

## Fertilisation with potato starch wastewater effect on the growth of Scots pine (*Pinus sylvestris* L.) forest in Poland

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### ABSTRACT

Fertilisation is often used to increase plant productivity in agriculture but has also been used in forestry. In our study, Scots pine forest growing in a nitrogen-poor environment was fertilised with NPK post-production wastewater from a potato starch factory. Our research aimed to investigate the dependence of tree growth on different NPK concentrations. Cell characteristics such as cell wall thickness (CWT), lumen diameter (LD) and tree-ring features such as ring width (RW), total number of cells in annual growth (nTotal), earlywood (EW) and latewood (LW) were investigated. Twenty-six years of regular fertilisation of the forest with different doses of wastewater rich in NPK elements have affected the anatomical structure of Scots pine trees. It is presumed that the reduction in CWT and LD on the fertilised site was due to deficiencies in plant water conductivity, which may have occurred due to physiological drought. The influence of nitrogen on unfertilised site from the wastewater area could contribute to the CWT thickening. The results confirm that the use of NPK in excessive doses is detrimental to trees' conductive system.

### Introduction

Forest fertilisation experiments have been conducted worldwide (Fox et al., 2007; Moller, 1992), mainly to increase productivity by enhancing the average wood biomass per unit area (Smethurst, 2010). However, results from these experiments have been mixed, with some studies reporting increased growth (Fox et al., 2007; From et al., 2015; Haverlaen and Frivold, 2015; Jacobson and Pettersson, 2001; Moller, 1992; Nilsson et al., 2021), while others found no significant effect or even reduced growth (Kytö et al., 1999; Seftigen et al., 2013; Wallace et al., 2007). Forest soil enrichment has become a common practice in northern Europe to increase tree biomass and gain economic benefits (Lindkvist et al., 2011). Nitrogen (N) is considered to be a major growth-limiting factor in many northern forests (Hedwall et al., 2014), including those with podzolic soils found in the study area in Poland (Chojnacka-Ozga et al., 2022). Those experiments have been carried out

with varying intensity in different northern countries, usually with positive results in the form of increased tree biomass (Balster and Marshall, 2000; Hedwall et al., 2014; Lindkvist et al., 2011). Stand fertilisation is known to have an almost immediate effect on tree growth and can be applied even in mature forests (Jacobson and Pettersson, 2010; Nohrstedt, 2001). The efficiency of soil enrichment in relation to the application rate is therefore strongly related to the nutrient demand and growth potential of the stand. It has been assumed that the effect on production decreases with repeated fertilisation (Jacobson and Nohrstedt, 1993), but (Jacobson and Pettersson, 2001) and (Pettersson and Högbom, 2004) found that while repeated, it maintained or even increased the effect of biomass growth, at least at longer intervals between nutrient supply. Sometimes additional growth was obtained when N was applied at relatively high and frequent rates and with phosphorus (P) and potassium (K) (Albrektson et al., 1977; Dralle and Larsen, 1995; Harrington and Wierman, 1990; Tamm et al., 1999). The reason for

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supplying forests with nutrients may not only be to increase yields but also to dispose of post-agricultural waste which was the case in the Ilawa forest area (Chojnacka-Ozga et al., 2022; Ciepielowski et al., 1999; Koprowski et al., 2015).

The sustainable management of post-production waste is a serious environmental issue. The potato processing industry requires large amounts of water. Depending upon the starch content of the potatoes and the techniques employed, the processing of potatoes typically requires 8–17 litres of water per kilogram of potato (de Haan and Van de Ven, 1973; Peters, 1972; Van Hung et al., 2006). The vast majority of polluted wastewater originates from starch production. These waters typically have a high chemical oxygen demand with high levels of N and P. The large volumes of wastewater generated can be problematic because, according to current legislation, the municipal wastewater system should not be used to discard such a large volume of waste. Some of the methods used to treat starch wastewater include biochemical treatments, biogas fermentation, adsorption, air flotation, flocculation, precipitation, sedimentation and recently developed combined treatments (Bouchareb et al., 2021; Cai et al., 2019; Wu et al., 2016).

In the past, an agricultural approach was adopted in Poland to dispose of this nutrient-rich wastewater including the irrigation of meadows (Hawelke and Sokołowski, 1999) and a forest located near a processing plant (Ciepielowski et al., 1999). It was thought that this approach would solve the problem of waste treatment and at the same time have a positive effect on the growth of plant biomass within the irrigated ecosystems. As the forest in the vicinity of the starch factory in Ilawa grows on the relatively nutrient-poor sandy soils, it was expected to increase tree biomass and improve stand productivity, as the potato starch effluent is rich in elements such as N, K and P, hence the expectation that it could also be used to improve the nutritional status of forest stands. Previous studies have shown that pine forests should not be irrigated with wastewater from potato starch factories, as this wastewater can damage trees within a few years (Peters, 1972). Nevertheless, these results were not considered, and contrary to these findings, wastewater with a high starch content was applied in the Ilawa Forest District in the years 1984–2012.

In the case of Ilawa, previous studies have shown that high-effluent rates reduced biomass (Gumnicka, 1999), and potato starch effluent reduced photosynthetic efficiency and disrupted Scots pine growth (Koprowski et al., 2015). A more recent study in the same forest also found that an increase in the concentrations of NPK elements in post-production wastewater decreases the radial growth of trees (Chojnacka-Ozga et al., 2022).

As previous studies based on rings-width measurement showed a decrease in radial growth, it was decided to investigate which anatomical features had changed in the rings due to the application of different amounts of wastewater, which, when overused, can lead to physiological drought in plants. The reason for water limitation in plants can vary, from lack of precipitation, soil type specifics or over-enrichment (Ward et al., 2015) and as a result can lead to disturbances in plant growth. Other studies with pine (Ward et al., 2015) showed that nutrient supply intensifies drought stress by decreasing water use and stomatal conductance of loblolly pine in a mid-rotation fertilisation experiment. (Song et al., 2022) found that climate-induced changes in the anatomical structure of Chinese red pine tree rings occur mainly in earlywood rather than in latewood. Frequent severe droughts in dry areas may induce Chinese red pine to form smaller lumens and thicker cell walls to survive.

