



Article A Tree Ring Proxy Evaluation of Declining Causes in Pinus sylvestris L. and Pinus nigra J.F. Arnold in Northeastern Romania

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Abstract: Drought-induced dieback has been extensively studied in various forests habitats. We used a retrospective tree ring width (TRW), basal area increment (BAI), oxygen isotope ratios in tree ring cellulose ($\delta^{18}O_{TR}$) and carbon isotope ratios in tree ring cellulose ($\delta^{13}C_{TR}$) to assess causes in declining Pinus sylvestris L. and Pinus nigra J.F. Arnold. The climate data analysis indicates a significant increased trend occurred after 1980 in minimum, mean and maximum temperature and a reduced amount of precipitation compared to the 1920–1980-time scale. According to the Palmer Drought Severity Index, we found two extreme drought years (1946 and 2000) and three years with severe drought (1990, 2003 and 2012). One-way ANOVA indicated no significant difference between P. nigra and P sylvestris tree ring width, basal area increment, but a considerable difference between $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$. Basal area increment evaluated the climate-growth relationship most accurately, comparing to $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$, which explained the influences of environmental factors in tree rings formation. The $\delta^{13}C_{TR}$ was mainly negatively correlated with high temperatures from April-August current growing seasons. The negative correlation between $\delta^{13}C_{TR}$ and NDVI indices (June, August) shows a decreased carbon uptake induced by drought from summer to early autumn. The low $\delta^{18}O_{TR}$ signal was associated with a complex of factors, including the strong influence of heavy precipitation occurring in the growing season and a weak reaction of declined trees to resources. Species-specific responses to drought in 1990, 2003 and 2012 indicated P. sylvestris as more sensitive to drought whit higher demand for water supply in the optimal compared with P. nigra. Weak and unstable correlations in time with increasing/decreasing values in drought periods were obtained more accurately using $\delta^{18}O_{TR}$ compared to $\delta^{13}C_{TR}$. The species-specific resilience response to drought years showed a weak resilience and resistance in *P. sylvestris* occurred more evident after the 2012 event compared to less sensitive P. nigra trees. Decision-makers can use presented results to reinforce specific management plans capable of protecting and changing local compositions where is the case with species more resistant to drouth.

Keywords: northeastern Romania; oxygen isotopes; carbon isotopes; climate change; resilience components

1. Introduction

In the last century, climate change in Europe raised faster than in other areas influencing plant species growth and response [1]. The recent heatwaves episodes revealed a new face of climate change's impact on terrestrial ecosystems that will not be extreme



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). in the future due to the high frequency of occurrence [2,3]. The extra-Carpathian region, especially sites from northeastern Romania, displays higher increasing temperatures in winter and spring and reduces solid rainfall in winter [4]. In contrast, a shift of maximum precipitation from spring to autumn in the east and west Carpathian Chain was observed, expanding the days without rain, mainly in the south and west region during the spring [5,6]. Climate influenced tree ring growth and response, observing divergent trends in various tree species for the last decades [7,8]. Thus, synchronous growth decline in spruce affected forest ecosystems after 2000 in the whole Carpathian Chain [9,10]. Climate increased sensitivity to drought in successional stages in natural Picea abies L. Karst., a forest located in Călimani Mountains (northern Romania), authors argued a shift in tree ring response to water availability from early spring to winter [11]. To claim that process is complex in broad areas, large-scale investigations demonstrated that warming and drying concurrence is irreversible in many ecosystems, as shown in a study for East Asia [12]. Nowadays, heatwaves more frequently reduce growth vigor, and recovery time diminishes, inducing more vulnerability to drought events at a global scale, which is the leading factor of decoupling stress memory after several extreme events followed by die-off [13].

The severe impact of 2000 and 2012 April–August high temperature cumulated with low precipitation was essential for *Pinus* sp. decline below 1000 m altitude in Romania [14]. In Northern Romania, stable carbon isotopes in cellulose and tree rings indicated that April-May relative humidity and regional summer precipitation were vital in *Pinus cembra* L. growth at high altitudes [15]. Furthermore, oxygen isotope ratios in tree ring cellulose $(\delta^{18}O_{TR})$ illustrated specific inferences with North Atlantic Oscillation and Mediterranean Oscillation temperature in the high-altitude Carpathians ecosystems [15]. The variability of multidecadal oscillation on summer east-central Europe temperature interacts with geophysical action of the Carpathian Chain in separating the climatic influences from north, east, and west [16,17]. Even maximum latewood density and blue intensity proxies indicate the divergent tree ring response to climate for *Pinus* sp. located on the eastern Carpathians [18]. Geophysical barriers and accelerating specific drought action change signals regardless of altitude, e.g., dieback in extra-Carpathians [19] and increasing forest disturbance at high altitude [20]. Even if various studies investigated *Pinus* sp. in Romania, a "black box" consists in using stable isotopes for understanding the response of planted tree ring patterns. Between 1950–1990, anthropic and managed stands was created nearby small towns with recreational and soil erosion stabilization all over the country. These ecosystems are subjected to a decline of human-induced stress, grazing, climate, biotic or abiotic factors. Investigating such stands can offer reliable knowledge regarding resilience in young, highly stressed trees and the future evolution of forests in nearby human communities.

