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Comparison of the Xylem Anatomical Traits of *L. gmelinii* and *P. sylvestris* var. *mongolica* at Three Longitudinal Sites

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Abstract

To find out the Xylem anatomical variations we analyzed the cell structure, lumen area early wood, lumen area late wood, ring width growth response relationship to climate factors in boreal forests. We present growth response from 3-sites KYH (Kheyihe), MEDG (Moredagha), ALH (Alihe) from central Daxing'an mountains China. Variations observed in Cell size from 2010-2016 years in *Pinus sylvestris* var. *mongolica* and *Larix gmelinii*. We analyzed growth response from 2010-2016. Results interoperates that significant growth of *Larixgmelinii* was lower at ALH-site than *Pinus sylvestris* var. *mongolica* as compared to KYH and MEDG-site. We measure the influence of temperature and precipitation which have been shown by correlations of different growing seasons. The warmest temperature from June to September at ALH-site growth of late wood show significant differences at ALH-site. It is also observed that summer temperature in late wood have a significant role in cell dimensions, while the influence of spring temperature frequently influences on tracheid size in early wood formation providing well-documented sound for anatomy and generally used for determining the relationship between maximum growth of tree ring density and effect of temperature variation.

Keywords

Tracheid Size, Ring Width, *Larix gmelinii*, *Pinus sylvestris* var. *mongolica*

1. Introduction

Plants have a variety of environmental strategies. These strategies are supported by measurable functional traits such as cell size, potential height, water conductivity, and leaf area mass [1] [2] [3]. The variety of strategies is dictated by the climate, nutrition, and competition and decodes into the incredible variability of the structures of the plant organs that we see in different climates, as well as in one place. Significant progress has been made in understanding leaf and seminal strategies [4] [5], but less with stem strategies [6]. However, the stems perform the vital functions of water transportation, mechanical maintenance, and storage. Three main tree tissues perform these functions in seed plants: water transport function by vessels, fibers provide mechanical support, and parenchyma transport and store nutrients and carbohydrates. The structure of these fabrics varies considerably between species [7] [8], suggesting a proliferation of environmental strategies applied at the anatomical level of wood. Potentially, by studying the structure of wood, we can learn more about the ecological strategies and plant functions.

In this study, I will focus on angiosperms because they form the vast majority of seed plants on Earth and their timber has a significantly different anatomy than that of the sapwood. Tree anatomy is by no means a new discipline. In other words, it was studied for many years, starting in the 17th century, when the first systematic descriptions of wood were made by pioneers such as Malpighi, Grew, and van Leeuwenhoek. No significant results were achieved in the 18th century, and it was not until the 19th century that the discipline of plant anatomy aroused new interest; in particular, with discussions on the value of anatomical features for systematic purposes (mainly the second half of the 19th century). During this period Rodkofer began a large-scale comparative study and his pupil Solerred analyzed the anatomy of the leaves and branches of over 1000 species from 140 families. The anatomy of plants has been extensively studied by other researchers; their work together with Rodkofer and Solar Eder is compiled in a complete volume, "Systematic Anatomy of Dicotyledons" [9]. This work is a further step forward compared to Metcalfe and Chalk's "Anatomy of the Dicotyledons" (1950).

The anatomy of plants has been extensively studied by other researchers these authors include anatomical studies from the first half of the 20th century and updated descriptions of wood with information on mature stems [10]. [11] have compiled comprehensive inventories of anatomical work done in the 20th century. At the same time, forestry research is proceeding in the description of the wood collected effectively in the forestry directives [12] [13] [14].

Consequently, an anatomy of the contrasting tree can be observed between different phylogenetic groups, but also between different individuals of the same group, between different organs of the same individual or even during the ontogenetic trajectory of a plant for the same organ. Furthermore, we know that the anatomy of wood also adapts to changes in environmental conditions either in space or time.

The formation of xylem cell, which formed first with small lumen size and they are closed to the center of stem (**Figure 1**). The primary xylem formed later towards the periphery with a larger size called meta xylem. The climatic conditions of the study area were followed by seasonal models representative of the cool and temperate area (Figure 2). We have observed different levels, but seasonal patterns are very similar between sites and years.

During xylogenesis, cell expansion and positioning and the alignment of secondary cell walls (hereafter referred to as thick walls) are two main secondary processes that determine cell size and structure [15] [16] [17]. The duration and speed of expansion determine the final size of the xylem cell, while the duration and speed of compression of the wall determine its final weight. The thickness of the cell wall is the result of the total amount of wall material deposited per cell relative to its final size. Finally, the complex interaction between the duration and speed of the xylogeny sub-process determines the changes in the characteristics

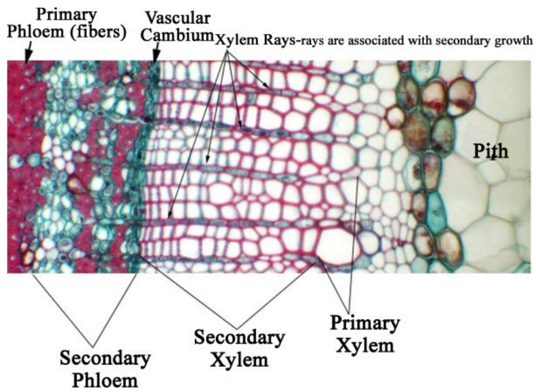

Figure 1. Represents the internal part of tree ring primary, secondary xylem primary, secondary phloem vascular cambium, and xylem rays.
https://www.plantscience4u.com/2017/11/difference-between-primary-xylem-and-secondary-xylem.

