The effect of meteorological factors on the formation of the anatomical structure of *Pinetum sylvestris myrtilloso-tufosum* on a drained peatland

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Abstract. This paper investigated wood structure formation of pure pine stands (*Pinus sylvestris* L.) depending on meteorological indicators. Influence of average temperature and sum of precipitation on wood structure at the level of annual growth and at the level of xylem cellular structure is presented. Three series of experimental objects in the boreal zone of the European part of Russia was observed: naturally formed mature stands, plots passed by thinning, plots passed by thinning with the fertilizers application. It is shown that fluctuations of average temperature have a more significant role in the formation of the zone of early wood than late wood. Statistical analysis has determined that the effect of average temperature and precipitation factors on the formation of macrostructure elements of wood is higher in the naturally formed stands, than in the stands that have been treated. It was identified that thinning and application of fertilizers increase the role of meteorological factors influence on changes in the number of xylem cells in annual growth. At the same time, the size of xylem cells and the thickness of cell walls are in a more significant correlation with weather factors in stands unaffected by treating.

1. Introduction

The effect of external meteorological factors on the dynamics of the radial increment of perennial woody plants has been studied in detail by many researchers [1-3]. The overall conclusions of the conducted studies were that the radial increment of wood varies significantly over the years and depended on many meteorological parameters. Nevertheless, the contribution of these factors to the annual radial increment of wood requires further investigation. The works of a number of researchers confirmed the influence of growing conditions on the annual growth rate of pine in different regions with similar fluctuations of weather factors [4-8]. The effect of temperature on the annual radial increment of pine trees has been assessed in numerous works both nationally and abroad. However, it is noted that the width of the annual growth rings cannot be considered as directly proportional to the annual amount of precipitation [3, 9-11].

Many authors have observed that in moist locations the diameter increment of pine is more affected by temperature, whereas on dry sandy soil – by the amount of precipitation during the growing season [5, 12, 13]. Depending on the habitat conditions, the annual growth rate of pine can help to estimate...
the dynamics of precipitation and temperature conditions. Silvicultural treatment, such as thinning, land improvement, and fertilizing significantly affect the quality of pine wood. At present, there was a lot of data confirming a noticeable increment of the radial growth of pine wood after silvicultural treatments [14, 15]. Nevertheless, there were very few reports on the combined impact factors, and especially factors influencing the long-term relationship between the external environmental factors and the magnitude of the radial increment.

Field measurements of the biological production process that have been carried out directly in the forest provide reliable information on the growth patterns, yet, such measurements are often hard to implement, since they require a significant investment and an observation continuity over several generations of researchers. Dendrochronology was a viable research alternative in this case. The width of an annual growth ring of a tree varies greatly from year to year due to environmental fluctuations [3-5, 9, 13]. This width can be interpreted either as an indicator reflecting the reaction of a plant to the given ecological background and served as a measure of appropriateness of different environments for the existence of plants, or served to compare an adaptation of different plants to a specific environmental background, including meteorological conditions during the growing season. Currently, this issue remains relevant and almost unexplored.

2. Methods and Materials

2.1. Objects of the study
The objects of the study were the stands of pine (*Pinus sylvestris*) at permanent accounting sites under long-term observation, located in Gatchina District Forestry of Leningrad Region, Russia. Two plots series of experimental sites No. 5 and No. 12 with similar soil conditions were used. Each series consisted of 3 plots: an untreated control plot and 2 treated plots – one was only thinned and one was thinned and treated with nitrogen fertilizer. The forest type in all locations was the *Pinetum myrtillos-tufosum* that united pine forests with *Vaccinum myrtillus* as the dominant in the dwarf-shrub-herb layer on the on drained peatland [16]. The composition and age of stands at the beginning of the experiment was the next: 100% pine, 45 years and solitary birch 45 years. The composition and age of stands at the end of the experiment (2013-2016) was the next: 100% pine, 90 years. Table 1 summarizes data on average height, diameter, and stand stock at the end of the experiment.