Nowadays, various studies demonstrated strong species-specific sensitivity to environmental drivers evaluated through tree ring stable isotopes [21,22]. The tree rings stable carbon isotopes are widely used to retrospect trees response to climate change due to carbon dioxide increase. The carbon isotope ratios in tree ring cellulose ($\delta^{13}C_{TR}$) reflects the balance between stomatal conductance and photosynthetic rate [23]. Plants exposed to drought stress reduce stomatal conductance to overhead hydraulic failure, which translates to liming the photosynthetic and carbon assimilation rate, further increasing $\delta^{13}C_{TR}$ [24]. The literature indicated a direct link between δ^{13} C and intrinsic water-use efficiency (iWUE), even if the relationship between this parameter and environmental disruption anthropically induced and trees dieback are poorly understood [25]. The stable oxygen isotopes are related to gas-exchange processes at the leaf level, reflecting changes in vapour pressure deficit, stomatal conductance and transpiration [26,27]. Inferences of atmospheric circulation patterns, soil evapotranspiration, seasonality of climatic conditions and back-diffusion of atmospheric water vapour are unseparated in $\delta^{13}C_{TR}$, representing a challenge for future experiments [28]. Combining $\delta^{13}C_{TR}$ and $\delta^{13}O_{TR}$ can respond to the complex process of gas exchange related to tree ring growth variation using the theoretical framework principles of the conceptual dual-isotope model suggested by Scheidegger et al. [29].

Here we investigate the resilience and response of the relationship between tree ring width, basal area increment, oxygen and carbon isotope in tree ring cellulose and climate over the period 1964–2018 for *Pinus sylvestris* L. and *Pinus nigra* J.F. Arnold in a planted stand growing in northeast Romania. Our specific objectives were (i) to evaluate the species-specific response to climate changes in the last decades using different tree ring proxies; (ii) to understand how the drought years 1990, 2003 and 2012 impacted the tree ring performance; (iii) to observe the trends in temporal stability of correlation coefficients between tree ring proxies and environmental variables, and (iv) to assess the species-specific resilience response to drought events over the studied period.

2. Materials and Methods

2.1. Study Site

The investigated tree stand is located in a nearby small locality (5400 inhabitants) in northeastern Romania (47.55 N; 25.66 E; 650 m altitude). The forest was planted around 1960 for soil erosion stabilization with P. nigra and P. sylvestris. After the 1990s, the anthropic pressure-induced through illegal cutting was the main factor in destroying the old forest. Stand progressively changed with a mixed combination of Pinus sp. and Picea *abies* L. Characterized by a strongly continental climate, the sampling sites are exposed to late freeze in spring and the last decades to drought in summer. The nearest European Climate Assessment & Dataset (ECA&D) data grid (E-OBS v23.1e) covering the period between 1920 to present [30] shows multiannual total precipitation of 627.73 mm/year, with 74.42% of occurrence during vegetation season (March–September) and 25.57% in the winter season (October-February). In June-July, we observed monthly maximum precipitation (99.32 and 92.10 mm/month) and minimum in January-February (28.29 and 27.94 mm/month). The extreme mean annual rainfall was noted in 1934 (409.20 mm/year) and 1955 (948.60 mm/year). The coldest and warmest months are January $(-5.86 \,^{\circ}\text{C})$ and July (17.08 °C). According to the climate dataset, the mean annual temperature varied between a minimum value of 4.30 °C (1980) and a maximum of 8.04 °C (1936).

2.2. Tree Ring Data and Climate

Sampling procedures were accomplished in 2020 when core samples of 31 Pinus nigra and 19 Pinus sylvestris were collected for dendrochronological investigations. Sampled trees were randomly selected, and cores were extracted at breast height (1.3 m) using Pressler 4.3 mm increment borer. Tree ring samples were prepared according to dendrochronological procedures [31]. Samples were scanned at 2400 dpi with Epson Expression 12000XL flatbed scanner calibrated using an IT8 calibration target coupled with SilverFast 8.0 software (LaserSoft Imaging, Inc., Kiel, Germany). Tree rings boundaries were established to the nearest 0.001 mm using Cdendro and CooRecorder image analysis software by CybisTM [32]. Ring widths were screened for missing rings and dating errors using COFECHA software [33]. Based on annual tree rings width (TRW) was constructed basal area increment (BAI) time series using R software version 4.0.2 [34] and dplR package [35]. Tree ring time series were standardized using a spline with a 0.50 frequency response cutoff at 67% of the series length. The individual residual series (RES) were averaged using Turkey's bi-weight robust mean. We tested the chronologies for accuracy with mean inter-series correlation (Rbar), expressed population Signal (EPS), and Gleichläufigkeit (GLK, sign test) agreement parameter based on sigh test for evaluating coherence among series. The mean, standard deviation, standard error of the mean, variance, minimum, maximum, 1st quartile, 3rd quartile, interquartile range and maximum statistics were computed using the direct weight method, the sum of weights degrees of freedom variance divisor moment and Turkey Hinges interpolation of quartiles statistics.

