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The Impact of Long-Term Fertilisation of Potato Starch Wastewater on the Growth of Scots Pines: A Retrospective Analysis

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Abstract: The article discusses the impact of the application of potato starch wastewater as a fertiliser on the growth responses of Scots pines at the Forest Wastewater Treatment Plant (FWTP) in Iława. More specifically, our study sought to determine the direction, extent, and duration of changes in the trees' growth responses caused by the application of fertiliser and the influence of climatic conditions on secondary growth in the trees to which the fertiliser had been applied. As part of the study, the extent of and changes in the growth responses were determined with reference to annual ring widths and earlywood and latewood widths using dendrochronological methods. The research was carried out in four pine stands: two stands of different ages (80 and 110 years) located within the FWTP site and two control stands of corresponding ages located outside that area. Core samples were collected from 12 trees in each stand. We found a two-way impact of potato starch wastewater on secondary growth in the trees under study, with a stimulatory effect (27%–30%) in the first decade of fertiliser application followed in the subsequent years by a strong reduction in growth (30%–45%, depending on the age of the trees). The trends of these changes could be seen in both the overall annual ring widths and the widths of earlywood and latewood. The direction of the changes was the same for trees of different ages, although age was found to have affected the extent and duration of the stimulatory or inhibitory effect. Over the entire period during which the fertiliser was applied, changes occurred in the structure of the wood as manifested in the increased share of earlywood. The sprinkler application of potato starch wastewater and the accompanying irrigation caused a shift in dendroclimatic relationships in comparison to the control plots. Surface irrigation and the resulting changes in water balance reduced the drought susceptibility of the pines under study. At the same time, however, trees weakened by the excessive concentration of toxic nitrates became more sensitive to temperature conditions in winter. The results confirm that the implementation of substances containing significant amounts of organic nitrogen and potassium into forest ecosystems may impair the vigour of trees, reduce stand productivity, cause an imbalance in the ecosystem and may consequently lead to forest degradation.

Keywords: tree ring widths; organic sewage; forest experiment; *Pinus sylvestris*; dendrochronology; Poland

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Citation: Chojnacka-Ożga, L.; Lendzion, J.; Ożga, W. The Impact of Long-Term Fertilisation of Potato Starch Wastewater on the Growth of Scots Pines: A Retrospective Analysis. *Forests* **2022**, *13*, 1575. https://doi.org/10.3390/f13101575

Academic Editors: Angela Lo Monaco, Rodolfo Picchio and Mark Vanderwel

Received: 28 August 2022
Accepted: 21 September 2022
Published: 26 September 2022

1. Introduction

Biogenic disposal of wastewater sludge is a relatively common solution used in agriculture, and in some countries also in forestry [1–8]. The potential benefits of utilising sludge as a forest fertiliser have been the subject of many studies conducted in the context of both increasing forest productivity and identifying potential environmental threats [2,5,9–14].

In Poland, land application of wastewater sludge has mainly been associated with agriculture [15,16]. In fact, it was in farming that wastewater generated by the potato starch industry found widespread use. In the 1970s and 1980s, attempts were also made to adapt the concepts of agricultural use of organic wastewater to the needs of forestry [17]. The positive preliminary results of both domestic and foreign studies provided a basis for the establishment of the experimental facility referred to as the Forest Wastewater Treatment Plant (abbreviation FWTP) in the Iława Forest District. The purpose of the experiment was to evaluate the possibilities for productive utilisation of organic wastewater in tree plantations. In this case, the waste to be treated in the forest environment was potato starch wastewater from the nearby starch factory (Zakłady Przemysłu Ziemniaczanego) in Iława. According to initial assumptions, the disposal of this type of wastewater in a forest ecosystem growing on relatively nutrient-poor sandy soils was expected to contribute to an increase in tree biomass and improvement in the productivity of forest stands. Potato starch wastewater is rich in such elements as nitrogen, potassium, and phosphorus; hence the expectation that it might also be used to enhance the nutrient status of forest stands. The research report from Peters [18] concerning the possible adverse effect of potato starch wastewater on the health of pine forests was not taken into account.

Upon the commissioning of the FWTP and throughout its operation, research was conducted to assess the impact of potato starch wastewater on the forest environment at the site. The effects being assessed included the impact of wastewater on physiological processes in pines [19], the health and sanitary condition of forest stands [20,21], the circulation of nutrients from wastewater via the soil and plants into groundwater [22], general changes in the soil and water environment [23–25], and changes in vegetation and condition of the habitat [26,27] As a result of these studies, Gumnicka [19] noted that high doses of wastewater resulted in biomass reduction, while Koprowski et al. [28] pointed out that potato starch wastewater caused a reduction in the efficiency of photosynthesis and disrupted the growth of Scots pines.

The observed ecological impact of the long-term operation of the FWTP, and especially the associated physiognomic changes in forest stands, became a starting point in the search for a record of the effects of the facility's operation in the secondary growth rings of Scots pine as the area's main forest-forming species. Since tree rings and their structure are regarded as bioindicators of vigour, a retrospective analysis of changes in the width and structure of the rings can provide detailed information regarding trees' sensitivity to environmental factors.

Tree-ring analyses have frequently been used to assess the condition of forest stands exposed to anthropopressure, including air pollution, fertilisation, and increased nitrogen deposition. The impact of fertilisation on the radial increment of trees was studied mainly in the context of the possibility of increasing the biomass production of forests for various trees and forest types. The studies on the growth response of conifer and deciduous trees to nitrogen fertilisation have shown various results: increased growth [29–32], no effect, and even decreased growth [33–35].

According to the research previously carried out at the FWTP site by Koprowski [28], nutrient enrichment with potato starch wastewater caused an increase in tree-ring width in the pines, which was later followed by slower growth and impairment of the trees' physiological condition. In our study, we decided to expand the existing knowledge on the subject by gauging the impact of sprinkler application of potato starch wastewater on the growth responses of pines of different ages and identifying other possible factors that model these responses. The aims of our study were to: (i) analyse the growth rhythm of trees from two stands of different ages subjected to sprinkler application of wastewater and trees from stands of corresponding ages which were not fertilised; (ii) determine the direction, extent, and duration of changes in the trees' growth responses caused by the sprinkler application of potato starch wastewater; and (iii) determine the influence of climatic conditions on secondary growth in the trees to which potato starch wastewater had been applied. The study focused on three characteristics of secondary xylem, namely (i) annual tree-ring width (TRW); (ii) earlywood (EW) and latewood (LW) width; and (iii) share of earlywood in the annual tree-ring width.

2. Materials and Methods

2.1. The Forest Wastewater Treatment Plant

The Forest Wastewater Treatment Plant was brought into operation in 1984 in the Iława Forest District of Poland's State Forests (located in the northern part of the country, in the Brodnica Lake District). The plant was designed as an experimental facility to test the disposal of wastewater from the potato starch industry in a forest environment (Figure 1). The facility was established on 216 hectares of mesic pine forest growing on a sandy outwash plain with a prevalence of brunic arenosols formed on slightly loamy and loose sands with low retention capacity [36]. The average annual air temperature and average annual precipitation for that region are 7.6 °C and 610 mm, respectively, for the period 1951–2020. A brief description of the climatic conditions of the study site is presented in Figure 2.