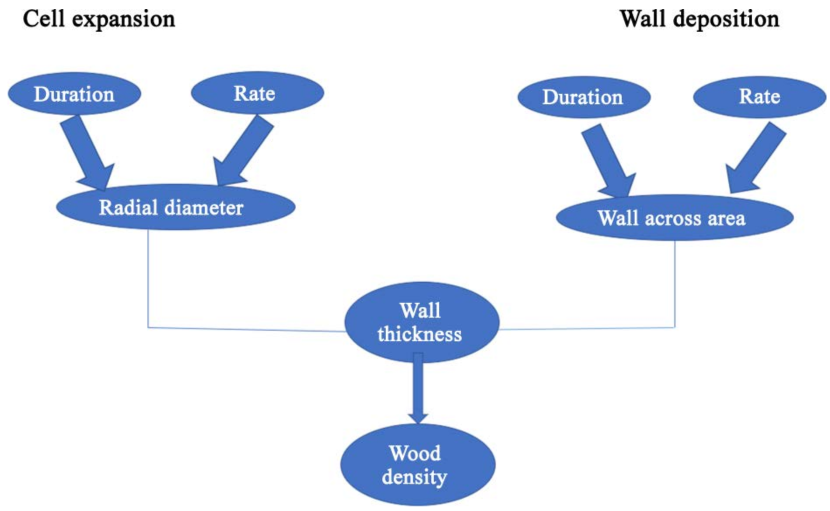

Figure 2. Graphical model which represents how environment influences on xylogenetic processes and subsequent cell dimensions' formation of latewood in conifer tree rings. For day length indirect effects on the duration of cell expansion, whereas for the temperature, the arrow indicates the direct effect on the rate of wall deposition. For wall deposition, the chain links represent coupling between duration and rate of the mechanism, this coupling is broken during the last latewood cell formation.

of the cell (*i.e.* the diameter and brightness of the cell, the surface of the light, and the thickness of the wall). Which in turn creates an anatomical structure that determines the density profile of the wood [15]. Conversely, the environmental theory holds that anatomical changes within the ring are primarily regulated by climate constraints. However, it is very difficult to accurately assess the impact of climatic factors on the formation of tree rings, because the processes involved are closely linked and depend on the physiology of the whole tree (carbon and hormonal balance), which in turn affects the environment. Therefore, the influence of climatic factors can be direct, inhibitory, or physically stimulating or indirect in the processes of halogenation (*i.e.* solved by carbon balance or growth regulators) [18].

Besides, cell expansion and wall thickening should be influenced by several factors as these are very different processes in nature (**Figure 1**).

Consequently, this process depends primarily on the carbohydrates produced by photosynthesis, for which light is a crucial source of energy. However, the thickening of the walls involves many complex metabolic processes that are considered to be the most sensitive to temperature among all the physiological processes linked to the carbon balance of trees [18]. In this study, we aim to evaluate how seasonal changes in environmental factors influence the dramatic changes in cell size observed on conifers. We determined the kinetics of cell development and the size of the resulting cells on the rings of two species of conifers: Dahurian larch (*Larix gmelinii*) and Scots pine (*Pinus sylvestris* var. *mongolica*). We have therefore used the capitals of recently published works that link the differentiation kinetics of cells to the structure of the tree ring [15] [19] to connect the kinetics (duration and speed) and the final (radial) result of the cell diameter and the transversal surface of the wall) of each differentiation process (cell expansion and cell wall thickening) according to the environmental factors that occur during the period under consideration. Based on the above knowledge, we reported that the soil water balance directly controls seasonal changes in the kinetics of cell enlargement and the size of the resulting cells, while seasonal changes in the kinetics of wall thicknesses and their resulting weights. Cells are directly limited by temperature.

2. Material and Methods

2.1. Study Sites

The study was carried out in the Daxing'an mountain in north east China in the Province of Heilongjiang (50˚13E, 123.2˚N), this natural biodiversity is considered hotspot; the contrasting climate conditions along the altitudinal gradient of the mountains allow growth of various vegetation types, this area extending about 1200 Km northeast to towards south east. Its average elevation is 573 m. The target species in this study area are *Pinus sylvestris* var. *mongolica* and *Larix gmelinii* which are distributed from 660 m to 842.7 m above sea level, and al-most cover the entire latitude range of the novel species of Mongolian Scots pine and Dahurian larch in northeast China [20]. It is covered with ice and snow for about half a year. Mean annual temperature ranges from −0.6˚C to −5.5˚C respectively. Annual total precipitation is 362.6 - 524.6 mm, with about 80% occurring from June to September. Annual mean temperature and total precipitation increase from north to south. Two pure stands of different altitudes were selected for each species, corresponding to the lower and higher altitudinal limits of *Pinus sylvestris* var. *mongolica* and *Larix gmelinii* forest on this mountain. The low elevation site was located at 660 m.a.s.l. and the high elevation site at 842.7 m.a.s.l.

2.2. Sample Preparation

The sampling of tissues for xylem-formation monitoring was performed from mid-March until late May in 2016. At each sampling date, five trees were randomly selected per species and site in a sampling plot. The selected trees were, dominant, healthy and similar, with a stem diameter at breast height of 35 - 50 cm, for *P. sylvestris* and 45 - 55 cm and for *Larix gmelinii*. From each tree, four microcores containing phloem, cambium and the last formed xylem growth ring were collected at breast height with a Trephor tool [21]. The sample had a diameter of 2 mm. For detailed anatomical studies transmitted light microscope is most commonly used in dendrochronological sciences. Xylem cell differentiates into different cell types, by observing cell division, cell differentiation, and programmed the death of a cell. For anatomical studies, cores were split into 4 - 5 cm long according to a longitudinal extent of cells. For the preparation of microcores hand sliding rotary microtome is used to obtain 10 - 20 μm thick sections and it is crucial for cutting microsections to facilitate high-quality sections for image analysis [22]. Depending on the specific aim, sections are cut parallel or perpendicular Different cell dimension of the microsections is then measured by taking photographed under the microscope with the use of specialized image analysis software. We measured the following wood-anatomical traits in the transversal xylem sections: mean LA and radial CWT. We choose these factors because it has higher year-to-year variability than the tangential dimension [23]. The time interval of sample collection is very short often for one or two weeks. So, for observations of cellular structures, small microcores are collected by using a tool that caused little damage on tree stem.