Cellulose oxygen and carbon isotope ratios in tree ring cellulose were based on a pooled-ring approach with three trees, even if other studies support the pooling of the rings of more than four trees [26]. We sustain our practice on the occurrence of little tree growth variability between species assessed through differences of mean and variance tests. We

expect to understand under which climatic conditions trees are subjected to vulnerability. Thus, three cores for each species were randomly selected for future $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$ analysis, considering clear detectable tree-ring boundaries avoiding missing or narrow rings to obtain a sufficient amount of cellulose. The cores were collected using a Pressler 12 mm increment borer. Individual rings of each core sample were separated using a razor blade under a binocular microscope (Leica MZ9.5 stereo-zoom microscope). Each tree ring was stored in a 2 mL Eppendorf vial as one sample base for α -cellulose extraction, measured according to the modified Jayme-Wise method [36]. The procedure uses sodium chlorite and sodium hydroxide, homogenized according to the standard ultrasonic protocol [37], dried at 60 °C for 24 h after which, the α -cellulose was weighed to determine the yield (a proper extraction with nearly 30% ($\pm 10\%$) α -cellulose yields). We used the International Atomic Energy Agency standards (IAEA-CH-3), Iso-Analytical Limited (IA-R001 and IA-R005) for δ^{13} C analysis, respectively, the International Atomic Energy Agency standards (IAEA-601, IAEA-CH-3 and USGS-32) for δ^{18} O measurements. The analytical precision of the measurements was $\pm 0.3\%$ for oxygen, respectively $\pm 0.2\%$ for carbon. The $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$ were measured by combustion/pyrolysis of α -cellulose to CO₂ and CO, using a Thermo Flash2000 elemental analyzer interfaced with a Thermo Delta V Plus IRMS (Thermo Scientific, Bremen, Germany). Three replicates were measured for both $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$. The isotope ratio values were reported using the δ notation in per mil units (‰) concerning the stable international oxygen and carbon isotope standards Vienna Standard Mean Ocean Water (VSMOW) and Vienna Pee Dee Belemnite (VPDB), respectively.

2.3. Climate Sensitivities and Extreme Drought Events

Bootstrap response correlation analysis of the Dendroclim 2002 program was used to investigate tree growth-climate relationship analysis [38]. We determine climatic influences on tree growth by quantifying correlation analysis between tree ring width, basal area increment, oxygen and carbon isotope ratios in tree ring cellulose and environmental data. Monthly mean self-calibrating Palmer Drought Severity Index (scPDSI) and Standardized Precipitation-Evapotranspiration Index cumulated for 1 and 3 months (SPEI) were extracted from the Climatic Research Unit database (CRU TS 4.04) [39], and climate data from E-OBS v23.1e grid dataset with a resolution differentiated for each parameter detailed in Table 1. Usually, negative and positive index of SPEI and scPDSI indicates the dry and wet condition. Here was selected scPDSI, SPEI1 and SPEI3 to evaluate dry/wet conditions and obtained annual values by averaging monthly values with a specific year. The climate data were investigated for trends using the Mann-Kendall trend test, and extreme values were considered those that exceeded two standard deviations.

Table 1. The characteristics of investigated environmental parameters.

| Parameter | Units | Resolution | Length |
|--|--------------------------|----------------|-----------|
| Precipitation (rr) | mm/day | 0.25° | 1920-2020 |
| Mean temperature (tg) | °C | 0.25° | 1920-2020 |
| Minimum temperature (tn) | °C | 0.25° | 1920-2020 |
| Maximum temperature (tx) | °C | 0.25° | 1920-2020 |
| Evapotranspiration (ET) | mm/day | 1° | 1979-2016 |
| Potential evapotranspiration (ETp) | mm/day | 1° | 1979-2016 |
| Atmospheric rain (RAIN) | mm/day | 1° | 1979-2016 |
| Self-calibrating Palmer Drought Severity Index (scPDSI) | indices | 0.5° | 1901–2020 |
| Soil liquid water 0–1 m (SOILLIQ 0–1 m) | kg/m ² | 1° | 1979-2016 |
| Soil liquid water 0–10 cm (SOILLIQ 0–10 cm) | kg/m^2 | 1° | 1979-2016 |
| Soil moisture content (SoilMoi00_10cm_tavg) | $m^{3} m^{-3}$ | 0.1° | 1982-2018 |
| Soil moisture content (SoilMoi00_40cm_tavg) | ${ m m}^{3}~{ m m}^{-3}$ | 0.1° | 1982-2018 |
| Standardized Precipitation-Evapotranspiration Index (SPEI1) | z-values | 0.5° | 1901–2018 |
| Standardized Precipitation-Evapotranspiration Index (SPEI3) | z-values | 0.5° | 1901–2018 |
| NOAA Climate Data Record of Normalized Difference Vegetation Index (NDVI) | indices | 0.1° | 1981–2019 |