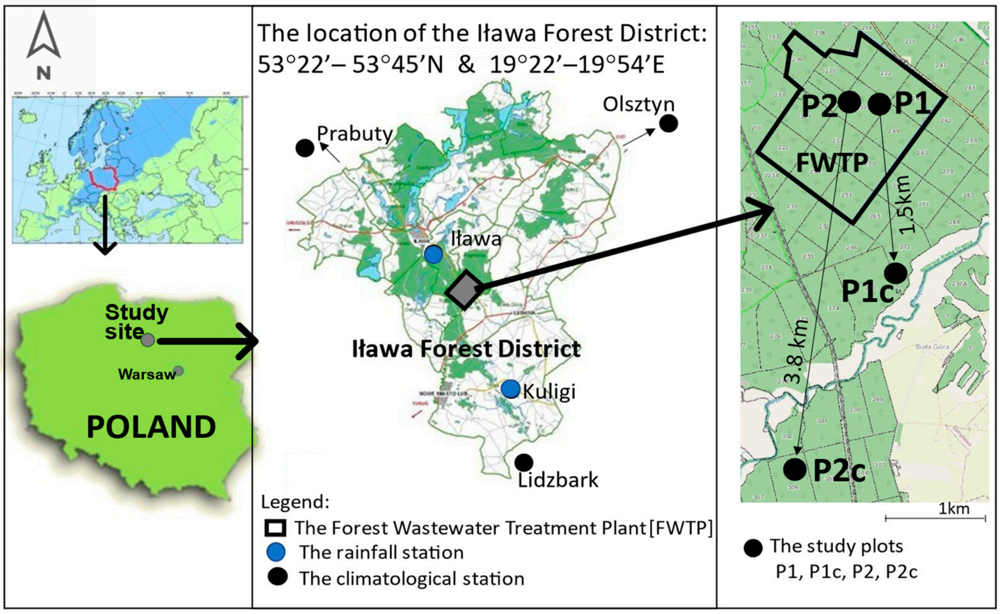

Figure 1. Location of the study plots (explanation of abbreviations in the figure on the right: P1 and P2—study plots within the FWTP; P1c and P2c—control stands).

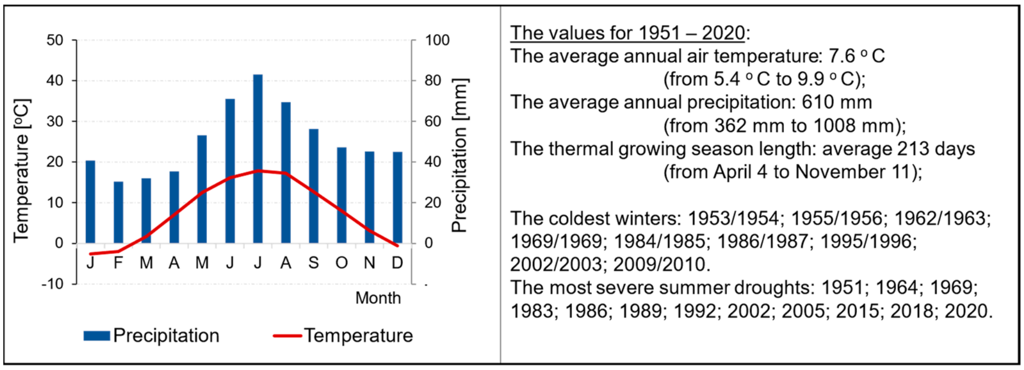

Figure 2. Climatic diagram (for the years 1951–2020) and the selected climatic characteristics for the study area (based on the data obtained from the Polish Institute of Meteorology and Water Management). Climatic characteristics were determined on the basis of the following criteria: thermal growing season: period from when daily temperatures are ≥5 °C for >5 days and when daily temperatures fall to <5 °C for 10 days [37]; the coldest winters: the mean temperatures of all the winter months were lower than the long-term mean value by at least 1.5 SD; severe droughts were determined on the basis of the SPEI value [38] calculated for the study site.

Potato starch wastewater from the starch factory (Zakłady Przemysłu Ziemniaczanego) in Iława was transported to the forest through an underground pipeline leading to a compensating reservoir from which it was distributed throughout the FWTP site using sprinkler devices (Figure 3, and Supplementary material Figure S1). The wastewater was distributed in the autumn period (September through December) at intervals of seven to eight days. The dose was 300 mm per year in most areas of the site, although, in some areas (18 ha), varied doses were also used (150, 300, 450, or 600 mm per year). According to research by Ciepielowski [20], however, the actual total annual doses of wastewater differed from those prescribed and ranged from 120 to 450 mm per year, depending on the size of the potato harvest. The wastewater contained large amounts of mineral nutrients, humic and organic. During the growing season, the forest area was irrigated with lake water (at rates of 140 to 320 mm per year, depending on the weather situation). In 2006, the amount of wastewater was significantly reduced, and in 2012, the FWTP went out of operation altogether. The many years of wastewater disposal led to numerous negative changes in the forest environment. Over-fertilisation of the soil and groundwater contamination (with increased concentrations of toxic compounds of nitrogen, potassium, phosphorus, calcium and magnesium) caused changes in the species structure of the undergrowth [27] as well as reduction of root systems, deformation of crowns, formation of longer needles and weakening of wood in pine trees. Furthermore, it resulted in inhibition of vertical growth in trees older than 70 years, accelerated suppression of trees [39], and resulted in a strong reduction of stand density (Figure S2).

Figure 3. Sprinkling with sewage of stands on the FWTP in 2000 (Photos: Archives of the Forest Management and Geodesy Office—Department in Olsztyn, Poland).

2.2. Research Material and Methods

The research material was collected in the Iława Forest District in December 2020. Two pine stands of different ages located within the FWTP site (P1 and P2) and two control stands of corresponding ages located outside the site (P1c and P2c) were selected for the study. The following criteria were used in the selection of the stands: (i) the same habitat type for all study plots; (ii) the same dose of fertiliser used on the study plots within the FWTP site; (iii) non-degraded condition of the control stands (N1); and (iv) the same level of tree density on all the plots prior to the commissioning of the FWTP (Table S1, Figure S3). Due to the possible differences in the amount of wastewater discharged in each year in different parts of the FWTP [20], the two study plots were located close to each other (Figure 1 and Supplementary material: Figure S1, Table S1). The idea to use a reference plot located within the FWTP site was abandoned due to the fact that wastewater could still have reached the soil on that plot (due to it being situated among fertilised plots) as a result of uncontrolled emergencies (Figure S1). For that reason, stands located in the buffer zone were selected for comparison instead.

Twelve trees were selected on each study plot and one increment core was taken from each of the trees at a height of 1.3 m above the ground using a Pressler increment borer. The samples were dried, placed in wooden mounts, and sanded using an angle grinder with progressively finer abrasive paper (80, 120, 180, 240, and 400 grit), then scanned at a high resolution (2400 dpi). Using the CooRecorder v.9.31 software [40], annual ring widths and latewood widths were measured (with 0.01 mm precision), and individual sequences (annual growth [TRW], latewood growth [LW], and earlywood growth [EW]) were created for each tree. All measurement sequences were pre-tested for correct dating and temporal synchronisation—both visually and statistically—using the CDendro software [40], and the final cross-dating was performed using the COFECHA software [41].

