despite a different impact of environmental changes between the groups, shifts in cell size distribution were emerging in both the

Dry and Wet tree's populations (Figs. 4 and 5) during the warm and dry summers. This pattern however occurred with differing magnitudes between the sites and among the species (stronger for *Larix*). Notably, anatomical shifts along with increasing drought stress were slightly diverging only for the wall thickness of the latewood tracheids, with decreasing cell wall in the Dry site and stable or increasing thickness in the Wet one. Being aware that—especially due to the short time window considered with only 27 years—we do not observe always significant climatic-growth responses (Table 2) and that we cannot exclude for an ontogenetic effect (Fig. 3) biasing our results, we nevertheless believe that the approach based on tracheid grouping according to summer climatic conditions over well distributed years (Fig. 1d) still supply enough confidence for a climatic background on the cell anatomical shift observed.

The reduction of earlywood cell lumen with increasing temperature and drought confirms results from studies in similar dry environments performed on both tracheids (Eilmann et al., 2009; Bryukhanova and Fonti, 2013; Heres et al., 2014; Olano et al., 2014) and vessels (Gea-Izquierdo et al., 2012). Also, the observation regarding the changes in cell wall thickness, which in this study was observed to be particularly reduced in the earlywood during dry years, but showed a divergent responses in the latewood, depending on the site (increases in Wet site, decreases in dry site), are also partially confirmed by other studies. For example, cell wall thickness was reduced in earlywood in unfavorable
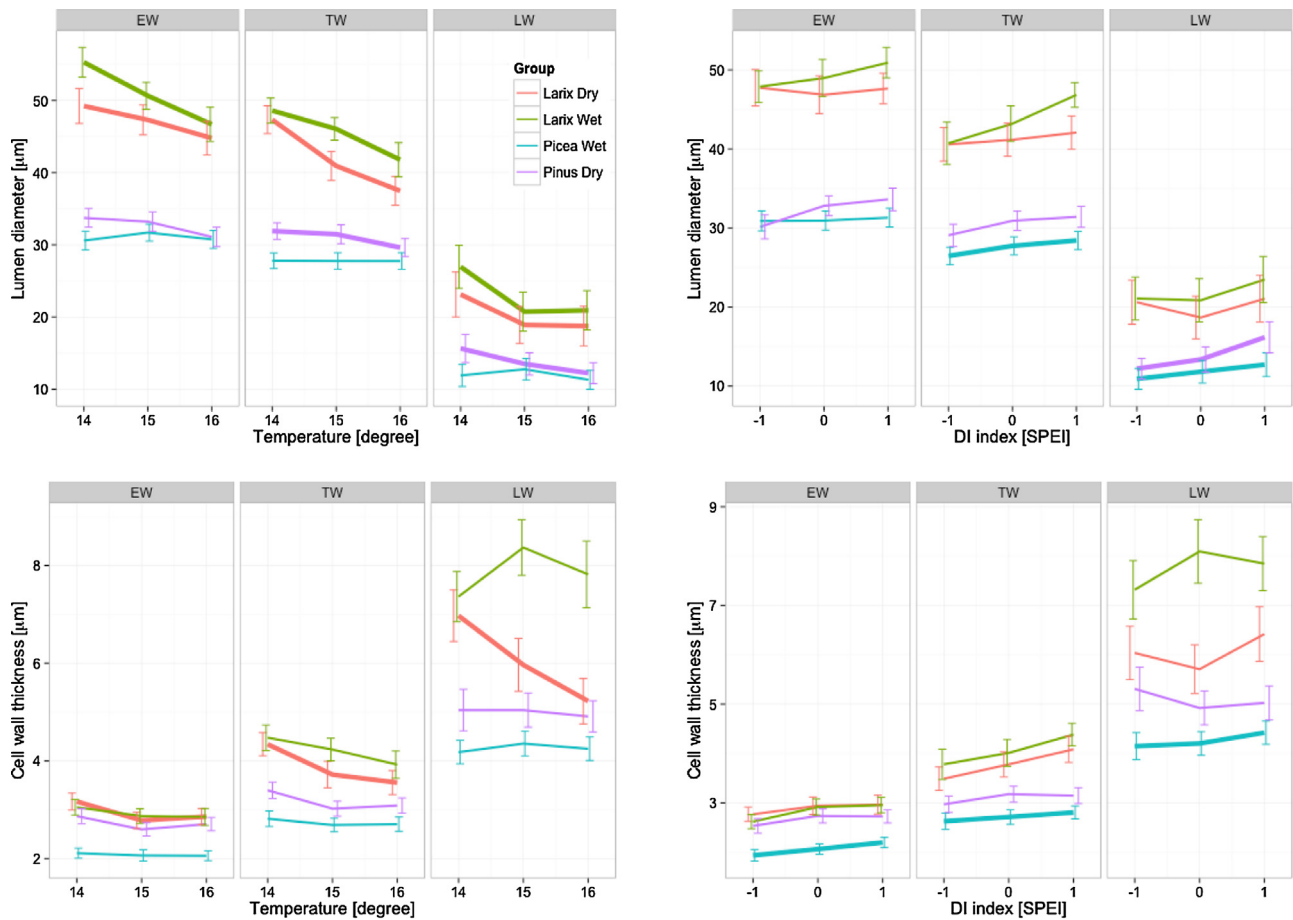
Fig. 5. Changes in mean lumen diameter and cell wall thickness grouped per species and ring sector (EW = earlywood, TW = transition wood, and LW = latewood) along a gradient of summer temperature and drought index. Vertical bars indicates the standard error. Thick lines indicate that the means significantly differs between the two more extreme classes (e.g.; 14 vs 16 for T and −1 vs 1 for DI) at level P < 0.05 using the non-parametric test Kruskal Wallis. Each group includes all the annual rings of a species formed within the given class of summer temperature or drought index. Climatic data are from the Shira meteorological station over the period 1986–2008. Negative values of DI index (SPEI) indicate dryness, positive values indicate wetness.