Correlation coefficients from August of the prior growth year to September current growing season were calculated for the periods defined by dataset length, according to Table 1, with similar time-interval for both species. A moving correlation analysis between the tree ring proxies and monthly climate variables was performed in two steps. First, we calculated correlation coefficients between August of the prior growth year to September current growing season, and we found consistent values only from March to July. The second step was conducted to reduce the baselenght of the window to 10 years, and the interval investigated ranged from March to July current growing season.

To identify the years with significant growth reduction, we evaluated the relative change of growth for each tree and year by comparing the previous five years using PointRes package in R [40]. We used the resilience indices [41] to evaluate the growth response to intense drought episodes. Tree resistance (Rt) consists of growth reduction during disturbance relative to pre-disturbance and the time expressed in a number of years needed to rich minimum growth value since drought event reflects the lag of the drought. The recovery (Rc) time demonstrates the number of years required for trees to return from the pre-disturbance growth level. Resilience (Rs) is a measure of estimating the difference in tree growth before and after the stress event year. We used a 5-year pre- and post-disturbance sliding window in each sample to identify growth release using PointRES package in R [40]. Linear trend in cellulose $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$ datasets was eliminated, and the years with more than two standard deviations of the series were defined as indicators of extreme droughts.

3. Results and Discussions

3.1. Climate Data

The site investigated is located in the optimum of species growth, and our analysis portrays the climate trend for an extended period of 1920–2020 to assess eventual climatic changes before 1960. We compared different time intervals to understand if the climate has been affected by changes in the last decade. Thus, the mean annual mean temperature calculated for 1920–2020 defined as mean and standard deviation were (tg = 6.13 ± 0.88 °C) was relatively higher compared with 1960–2020 (tg = 5.90 ± 0.85 °C). The mean annual maximum temperature in change slight increase in 1960–2020 (tx = 10.84 ± 1.10 °C) referred to 1920–2020 (tx = 10.63 \pm 1.06 °C). The statistical parameters show notable differences between mean and maximum temperature only in the case of variance ($\sigma^2 = 0.77/1.13$) and an interquartile range (Q3-Q1 = 1.30/1.24) (Figure 1c). The trend analysis indicates no trend in tg dataset for the interval 1920–2020 (Kendall's tau = -0.05, *p*-value two-tailed = 0.47). Still, after 1950 and even more, pronounced between 1980–2020, we observed a significant positive trend (Kendall's tau = 0.26/0.55, *p*-value two-tailed = 0.001/0.0001). For maximum temperature, the trend analysis shows higher values than the mean temperature for intervals 1950–2020 and 1980–2020 (Kendall's tau = 0.38/0.58, *p*-value two-tailed < 0.0001). The basic statistics (mean, standard deviation, variance and interquartile range) indicated lowest values in annual minimum temperature (1.74, 0.83, 0.69, 1.02) and a slow increasing trend since 1920 (Kendall's tau = 0.14, *p*-value two-tailed = 0.03) (Figure 1b). Statistical analysis applied on 1950–2020 and 1980–2020 datasets demonstrated incremental trends (Kendall's tau = 0.38/0.55, *p*-value two-tailed < 0.0001/0.001).

Similar shifts in climate conditions were previously discussed in Romanian literature [42–44]. The mean annual values revealed a significant increasing trend for temperature and negative for precipitation registered after the 1980s, which resulted in extreme negative anomaly recorded in the drought years 1990, 2000, 2003, 2007 or 2012 [45]. Our results are consistent with previous worldwide reports from the United States [46], Europe [47], China [48]. However, extreme indices' changes in intensity and magnitude are not similar. The explanation can be found in the different local geographical patterns of intramountain region sensitivity compared to global warming. The time-series data analyzed in the present study indicated no significant trend in the precipitation regime (Figure 1a). Even so, the January–June (JFMAMJ) and July–December (JASOND) sum of precipitation for the intervals 1920–1949, 1950–1979, 1980–2020 indicated a reducing amount expressed in mm/month (JFMAMJ = 308.96/347.16/285.78, and JASOND = 316.10/342.19/283.95). The precipitation regime in the Carpathian region shows divergent trends following a geographical influence, in appearance is more robust in the areas more exposed to increasing maximum temperature [49]. Other authors observed an increase in the frequency of extreme events regulated by the elevation, lower at high altitudes than in the plain [50], frequently intercorrelated with the atmospheric circulation pattern [51].