2.3. Protocol for Preparation of High-Quality Slides

For making clear and high-quality images of microcore slides we dip microcores in a series of ethanol solutions, anhydrous ethanol, Dimethyl benzene respectively for specific times of 4-days. For this purpose, put the tissue in 30%, 50%, 70%, 85% ethanol solution for 90 - 120 min, while 95% ethanol solution was used for 60 - 90 minute. Anhydrous ethanol solution was used for 60 - 90 minutes, then kept all the samples for a night to become air-dried, after that the dimethyl benzene and Ethanol were used at the concentration of 1:1 for one hour. In the end, paraffin blocks were made for cutting the microcores sections by a rotary microtome. Softening solutions and stain also made for slide preparations. For making microcore wood soft to put it into softening solutions (glycerine: 70% ethanol). For good quality image analysis of xylem cell structures, first of all, we select the blades Lecia 819. We use gelatin solution (gelatin, glycerol, and water) to fix cores properly which split microtomes and positioned the microtome blade. We cut the transversal microcore slices between 10 - 20 μm thick using Leica rotary microtome (KEDEE) (Leica Biosystem, Buffalo Grove, Illinois USA). For fixation of the cut section on the slide, first, we heat cutting sections at 45˚C an oven for about 12 hours. Cover the slide with natural balsam for preservation (**Table 1**).

Then measurements of high-quality images were taken by IPWIN32 (Image-Pro plus). Each microcore includes several xylem layers 4 - 5 in fast-growing seasons and up to 37 - 45 in slow-growing seasons. The average number of tree rings in all species 0 - 11. So, period 2006-2016 was selected for comparison purposes. Moreover, tree rings were visually cross dated along 5-lines in each annual ring. Anatomical parameters were included ring width (RW), earlywood, and latewood width (EW, LW), lumen area (LA), cell wall thickness (Cwt). Earlywood and latewood were measured by averaging the sum of five-lines of tracheid diameter classified as earlywood and latewood.

Following protocol is used to produce high quality images. 1st for collecting samples the equipment needed in the fields are.

- Hammer
- Chisel
- Trephor
- Eppendorf tubes
- FAA (500 ml of FAA contains: 25 ml of 37% formalin, 450 ml of 50% ethanol and 25 ml of 100% acetic acid)

For sample collection we used a tool (trephor) or needle with an opening of 2 mm. depending on thickening of bark chisel is used to remove bark so very less

Table 1. Sampling information of the two species in three sites along longitudinal gradients.

Sites	*Larix gmelinii*			*Pinus sylvestris* var. *mongolica*		
	ALH	KYH	MEDG	ALH	KYH	MEDG
Elevation (m)	660	719.4	842.7	660	719.4	842.7
Latitude (E)	50.13	50.65	51.16	50.13	50.65	51.16
Longitude (N)	123.2	121.36	120.55	123.2	123.2	120.55
Annual mean temperature (˚C)	−0.6	−5.5	−1.6	−0.6	−5.5	−2.5
Annual total precipitation (mm)	524.6	460.3	362.9	524.6	460.3	362.6

damage to tree. After removal sections from tree preserved immediately in Ependorf tube which filled with the preserving solution of formalin acetic acid-ethanol solution [24]. Usually 4-samples were collected from each tree from 5 tree of same species we collected from one site about 20 samples for study of xylem anatomy.

2.4. Dehydration

For preparation of slides of microcore sections dehydration steps involves following procedure.

For making clear and high-quality images of microslides we dip microcores in a series of ethanol solutions, anhydrous ethanol, D-methyl benzene respectively for specific times of 4 days. At the end paraffin blocks were made. For cutting the microcores sections by rotary microtome. Softening solutions and stain also made for slide preparations. For making microcore soft put into softening solutions (glycerine: 70% ethanol).

2.5. Fixation

Now samples are in crucible moved into oven at 65˚C for one an hour, then pour out liquid paraffin and used pure liquid paraffin which is melt on 70˚C and replace paraffin twice. Keep the crucible in oven at 45˚C and then 12 a.m. to 4 p.m. keep in an oven. After that put outside for solidify the paraffin. Now cut paraffin blocks into suitable blocks for cutting slices by microtomes. The surface should be cut in trapezoid shape for the formation of ribbon at microtome [25].

2.6. Preparation of Microslides

For good quality image analysis of xylem cell structures first of all we select the blades Lecia 819. We use gelatin solution (gelatin, glycerol and water) to fix cores properly which split microtomes and positioned the microtome blade. We cut the transversal microcore slices between 10 - 20 μm using Leica rotary microtome (KEDEE) (Leica Biosystem, Buffalo Grove, Illinois USA). For fixation of cut section on slide heat cutting sections at 45˚C on an oven about 12 hours. Cover the slide with natural balsam for preservation. **Table 2** represents the timing and methodology used for staining the microcores to make the clear images.

Table 2. Steps and timing for paraffin embedding from tissues of microcores with safranin.

	Reagent	staining time
1	Dimethyl benzene	12 minutes
2	Anhydrous ethanol:dimethylbenzene 1:1	6 minutes
3	Anhydrous ethanol	6 minutes
4	95% ethanol	6 minutes
5	85% ethanol	6 minutes

Continued

6	75% ethanol	6 minutes
7	1% safranin solution	4 hours
8	water	60 sec.
9	30% ethanol	60 sec.
10	50% ethanol	60 sec.
11	70% ethanol	60 sec.
12	80% ethanol	60 sec.
13	anhydrous ethanol	60 sec.
14	Anhydrous ethanol:dimethylbenzene 1:1	60 sec.
15	Dimethyl benzene	60 sec.

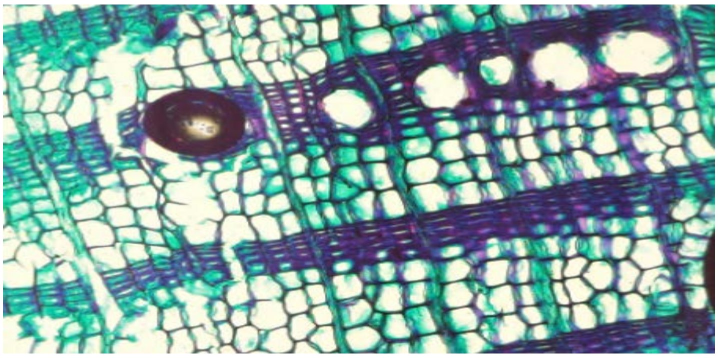

Figure 3. Microcore image of Dahurian larch resin ducts.