| Plot number | Average height, m | Average diameter, cm | Stand stock, m³/ha | Forestry impact               |
|-------------|-------------------|----------------------|--------------------|------------------------------|
| 12-1        | 23                | 24                   | 326                | None                         |
| 5-1         | 26                | 24                   | 355                | None                         |
| 12-2        | 24                | 26                   | 355                | 30% intensity thinning       |
| 12-3        | 25                | 29                   | 354                | 30% intensity thinning and N₁₂₀ fertilization |
| 5-2         | 27                | 26                   | 450                | 30% intensity thinning       |
| 5-3         | 28                | 28                   | 470                | 30% intensity thinning and N₁₂₀ fertilization |

2.2. Methods
Stand indicators were assessed using generally accepted forest mensuration methods. Samples (wood cores) were taken from at least two trees of each diameter class, taking into account the quantitative representation of the diameter class in the stand. The samples were taken at the height of 1.3 m from the root collar. The core samples were extracted in the radial direction using Pressler’s increment borer. At least 20 samples per tested plot were taken, summing up to a total of 120 samples.
2.2.1. **Macrostructural analysis of xylem** The macrostructural properties of wood – the annual growth width, earlywood and latewood width, and the total radial increment, were investigated using a high-resolution scanner, which, according to latest research, provides ± 0.01 mm measurement accuracy. Despite the often positive experience in software-assisted semi-automated calculation of the anatomical properties of wood, the manual method was used because of its higher accuracy in processing digitized images with inhomogeneous brightness gradient within the diameter increment area [17].

Previously prepared and sanded wood sample was glued into the holder and placed in the high-resolution scanner; the surface of the sample was scanned with an accuracy of 1200 dpi in full color mode (16.7 million colors). The brightness and contrast of the digitized scans was then adjusted to ensure better differentiation of early- and latewood areas in the sample [18].

The macrostructural wood properties in the resulting images were measured using a toolkit ‘Geo-information system Panorama 10’. The image was converted into a geo-information system (GIS) with a resolution equal to the scanning resolution (a relative 1 m on an electronic map was considered equal to 1 mm). Using GIS tools, a line was drawn along the longitudinal axis of the core sample. The limits of the late- and earlywood areas were set at each annual growth ring. All measurements were done to the middle of the sample (center of the wood). The obtained data were verified using a high-precision electronic caliper with digital screen; the difference in measurement results between the two methods statistically was not significant. The created line was used to prepare a report in GIS, containing information on the distance between the points (figure 1), i.e. in this case, values of late- and earlywood widths in mm [18].

![Figure 1. Annual growth ring limits in wood sample.](image)

Thus, wood macrostructure properties were expressed in terms of the average width of annual growth rings and the amount of late- and early xylem in the growth rings over the life of the tree. These values were estimated for all wood samples taken from tested trees in the experimental plots. After that, the obtained quantitative and qualitative data on pine and spruce wood macrostructure were systematized using ‘Microsoft Excel’ spreadsheet editor.

2.2.2. **Microstructural analysis of xylem.** To study the microstructural properties of xylem, wood samples were taken from each diameter class of pine at the sites. In total, 10 samples were collected from control plots and 20 samples from treated plots. The anatomical microstructure of xylem was analyzed using a ‘Leica DVM5000’ digital microscope. The design of the microscope allows studying samples both in reflected and lateral light. The measurements were carried out in the annual growth rings from the last 45 years; in some cases, when it was not possible to cover 45 years (on the samples from small diameter trees aged up to 45 years), the measurements were done over a shorter period of time. Using ‘Leica Application Suite’ software, the annual growth increments were captured at a given scale that allowed identification of wood anatomical features. If the width of the annual increment was too big for processing it at a single image, it was marked and digitized over a set of images. This set of images was then combined into one single digital image in accordance with the marks.
The subsequent measurements were done using the ‘Geo-information system Panorama 10’ toolkit. The digitized image of the sample xylem was converted into a raster map for GIS at the appropriate scale (figure 2).

![Figure 2. Measurement of sample xylem anatomical structure.](image)

To avoid errors caused by image distortion, the GIS rasters were checked against sample measurements with ocular micrometer. Then a line was drawn in GIS through a row of cells of one annual growth ring, in some cases the line was broken on account of the curvature of the ring. The measured rows were chosen at random. A number of cells was counted in each row, and also the radial size of a lumen and a thickness of radial walls in early and late tracheids were measured by setting marker points on the line drawn in GIS. The distance between the points, presented as a report, was the desired xylem microstructure data. Based on the previously proposed methodology, the parameters of every tenth cell in the area of early- and late growth were measured, in case of a smaller number of cells, the measurements were done for at least three cells. The two limit cells on each side of the early and late xylem area were not measured, because they could belong to the transition zones between the structural elements. To speed up the measurements of cells and improve the accuracy, the measured thickness was the thickness of the double cell wall on both sides of a lumen along the line. After that, the thickness of a single cell wall was calculated as a quarter of the obtained value (figure 3).