Surprisingly, from the perspective of the wood industry, a lower growth rate may affect wood quality, e.g., tracheid properties, in a positive way (Mäkinen et al., 2002a, 2002b; Saranpää, 2003). As (Jaakkola et al., 2006) note, wood properties determine the suitability of wood as an end product in the pulp and paper industry, wood panel industry and solid wood production. Tracheid properties express the density of the wood, which is widely used as a measure of wood quality (e.g. (Panshin and de Zeeuw, 1980; Saranpää, 2003)). Because the

enrichment of coniferous forests is currently used worldwide to increase wood production (Augusto and Boča, 2022; Durand et al., 2020; Jaakkola et al., 2006; Ward et al., 2015), any research that increases knowledge of wood cell growth, especially of species highly used in forest plantations such as Scots pine, can contribute to a better understanding of their growth mechanisms and therefore more efficient management. Despite the growing interest in cell anatomy in dendrochronology, little is still known about interpreting the variability in cellular anatomical responses observed in different fertilised environments and species. As previous studies have shown declines in tree ring growth (Chojnacka-Ozga et al., 2022; Koprowski et al., 2015), we focused on finding out what changes have taken place within the annual increment that are reflected in its reduction. We hypothesised that fertilisation has a negative impact on the tree radial growth by reducing cell lumen diameter (LD) in tree-ring width (RW).

This study aims to investigate how the different concentration of N, P and K elements in post-production wastewater affects the anatomical structure of tree rings at the cellular level. We measured cell characteristics such as cell wall thickness (CWT), LD and tree-ring features such as RW, total number of cells in annual growth ring (nTotal), earlywood (EW) and latewood (LW) to investigate how these variables responded to the nutrient supply regime with added amounts of the N, P and K for the period 1984–2009. Although fertilisation took place between 1984 and 2012, the period analysed only covers the years 1984–2009. This is due to the timing limitation of the chemical data provided by Chemirol Sp. z o.o., the company that processed potatoes into starch, indicating the content of N, P and K in the post-production effluent.

## Materials and methods

### Site characteristics

The Forest Wastewater Treatment (FWT) commenced operation in 1984 in the Ilawa Forest District, located in the northern part of Poland, in the Brodnica Lake District, a few kilometres from the Ilawa town (Fig. 1). The FWT was designed to discharge wastewater from the potato starch factory into the forest soil. The aim was to distribute wastewater

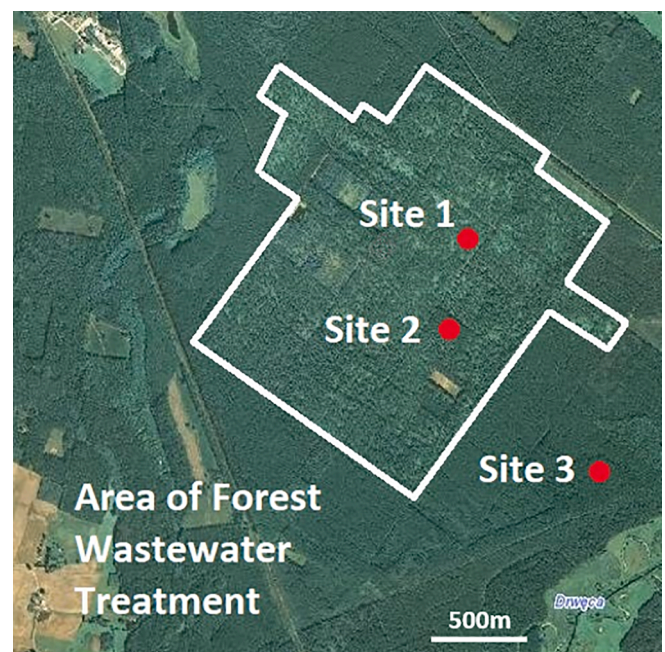


Fig. 1. Location of test sites (red circles) at the Ilawa Forest. Site 1: directly fertilised by wastewaters from starch factory. Site 2: not fertilised, despite being in the FWT zone. Site 3: control, not fertilised (adapted from Koprowski et al., 2015).

to purify it and contribute to the stand productivity. The facility was established at 216 hectares of pine forest growing on podzolic and rusty soils, formed on slightly loamy and loose sands with low retention capacity (Brandyk et al., 1999). The soil was the same type at each of the three sites. The mean annual air temperature and mean annual precipitation for that region are 7.6 °C and 610 mm for the period 1951–2020.

### Sampling

In this study site, during the operation of the potato starch factory, the effluent was applied every 7–8 days, up to a maximum of 10 times per season. Wastewater application started after the potato harvest, which was usually in early September. The experimental forest area was 174 ha, located 7 km apart from the potato starch factory. The total length of the pipe system used for effluent application exceeded 40 km. Three sites were selected to test the effect of forest fertilisation (Fig. 1). Sites 1 and 2 were located in the FWT zone, while site 3 was a control stand located outside this zone. Site 1 and site 2 are 0.5 km apart, while site 1 and site 3 are 1.5 km apart measured in a straight line. The trees growing at site 1 were fertilised, while the trees growing at site 2 were not fertilised (Fig. 1). Although stand 2 was not fertilised, a certain amount of N, P and K must have migrated to this stand, most likely with the flow of water in the soil, as these elements were found in higher amount in the trees (Ostrowska et al., 1999). Furthermore, they were found to have a positive effect on tree growth at that site. According to the information provided by the Ilawa Forest District, the trees were of similar age, around 80 years in 1984. The trees showed no visible signs of disease. However, on the fertilised site 1, tree density was lower due to the snags. Using a standard 5 mm diameter Pressler borer, 120 cores were taken from pine trees growing on three sites (40 cores per site). Two core samples were taken from each tree, one from the west side and one from the east side, at a height of approximately 1.30 m above the ground.