Next, TRW, EW, and LW chronologies were constructed in raw and indexed (standard and residual) versions for each study plot using the ARSTAN software [41]. The raw chronologies (in mm) were constructed by averaging a series of tree-ring widths (TRW, EW, and LW) for each sampling site. The indexing process used a negative exponential function, a spline function and an autoregression model [41,42]. To remove biological trends, each individual raw data series was transformed into a growth index series. All tree-ring component series (EW, LW, and TRW) were detrended using an age-dependent spline [43] using the ARSTAN software [41]. An "n-year spline" at 2/3 the wavelength of n years was used in accordance with Cook et al. [43]. The indexed tree-ring series were used to build standard-version chronologies (TRW, EW, and LW) for each site. Then, the indices were prewhitened using an autoregressive model and averaged across all series with a bi-weight robust mean estimation to produce mean residual chronologies [42,43].

The strength of the environmental signal in the chronologies was estimated using the *Expressed Population Signal* (EPS) parameter [44,45]. The EPS threshold was set to the widely accepted value of 0.85. The level of similarity between the growth chronologies (within each study plot and between the different plots) was determined using the *Gleichläufigkeit* convergence coefficient (GLK), correlation coefficient, and t values between all pairs of series comprising the chronologies [46]. Additionally, standard dendrochronological statistics were computed, for example mean values, mean sensitivity (MS), first-order autocorrelation (AC1), and inter-series correlation (rbar).

A non-parametric test, Kruskal–Wallis, was used to determine the significance of differences between the pine growth on respective research and control plots [47]. Changes in the tree growth at the fertilised plots, as in earlier studies [28,31–33], were evaluated by statistically comparing the distribution of tree-ring chronologies (raw and standard version) with those of the control plots. Comparisons were performed for the period prior to the launch of the FWTP (1960–1984), the period of active operation of the FWTP (1985–2012), and the period following the decommissioning of the plant (2013–2020). During the FWTP activity period, trees growing on the fertilised plots showed two different growth phases, initial intensive growth and then growth suppression. Therefore, it was decided to analyse the differences in growth between the fertilised and control plots in two shorter time intervals: 1985–1995 (intensive growth, exceeding the growth of trees in the control plots) and 1996–2012 (reduction of growth compared to the control plots). In 1996, the growth of trees on both fertilised plots was, for the first time since 1985, lower than on the control plots. Therefore, it was decided to analyse the differences in growth between the fertilised and control plots in two shorter time intervals: 1985–1995 (intensive growth, exceeding the growth of trees in the control plots) and 1996–2012 (reduction of growth compared to the control plots). In 1996, the growth of trees on both fertilised plots was, for the first time since 1985, lower than on the control plots. Thus, the differences between the growth of pine on the fertilised plots and those on the control plots were calculated for four periods: (a) 1960–1984; (b) 1985–1995; (c) 1996–2012; and (d) 2012–2020. The statistical analysis was conducted using the STATGRAPHICS 19 software [48].

The climate–growth relationships were investigated using residual chronologies (TRW, EW, LW) and mean monthly values of air temperature (T) and precipitation (P).

Residual chronologies are most often used in analyses of the climate–growth relationship [42,43]. They are calculated by removing the temporal autocorrelation, which allows highlighting of the high-frequency variability and enhances the climate-related year-to-year tree-ring signal. Hence, the residual version usually shows a stronger association with climatic factors compared to the standardized version.

Due to the lack of long-term meteorological observations in the immediate vicinity of the research site, the monthly values of climatic parameters were calculated using the K-nearest neighbours weighted averaging method after [49,50] on the basis of data from climatological and rainfall stations closest to the study site (Figure 1). The meteorological data used in the calculation were obtained from the database of the Polish Institute of Meteorology and Water Management (IMGW-PIB) [51].

The influence of climatic conditions on radial growth was investigated using the response function analysis method [42,52], which is a correlation and multiple regression model that links growth indices (as dependent variables) with climate parameters (as explanatory variables). The sixteen-month climatic window from June of the year preceding tree-ring formation to September of the year ring formation was used. The temporal range of climate data defined in the above manner make it possible to assess the relationship between radial growth and climatic factors both in the preceding growing period and immediately before winter dormancy [42]. Taking note of reports in the literature that the deposition of pollutants may potentially be a factor in the varying sensitivity of pine to climatic conditions [53,54], we performed dendroclimatic analyses for all plots separately for two periods: the period preceding the commencement of fertiliser application (1951–1984) and the period following the commissioning of the treatment plant and the beginning of additional surface irrigation (1985–2012). Based on the above assumption, it was possible to detect any differences in dendroclimatic responses resulting from sprinkler application of wastewater. Sprinkler fertilisation was first conducted in the autumn of 1984; hence the assumption that the application of wastewater in that year could not have affected the width and structure of the secondary xylem. The climate–growth relationships were assessed by bootstrapped Pearson correlation and response function analysis using the program DendroClim2002 [55].

3. Results

3.1. Analysis of Tree-Ring Widths

EPS values for all chronologies—that is for both the overall tree-ring widths and the earlywood/latewood widths (TRW, EW and LW)—were above 0.90, which indicates a high degree of common coherence [44]. The basic characteristics of the chronologies calculated for the research periods adopted in the study are presented in Table S2. In the year in which the treatment plant was brought into operation (1984), the trees growing on the P1 study plot and the P1c control plot were approximately 40–45 years old, while those growing on the P2 and P2c plots were aged around 70–75. Until 1984, the growth characteristics of the trees on the study and control plots were similar for both younger and older trees (Table S2; Figure 2), with the GLK, intra-series intercorrelation and inter-series correlation values as well as t values indicating a high degree of homogeneity in the growth responses of the pines under study. Differences in annual ring structure measured in terms of the share of latewood in the overall annual ring width and the ratio of earlywood to latewood were also insignificant for all the pines in question. Once sprinkler application of potato starch wastewater began, the growth responses of the pines located on the two study plots began to diverge from those of the trees located on the control plots. This divergence in growth responses can be seen in the courses of the growth curves and chronologies and in the mean values and statistical characteristics, although the differences manifest themselves in slightly different ways in the younger and older trees (Table S2; Figures 4–7 and Supplementary material Figure S4).