growing seasons in both drought and temperature limited environments (e.g.; Eilmann et al., 2009; Liang et al., 2013; Heres et al., 2014; Bryukhanova et al., 2015) as well as in the latewood (e.g.; Fonti et al., 2013; Pritzkow et al., 2014), even though the latter is less documented due to the reduced number of studies. These few observations are supported by indications that maximum latewood density is often a good proxy for favorable growing season conditions (e.g.; Buntgen et al., 2010).

4.2. Anatomical shifts suggest carbon limitation

The results of this study suggest that the anatomical shifts—namely towards a downscaling of cell size and wall thickness with increasing temperature and drought—might be principally associated with a differing carbon economy induced by warmth and drought, confirming previous results on internal storage and dynamics of carbohydrates (Galiano et al., 2011; Sala et al., 2012; Poyatos et al., 2013). On the one hand, the observed decreased lumen size match with a reduced water availability, since the ∼5% reduction rate in earlywood tracheid cell lumen per degree Celsius causes an important downscaling of the stem hydraulic conductivity. According to the Hagen–Poiseuille law (Tyree and Zimmermann, 2002) conductivity decreases with the fourth power of the lumen diameter. For the *Larix* Dry tracheid lumen reduction implies a loss of ∼ −32% of hydraulic conductivity. On the other hand, however, the increased flow

resistance to maintain a similar hydraulic efficiency is not compensated by an increase in hydraulic safety against cell implosion by thickening the cell walls. Although a reduction in tracheid cell lumina is also beneficial to increase hydraulic safety (Hacke et al., 2001), the paralleling reduction in the cell number (ring width) and cell wall thickness, especially in the water-conductive earlywood, suggests that the anatomical adjustments might mainly be forced by limited carbon availability rather than by increased demand of hydraulic safety. These observations are also supported by results that conifers species in non-carbon limited condition, usually form thicker cell walls during drought (e.g.; DeSoto et al., 2011; Bryukhanova and Fonti, 2013; Liang et al., 2013), while they did not for the *P. sylvestris* on a very drought exposed site in Switzerland (Eilmann et al., 2009). In fact, limited water availability not only reduces cell expansion (Von Wilpert, 1991), but also limits gas exchange at the leaf level, shorten the growing season and increases respiration (Eilmann et al., 2011). The observation that in the deciduous *Larix*, which strongly relies on stored carbon assimilates to fully rebuild the needle mass at every beginning of the growing season, seems to support our drought-driven carbon deficiency hypothesis (to maintain optimal functional xylem properties). The divergent response in the latewood cell wall thickness between dry and wet sites can thus imply that in extreme conditions (i.e.; at the dry site), the limited carbon availability strongly reduces the wall thickness of the latewood cells. The negative trend in *Larix* Dry tracheid wall thickness, at

least over the period 1986–2008 (Fig. 3), might also suggest that there is a negative feedback between wood structure and future tree performance (net carbon assimilation), which might be the cause for a slow but constant tree decline in growth and vitality.

5. Conclusions

In this case study we observed that, independently from site conditions, all trees simultaneously down-regulated both the cell lumen and wall thickness in the earlywood in relation to the increasing climatic stress (e.g.; in some cases even at a rate of ~5% per °C). Considering that the efficiency and safety of water transport through the xylem is critical for tree performance and survival, it is fundamental that trees growing in dry areas are able to maintain a functional and efficient water transport system. Based on our results, we suggest that increasing environmental constraints as drought and warmth might hamper the assimilation of sufficient (carbon) resources for building a xylem structure, meeting an optimal functional balance between hydraulic safety and efficiency. This limitation might be a possible trigger for a functional xylem miss-acclimation that might trigger a long-term decline and higher exposure to hydraulic failures.

Moreover, from a methodological point of view, with this study we could demonstrate that the applied approach allows to (1) identify the main environmental drivers affecting tracheid anatomy and (2) in particular to quantify the rate of change (in terms of micrometer per degree Celsius) within the available climatic gradient of 3 degree Celsius. Although this study approach of comparing distributions of tracheid anatomical properties seems to provide valuable results to quantify rates of responses to environmental change, its general validity needs to be further tested with more species, sites, climates, and by also including quantification of cell numbers for a better quantification of carbon allocation in the rings. In particular, by increasing the climatic gradient and/or the range of site conditions (e.g. along topographic contrast) might allow to even identify thresholds of responses. This approach applied for more sites and species might thus be very helpful to identify general pattern of responses.

Acknowledgements

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