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Assessing climate constraints on *Fagus sylvatica* L. in the
Northern Apennines through the analysis of xylem anatomical
traits

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ABSTRACT

European beech (*Fagus sylvatica* L.) is the most widespread broadleaf tree in Italy and its higher sensitivity to the climatic variables and especially toward low water availability and longer drought periods than other deciduous species, makes it one of the most studied species in dendrochronology. Since faster increase in temperatures and decrease in precipitation are expected in the near future due to climate change, in this study I investigated the performance of European beech in the Northern Apennines, which falls within the optimum climatic area of this species.

The study was conducted in a beech stand at the summit of Monte Giovarello, part of a mountain complex located within the National Park of the Tuscan-Emilian Apennines.

A combined methodological approach of dendrochronology, quantitative wood anatomy and remote sensing was adopted: tree-ring width was measured on increment cores from 13 trees at the timberline distribution of the beech stand, then on 8 selected cores the anatomical wood analysis was applied.

Inter and intra-annual analysis was computed between the standardised mean chronologies of the anatomical parameters (mean ring width-MRW, cell number-Cno, theoretical hydraulic diameter-KH, mean lumen area-MLA) and the climatic variables (maximum and minimum temperature, precipitation and SPEI03).

Both the monthly and the 20 days moving average windows correlations indicated a reduced sensitivity of the species respect to the effects of climatic variables, especially in the summer months.

For a drought-sensitive species as *F. sylvatica*, a focus on the two characteristic years as 2003 and 2018 seemed important.

Wood anatomy and satellite (Normalized Vegetation Index) data were used to investigate the characteristic years, together with a buffer of one year (2002-2004; 2017-2019). A decreased in the NDVI values occurred in 2003 but not in a significant way and a significantly decreased of the anatomical traits occurred one year later, in 2004. In parallel, no significant decreased was showed in 2018, for both anatomical values and NDVI.

These results suggest a low sensitivity of the beech stand to drought that is likely linked to site conditions characterized by wet winters and cool summers. Therefore, I can assume that Monte Giovarello is still an optimum climatic area for European beech.

RIASSUNTO

Il faggio (*Fagus sylvatica* L.) è la latifoglia più diffusa in Italia e la sua sensibilità alle variabili climatiche, in particolare alla scarsa disponibilità idrica e ai periodi di siccità più lunghi rispetto ad altre specie decidue, lo rende una delle specie più studiate in dendrocronologia. Poiché a causa del cambiamento climatico un aumento rapido delle temperature e una diminuzione delle precipitazioni sono previsti nel prossimo futuro, in questo studio si è deciso di valutare le prestazioni del faggio nell'Appennino settentrionale, area che rientra nell'optimum per questa specie.

Lo studio ha riguardato una faggeta che occupa il limite superiore del bosco del Monte Giovarello, parte di un complesso montuoso situato all'interno del Parco Nazionale dell'Appennino Tosco-Emiliano. È stato adottato un approccio metodologico combinato di dendrocronologia, anatomia quantitativa del legno e remote sensing: l'ampiezza degli anelli è stata misurata su carote provenienti da 13 alberi, mentre sono state selezionate 8 carote su cui svolgere l'analisi anatomica.

Le analisi inter- e intra-annuale hanno riguardato le cronologie medie standardizzate dei parametri anatomici (mean ring width-MRW, cell number-Cno, theoretical hydraulic diameter-KH, mean lumen area-MLA) e le variabili climatiche (temperatura massima e minima, precipitazioni e SPEI03). Sia le correlazioni mensili che quelle con le finestre mobili di 20 giorni hanno mostrato una ridotta sensibilità della specie rispetto agli effetti delle variabili climatiche, soprattutto nei mesi estivi. Si è inoltre ritenuto di studiare più nello specifico la sensibilità della specie alla siccità attraverso l'analisi delle risposte nei due anni caratteristici del 2003 e del 2018. L'analisi anatomica e i dati satellitari (Normalized Vegetation Index) sono stati quindi utilizzati per indagare i due anni caratteristici rispetto a quelli immediatamente precedenti e successivi (2002-2004; 2017-2019). Nel 2003 si è verificata una diminuzione dei valori di NDVI, ma non in modo significativo, mentre nel 2004 la diminuzione dei valori riferiti ai tratti anatomici è stata significativa. Nel 2018 non è stata evidenziata alcuna diminuzione significativa, sia per i valori anatomici sia per l'NDVI.