Image Analysis

Images were captured by OLYMPUS DP73 microscope (Olympus U-TV0.5XC-3) SN 4A01028 mounted on a computer. OLYMPUS cell Sens standard software is used for capturing image of microsections. Then these sections are stitched by Adobe photoshop software. Then measurements of high-quality images were taken by IPWIN32 (Image-Pro plus). Figure 3 represents the clear image of resin ducts in Dahurian larch in xylem structure. Each micro core includes several xylem layers 4 - 5 in fast growing seasons and up to 37 - 45 in slow growing seasons. Average number of tree rings in all species 11—so period 2016-2006 was selected for comparison purpose. Moreover, tree rings were visually cross dated along 5-lines in each annual ring. Anatomical parameters were included ring width (RW), early wood and late wood width (EW, LW), lumen area (LA), cell wall thickness (CWt). Early wood and late wood were measured by averaging the sum of five-lines of tracheid diameter classified as early wood and late wood.

3. Results

3.1. Comparison between Earlywood and Latewood of the Two Species

Wood density is the expression of the total ratio of dry wood to its volume. So, in this study, we determine the combination of several tree ring features of earlywood, latewood. It varies between the tree species along with the environmental conditions. The growth of the trees may also be varied between organs of similar individuals. Table 1 represents the site characteristics along longitude. The mean seasonal temperature at the three climate stations along longitude in Table 3 reflect temperature gradients in summer, fall, winter and spring seasons.

The average temperature of July and August was higher at ALH-site than KYH and MEDG. Total annual temperature and the seasonal distribution of temperature was different between the three sites.

In Figure 4, both the species *Pinus sylvestris* var. *mongolica* and *Larix gmelinii* were sampled and considering all three sites (ALH, KYH, MEDG) along the longitude. At KYH site in 2013 there is a decreasing trend in growth in both the species, while at ALH site there is a decreasing trend in 2014 in both *Larix gmelinii* and *Pinus sylvestris* var. *mongolica*.

We used two-way ANOVA to determine the effects of tree species and sampling sites on mean tracheid size of coniferous trees in Table 4.

We examine the effects of cell size growth and sites on traits variations using a one-way ANOVA and post hoc test (Table 5). The results interpreted that the size of sampled species with in-site combinations show the variations. The relative growth rate of *Larix gmelinii* significantly lower than the *Pinus sylvestris* var. *mongolica* growth in Figure 5 at ALH site, however there were no significant differences in growth pattern on KYH and MEDG sites in *Pinus sylvestris* var. *mongolica*. Table 6 showed the tree species and sampling site characteristics effects on mean tracheid growth of earlywood and latewood cells size of coniferous trees by two factor ANOVA, where the $p < 0.0001$. Early wood shows higher significant level then late wood.

Table 3. Seasonal mean, minimum, and maximum temperatures at the 3-weather stations in the northeast China (2010-2016).

Weather station			ALH	KYH	MEDG
Summer temperature (˚C)	Jun-Aug	Mean	18.7	14.3	12.6
		Minimum	16.2	11.8	10.0
		Maximum	21.1	17.0	16.0
Autumn temperature (˚C)	Sep-Nov	Mean	−0.2	−4.7	−6.6
		Minimum	−17.4	−21. 3	−22.2
		Maximum	12.5	8.5	7.5
Winter temperature (˚C)	Dec-Feb	Mean	−20.8	−27.5	−31.1
		Minimum	−26.8	−33.3	−37.5
		Maximum	−14.1	−20.2	−24.4
Spring temperature (˚C)	Mar-May	Mean	2.1	−3.6	−6.3
		Minimum	−12.0	−19.5	−22.8
		Maximum	13.5	8.3	7.2

Table 4. Effects of tree species and sampling sites on mean tracheid size of coniferous trees by two factor ANOVA.

	SS	Degree of freedom	MS	*F*	*p*
Intercept	1,392,011	1	1,392,011	9714.7	<0.0001
Species	21,588	1	21,588	150.7	<0.0001
Site	64,812	2	32,406	226.2	<0.0001
Species × Site	18,085	2	9042	63.1	<0.0001
Error	5158	36	143		

Table 5. Mean size, earlywood, and latewood growth size of *Larix gmelinii* and *Pinus sylvestris* var. *mongolica* along latitude across different elevations. Different letters indicate significant differences at P < 0.05 across sites according to post hoc LSD mean comparison.

Species	Sites	La EW (μm^2)	La LW (μm^2)	Mean La (μm)	RW EW (10^{-2} mm)	RW LW (10^{-2} mm)	Mean RW (10^2 mm)
L. gmelinii	KYH	240.9 ± 16.9^a	95.0 ± 17.6^a	146.3 ± 33.2^a	1.28 ± 0.13^b	0.21 ± 0.09^b	0.75 ± 0.07^{ab}
	MEDG	122.5 ± 12.9^{ab}	48.1 ± 8.9^{ab}	76.8 ± 14.69^{ab}	0.61 ± 0.17^c	0.17 ± 0.08^{ab}	0.39 ± 0.11^b
	ALH	149.5 ± 22.8^b	45.4 ± 17.9^b	88.1 ± 21.4^b	2.50 ± 0.29^a	1.48 ± 0.38^a	1.99 ± 0.26^a
P. sylvestris var. *mongolica*	KYH	346.2 ± 27.5^a	121.4 ± 18.8^b	238.8 ± 17.6^a	1.56 ± 0.38^b	0.71 ± 0.16^a	1.13 ± 0.22^b
	MEDG	137.3 ± 20.8^{ab}	63.5 ± 11.0^c	100.4 ± 10.7^{ab}	0.51 ± 0.13^{ab}	0.20 ± 0.12^b	0.36 ± 0.10^{ab}
	ALH	241.3 ± 38.2^b	109.8 ± 11.9^a	175.6 ± 0.31^b	2.32 ± 0.57^a	1.49 ± 0.20^a	1.91 ± 0.32^a

Notes: La—Lumen area; EW—early wood; LW—late wood; RW—ring width.