### Data treatment

The cores were prepared for measurement using standard dendrochronological procedures (Zielski and Krapiec, 2004). Tree rings were analysed by Koprowski et al. (2015). COFECHA 6.06P (Holmes, 1983) software was used to check series intercorrelation. Each sample was analysed by means of the skeleton plot method according to (Stokes and Smiley, 1996). To analyse the effect of meteorological parameters on tree ring widths the long-term trends in tree ring widths were removed. The smoothing spline option of the dplR package (Bunn, 2008) from R (R Core Team, 2021) was used to detrend the temporal data series. The “nyear spline” at 2/3 the wavelength of n years was used (Cook et al., 1990). The effect of outliers was minimised using Tukey’s bi-weight robust method (Cook et al., 1990). A residual version of the chronology was built by pre-whitening after fitting an autoregressive model to the data using the Akaike information criterion (AIC) for variable selection (Bunn, 2008). At each site, the Expressed Population Signal (EPS) index was above the frequently used threshold of 0.85, indicating a high degree of common cohesion (Wigley et al., 1984). At site 1, the EPS was 0.94; at site 2, the EPS was 0.92; and at site 3, the EPS was 0.91. Analysis of site chronologies shows that before fertilisation with potato starch effluent, between 1944 and 1983, tree growth at all three sites was comparable with t-values between sites ranging from 5.8 to 7.8. For site 1 the mean of all the correlations between different cores (total rbar) was 0.52, the mean of the correlations between series from the same tree over all trees (rbar.wt) was 0.65 and the mean interseries correlation between all series from different trees (rbar.bt) was 0.52. For site 2 total rbar = 0.36, rbar.wt = 0.63, rbar.bt = 0.36. For control site 3 total rbar = 0.34, rbar.wt = 0.46, rbar.bt = 0.34.

### Choosing trees for quantitative wood anatomy (QWA)

In total three trees were chosen for wood anatomical study. For each site, one tree with the best correlation with the mean chronology was chosen. A similar approach was applied by (Koprowski et al., 2018). To choose the most representative and suitable trees for QWA, the correlation of the selected sample with the master chronology (without the selected sample) was checked. For site 1 the highest correlation of the selected sample was 0.79, at site 2 = 0.74 and site 3 = 0.77 ( $p < 0.05$ ).

### Microslide preparation and wood anatomical analyses

Three selected trees were felled, and one disc was taken from each tree at breast height, 1.30 m above the ground. From each disc, a radius was selected and divided into wooden cubes of approximately  $1 \times 1 \times 1$  cm. This allowed the preparation of a series of microscope slides covering all tree rings from pith to bark. Microscope slides were prepared according to the methodology proposed by (Schweingruber et al., 2006). Thin (approx. 15  $\mu\text{m}$ ) sections of the samples were prepared using a GSL 1 core microtome (Gärtner and Nievergelt, 2010) and then stained with safranin for cell wall staining. The sections were then mounted using a Heft Histokitt and photographed using a Canon DS126171 digital camera connected to a microscope (Olympus BX41). The microsection photographs were processed using Adobe Photoshop software (ver. 22.5.5.) to enhance the contrast between the cell wall and the lumen of the cells. In each ring (year) it was decided to select a block of five transects of cells lying directly next to each other to analyse cellular parameters. The number of cells in one transect varied from year to year and ranged from 5 to 59 cells at site 1, 29–90 cells at site 2 and 24–82 cells at site 3. Dendroanatomical calculations were performed in R (R Core Team, 2021), R version 4.2.2 (2022–10–31 ucrt) using different packages. The R package tracheideR (Campelo et al., 2016) was used to transform the raw data obtained from the image analysis into a tracheidogram to better visualise the radial intra-ring variation of the histometric parameters and to get the following parameters: CWT, LD, RW, nTotal, EW and LW (Fig. 2). Each parameter is illustrated in Figs. 4 to 9, where every point on the graph describes the average value for the entire ring. To analyse EW and LW, it was decided to use the relative percentage of earlywood (relEW) and the relative percentage of latewood (relLW), so that all annual increments can be easily compared with each other according to the method described and applied in the tracheideR package by (Campelo et al., 2016).

### Periods of different fertilisation intensities

As the method of fertilisation, the water supply and the concentration of supplied elements varied over time, three 12-year periods were distinguished in which the effects of elements and selected climatic periods on the cellular parameters of the trees were analysed. The first period was before fertilisation, the so-called ‘no’ period (1971–1983), in which only climatic data were investigated.

The second period was, the so-called ‘low’ period (1984–1996), in which the utilisation of production waste and its distribution to the forest through a pipe system began. The mean amount of fertiliser applied, in the form of water solution, was 659  $\text{m}^3$  per year, while the mean amount of N, P and K applied was  $N = 195 \text{ mg}/\text{dm}^3$ ,  $P = 32 \text{ mg}/\text{dm}^3$  and  $K = 318 \text{ mg}/\text{dm}^3$  per year respectively.

The third period was, the so-called ‘high’ period (1997–2009), in which the availability of water was considerably decreased and the relative concentration of elements supplied to the environment increased. The mean amount of fertiliser applied, in the form of water solution, was 303  $\text{m}^3$  per year, while the mean amount of N, P and K applied was  $N = 364 \text{ mg}/\text{dm}^3$ ,  $P = 43 \text{ mg}/\text{dm}^3$  and  $K = 521 \text{ mg}/\text{dm}^3$  per year respectively. The Holm-Bonferroni method was used to test the statistical relationship between two time periods within the sites. Calculations were based on the stepAIC function and mass package



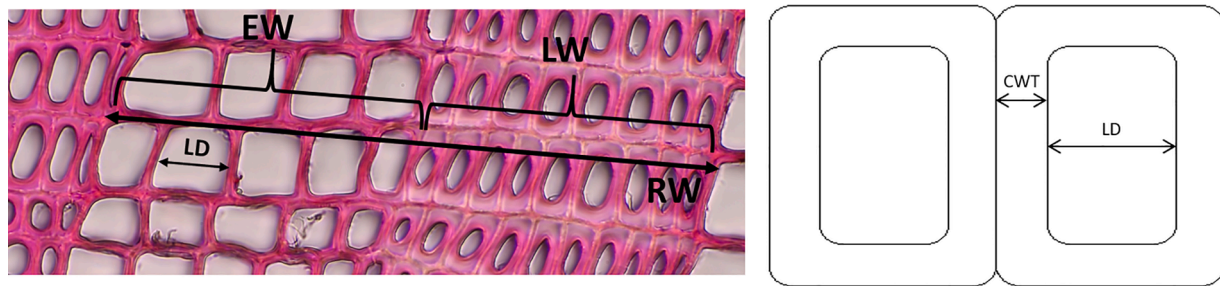


Fig. 2. Schematic representation of anatomical variables. The radial lumen diameter (LD), the cell wall thickness (CWT), ring width (RW), earlywood (EW) and latewood (LW).