Figure 1. The climate regime in the studied area is based on CRU TS 4.04 and E-OBS v23.1e grid datasets. (a) annual total precipitation, (b) average annual minimum temperature, (c) average annual maximum temperature, (d) average annual mean temperature, (e) average scPDSI, SPEI1 and SPEI3 indices.

Frequently discussed in the literature are drought-triggered decline in forest growth and productivity [52], climatic events responsible for flash floods [53,54], or disfunction between physiological and functional processes at various levels in the ecosystems [55]. Severe drought caused lagged effects on trees physiology with post-drought dysfunction of carbon assimilation associated with die-off events [56]. Based on the Palmer Drought Severity Index classification, the values between -3.0 and -3.99 are interpreted as severe drought and less than -4.00 extreme drought. In the present study, two values were found as extreme drought in 1946 (-5.12) and 2020 (-4.01). Between 1980–2020 severe drought was noted in 1990 (-3.60), 2003 (-3.36) and 2012 (-3.75) (Figure 1e). According to McKee et al. [57] and Paulo et al. [58], no extreme and severe drought-event were found, only moderate according to SPEI3 in 1946 (-1.21) and 1990 (-1.16). After the 2003 drought, the extreme events became more frequent, and water stress was caused by limited subsurface water supplies that affected forest ecosystems worldwide [59]. Due to early exposure, extensive forest die-offs were intensively studied and monitored to understand the causes of drought-induced changes in plant hydraulic functioning as a principal cause of mortality [60]. Discussing the comparison between SPEI and scPDSI indices shows different drought results explained through different physical mechanisms of each index in evaluating drought conditions. Thus, the scPDSI indices are more relevant to evaluating drought duration and drought intensity based on the supply-and-demand concept of the water balance equation [61]. A comparison between SPEI indices that include impacts of evapotranspiration on drought conditions and scPDSI showed that water deficit/surplus conditions are reflected more reasonably using the Palmer Drought Severity Index [62].

3.2. Tree Ring and $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$

The Pinus nigra J.F. Arnold tree ring chronology was formed from 31 individual time series spanning between 1964–2020, with an average mean sensitivity of 0.26 and a mean length of 52.2 years. The mean tree ring was 3.35 ± 0.77 mm/year ranging between 1.96 and 5.37 mm/year (Figure 2a). After the standardization, the mean rbar and EPS values were 0.23 and 0.88. The Pinus sylvestris L. tree ring chronology consists of 19 individual time series ranging between 1961–2020. The average mean sensitivity was 0.25, and the mean length of the series of 51.3 years. The arithmetic mean of tree ring width were 3.40 ± 0.97 mm/year (2.09–6.43 mm/year), for residual chronology was calculated a mean rbar and EPS of 0.24 and 0.86. Tree rings statistics show the sustained growth of trees investigated compared with managed stands from south Romania [63] and comparable results with *Pinus* sp. growing in the lower limit of the optimum [14]. The mean sensitivity oscillates with age, even the mean correlation between individual tree ring chronologies [64]. The previous studies indicated that tree rings statistics in the comparable environment are uncertainly influenced by climate variability in the case of younger trees [65]. The one-way ANOVA using Bonferroni means comparison and Levene's test for the equal variance for p < 0.001 indicate no significant difference between *P. nigra* and *P sylvestris* chronologies (*F*-value = 0.28, p = 0.59 and *t*-value = 0.25, p = 0.79). The Gleichläufigkeit value measures high-frequency coherence between tree ring data [66] and, in our case, shows standard statistically significant signals between mean P. nigra and P. sylvestris chronologies (GLK = 79%).



Figure 2. The growth trend for *Pinus sylvestris* L. and *Pinus nigra* J.F. Arnold. (**a**) tree ring width (TRW), (**b**) basal area increment (BAI), (**c**) carbon isotope ratios in tree ring cellulose ($\delta^{13}C_{TR}$), and (**d**) oxygen isotope ratios in tree ring cellulose ($\delta^{18}O_{TR}$).

The dendrochronological standardization models are applied to remove the biological trend. Still, they are suspected of having numerical and conceptual drawbacks [67], which is why the basal area increment estimation is widely used to evaluate climate influences on tree growth [68,69]. In the present study, the basal area increment does not differ substantially between species. The mean values were $1.78 \pm 1.09 \text{ m}^2$ (*P. nigra*) and $1.71 \pm 0.77 \text{ m}^2$ (*P. sylvestris*). We observed a higher variance and interquartile range for annual *P. nigra* BAI (1.19/1.60 m²) than *P. sylvestris* (0.60/1.01 m²). The one-way ANOVA shows that inter-species chronologies are not significantly different (*F*-value = 0.15; *p* = 0.69), even if, the Levene's test demonstrate that population variance are significantly different (*F*-value = 7.98; p = 0.005). A significant positive trend for both chronologies (Mann-Kendall analysis, only for a 95% confidence interval) is explained by an increased growth until 2008 followed by an abrupt decline in both species BAI time series values (Figure 2b).