Questi risultati ci portano a ritenere che il faggio nel Monte Giovarello evidenzi una ridotta sensibilità alla carenza idrica e che l'area, caratterizzata da inverni umidi ed estati fresche, rappresenti ancora una stazione climaticamente ottimale per il faggio europeo.

1.INTRODUCTION

Climate change is occurring at an unprecedented rate with higher atmospheric CO₂ concentrations than at any other time in the Quaternary (Holden, 2017). Indeed, according to the IPCC 2014 report, the industrial revolution and high population growth increased the emission of carbon dioxide, methane, and nitrous oxide bringing them at the highest concentration levels in history, at least during the last 800,000 years.

Real-time data indicate that global greenhouse gas emissions continued to increase in 2022. As greenhouse gas concentrations rise, so does global mean surface temperature (GMST). As a result, from January-September 2022, GMST (Global Mean Surface Temperature) was 1.15 ± 0.13 °C warmer than the pre-industrial baseline (1850-1900) (World Meteorological Organization, 2022). The mediterranean region is predicted to be the most vulnerable of the European regions to global change (Schröter et al., 2005).

Such changes in the climate are likely to strongly affect forest ecosystems by altering the physiology, growth, mortality and reproduction of trees, the interactions between trees and pathogens, and ultimately the disturbance regimes (winds, drought, wildfires, insect outbreaks, etc.).

Given the longevity of trees and the pace at which the climate is changing, it is almost certain that many individuals living nowadays have been or will soon experience abrupt changes of the climate before being replaced by the next generation. Hence, it is fundamental to assess the ability of trees and forest ecosystems to cope with a changing climate (Bugmann et al., 2014). In this sense, it's crucial to understand the responses of forest ecosystems to different climatic conditions, as they could affect ecosystems functioning and services such as carbon storage, hydraulic regimes but also timber production and touristic function (Peters et al., 2013). The crucial fingerprints of climate change are mostly associated with changes at the species distribution margins (i.e., trailing edge, rear edge); however, recognizing changes in the species optimum (i.e., maximum occurrence probability and maximum abundance) is also essential (Klopčič et al., 2022).

A common method for monitoring forests health is the Normalized Difference Vegetation Index (NDVI), thanks to its sensitivity to photosynthetic activity. Indeed, with the growing maturity of remote sensing technology, more and more vegetation monitoring indicators have emerged, but the Normalized Difference Vegetation Index is the most widely used. It is considered to be the best indicator for studying surface vegetation cover and

analysing the response of vegetation cover to climatic conditions at global and regional scale (Zheng et al., 2022).

Working on smaller spatial frameworks, but at rather longer temporal scales, dendrochronology and its applications, such as dendroecology and dendroanatomy, are used to investigate forest responses to climate variability.

Classical studies are based on dendroclimatological approach, where tree-ring width represents the key variable to analyse (Schweingruber, 1996), while in the dendroanatomy are the wood anatomical traits to be analysed along series of dated tree rings (Solana, 2016). Wood anatomical features represent a natural archive for growth environment relationships and plant functioning with intra-annual resolution (Fonti et al., 2010). Furthermore, xylem anatomy preserves information related to tree allometry and Eco physiological performance, which is not available from the more customary ring-width or wood density proxy parameters (Carrer et al., 2014). Consequently, a multiproxy approach using various growth parameters would thus permit a more holistic understanding of the influence of climate on tree growth and function (Prislan et al., 2018). Thus, dendrochronological and dendroanatomical analyses show up as useful approach to investigate the ecological implications of climate driven changes on forest dynamics and to forecast tree decline (Dorado-Liñán et al., 2017).