(Venables and Ripley, 2002) in R. Linear regression together with AIC was used to select the best fitted model. The highest t value coefficient was chosen based on the statistically significant p-values > 0 and < 0.05. P-value significance was divided as follows: 0 < \*\*\* < 0.001 < \*\* < 0.01 < \* < 0.05. During both periods, pH was, in general constant, with a mean of 6.0, ranging from 5.5 to 6.0 in a second 'low' period (1984–1996) and 5.1–6.0 in a third 'high' period (1997–2009).

As there was no statistically significant (p-value < 0.05) pattern in relation between ring-widths and both daily and monthly climatic data checked for periods from 14 days to 90 days, we investigated the influence of climate from periods which are known to be important for Scots pine growing in Northern Poland (Zielski et al., 2010): the mean temperature of February and March and the sum of precipitation of May, June and July. Multiple linear regression was used to investigate the relationship between wood anatomical parameters with climatic variables, elemental concentrations (N, P and K) and wastewater volumes. The AIC was used to select the critical factors determining the width of tree rings. At first, all independent variables were used, and then a regression model with selected climatic variables and N, P and K concentrations, was applied. We used AIC to select the model that best describes the relationships between climatic factors and the effects of N, P and K on tree ring anatomy. In the first period, we only analysed the

influence of climate factors, but in the second and third periods, we added analyses of the following parameters: CWT, LD, RW, nTotal, EW and LW.

**Results**

Two forms of data control were chosen for this experiment: (i) control site 3, located outside the FWT, where post-production wastewaters did not infiltrate (Fig. 1) and (ii) the period of tree growth before the establishment of the FWT zone (1971–1983) and the addition of wastewater.

*Comparison of the sites over time*

In the 'no' fertilisation period, at site 1 (1971–1983), there was a positive effect of February-March mean temperature on nTotal (Fig. 3) (specific numeric values in Table 1 in Supplementary Materials), while at site 2 May-July precipitation positively influenced total annual RW, nTotal and LD.

During the 'low' fertilisation period, at site 1 (1984–1996), a highly significant, negative effect of N (average supply 195 mg/dm<sup>3</sup> per year) on RW is observed, while the presence of P (average supply 32 mg/dm<sup>3</sup>

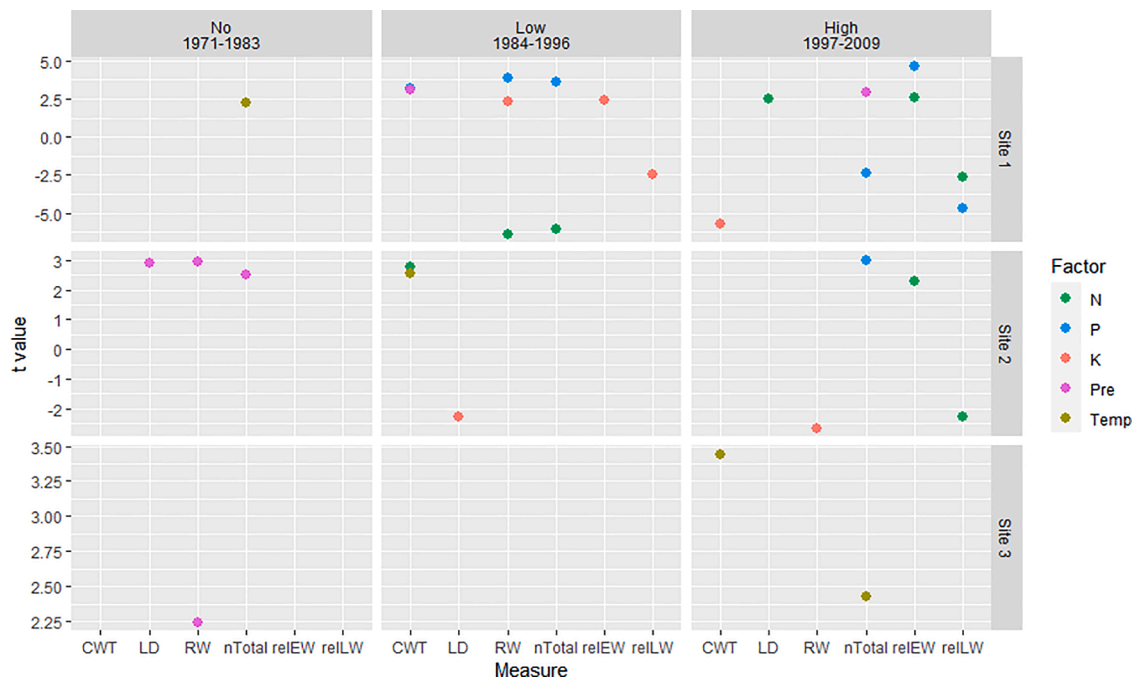


Fig. 3. Effects of N, P and K and selected climatic intervals (Pre - sum of precipitation from May to July; Temp - mean temperatures from February to March) on the cell parameters of Scots pine growing at sites 1, 2 and 3 in the Ilawa Forest. Dots represent the highest t values based on the statistically significant p-values: \*\*\* < 0.001, \*\* < 0.01 and \* < 0.05. Cell Wall Thickness (CWT), Lumen Diameter (LD), Ring Width (RW), Total Number of Cells in a ring (nTotal), Relative Percentage of Earlywood (relEW) and Relative Percentage of Latewood (relLW) (Campelo et al., 2016), Nitrogen (N), Phosphorus (P) and Potassium (K) values are indicated.

per year) and K (average supply 318 mg/dm<sup>3</sup> per year) positively impacted RW. A similar pattern of t values coefficient is observed in nTotal. A positive effect of P was also observed for CWT. There was a positive effect of K on EW width. At the same time, at site 2, there was a negative effect of K on LD and a positive effect of N and mean February-March temperatures on CWT.