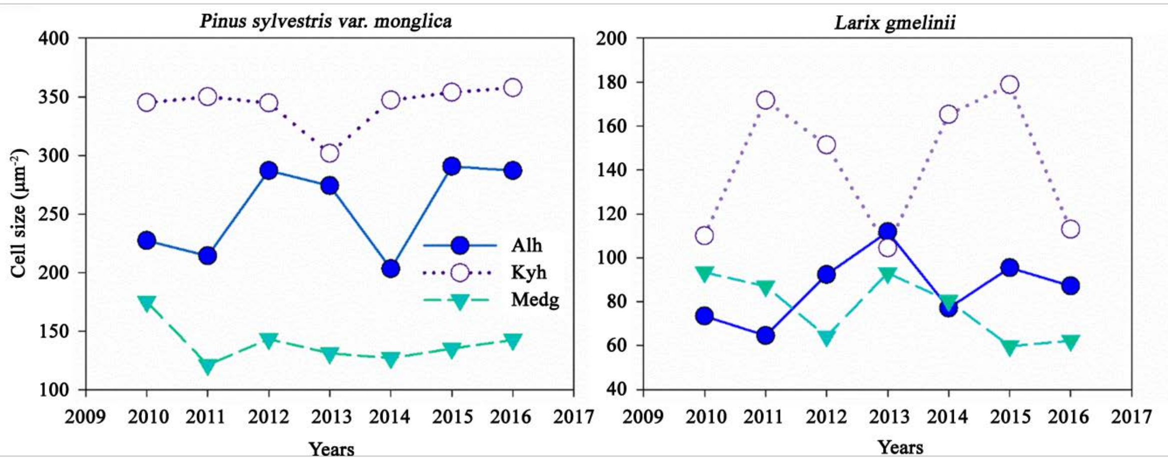

Figure 4. Represents the variations in cell lumen diameter from 2010-2016 in *Pinus sylvestris* var. *mongolica* and *Larix gmelinii*.

After we divide the data into different growth periods earlywood growth and latewood growth for each habitat site, average growth rates are significantly different among different sites (Figure 6). There were no significant differences in latewood growth among 2-sites KYH and MEDG (Figure 6). At ALH site-latewood growth of scots pine significantly different than the other two sites ($P < 0.01$, Figure 6).

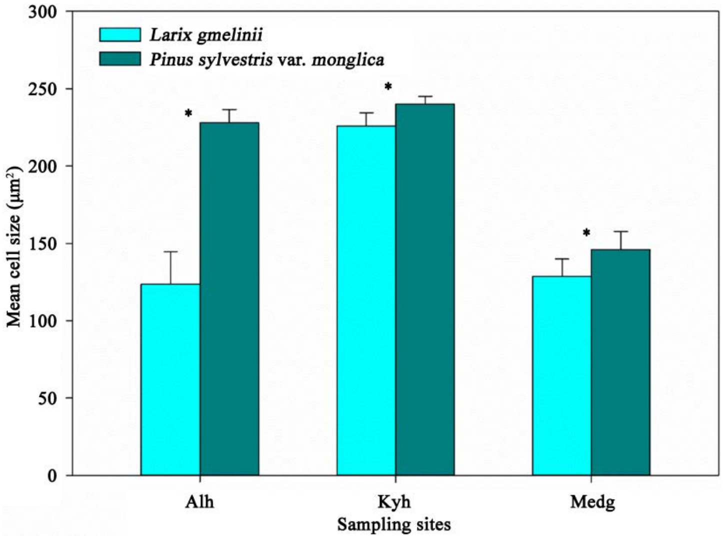

Figure 5. Comparison of sites and mean cell size growth of *Pinus sylvestris* var. *mongolica* and *Larix gmelinii* Bars indicate trait averages and whiskers indicate standard deviation. Lightbars—*Larix gmelinii,* dark bars—*Pinus sylvestris* var. *mongolica.* The boreal rainforest is a cool and wet site. Along 3—longitude sites ALH, KYH, and MEDG. The results show a t-test mean comparison and level of significance with * and "ns" indicates $p < 0.05$ and $p > 0.05$ respectively.

Table 6. Effects of tree species and sampling sites on mean tracheid growth of earlywood and latewood cells size of coniferous trees by two factor ANOVA.

	Earlywood					Latewood				
	SS	Df	MS	*F*	*p*	SS	Df	MS	*F*	*p*
Intercept	1,786,405	1	1,786,405	9247.695	<0.0001	223,029	1	223,029	3548.8	<0.0001
Species	65,326	1	65,326	338.174	<0.0001	8350	1	8350	132.9	<0.0001
Site	140,802	2	70,401	364.445	<0.0001	10,008	2	5004	79.7	<0.0001
Site × species	5334	2	2667	13.807	<0.0001	6239	2	3119	49.6	<0.0001
Error	6954	36	193			2262.5	36	62.8		

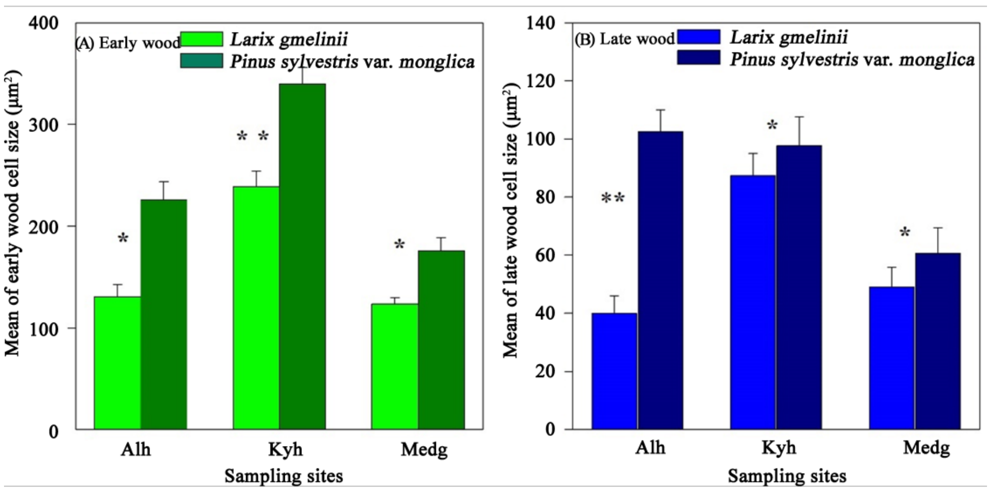

Figure 6. Sites and mean cell size growth of earlywood and latewood cells in *Pinus sylvestris* var. *mongolica* and *Larix gmelinii.* Bars indicate trait averages and whiskers indicate standard deviation. Lightbars—*Larix gmelinii,* dark bars—*Pinus sylvestris* var. *mongolica.* The boreal rainforest is a cool and wet site. The results show a t-test mean comparison and level of significance * and ns indicates $p < 0.05$ and $p > 0.05$ respectively. Along 3-longitude sites KYH, Alh, and MEDG.