European beech (*Fagus sylvatica* L.) is a key species in European forests: its natural distribution spans most of continental Europe making it the most abundant broad-leaved forest tree in Central Europe (Dittmar et al., 2003) (*Fig. 1*). Physiological performance and growth of beech are thought to be adversely affected by changing environmental conditions (long dry periods, more frequent frosts etc.) because of its higher sensitivity toward low water availability and longer drought periods than other deciduous specie (Miranda et al., 2022). Model-based projections predict that climate change will likely induce a reduction in beech-dominated forests in southern Europe (Hanewinkel et al., 2013). Indeed, growth declines for beech have already been reported in recent decades (Charru et al., 2017), especially at its southern range limits (Jump et al., 2006).

In this context, beech forests in the Northern Apennines may be a suitable experimental site to investigate the species sensitivity to the climate variables identifying any limiting factors.

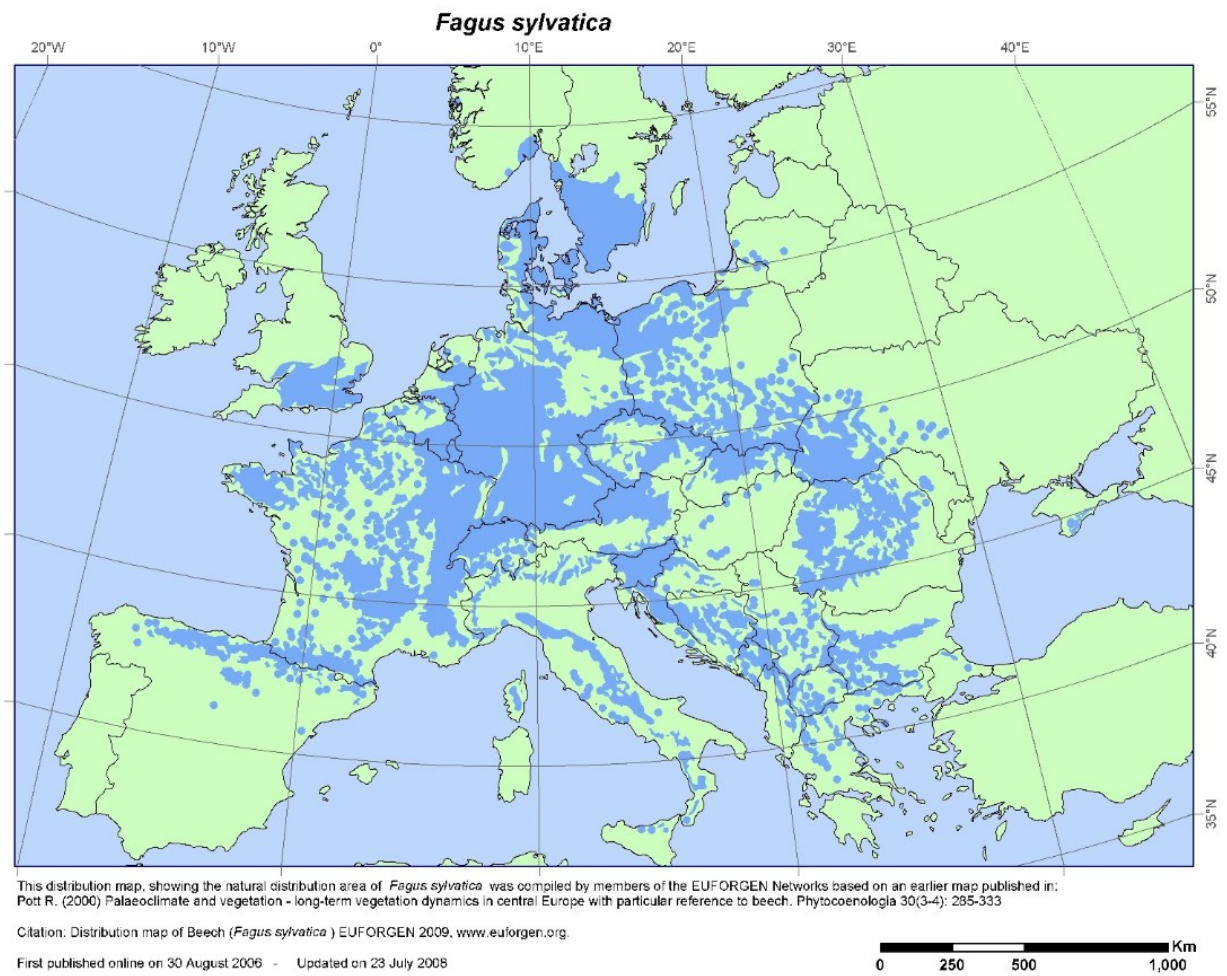


Figure 1 Map depicting the entire distribution range of beech in Europe (EUFORGEN 2008)