In the 'high' fertilisation period (1997–2009), characterised by high nutrient concentration, a significant positive effect of N (average supply 364 mg/dm<sup>3</sup> per year) on LD and EW width was observed on site 1 (fertilised). A particularly positive effect of P (average supply 43 mg/dm<sup>3</sup> per year) on EW was also noted. At the same time, the presence of P had a negative effect on nTotal and LW thickness. A strong negative effect of K (average supply 521 mg/dm<sup>3</sup> per year) on CWT was noted. A positive effect of May-July precipitation on RW and nTotal was observed. At the same time, site 2 recorded a negative effect of K on RW, a positive effect of N on EW width and a positive effect of P on nTotal.

At the control site, S3 (Fig. 3), during the 'no' fertilisation period (1971–1983), annual precipitation affected only RW. During the 'high' fertilisation period (1997–2009), average February-March temperatures significantly impacted CWT and nTotal.

A highly significant effect ( $p < 0.001$ ) of fertiliser elements on pine cell parameters was observed at the site 1. At the same time, a lesser but significant (Fig. 3) effect of fertilisers on individual parameters, was observed at site 2 ( $p < 0.05$ ).

*Comparison of the periods within a single site*

At the fertilised site 1, a statistically significant decrease ( $p < 0.01$ ) in RW was observed between the control period "no" (before the start of fertilisation) and the period characterised by a high concentration of elements N, P and K denoted 'high' owing to their high supply and a drastic reduction in the water delivered to the ecosystem (data from the Chemirol Company Ltd.). In addition, a significant decrease ( $p < 0.01$ ) of RW was also observed between the 'low' period, with low pollutant concentration, and the period with high pollutant concentration 'high' (Fig. 4).

At site 1, there was a significant decrease in CWT between the "no"

and "high" periods with a  $p < 0.01$ , as well as between the 'low' and 'high' periods with a  $p < 0.05$ . A successive decrease in cell wall thickness with an increasing nutrient concentration in the wastewater was observed. In addition, at site 2, significant thickening of the CWT was observed between the control period "no" and the first fertilisation period "low" with a  $p < 0.01$ . At site 3, no significant change in CWT was observed over the period studied (Fig. 5).

At site 1, a significant decrease in the size of the LD was found between the 'no' and 'low' periods ( $p < 0.05$ ) and between the 'no' and 'high' periods ( $p < 0.05$ ). A successive decrease in the size of the LD is observed as the concentration of nutrients in the wastewater increases (Fig. 6). Although a reduction of the size of both LD and CWT is observed, it is noteworthy that the p-values are significantly smaller when tests are performed for CWT. This indicates more significant changes within the CWT.

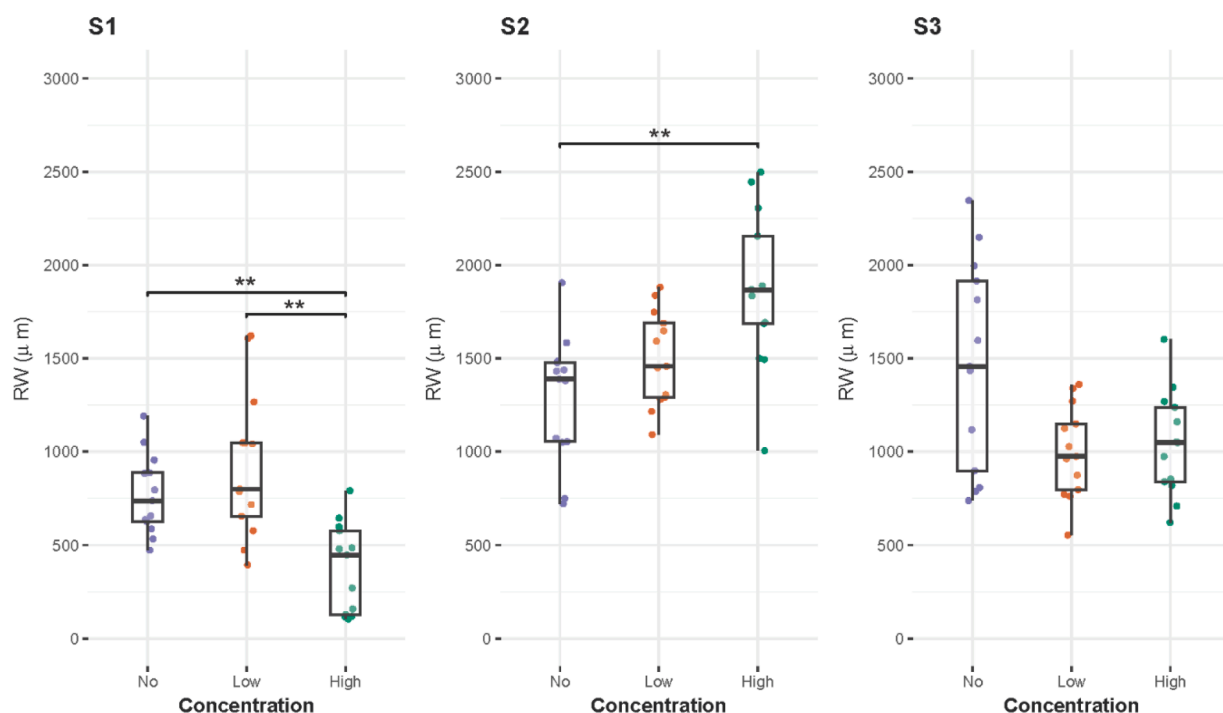
No significant differences ( $p > 0.05$ ) in RW and LD were observed at the unfertilised sites 2 and 3. For the nTotal parameter, no significant differences are observed within sites. The same growth pattern between RW and nTotal is observed across all sites and periods (Fig. 7). No significant differences are observed between the reLEW and the reLLW within sites (Figs. 8, 9 Supplementary material).

It was observed that at the fertilised site 1, the supply of nutrients delivered with wastewater resulted in a decrease of RW, CWT and LD. Only at site 2 is an increase in CWT observed due to the supply of N during the initial period of FWT functioning.

**Discussion**

*Fertilisation alters CWT*

Previous experiments with forest fertilisation have focused mainly on observing changes in stem biomass growth and have relied on measurements of tree girth or annual increments (Balster and Marshall, 2000; Hedwall et al., 2014). The novel contribution of this study is it presents the response of individual cellular characteristics to N, P and K, separating periods of different fertilisation intensities. Moreover, it describes an experiment with an extremely high supply of nutrients to the



**Fig. 4.** Ring Width (RW) during periods with different concentrations of supplied wastewater. S1 - site 1 fertilised, S2 - site 2 unfertilised but within a zone of the Forest Wastewater Treatment (FWT), S3 - control site 3. Each point describes the average value for the entire ring.