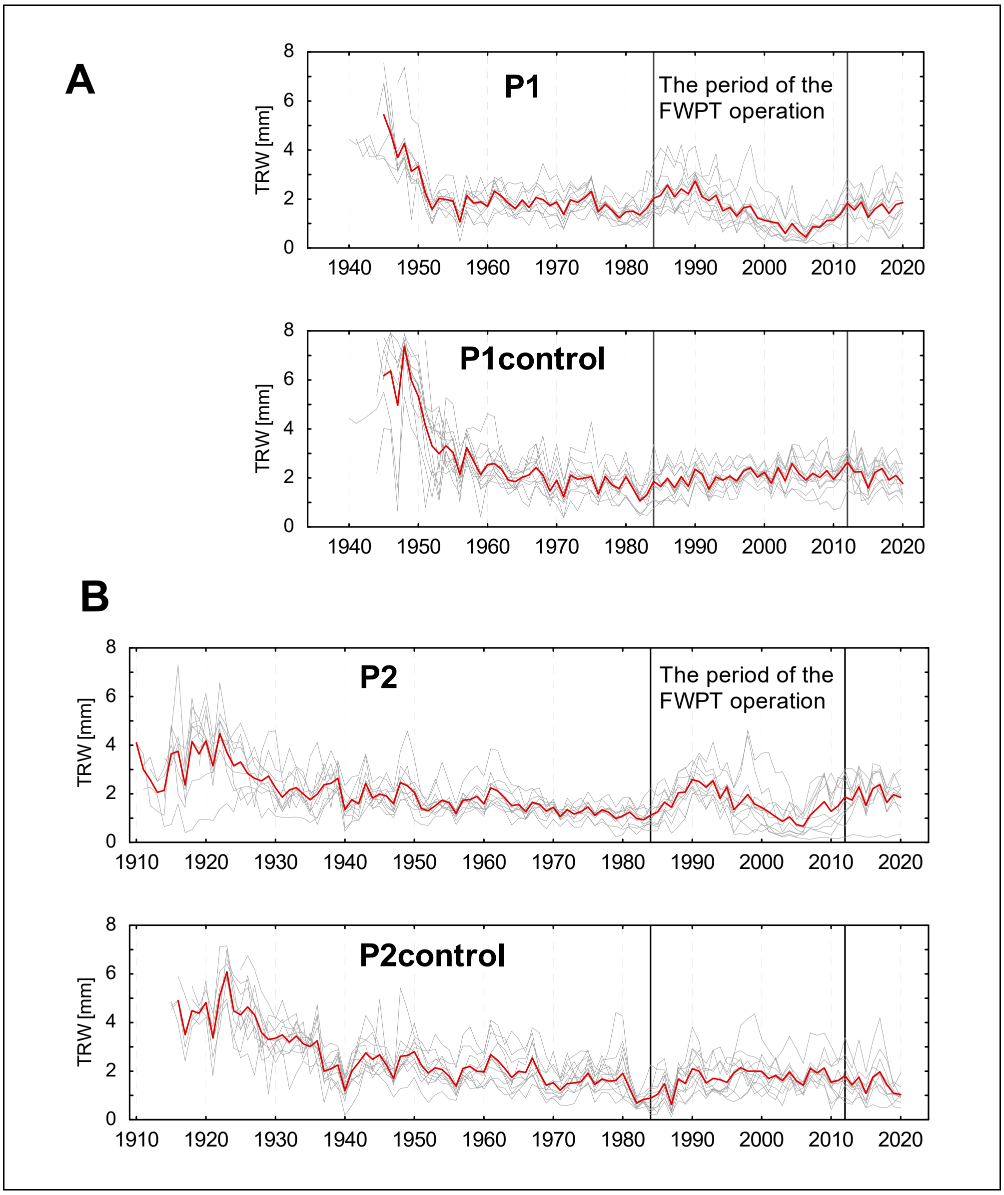

Figure 4. Sequences of tree-ring width (TRW) Scots pine growing on the study plots (thin grey lines—individual series, thick red line—raw chronology); (**A**)—for younger trees (P1 and P1 control plots); (**B**)—for older trees (P2 and P2 control plots). The vertical lines indicate the period of operation of the Forest Wastewater Treatment Plant [FWTP].

The growth response of the pines under study to fertilisation and irrigation was bidirectional, with an increase in growth during the initial period followed by a severe suppression/reduction (Figure 5).

Younger trees (P1 and P1c plots) responded with changes in the level of incremental growth as early as during the first growing season after the commencement of wastewater application. The response was abrupt, with a marked increase in growth that could be observed over a period of eight years on average (1985–1993). The mean value of annual growth in the pines under study during that period was 27% per cent higher than in the period preceding fertiliser application and 25% higher than on the control plot (p = 0.0003 and p = 0.0192, respectively). At the same time, there was an increase in the share of earlywood in the structure of the tree–rings being formed (Figure 7A), and the difference in the share of EW in the TRW between the study plot and the control plot was statistically significant (p = 0.0374). In 1994, the trend in growth response shifted from upward to downward, with a marked increase in intensity from 1999 onwards (Figure 5A).

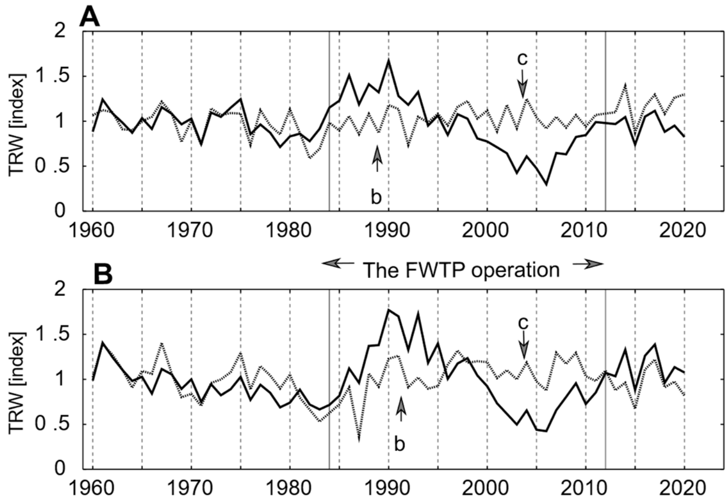

Figure 5. Comparison of standard chronologies of Scots pine from study plots. (**A**)—plots with younger trees (P1, P1 control); (**B**)—plots with older trees (P2, P2 control). Black lines—chronology for P1 and P2, respectively, dashed grey lines—chronology for control plots, P1 control and P2 control, respectively. The vertical lines indicate the period of operation of the Forest Wastewater Treatment Plant [FWTP]; the letters marked: b—the first decade of sprinkling with sewage of stands; c— subsequent years of the fertilization with sewage.

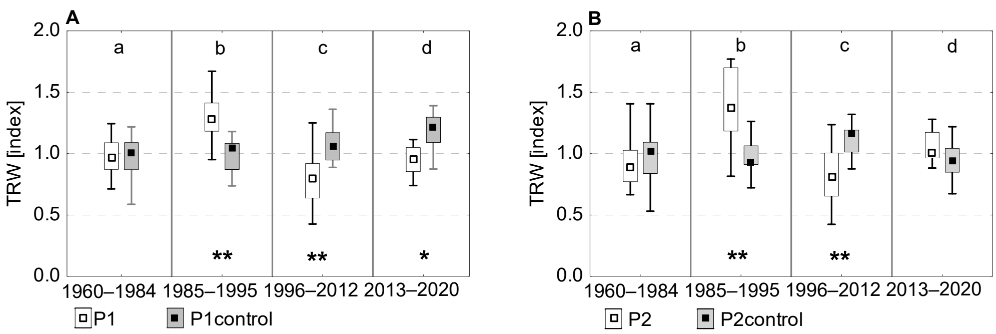

Figure 6. Box plots showing differences in means TRW (median) between the study stands in the different tree growth periods: a—25 years before the start of FWTP activity; b—the first 11 years of the FWTP operation; c—next years of the FWTP operation; d—years after the FWTP was closed. (**A**)—plots with younger trees; (**B**)—plots with older trees. Asterisks indicate a significant difference amongst mean tree-rings widths of Scots pine on study plots; * represents that 0.01 < p-value < 0.05; ** represents that 0 < p-value < 0.01.