![Figure 3. Double cell wall (CW) and lumen measurement in xylem.](image)
2.2.3. Meteorological data. Meteorological data – the average air temperature and the amount of precipitation during the growing season – were collected at weather station ‘Belogorka’, the village of Belogorka, Siversky urban settlement, Gatchina District, Leningrad region, Russia. The effect of meteorological factors on the macrostructure of wood was investigated on the basis of data from 1960 to 2012. The weather station began precipitation measurements in 1960. For the microstructure studying the data from 1970 to 2012 were used, because it was intended to assess microstructure over the period of 45 years, and the samples from series 5 and 12 were collected with 2 years interval.

In the study, a Hydro-thermal Coefficient of Selyaninov (HTC) was used, as it is a most common measure for forestry studies in the boreal zone [12, 19]. The HTC describes moisture supply of a territory and is given as (1):

\[ K = \frac{R \times 10}{\sum T} \]  

where, \( R \) – is the precipitation in millimeters for a period with temperatures above + 10°C; \( \Sigma T \) - is the sum of temperatures in degrees Celsius (°C) for the same period of time.

2.2.4. Statistical analysis. The statistical analysis of the obtained qualitative and quantitative data was done using the ‘Statistica 10’ and ‘Microsoft Excel’ software packages. To assess the strength of a relationship between nonparametric values and in cases where the distribution of the studied variables was significantly different from normal [19], a ranking analysis method was used. As a quantitative measure of the relationship between the studied phenomena, Spearman's rank correlation coefficient \( R_s \) (2) was calculated:

\[ R_s = 1 - \frac{6 \times \sum d_i^2}{n \times (n^2 - 1)} \]  

where, \( d_i \) – are the differences in the ranks of each pair of linked values; \( n \) – is the number of measurements.

The coefficient reflects how the sum of the squared differences in the ranks obtained during observation differs from the case when there is no relationship. The rank correlation coefficient assesses the relative strength of the relationship between the variables, considering that \( R_s \) coefficient values equal to 0.3 or less mean a weak relationship; values of 0.4-0.7 – indicate a moderate relationship, and values of 0.7 and higher – indicators a strong relationship [20]. A negative coefficient value indicates a possible negative correlation. The significance level of the correlation was checked with \( p \)-value at the confidence level of 95%.

3. Results and Discussion

Figure 4 shows a graph of growth increment and average temperatures during the growing season.

The effect of silvicultural treatment is observed during 40 years after the treatment, as evidenced by the difference in the results at the experimental sites. In recent years, the growth increment at all sites has become uniform as a result of the long time that has passed since the silvicultural treatment on the stand.

The coefficients show that earlywood significantly correlates with the average temperature over the growing season in all experimental cases. The annual growth width correlates significantly only at control stands; in thinned and thinned and fertilized stands, the coefficient is high, but not significant at a confidence level of 95%. At the confidence level of 90%, the correlation of the annual growth width and the average temperature is also significant for the plots that underwent a combined treatment \( (R_s = -0.25) \).

The correlation coefficients between the meteorological factors and the cellular structure properties of pine wood from the experimental sites were also calculated. The results show that at the xylem
microstructure level, the average temperature during the growth period has the most significant effect on all sites. Table 3 summarizes these data.

![Figure 4. Average air temperature and growth increment in pure pine stands at treated and untreated experimental sites.](image)

Table 2 shows the calculated Spearman’s rank correlation coefficients for the experimental sites.