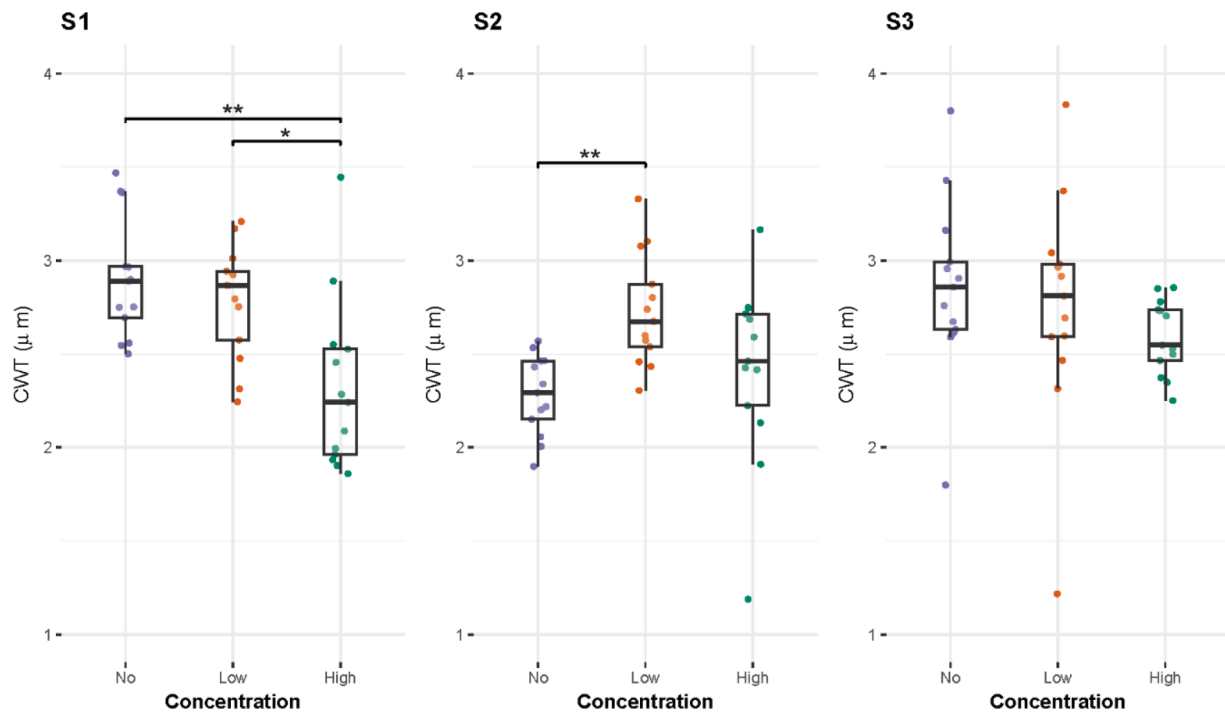


Fig. 5. Cell Wall Thickness (CWT) during periods with different concentrations of supplied wastewater. S1 - site 1 fertilised, S2 - site 2 unfertilised but within a zone of the Forest Wastewater Treatment (FWT), S3 - control site 3. Each point describes the average value for the entire ring.

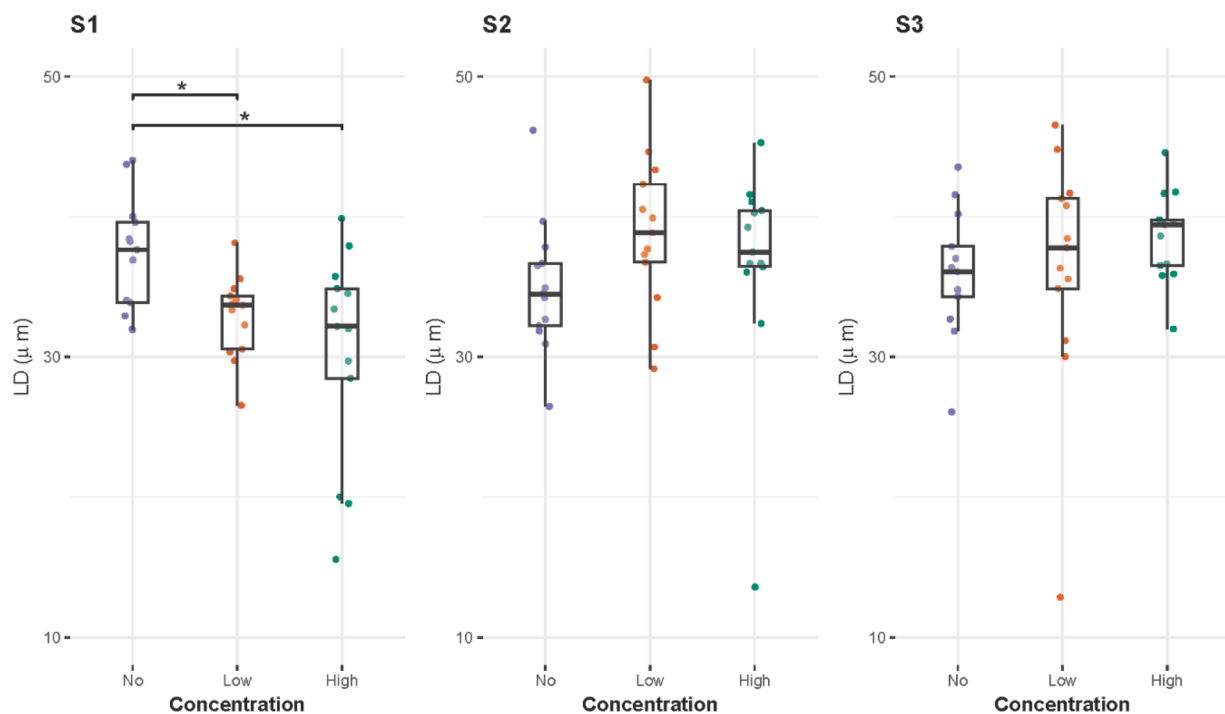


Fig. 6. Lumen diameter (LD) during periods with different concentrations of supplied wastewater. S1 - site 1 fertilised, S2 - site 2 unfertilised but within a zone of the Forest Wastewater Treatment (FWT), S3 - control site 3. Each point describes the average value for the entire ring.

ecosystem.