The downward trend continued until 2006 and was then followed by a gradual increase in ring width. However, the radial growth of these pines remained smaller than that of the pines growing on the control plot up until 2012, when the FWTP ceased its operations. The mean value of annual growth in the pines under study during that period was significantly (45%) lower than in the pines on the control plot (p = 0.0000). During the period of reduced incremental growth, the share of earlywood in the total growth remained high. Throughout the period affected by the anthropogenic stressor, the pines growing on study plot P1 showed increased "individuality" in terms of the growth response of each tree (i.e., reduced intercorrelation within the series), heightened sensitivity, and reduced similarity of growth patterns when compared with trees growing on the control plot (i.e., a decrease in the values of both GLK and T). The discontinuation of wastewater application (in 2012) resulted in an increase in the share of latewood, although the annual growth values remained lower than on the control plot ($p = 0.0117$), (Figure 7).

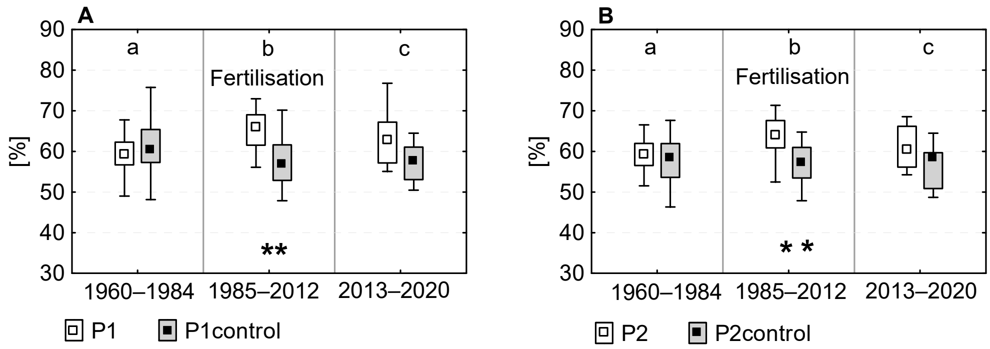

Figure 7. Box plots showing the share of earlywood to total annual ring width of Scots pine on study plots in different tree growth periods, the lowercase letters marked: a—25 years before the start of FWTP activity; b—the period of the FWTP operation (fertilization); c—years after the FWTP was closed. (**A**)—plots with younger trees; (**B**)—plots with older trees. Asterisks indicate a significant difference between the study and control plots; ** represents that $0 < p$-value < 0.01.

The response of older trees (aged 70–75 when fertiliser application began) to the anthropogenic interference with the environment was similar in terms of the direction of the changes, albeit delayed. A significant increase in the radial growth of these pines in 1988–1995 can be observed: the growth values were 30% higher than on the control plot and more than 60% higher than in the period preceding fertiliser application ($p = 0.0328$ and $p = 0.0004$, respectively). In 1996, the trend in growth response shifted from upward to downward, with a marked increase in intensity from 1999 onwards. As in the case of the younger trees, the downward trend continued until 2006 and was then followed by a gradual increase in ring width. Thus, the period of reduced growth lasted from 1996 to 2009, with the average values of annual growth in the pines under study being significantly lower than on the control plot (by more than 27%, $p = 0.0013$). As in the case of the younger trees, fertiliser application caused the increase in the share of earlywood in the structure of the rings being formed. There were significant differences between the pines on the two plots in terms of the share of earlywood in the overall annual ring width (Figure 6B).

As with the younger trees, throughout the operation of the treatment plant, the trees subjected to fertiliser application exhibited reduced homogeneity of growth responses, heightened sensitivity, and reduced similarity of growth patterns in comparison with trees growing on the control plot (Table S2; Figures 4B, 5B and 6B). When fertiliser application ceased (in 2012), the annual growth values began to increase at a higher rate than on the control plot ($p = 0.0741$), (Figures 5B and 6B and Supplementary material Figures S4 and S5).

3.2. Climate–Growth Relationships

In accordance with the assumptions adopted, the climate–growth relationships in the pines growing on the plots under study were determined for two time periods: 1951–1984 and 1985–2020. In the former period, the impact of climatic factors on growth in terms of tree-ring width and earlywood and latewood widths was similar in trend for all plots, the differences between the plot relating to the strength of linkages (Figures 8 and 9). The growth responses of all the trees under study were largely linked to temperature conditions in late winter and early spring (February and March). The above-average air temperatures in these months contributed to the formation of wide annual rings (TRW, EW, LW) in the coming growing season. The younger trees showed stronger linkages between growth and temperature conditions in February and March, whereas in the case of the older trees, negative relationships were also found between TRW and air temperature in May of the year of ring formation. The influence of the previous year's temperatures was smaller, and the relationships were distributed depending on the study plot involved. A further factor determining the formation of wide annual rings was the supply of water in the growing season. Precipitation in May and July (and also in February in the case of the younger trees) was found to have a significant positive impact on the amount of growth. The width of earlywood showed a significant positive linkage to precipitation in May, while the width of latewood was similarly linked to precipitation in winter (February), spring (May), and summer (July and August). The climate–growth relationships in the latter period (1985–2020) were different in the plots subjected to irrigation and fertiliser application and in the control plots.

On the irrigated plots, the most important climatic factors determining growth in that period were temperature conditions in winter and early spring (December to March), with no significant impact of precipitation. On the control plots, the influence of temperature conditions on annual growth was limited, with a significant (negative) linkage only found for July. Stronger linkages were found for earlywood (positive effect of temperature in March) and latewood (positive effect of temperature in June). In contrast to the pines growing on the plots where fertiliser was applied, the growth response of the pines growing on the control plots was strongly linked to precipitation in May (TRW, EW, and LW), July (TRW, and LW) and February (TRW, and LW).