Changes in stable isotopic variability are induced by advanced interaction among climate, ecological and physiological processes, respectively, the cambial phenology [70]. Some studies consider even two trees samples representative for the stable isotopic analysis [71]. Others suggest using expressed populational signal values higher than the guide threshold of 0.85 to estimate the correct number of trees investigated [26]. The low sample replication was often associated with the pooling practice (mixing material from different trees before mass spectrometry analysis to reduce signal variability). In this study, all EPSs values of the tree rings, $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ chronologies were above 0.90, suggesting that three incremental cores collected from different trees are representative for investigating site-species signals. Since individual samples of the same species and isotope analysis were significantly correlated, we averaged the three series of $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ for each species in a mean chronology which was further analyzed. The average $\delta^{13}C_{TR}$ value was $-25.47 \pm 0.50\%$ ranging (min = -27.00%, max = -24.22%) for *P. sylvestris* and $-25.09 \pm 0.47\%$ (min = -26.47%, max = -24.15%), respectively, in the case of *P. nigra*. The two-standard deviation of -26.47 indicated two extreme years in 1980 (-22.55%), 2018 (-27.00‰) for *P. sylvestris* and 2004 (-26.18‰), 2018 (-26.47‰) for *P. nigra*. We found a positive week correlation coefficient between the two interspecific chronologies of r = 0.23, p = 0.09 (two-tailed test of significance). The one-way ANOVA shows significant differences between means (*F*-test = 15.39, *p* < 0.0001). In oak samples, Ponton et al. [72] discussed higher discrimination between species, correlated with functional anatomical traits and genotypes influenced by environmental conditions. The literature also mentioned an age effect reflected on stable isotope values related to different stages of plant development of the total leaf area variations, root system morphology system, and photosynthetic capacity [71]. In our study, the minimum and maximum values can be more closely associated with climate variability than tree age.

In the present study, we obtained a mean $\delta^{18}O_{TR}$ of 29.10 \pm 0.67‰ for *P. sylvestris* and $29.55 \pm 0.69\%$ for *P. nigra*. The values oscillated between 27.56–30.79‰ and 28.12–31.15‰. The two-standard deviation was calculated as 27.77‰ and 28.16‰, bases for identifying two extreme years in *P. sylvestris*, 1995 (27.56‰) and 2001 (27.67‰), respectively, 1984 (28.12‰) in *P. nigra*. Significant correlation among oxygen chronologies were calculated (r = 0.33, p = 0.01) and one-way ANOVA demonstrated significant differences between means populations (*F*-value = 10.81, p = 0.001). We noted a slight increase in $\delta^{18}O_{TR}$ after growth release, also observed in other studies that associated this behaviour with various factors [73,74]. The stable oxygen isotopes age trend appears contradictory reported, both increasing [74] and decreasing [73]. The water dependence of the evolving system roots is responsible for negative $\delta^{18}O_{TR}$ values [75]. Declined trees are associated with lower $\delta^{18}O_{TR}$ values caused by seasonal differences in carbohydrates use. With the higher sensitivity for spring climatic conditions, rather than excessive drying from summer in the formation of the tree growth, the $\delta^{18}O_{TR}$ reflects the signature of carbohydrates produced in spring [76]. Thus, lower $\delta^{18}O_{TR}$ values can be interpreted in stressed trees as a response to different rooting adaptation patterns, excluding the high capacity of developing deeper roots systems unexpected to trees with chronic decline [77].

3.3. Relationship between Environmental Variable and Tree Ring Proxy

The bootstrapped correlation coefficients were assessed between 15 environmental variables and TRW, BAI, $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ chronologies, and results are illustrated in Figure 3. *P. nigra* tree ring chronology correlated with atmospheric rain (r = 0.39), precipitation (r = 0.35) and SPEI1 (r = 0.29) of June in the previous year. In addition, TRW correlated with SPEI3 in August year preceding tree growth (r = 0.28) and SoilMoi00_10cm_tavg, SoilMoi00_40cm_tavg in September of the previous year (r = 0.31) (Figure 3a). In contrast, *P. sylvestris* TRW chronology demonstrated a significant positive relationship with RAIN,

rr, soil moisture content, SPEI1 and SPEI3 in July of the previous year. There is a negative relationship of TRW with mean and minimum temperature in November of the previous year (Figure 3b). During the growing season for *P. nigra*, were quantified a significant relationship between TRW and ETp in February (r = 0.36) and NDVI in May (r = 0.33). Analyzing the case of *P. sylvestris*, we noted that trees responded to ET in February and ETp in February, May and June. There was also a negative relationship between TRW and atmospheric rain in May (r = -0.32), respectively, with soil moisture content in June (r = -0.29). Negative was even the relationship with scPDSI in July and August, respectively, with SPEI3 in June-August. The results obtained in the present survey indicate that *P. sylvestris* is more sensitive to drought and reclaims more water demands compared with *P. nigra*. In years with warm winters (as is the case of the last two decades in our region), this positive influence can significantly threaten tree growth vigor in an unusual temperature boost through summer and autumn [78]. In contrast, *P. nigra* is characterized by a wide tolerance range from sea level to high altitude [14], the reason why we evaluated those environmental conditions to fulfil species-specific requirements.