In this study, the main objective was to investigate how high concentrations of N, P and K in post-production wastewater affect the anatomical structure of tree rings at the cellular level. As previous studies at this site have shown a decline in tree ring growth (Chojnacka-Ozga et al., 2022; Koprowski et al., 2015), we focused on investigating the changes that have taken place within the annual increment

that are reflected in its reduction. Because this study was initially established to test the hypothesis that fertilisation can have a negative impact on tree growth by reducing LD in RW, we analysed different cell characteristics such as CWT, LD, RW, nTotal, reLLW and reLEW to find out which factor was mostly affected by fertilisation. We observed a negative effect of fertiliser on all Scots pine cell characteristics at site 1 (fertilised), but at the same time, a lesser but significant (Fig. 3) and

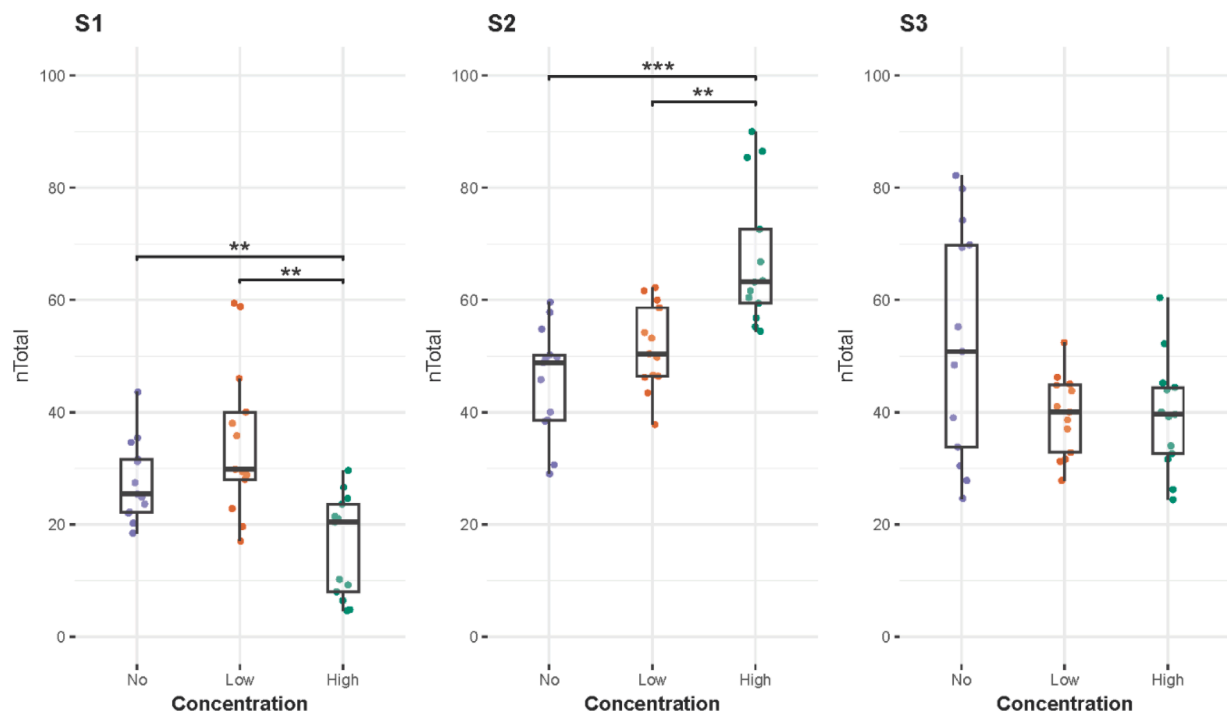


Fig. 7. Total number of cells in a ring ( $n_{Total}$ ) during periods with different concentrations of supplied wastewater. S1 - site 1 fertilised, S2 - site 2 unfertilised but within a zone of the Forest Wastewater Treatment (FWT), S3 - control site 3.

positive (Figs. 4,5 and 6) effect of fertilisers on individual cell parameters, was observed at site 2. Technically, site 2 was not fertilised, but was located within the wastewater treatment zone. This indicates migration of contaminants within the whole FWT zone and shows the effect of fertilisation on the neighbouring plant populations, as confirmed by (Dyguś, 1999; Koprowski et al., 2015) who observed intensive growth of undergrowth forest plants. On site 2, a significant thickening of RW and an increased  $n_{Total}$  were observed, with a concomitant increase in CWT. At the same time, no significant changes in LD or changes in reLW or reLLW were observed.

In this study, N and P are considered the most influential parameters on RW and  $n_{Total}$  during the first phase (1984–1996) of fertilisation (Fig. 3), while during the second phase (1997–2009) their influence switch on the CWT and reLW and reLLW. At the site 1 it is observed that RW correlates well with  $n_{Total}$  during both 'low' (1984–1996) and 'high' (1997–2009) periods. K seems to have a negative effect on the CWT during the second phase of fertilisation (1997–2009). Although forest fertilisation experiments, where the effect of individual elements on tree growth is considered, are not rare, there are few papers where cellular parameters such as CWT, LD,  $n_{Total}$  together with EW and LW of maturity Scots pine forest have been analysed. In the case of EW and LW, our analysis focuses on differences between sites with different fertilisation levels and showed no statistically significant differences in the proportion of EW and LW. Recently, however, an examination of trees from exactly this location was made by (Chojnacka-Ozga et al., 2022) taking into account RW, EW and LW. They analysed the proportion of early/late wood in different periods of the FWT but divided the time into heterogeneous periods dictated by changes in the proportion of earlywood. In this approach, there was an alternating increase and decrease in the proportion of earlywood depending on fertiliser supply over 5–8 years. The difference between studies may also be due to the different time intervals adopted.