3.2. Effects of Recent Climate Changes on Wood Formation

During the 7-year study period, large variations between years in temperature and xylem cell formation along the longitude until start of the growing season were recorded. We perform Pearson correlation between tree ring density versus monthly climate variables relationships presented in (Figure 7). Tree ring growth was mostly positively correlated with the temperature as in (Figure 7(D)) at ALH-site in *Pinus sylvestris*. EWD shows positive correlation at KYH site in (Figure 7(B)) in *Larix gmelinii* in preceding year. The sign and level of correlation between LD and EWD shows positive correlation with temperature, in (Figure 7(B)). LWD also shows an alteration in preceding year from negative to positive correlation. Tree ring parameters were consistently and positively correlated to winter temperature and its intensity. Significant positive correlations with temperature, in RW, EW, were mostly concentrated in November, January preceding the ring formation. TWG and LWG were the most sensitive to winter season as in (Figure 7(D)). EWD was the most sensitive to the lack of water availability during summer while, LWD benefit from the lack of precipitation. Wood properties showed only few significant correlations to bioclimatic and geographic variables. Whereas both inter- and intra-specific ring density variation was negatively correlated to longitude ($p < 0.01$), suggesting that provenances and

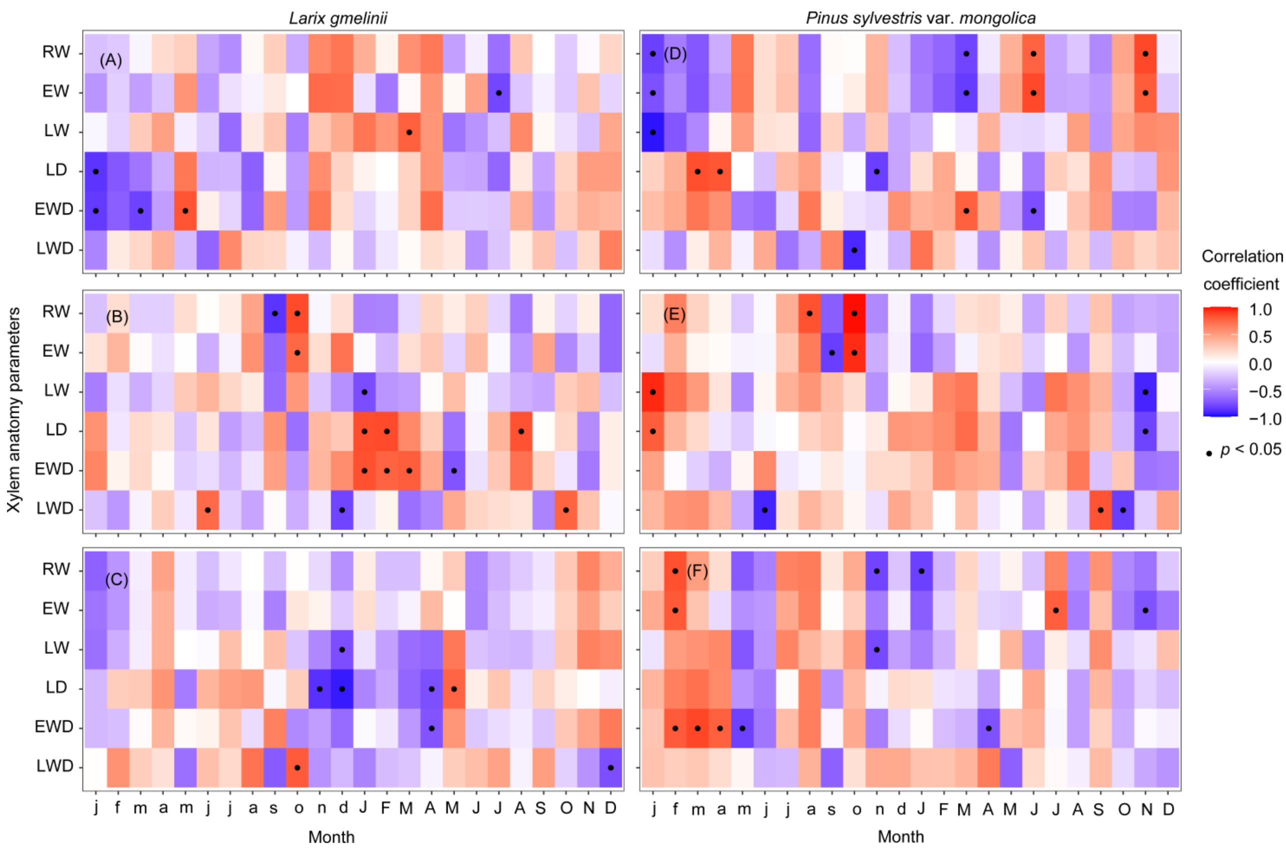

Figure 7. Heatmap showing the correlation between climatic variables mean temperature and (right) xylem parameters ring width RW, EW, LW, and LD, EWD, LWD drought response measures level from 2010-2016. Asterisks indicate that correlation is significant on p < 0.05, p < 0.01. Small letter indicates (j, f) previous months, capital letter represents the current months of the year.

species from more eastern origin have lower wood density. Positive correlations were observed between LD and T-based variables in Jan, Feb, Mar of the current year at KYH site in *Larix gmelinii.*

4. Discussion

4.1. Influence of Environmental Factors on Xylem Anatomy

Tree growth is largely influenced by different climatic factors, which become more restrictive in harsh weather conditions, some studies based on tree ring width in northeast China specified that the forest growth dynamics are highly sensitive to climate change [26] [27]. These studies revealed that the forest has been early-successional condition. The *Pinus* genus has been established as very plastic and capable of adapting its growth to changing climatic conditions. *Pinus sylvestris* var. *mongolica* show significant growth with large diameter at ALH and KYH site in **Figure 6**.