2. OBJECTIVES

We used an interdisciplinary approach involving dendrochronology, wood anatomy and remote sensing to study the performance of European beech in relation to the current climate change in the northern Apennines.

Particularly, the main aim of this study was to identify current and future climatic threats potentially challenging the persistence and survival of beech at high elevation, detecting any potential early-warning signal of decline or maladaptation of the species.

Wood anatomy and dendrochronology have been used to (I) assess climatic influences on beech radial growth; (II) analyse whether the difference in radial growth are paralleled by corresponding changes in wood-anatomical traits as lumen area, cell number and theoretical hydraulic conductivity and (III) to analyse, in this case together with satellite data (Normalized Difference Vegetation Index), the extreme drought events of 2003 and 2018 and compare the performances of these two different approaches in detecting forest response.

3. MATERIALS AND METHODS

3.1 Study site

The sampling site is located on the summit area of Monte Giovarello (1760 m a.s.l.) at an elevation range from 1642 to 1662 m a.s.l. (Fig.2).

Monte Giovarello is part of a mountain complex located within the National Park of the Tuscan-Emilian Apennines (Parco Nazionale dell'Appennino Tosco-Emiliano).

The study site is located in the protected area IT4030005 - ZSC-ZPS - Abetina Reale Alta Val Dolo (Fig.3) where the beech forest (habitat 9110) is the predominant tree formation interspersed with spruce and silver fir trees along the sides covering 382,99 ha. Here, beech grows mainly from 900 to 1.800 m a.s.l. in rather old and dense coppice or transition stands, where tree height is controlled by the fertility of the soil, reaching a maximum of 25-30 m in deep and fresh soils. As the altitude gets higher, they gradually reduce in size in order to cope with even the most extreme environmental conditions, until they become rather short and twisted near the tree line (Ente Parco Nazionale Appennino Tosco-Emiliano, n.d.).

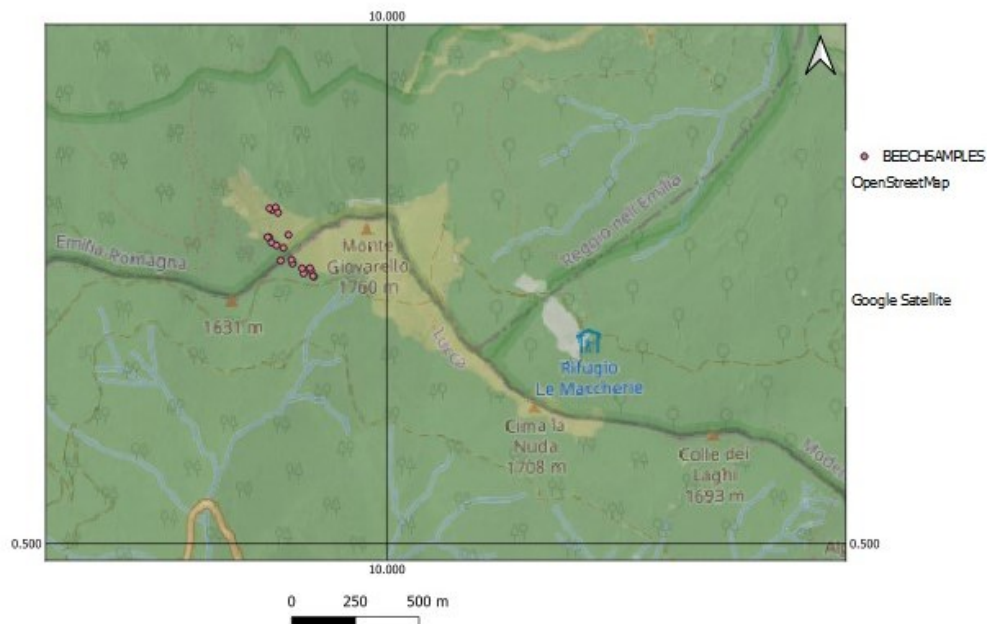


Figure 2 Map of the collected samples along the timberline of Monte Giovarello (source: QGis)