**Table 2.** Spearman’s rank correlations coefficients for macrostructural wood parameters and weather conditions at pine stands.

| Sample plot | Variables           | Latwood width | Earlywood width | Annual growth width |
|-------------|---------------------|---------------|-----------------|---------------------|
| 100% Pine   | Average temperature | -0.18         | -0.33<sup>a</sup> | -0.31<sup>a</sup>   |
| control plot(5-1, 12-1) | HTC | -0.03 | -0.10 | -0.08 |
| 100% Pine   | Average temperature | -0.19         | -0.28<sup>a</sup> | -0.23               |
| 30% thinning | Amount of precipitation | 0.00          | -0.01          | 0.00                |
| (5-2, 12-2)  | HTC | 0.07 | 0.06 | 0.07 |
| 100% Pine   | Average temperature | -0.19         | -0.29<sup>a</sup> | -0.25               |
| 30% thinning, N120 (5-3, 12-3) | Amount of precipitation | -0.01 | -0.03 | -0.03 |
| HTC | 0.03 | 0.04 | 0.03 |

<sup>a</sup> Marked bold correlations are statistically significant at p<0.05.
Table 3. Rank correlation coefficients for wood microstructure properties and weather conditions at the plots in 1970-2012 years.

| Variables                  | Late xylem cell count | Early xylem cell count | Latewood cell wall thickness | Earlywood cell wall thickness | Lumen of late xylem cells | Lumen of early xylem cells |
|----------------------------|-----------------------|------------------------|------------------------------|------------------------------|---------------------------|---------------------------|
| For the pure control pine stand |                       |                        |                              |                              |                           |                           |
| Average temperature       | -0.09                 | -0.27                  | -0.06                        | **-0.39**<sup>a</sup>         | **-0.46**<sup>a</sup>     | -0.27                     |
| Amount of precipitation   | -0.11                 | -0.13                  | -0.30<sup>a</sup>            | 0.09                         | -0.02                     | -0.20                     |
| HTC                       | -0.07                 | -0.02                  | -0.23                        | 0.23                         | 0.13                      | -0.04                     |
| For the pure pine stand with thinning |                 |                        |                              |                              |                           |                           |
| Average temperature       | **-0.42**<sup>a</sup> | **-0.39**<sup>a</sup>  | -0.19                        | 0.05                         | -0.18                     | 0.01                      |
| Amount of precipitation   | 0.03                  | -0.08                  | 0.15                         | -0.20                        | 0.02                      | -0.10                     |
| HTC                       | 0.16                  | 0.06                   | 0.17                         | -0.20                        | 0.09                      | -0.06                     |
| For the pure pine stand with thinning and fertilizing |                 |                        |                              |                              |                           |                           |
| Average temperature       | **-0.34**<sup>a</sup> | **-0.37**<sup>a</sup>  | -0.29                        | -0.07                        | 0.03                      | 0.23                      |
| Amount of precipitation   | 0.07                  | -0.02                  | -0.03                        | -0.21                        | -0.08                     | -0.12                     |
| HTC                       | 0.15                  | 0.09                   | 0.07                         | -0.15                        | -0.05                     | -0.12                     |

<sup>a</sup> Marked bold correlations are statistically significant at \( p<0.05 \).

The analysis of the relationship of xylem microstructure showed a significant relationship with average temperature during the growing season and, in one case only, with the amount of precipitation. Apparently, in the unthinned stand the thickness of the cell wall of the latewood shows a more pronounced relationship with the amount of precipitation, whereas for the earlywood the average temperature plays a more important role. A different physiological dependence of the xylem growth was manifested from the point of view of microstructural development. For treated stands these relationships have not been statistically proved. In these stands, the number of late- and earlywood cells in annual growth increment depended on the more average temperature, which was probably due to favorable soil and hydrological conditions obtained through the land improvement. No statistically significant relationship between the combined effect of temperature and precipitation during the growing season and the microstructure of wood at treated and untreated experimental plots have been found.

4. Conclusions

The results of the study have demonstrated that:

- The average temperature during the growing season has the most significant effect on the development of the macrostructure of wood.

- The average temperature during the growing season affected the earlywood areas of wood structure more than the latewood areas.

- The effect of meteorological factors on the macrostructure of wood was stronger in untreated stands than in treated stands.

- The combined effect of the average temperature and precipitation on the structural development of wood in the experimental stands was not statistically proved.

- In the treated stands, the influence of meteorological factors on the number of late and early xylem cells in annual growth was more significant than in the unthinned stands.
- In the unthinned stands, the effect of meteorological factors on microstructural properties of pine wood cells was very significant.
- The combined thinning and treatment with nitrogen fertilizer resulted in a lower dependence of xylem cellular formation.

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