Mechanical properties of tracheids are associated with an increase in the thickness/span ratio linear with cell wall thickness and decreasing lumen diameter [28].

Our results also displayed that the largest ring widths comparable to the largest density of latewood (**Figure 6(B)**). So, the latewood is considered the best section more resistant to the risk of explosion of an underwater cell tension [26] [29]. Björklund *et al.* [30] show that latewood, density is closely related to the number of cells, the thickness of the cell wall. The density of the trees must, therefore, be increased leads to a proportional increase in conductivity safety. More works by Bjorklund and others opens a promising avenue of exploration use of ring density parameters as a mediator for the functioning of the three reactions to a changing environment. The earlywood and minimum density indicate constant experiencing a warming environment and increasingly strong water stress [28] [29] [31].

Our results are demonstrated that climate in northeast China has less impact on *P. sylvestris* var. *mongolica* than on *Larix gmelinii*, despite this

positive responses to the temperatures of the previous year, while the latewood density and the maximum density are exhibit early spring responses, suggest a quantity of the carbon used to form the cell wall was previously captured by growing season or even the previous year. As in our study EWD also illustrates positive correlation at KYH site in **Figure 7(B)** in *Larix gmelinii* in preceding year. This interpretation is reinforced by numerous reports from recent experimental studies that early wood formation relies heavily on photosynthesis of the previous year and this secondary growth could even be mobilized carbon stocks stored for several years if needed [32] [33] [34].

Different tree species are affected differently by the climate, for example, deciduous or evergreen species, late successions or early successions context, previous researches proposed that conifers are adaptable better for the environmental and climatic conditions of the Mediterranean compared to the deciduous species

[35] [36], while the species which are early successional adopt life-threatening strategies, which makes them more adaptable but also more susceptible to the highly variable Mediterranean climate. These differences can activate phenology different from the formation of xylem. Our results suggest that *Larix gmelinii* and *Pinus sylvestris* var. *mongolica* responds differently to the local conditions of the northeast China. Therefore, phenology (the formation of xylem) is significantly different between the two species because the period of development of *Pinus sylvestris* var*. mongolica* much longer.

4.2. The Relationship between Growth and Wood Density

In forestry science, the growth rate is considered the most influential factor in wood density. It is generally accepted that in conifers when increasing the width of the density of rings decreases in return [36] [37]. With the power of a global dataset, Bjorklund *et al.* it has been observed that correlations between the ring width and the density generally changes from negative to earlywood—other the width of the earlywood corresponds to the lower density of the early tree—up to positive in latewood. Our results also showed that the largest wood widths correspond to the largest density of latewood (**Figure 6(B)**). These results show that width/density structural relationships should be studied intra-ring up to draw stable conclusions and advocate new investigations at the anatomical level. Furthermore, Bjorklund *et al.* confirm globally scale and for a large number of conifers this size of tracheid the main driver of the annual variability for the density of the earlywood, while the quantity of cell wall has a greater influence on the density of latewood. Gradual shift passing, from the earlywood to latewood, tracheid allometry is the key to understanding the structure links between the width of the rings and the density of the wood, in case of early woods, the cell wall is quite independent of the tracheid area; while in latewood, the cell wall is positively associated with the tracheid region. The xylogenesis processes at the beginning of this allometric change have recently been discovered [18]. **Table 7** represents the xylogenesis process duration in this study and other studies over all Europe.

During the early growing season, cell division is intense, while the duration of cell expansion is long and the walls are thick comparatively short, producing a large number of large thin-walled cells early light wood; throughout the second part of the growing season, cell division slows down as the duration of expansion decreases the compression time is extended, producing a moderately small number of narrow cells with thick walls of dense wood (**Figure 8**) [15].

Density is generally considered the main characteristic used to evaluate wood quality because it has a great effect on yield and quality of wood fiber and solid wood products and so that it can be manipulated by forestry practices and genetic selection [38] [39]. The density of the wood is an important feature of the radial growth of trees that is necessary for a precise description of biomass production and carbon capture in forest ecosystems [37] [40]. It is also considered

Table 7. Mean xylogenesis duration values of *Pinus sylvestris* from various wood-formation studies in Europe. And in this study (Martinez del Castillo *et al.*, 2016).

Country	Elevation (m)	Longitude (˚)	Latitude (˚)	Mean xylogenesis Duration (days)	References
China	842	20.5	51.16	150	This study
Spain	1560	−1.82	41.79	215	del Castillo *et al*, 2016
Spain	1180	−1.81	41.80	217	del Castillo *et al.*, 2016
Austria	750	10.84	47.23	160	Gruber etal., 2010
Austria	750	10.84	47.23	172	Swidrak *et al.*, 2014
Austria	750	10.84	47.23	137	Gruber *et al.*, 2010
Austria	750	10.84	47.23	170	Oberhuber *et al.*, 2011
France	270	6.32	48.74	199	Rethgeber *et al.*, 2011a
France	643	7.15	48.48	189	Cuny *et al.*, 2012, 2014
Finland	602	5.00	60.20	91	Jyske *et al.*, 2014
Finland	120	25.60	61.20	79	Jyske *et al.*, 2014
Finland	140	26.40	66.20	64	Jyske *et al.*, 2014
Finland	181	24.30	61.90	73	Jyske *et al.*, 2014
Finland	140	26.70	66.30	63	Seo *et al.*, 2011
Finland	300	27.40	68.30	49	Seo *et al.*, 2011
Finland	110	27.30	62.40	63	Jyske *et al.*, 2014
Finland	390	29.40	67.50	54	Jyske *et al.*, 2014

https://www.frontiersin.org/articles/10.3389/fpls.2016.00370/full.