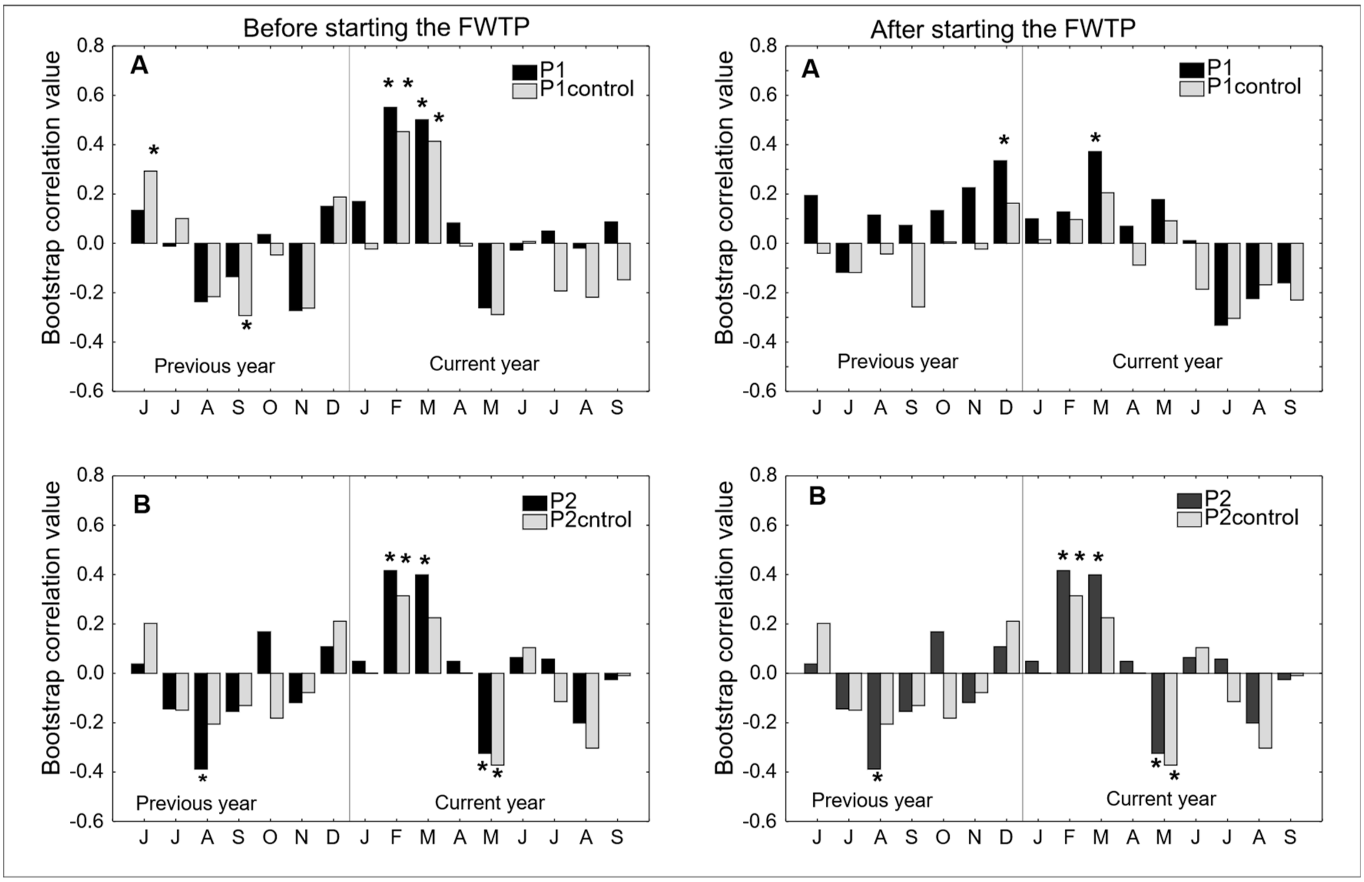

Figure 8. Relationships between air temperature and radial growth of Scots pine growing on the study plots (residual chronologies) for the period before fertiliser application and the period after the starting operation of the Forest Wastewater Treatment Plant. (**A**)—plots with younger trees; (**B**)—plots with older trees. Asterisks (*) indicate significance at $p < 0.05$.

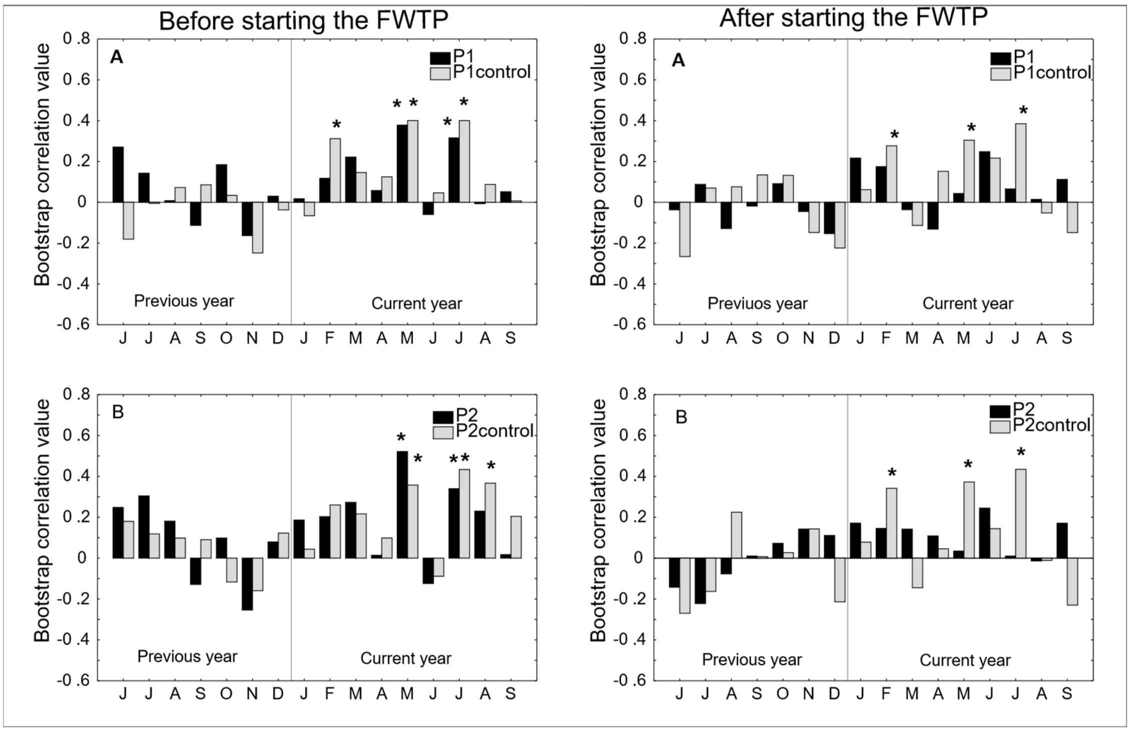

Figure 9. Relationships between precipitation and radial growth (TRW) of Scots pine growing on the study plots (residual chronologies) for the period before fertiliser application and the period after starting operation of the Forest Wastewater Treatment Plant). (**A**)—plots with younger trees; (**B**)—plots with older trees. Asterisks (*) indicate significance at $p < 0.05$.

4. Discussion

In forests growing on nutrient-poor soils, various types of fertiliser are often applied in order to increase biomass production [56–60]. Where fertiliser application is performed on a regular basis and under controlled conditions, both positive [30,31,56,57] and negative [53] impacts on growth in conifers have been found. For example, Scots pines growing in nutrient-poor or moderately fertile habitats in South-Eastern Norway have been shown to respond to improved nutritional conditions with increased tree-ring widths as early as in the first year following the application of nitrogenous fertiliser, reaching a maximum after three or four years, with a stimulatory effect also visible after each subsequent application [31,57]. The additional radial growth due to fertiliser application in mature Scots pines reached 25%–30% within 10 years [57] and exceeded 50% within 20 years [59]. In contrast to the above, however, research by Seftigen et al. [33] showed a significant negative effect on pine growth due to the use of fertilisers over an extended period of time (16 years).

Poland's experiment with the FWTP sought to determine whether biogenic treatment of wastewater could provide similar benefits as the use of fertilisers in terms of contributing to an increase in tree biomass and improving the productivity of forest stands [17].