Basal area increment revealed a strong dependence on the amount of water from the soil and the atmosphere and comparable interspecies effects of drought on trees growth. Thus, BAI showed for *P. nigra* a solid relationship positive with ET, ETp, soil moisture content and atmospheric temperature, with values ranging between r = 0.24 and r = 0.67 both in the previous and current growing season (Figure 3c). More specifically, in the current growing season from March to August ET, ETp, NDVI, tn, tg and tx correlated positive with BAI with coefficients from r = 0.22 to r = 0.74. The high positive correlation coefficients between TRW and NDVI (up to r = 0.74) in different intervals of the growing season indicate more considerable carbon uptake [79].

The current growing season's atmospheric rain and soil water supply negatively influence BAI formation (r = -0.50 and r = -0.24). A negative relationship was established between scPDSI in the June-July (r = -0.29) and SPEI indices in June-August current growing season (r = -0.36). In the case of *P. sylvestris* BAI chronology, the correlation was comparable with *P. nigra*, mentioning only the coefficients' intensity was higher. The minimum and maximum correlation coefficients range between r = -0.45 (soil liquid water from 0 to 10 cm in June current growing season) and r = 0.61 (minimum temperature in August previous growing season) (Figure 3d). The significant correlation values were mainly positive, indicating a robust cumulative effect of evapotranspiration, temperatures and water supply in April-July. The mixed responses of trees to environmental factors must be considered under the particular condition of illegal logging found in sites near the human village, as is our case. The literature discusses the influence of thinning in diminishing the competition among trees and inducing a lower climate sensitivity, reducing stress during extreme events. After thinning, a notable difference in evaluating tree ring sensitivity compared to BAI can decrease dependence on the previous year-growth condition [80]. Even if we noted boost coefficients of correlation using BAI proxy, according to this statement, the possible effects of drought could be even more robust in the studied area. Similar results in Hungary raised the question regarding the high tolerance of P. nigra to climate change after the last decades of increasing temperatures [81].

The tree's response to environmental factors evaluated through stable $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ chronologies indicate a week signal in *P. nigra* (Figure 3e,g) compared with *P. sylvestris* (Figure 3f,h). In our region, earlywood production in tree rings starts in mid-April when positive correlations are found. Thus, for *P. nigra* can be noted the values calculated in the current growing season between $\delta^{13}C_{TR}$ and RAIN in May (r = -0.29) and SOILLIQ 0–10 cm in April (r = 0.36), respectively, between $\delta^{18}O_{TR}$ and scPDSI in March (r = -0.20) and SPEI1/SPEI3 in June (r = -0.26/-0.22). The correlation coefficients calculated using *P. sylvestris* $\delta^{13}C_{TR}$ chronology show an intense negative relationship with ET, ETp, soil moisture content, tg, tn and tx (up to date r = -0.56). During April–June, positive values were calculated with RAIN, rr, and soil liquid water. The scPDSI and SPEI3 drought indices were positively correlated with $\delta^{13}C_{TR}$ in June (r = 0.34 and r = 0.30).



Figure 3. Bootstrapped correlation matrix between tree ring proxy (**a**) TRW for *P. nigra*, (**b**) TRW for *P. sylvestris*, (**c**) BAI for *P. nigra*, (**d**) BAI for *P. sylvestris*, (**e**) $\delta^{13}C_{TR}$ for *P. nigra*, (**f**) $\delta^{13}C_{TR}$ for *P. sylvestris*, (**g**) $\delta^{18}O_{TR}$ for *P. nigra*, (**h**) $\delta^{18}O_{TR}$ for *P. sylvestris* and the environmental variables.

These results are consistent with other studies conducted in the region where a decreased stomatal conductance caused by a low relative humidity (atmospheric drought) induce more $\delta^{13}CO_2$ fixation and simultaneously higher $\delta^{13}C_{TR}$ absorption rates [26]. Positive correlation coefficients calculated between $\delta^{18}O_{TR}$ chronology and ET, ETp and maximum temperature in February-September current growing season sustain this affirmation. The oxygen composition of tree rings is conditioned by soil water isotope changes induced by the pattern of the precipitation isotope composition and soil evapotranspiration [82]. Climatic data indicate some influences of previous summer/autumn and evapotranspiration for $\delta^{18}O$ signal derived from tree rings. The literature suggests stronger trees dependence on the water regime from the year after the drought [82], which was not observed in our case. Our results containing a weak signal can be associated with declined trees where δ^{18} O is lower in the years with heavy precipitation [80], which characterize the study site (Figure 1). The $\delta^{13}C_{TR}$ was strongly influenced by the relationship between soil water and atmospheric drought stress in previous and current growing seasons. The scPDSI (a sum of effects between precipitation, evapotranspiration, surface runoff and groundwater) indicates the local moisture drought stress reflected through *P. nigra* $\delta^{18}O_{TR}$. The high temperature and low precipitation amount decrease the relative humidity, accelerating soil water evapotranspiration and limiting trees water supply. This combined action can increase water vapour pressure differences in leaves and reduce water evapotranspiration. The effect of $\delta^{13}C_{TR}$ increase results from air relative humidity decreasing, indicating this proxy signal is dominated by stomatal conductance and environmental variables responsible for soil moisture content [83].