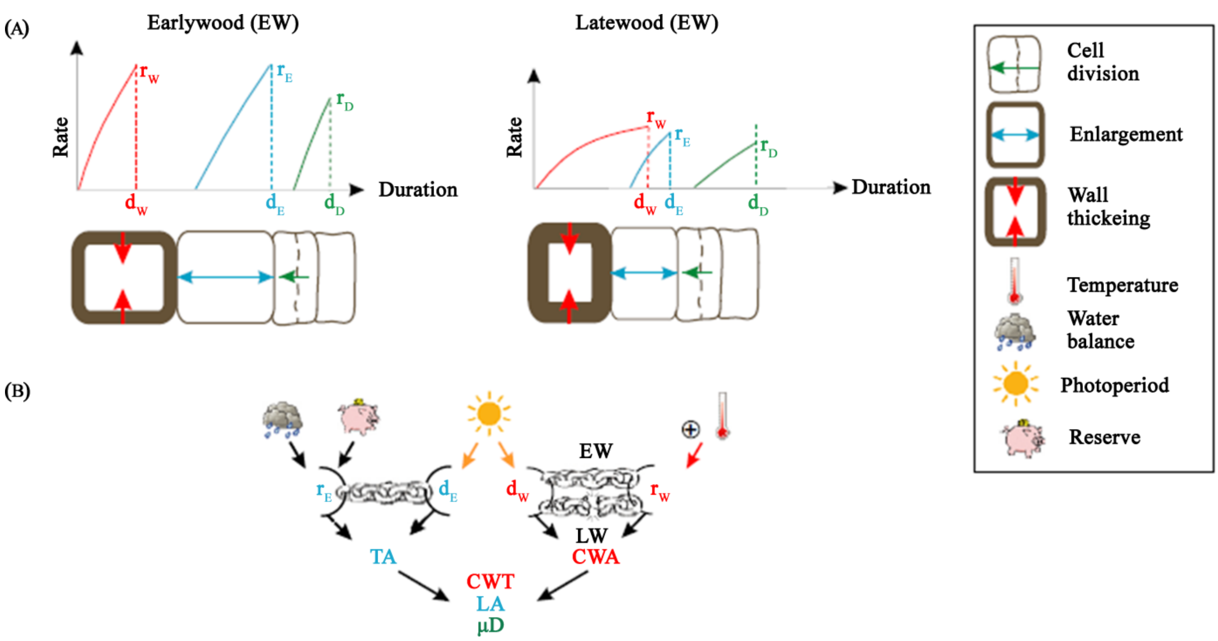

Figure 8. Process of differentiation in tracheid in earlywood and latewood parts. Duration of d_E, cell enlargement, d_W, cell-wall thickening; d_D, cell division; Rate of RW, wall thickening; r_E, cell enlargement; r_D, cell division; (B) Cascade of stimuli of external and internal factors on the evolutions of cell differentiation and subsequent tracheid features and tree-ring structure. CWT, cell-wall thickness; LA, lumen area; CWA, cell-wall area; TA, tracheid area; µD, micro density.
https://pubmed.ncbi.nlm.nih.gov/29034974/.

an important functional attribute by environmentalists [6]. However, the density of the wood is a complex property by the combination of different structural tree ring characteristics (e.g. the relationship between early and latewood) and the anatomical xylem e chemical characteristics.

4.3. Environmental Imprinting in Wood Cell Anatomy

Factors that strongly influence on secondary growth restore the imprints in anatomical features and is reflected in the structure of the tree ring. During the wood formation, xylem cells are differentiated from the complex process that involves determining the cell type, cell differentiation, cell division, and programmed cell death [39] [40] [41]. These processes are affected directly or indirectly by environmental conditions, but are also genetically controlled and depend on the ontogenetic state of the tree [42] [43].

These results may also reflect changes in source-delineation relationships during the growing season, latewood will directly be affected by current weather conditions on cambium activity (sink restriction). While the earlywood will experience the indirect effects of previous climatic conditions on photosynthesis activities. It also observed that in latewood summer temperature play important role in cell dimension while spring temperature frequently influences on tracheid size in earlywood formation providing a well-documented sound for anatomical basis, and widely used for exploring the relationship between maximum tree ring density and summer temperature (**Figure 6(B)**). Generally, transition from primitive wood is towards the latewood is relatively independent of environmental factors. But it can be prompted by certain climatic conditions such as drought or cold [43]. The transition from the early to latewood, therefore, must be under genetic control and strong monitoring development is needed to a recording of the physiological state and the growing season of trees and changes in the coordination between progression and xylogenesis process accordingly. The most conspicuous result of the present study is the great variances in growth patterns among the years, highlighting a plastic response of anatomical growth in *Larix gmelinii,* similarly as in *Pinus sylvestris* var*. mongolica.*

Recent studies that measure the anatomical variables of wood using a series of rings have shown that there is also the potential for extracting information from paleo ecological chronologies [44] [45]. These chronologies allow statistical models to be used to relate tree anatomical variables to continuous and strictly resolved environmental variables, and can be used for reconstructions before instrumental data using transfer functions. It really will open the door for a mechanical approach in the science of tree ring, which will allow a combination of short, medium- and long-term responses to the fundamental environment to restore the past environmental conditions and forecast for future exposure changing of the climate.

5. Conclusions

We observe the effects of cell size growth and sites on trait variations. The results interpreted that the size of sampled species with in-site combinations show the inconsistencies. The relative growth rate of *Larix gmelinii* is significantly lower than the *Pinus sylvestris* var. *mongolica* growth at ALH site. However, there were no substantial differences in growth patterns on KYH and MEDG sites in *Pinus sylvestris* var. *mongolica*. With respect to tree ring variables, the chronology of tree-anatomical variables can provide new information that is not limited to trees growing in harsh conditions in areas with limited habitat.

Tree density and xylem anatomy echo are tree strategies for better distribution during the growing season, and year after year—available resources change environmental conditions. Our findings generally suggest that the transition to primitive wood is towards the latewood is comparatively independent of environmental factors. It was found that the variability of the anatomical variables of wood is mainly related to seasonal climatic conditions, such as the presence of temperature or water, and the strength and quality of the signal vary depending on the species, season climatic zone, and anatomical variable.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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