Our study found that the application of potato starch wastewater combined with additional irrigation in the growing season had a significant impact on secondary growth in the Scots pines subjected to such treatment, with the changes in growth being bidirectional. The fact that nutrients were supplied with the wastewater caused an initial rapid increase in growth, which was then followed by a strong reduction in the next period (Figures 5 and 6). This pattern of changes indicates that long-term fertiliser application—and thus the supply of an additional pool of nutrients to the soil—results in severe over-fertilisation with one of the nutrient components. Indeed, earlier studies conducted at the FWTP demonstrated that the systematic introduction of wastewater to the forest environment resulted in over-fertilisation of the soil and contamination of groundwater, in particular with toxic nitrates [23,24]. On average, at a dose of 300 mm per year, 560 kg of nitrogen and 680 kg of potassium were supplied with the wastewater to each hectare of the stand (with values ranging from 176 to 1325 kg of nitrogen and 250 to 1846 kg of potassium depending on the year). Such cumulative doses are in fact much larger than those applied with typical fertilisation practices used in forest plantations, where—with a single application once every few years—the doses of nitrogen range from 150 to 200 kg ha^{-1}, and the doses of potassium range from 40 to 200 kg ha^{-1} [57,61]. Likewise, the study conducted by Seftigen et al. [33] involved nitrogenous compounds being distributed on a weekly or biweekly basis via a sprinkler system at an annual dose of 40 kg ha^{-1}. Hence, it is relatively difficult to compare our results concerning the growth responses of the pines located at the FWTP site with research on the growth responses of trees subjected to the controlled application of fertiliser in much smaller doses.

The directional changes in the secondary growth of the pines under study as determined by our research proved consistent with the findings of previous studies conducted at the FWTP site [28]. However, in comparison with the previous study, our research extends the existing knowledge regarding the impact of potato starch wastewater on growth responses in Scots pines.

Firstly, we found that the age of the trees mainly affected the timing of the stimulatory effect, with younger trees responding in the first year following the fertiliser application and older trees only responding after a time lag of two or three years (Figures 5, 6 and 10). This is in fact consistent with the observations made as part of the Swedish research [29,62]. According to our research, the duration and scale of the growth stimulus were similar in both age groups of trees, with a mean duration of approximately 10 years and an increase in growth of around 30% in comparison with the control plots. This is comparable with the experiences of the Scandinavian countries [57]; therefore, it can be assumed that in the early years of the experiment, the potato starch wastewater in fact caused nutrient enrichment of the forest environment, as reflected in the growth responses of the trees.

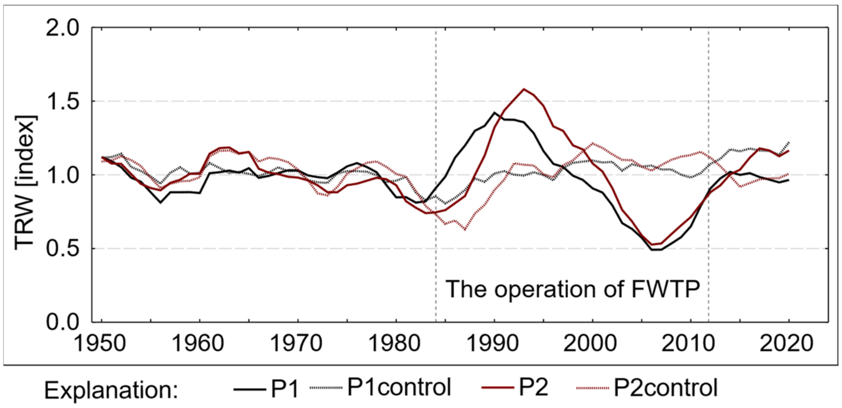

Figure 10. Growth responses of the Scots pines growing at the Forest Wastewater Treatment Plant. The figure shows standardised chronologies after the application of a 5 year filter.

Secondly, we were able to determine the extent of the decline in tree growth that resulted from excessive fertiliser application. In previous studies on the impact of fertilisation on the growth of tree stands, the reduction in growth was not quantified. While the character and mean extent of the reduction in growth were similar in both stands under study, the reduction began at an earlier point in time and lasted longer in the younger stand (Figure 10). The onset of the reduction in growth coincided with the years in which the applicable limits of the concentration of nitrates in the soil and groundwater were exceeded to a marked degree [23]. According to Ciepielowski et al. [20], from the mid-1990s onwards, there was a major increase in the number of snags (standing dead trees) created in the pine stands under study (with an average of 20 trees per unit per year as compared with around 4 trees per unit per year in the buffer zone). As the increase in the creation of snags coincided with the onset of the reduction in growth, the utility of secondary growth as a bioindicator appears to have been confirmed. Furthermore, the reduction also coincided with a period of a complete retreat of native vegetation in the undergrowth [27]. It is therefore our view that the marked reduction in annual ring widths in the pines growing at the FWTP site can be compared to the growth response of trees growing near nitrogen factories and exposed to high deposition levels of nitrogenous compounds [63]. However, it is important to note that the extent of the stimulatory or inhibitory effect of potato starch wastewater on growth—as presented in our study—was not determined in previous research.

Thirdly, we preliminarily identified the changes in the structure of wood that occur as a result of sprinkler application of potato starch wastewater. We found fertiliser application caused the share of earlywood in the annual ring width to increase by an average of 6% in comparison with the control plots. A similar observation concerning fertiliser use in loblolly pine (*Pinus taeda*) was made by Antony et al. [64]. Furthermore, in our study, the increased share of earlywood could be observed in periods of both increased and reduced growth and was more pronounced in the younger trees.

In the period prior to the commencement of nutrient enrichment, all the trees under study exhibited similar growth patterns, whereas in the period during which the fertiliser was being applied—and in particular in the phase of reduced growth—a high degree of heterogeneity in growth behaviour could be observed (Figure S4). Reduced coherence of tree-ring chronologies during fertiliser application was also noted by Koprowski et al. [28], while Szychowska-Krąpiec [65] pointed out that heterogeneity of trees' growth responses increases as a result of strong exposure to industrial pollution. However, we believe that in the case of the trees covered by our study, the divergence of growth responses between stands could also have been influenced by the disparate water balance of the plots to which the wastewater was applied. During the first 10 years, in addition to precipitation at an annual average rate of 550 mm, including 350 mm in the growing season, the fertilised stands also received an average of approximately 300 mm of water in total and 230 mm in the growing season. In consequence, the total input during the growing season was 880 mm (which is 2.5 times the amount received by the control stands in the buffer zone). After that period, the reduction and subsequent discontinuation of irrigation caused another disturbance in the water balance and, at the same time, resulted in a significant increase in the concentration of toxic substances in both the soil and the groundwater (Figure 11).