#### Radial growth depends on fertiliser concentration

Fertilisation is generally considered beneficial for plant growth, but as it is shown in our studies the positive effect depends on the

concentration of the fertiliser. In our study, N application ranged from 176 to 1325 kg ha<sup>-1</sup> depending on the year (data from the Chemirol Company Ltd.) (Koprowski et al., 2015), while published rates range from 100 to 300 kg ha<sup>-1</sup> for established forests (Binkley et al., 1994). In our study, P and K were also applied in higher amounts than in other experiments. For example, in a study on the effect of fertilisation in Sweden in 1988–1989, the amount of K applied was 43 kg ha<sup>-1</sup> (Kårén and Nylund, 1996), whereas in our case in 1988 about 1846 kg ha<sup>-1</sup> was applied, exceeding this dose by more than 40 times. From 1999 onwards, the volume of water applied decreased while the amount of N, P and K remained unchanged, resulting in an increase in the relative concentration of these elements and consequently a decrease in radial growth. Reviews of fertiliser experiments conducted in northern countries (Denmark, Finland, Iceland, Norway and Sweden) revealed that a single application of 150 kg N ha<sup>-1</sup> increases standing timber growth in mature stands of Norway spruce and Scots pine in areas with low anthropogenic N deposition (Nilsen, 2001; Nohrstedt, 2001; Óskarsson and Sigurgeirsson, 2001; Saarsalmi and Mälkönen, 2001; Vejre et al., 2001). Similar results were obtained by (Balster and Marshall, 2000) in North America where a single application of 178 kg N ha<sup>-1</sup> led to a greater standing timber production in 85-year-old Douglas-fir stands in the northwestern USA and 336 kg N ha<sup>-1</sup> increased tree growth in 45-year-old Ponderosa pine stands in Ontario (Groot et al., 1984). These findings were later confirmed by (Newton and Amponsah, 2006) for 21- to 100-year-old Black spruce and Jack pine stands in Canada. Most of the fertilisation studies in Scandinavia and the USA were conducted in middle-aged or older coniferous forests, which are, in terms of age, comparable to the forest we studied. According to the information provided by the Itawa Forest District, the trees while sampled were of a similar age, reaching about 80 years (Koprowski et al., 2015).

#### Over-fertilisation might affect in physiological drought

Several studies found that over-fertilisation can lead to physiological drought, which can cause trees to die despite intensive irrigation (Ahanger et al., 2016; Ward et al., 2015). The use of excess fertilisers can be harmful to plants, depending on the type of fertiliser and time of

application. Very high salt concentrations in the soil solution can reduce its osmotic potential enough to reduce water absorption, leading to leaf dehydration, closure of stomata, reduced photosynthesis, leaf damage and plasmolysis of root cells (Kozłowski et al., 1991). Previous research on this study site using RW measured from the same trees (Koprowski et al., 2015) observed that once the concentrations of N, P and K were increased in the effluent it caused a growth decrease and a weakening of the physiological condition of the trees. They concluded that the change in physiological condition can be a result of nutritional imbalance and caused osmotic stress in soil caused by the high level of nutrients itself (Elliott and White, 1994).

We found that due to the applied fertilisation regime, the Scots pine trees reduced RW, LD and CWT at site 1 (fertilised) over 12 and 24 years periods (Fig. 3). We assume that the reduction was caused by the shortages in water conductivity in plants, which might appear due to the physiological drought (Ward et al., 2015). These results confirm that the application of N, P and K at excessive rates is detrimental to forest health (Koprowski et al., 2015).

The observed wood anatomical responses are similar to those produced by drought which can be caused by the experimental conditions or occur naturally. Despite the high presence of water in the ground, due to the regular watering with water aqueous fertiliser, the amount of nutrients could cause water stress and prevent plants from taking up water from the soil. One of the indicators of normal plant development is the water content of plant tissues, which should reach 75–95 % of the mass. Using pine seedlings and soil from the FWT research zone, the water content level was determined to be 48 % of the total mass (Gumnicka, 1999). It has been reported that a too high concentration of elements in the soil solution and oxygen deficiency could cause the phenomenon of physiological drought (Ahanger et al., 2016) and lead to wilting of the seedlings. The same phenomenon may have occurred in the mature stand at the FWT zone.

We compared our results with research analysing the response of Scots pine growing under different hydrological conditions. Since the observed decrease in the size of RW, LD and CWT might be explained by the possibility of provoked osmotic stress in soil caused by the high level of nutrients itself leading to physiological drought phenomenon (Koprowski et al., 2015), we found such a comparison reasonable. (Eilmann et al., 2009) calculated the response of the same set of parameters (RW, EW, LW, CWT, LD and nTotal) to drought in Scots pine growing under relatively comparable environmental conditions and found a decrease in CWT and RW, but a slight increase in LD which is the opposite to what we observed. They also found a significant decrease in nTotal, EW and LW, which we did not observe. (Montwé et al., 2014) analysed the response of the cellular parameters of Norway spruce in an experimental drought. They observed that under drought, trees had a larger proportion of LW, smaller LD and thicker CWT, whereas in our case, no larger proportion of LW was observed, while the decrease in LD width was the same. Although we observed a decrease in CWT at the fertilised site 1, a thickening of the CWT was observed on site 2, where fertiliser might have entered the area in smaller amounts through soil water migration.

## Conclusion

Despite growing on nitrogen-deficient soil, Scots pine respond negatively to frequent very high nitrogen-rich fertiliser applications. This suggests that the frequency and amount of fertiliser applied are more important for pine growth than the supply of essential nutrients. Over-fertilisation can lead to physiological drought, which can cause the trees' growth to decrease, despite intensive irrigation. While the initial fertilisation period appeared to have a positive effect on earlywood growth and cell wall thickness, the excess of nitrogen resulted in growth reduction. Our results provide a starting point for further analysis of the effects of fertilisation on tree growth, with a particular focus on its effect on individual cell parameters. Such plant physiology knowledge could

be important for optimization of production of high technical quality wood.

## CRedit authorship contribution statement

**Nella Waszak:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Filipe Campelo:** Writing – review & editing, Software. **Iain Robertson:** Writing – review & editing, Resources. **Radosław Puchalka:** . **Fatima-Zahraa El Balghiti:** Resources, Data curation. **Jozica Gričar:** Writing – review & editing, Validation. **Ali Boularbah:** Writing – review & editing. **Marcin Koprowski:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tfp.2023.100480.

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