3.4. Temporal Stability of the Climate-Growth Relationship

The temporal stability of the climate signal was tested using different lengths of moving windows, and results demonstrated an unstable correlation relationship in time. Thus, we observed a strong influence of drought in 1990, 2003 and 2012 on changing trees response to climate. In the case of tree rings, only for *P. sylvestris* was noted sequences of stable significant coefficients during (considering the 10-year moving window correlation) 1980–2004 (*r* average = -0.41) and 1997– present (*r* average = -0.28) (Figure 4a). The BAI time datasets for both species highlighted positive coefficients for May, the mean and maximum temperature during 2003—present, ranging between *r* = 0.25 and *r* = 43 (Figure 4c). Time-dependent variability of limiting climatic factors showed a shift in tree response after extreme events, a fact observed even in other studies [84,85].



Figure 4. Temporal stability between *P. nigra* and *P. sylvestris* basal area increment chronologies and the May mean and maximum temperature calculated as a 10-year moving correlation. The statistically significant correlation is represented with continuous lines. Each panel illustrates significant correlation coefficients between climate date and (**a**) tree ring width, (**b**) carbon isotope ratios in tree ring cellulose, (**c**) basal area increment, and (**d**) oxygen isotope ratios in tree ring cellulose.

The $\delta^{13}C_{TR}$ was the optimal proxy for understanding the influence of minimum, mean and maximum temperature on tree growth. We noted a shift in time of stable correlation from May to July (Figure 4a). Thus, the significant correlation values were continuous for tg in May during the interval 1973–1985 (*r* average = -0.53), 2007–present (*r* average = -0.30), and for tn 1973–1994 (*r* average = -0.49), 2005–present (*r* average = -0.29), respectively, for tx 1973–1985 (*r* average = -0.50). In contrast, during July, the intervals with significant coefficients started for mean temperature from 1991–present (*r* average = -0.41), minimum temperature since 1990–present (*r* average = -0.38), and for maximum temperature from 1994–present (*r* average = -0.40). Through $\delta^{18}O_{TR}$, the positive effect of April extreme temperature on tree growth was noted between intervals 1982–1996 (tx *r* average = 0.38) and 1986–2018 (tn *r* average = 0.27). In addition, a negative drought influence in March was emphasized by the scPSDI index during 1973–1994 (*r* average = -0.44) and 2012-present (*r* average = -0.24). Similar results were noted in various studies which investigated trees located in a wide range of environmental conditions [86–88]. The $\delta^{18}O_{TR}$ correlated significantly with scPDSI in June since 1977–present (*r* average = -0.38) and SPEI3 since 1984–present (*r* average = -0.37).

3.5. Resilience Components

The pointer years analysis showed no extreme years. Still, the fluctuation of the growth trend indicates different resilience components from P. nigra and P. sylvestris TRW chronologies. The mean yearly results showed a changing response in tree ring resistance, resilience, and recovery, higher positive values in *P. sylvestris* (Figure 5a,b). The two standard deviations emphasized lower release in resilience and recovery indices $(2\sigma^2 = 1.89/1.65)$ in *P. nigra* than *P. sylvestris* $(2\sigma^2 = 2.32/1.79)$. The maximum two standard deviations of resilience were found in 1992 for *P. nigra* ($2\sigma^2$ >1.89) and 1997–1999 for *P. sylvestris* $(2\sigma^2 > 2.32)$. The highest resistance for both species (>1.49) was noted during 2001–2002 (P. nigra) and 1999–2001 (P. sylvestris). The misdistribution model of resilience indices illustrated in Figure 5c,d demonstrates lower mean values in *P. sylvestris* tree ring chronology with several extreme values discussed previously. Lower resilience indices in *Pinus* sp. planted stands show reduced growth vigor [41] and indicate a cumulative drought effect on resilience and resistance. These findings suggest that present climate conditions are probably responsible for growth and signal loss, equivalent to increasing dieback and die-off in planted stands. Future climate warming will increase drought stress. Lagged drought effects can be associated with preceded dieback process expressed through marked growth decline associated with signal loss.



Figure 5. Resilience indices (**a**) *P. nigra* yearly mean site indices, (**b**) *P. sylvestris* yearly mean site indices, (**c**) distribution of resilience indices for *P. nigra*, and (**d**) for *P. sylvestris*.

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