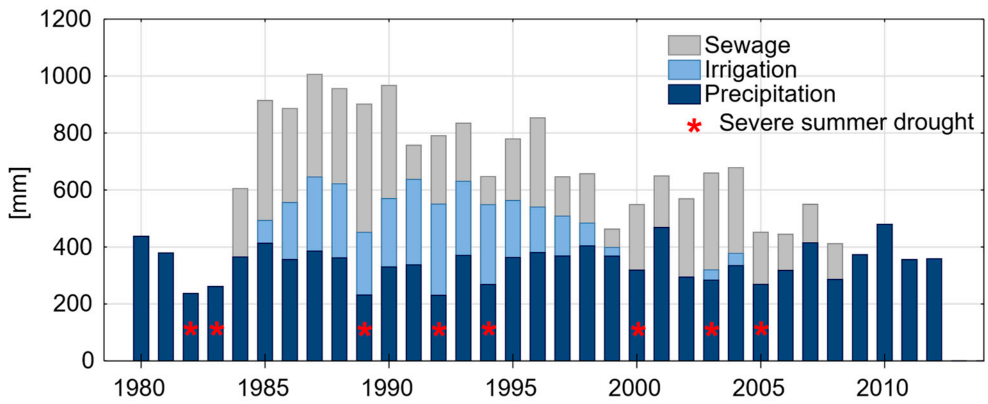

Figure 11. The amounts of water supplied to the stands at the Forest Wastewater Treatment Plant during the growing season in the period 1984–2010. Data on sewage and irrigation were obtained from the Iława Forest District. The years in which droughts occurred in the Iława Forest District are marked.

The results confirmed our initial assumption that the sprinkler application of potato starch wastewater could have influenced the dendroclimatic responses of Scots pines subjected to this type of treatment. In the period prior to the application, all the trees under study showed similar sensitivity to climatic factors (Figures 8 and 9). Specifically, temperature conditions in late winter and early spring (February and March) and heavy precipitation in the growing season (May to July) had a positive effect on the formation of secondary xylem cells. The relationships we identified are further confirmed by other dendroclimatic studies involving pine, with the dominant role of late-winter and early-spring temperatures in the development of annual rings having been noted by a number of authors including [45,66–68]. Warm temperatures at the end of winter and the beginning of spring trigger physiological processes that lead to earlier and faster cambial division [69]. The significant impact of precipitation in the growing season (May to July) has also been confirmed by dendrochronological studies involving pines growing in Poland [45,66,67].

Sprinkler application of wastewater and irrigation caused a divergence in dendroclimatic responses between the trees growing within the FWTP site and those in the buffer zone. During the period in question, the latter showed—above all—high sensitivity to precipitation in the growing season. In fact, Poland experienced a number of severe summer droughts in 1985–2012, which were reflected in the growth responses of the trees and in the high value of the sensitivity indicator (Table S2). Such relationships, however, were not noted among the pines growing within the FWTP site, which in our view is mainly due to the different water balance that resulted from the introduction of large amounts of additional water to the site in the growing season. In the drought years mentioned above, the amount of irrigation water was equal to or greater than the precipitation totals over the entire growing season (Figure 11). These relationships are particularly evident in the period of increased growth. The relationships concerning the trees' sensitivity to air temperature are also different. The trees growing in the buffer zone (on control plots) did not show significant sensitivity to temperatures in winter and spring from the late 1980s onwards, which can be attributed to climate change and the statistically significant increase in air temperature in those months. Similar observations concerning the decreasing impact of temperature conditions in winter on radial growth in pine have also been made by Misi et al. [70]. Conversely, the trees growing within the FWTP site still showed high sensitivity to temperature conditions in winter and early spring (December to March). The causes of this heightened sensitivity probably lie in the pines' physiological condition, which represents a promising area for further research. A study by Dyguś [27] revealed that in a forest habitat fertilised with wastewater, the concentrations of most elements (N, P, K, Na, and Cl) in the affected plants were greater than those found in the tissues of non-fertilised (control) plants. Furthermore, according to Oleksyn [71], industrial pollution also significantly reduces the resistance of pine trees to low air temperatures.

The picture we have obtained in our study as regards the relationship between climate and secondary growth in pine stands fertilised with wastewater is only partially consistent with earlier findings [28]. The differences concern, in particular, the relationship between precipitation and growth. This may potentially stem from the disparate timeframes of the analyses: in the study cited above, the climate–growth relationships were determined for the entire 1951–2012 period, with no distinction drawn between the time prior to and during the application of the nutrients, and thus with no account taken of the changes in the water regime over time. We believe, however, that the timeframe adopted in our study permits a more accurate determination of the impact of sprinkler application of potato starch wastewater on the dendroclimatic relationship.

5. Conclusions

Our study offers a broad overview of the impact of sprinkler application of potato starch wastewater on growth response in Scots pines. By analysing changes in annual ring widths and earlywood and latewood widths, we have been able to demonstrate a two-way impact of this type of wastewater on secondary growth in the trees under study. The initial (almost decade-long) upward trend in radial growth came to an abrupt halt, giving way to a strong reduction in growth that persisted for more than ten years. The trends of these changes could be seen in both the overall annual ring widths and the widths of earlywood and latewood. Sprinkler application of potato starch wastewater caused changes in wood anatomy that manifested themselves in the increased share of earlywood in TRW. The direction of the changes was the same for trees of different ages, although age was found to have affected the extent and duration of the stimulatory or inhibitory effect. Specifically, younger trees displayed a stronger and more prolonged negative growth response. The sprinkler application of potato starch wastewater and the accompanying irrigation caused a shift in dendroclimatic relationships in comparison to the control plots. Surface irrigation and the resulting changes in water balance reduced the drought susceptibility of the pines under study. At the same time, however, trees weakened by the excessive concentration of toxic nitrates became more sensitive to temperature conditions in winter.

The record of the growth responses in the stands subjected to sprinkler fertilisation with potato starch wastewater confirms that the introduction of substances containing significant amounts of organic nitrogen into forest ecosystems may impair the vigour of trees, reduce stand productivity, and cause an imbalance in the ecosystem, and consequently entail the need for forest regeneration.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f13101575/s1, Figure S1: Layout diagram of pipelines in the area of the Forest Wastewater Treatment Plant [FWTP], (source: Iława Forest District). Figure S2: The local tree crown map for the Forest Wastewater Treatment Plant and adjacent forest areas (current condition, research plots are marked with circles, the density of pixels reflects the density of the stand (source: https://mytreemap.com/, accessed on 15 June 2022). Figure S3: Photos of study plots (May 2020, all photos J. Lendzion, the arrow marks the traces of the pipeline). Figure S4: Comparison of raw chronologies of Scots pine from study plots. The vertical lines indicate the period of operation of the Forest Wastewater Treatment Plant. Figure S5: Mean tree-ring width [mm] of the study pines in the different tree growth periods: a—25 years before the start of FWTP activity; b—the first 11 years of the FWTP operation; c—next years of the FWTP operation; d—years after the FWTP was closed. Table S1: Description of study plots. Table S2: The basic dendrochronological statistics of tree-ring width chronologies of Scots pine for the different research periods.

Author Contributions: Conceptualization, L.C.-O. and J.L.; methodology, L.C.-O.; software, L.C.-O. and J.L.; validation, L.C.-O. and J.L.; formal analysis, L.C.-O. and W.O.; investigation, L.C.-O. and J.L; writing—original draft preparation, L.C.-O.; writing—review and editing, L.C.-O. and W.O.; visualization, L.C.-O.; supervision, L.C.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The detailed data supporting reported results are available upon request to the corresponding author.

Acknowledgments: The authors thank Tymoteusz Kuczyński for his English editing. We acknowledge the Iława Forest District's assistance during the obtaining of archival material and collecting of research material. We also thank the anonymous reviewers for their valuable comments and suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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