Figure 3 National parks, Regional Parks, Natura 2000 sites, reserves and protected area of the Emilia Romagna region (PROGETTO LIFE14 NAT/IT/000209 EREMITA)

According to the Koppen-Geiger scheme, the study site falls within the mid-Appennine region with cool temperate climate (Cf), sometimes with subcontinental characteristics.

The soil of the study area belongs to the 7 Cd class according to the “**Carta dei suoli 1:50.000**” provided from the “Regione Emilia-Romagna” website (<https://datacatalog.regione.emilia-romagna.it/catalogCTA/geoviewer2>).

This class includes steep or very steep soils; stony; deep or very deep; medium texture, very stony in depth; good oxygen availability; weakly acid to strongly acid in depth. Locally non-calcareous, neutral or weakly alkaline in depth. Originated from moraine deposits and materials derived from stratified arenaceous and subordinately arenaceous-pelitic rocks.

3.2 Sample collection and processing

During the field work, one increment core was extracted at breast height with a Pressler borer from 13 beech trees with slightly twisted stem and mean DBH of 20-30cm. Selection was done randomly along the timberline. At the lab, cores were sanded with progressively finer gridded sandpaper for a clear visualization (*Fig.4*) of the rings and measured to the nearest 0.01 mm (Carrer et al., 2019) using TsapWin. Ring-width series were cross-dated to match each tree ring with its year of formation and dating was checked with COFECHA software (Holmes, 1983).

For the anatomical analysis, 8 cores were selected for each tree based on the absence of visible defects such as nodes, or rotten and missing parts. We used a bandsaw to split the selected cores into 4-5 cm long pieces paying attention to have rings in common with the previous and the following segments and to being able to reconstruct the whole temporal series afterwards.

The subsamples were boiled for 10-15 minutes and then cut with a rotary microtome (Leica, Heidelberg, Germany) to obtain 10 μ m thick transversal micro-sections with undamaged cells.

Samples were then washed with distilled water, stained with safranin and astra blue and washed again with 50 and 100% ethanol. Finally, the stained micro-sections were fixed with Euparal mounting medium (*Fig.4*).

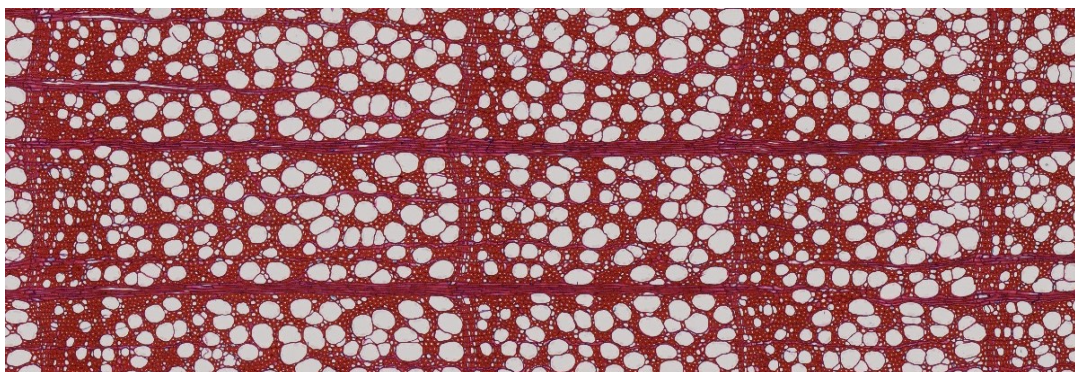


Figure 4 picture of one of the sample after the laboratory processing

Axioscan 7 (ZEISS) slide scanner was used to capture digital images of the microsections which were subsequently split with Zen microscopy software in 4-5 parts. The images were then processed with the image analysis software ROXAS (*Fig.5*) (von Arx

et al., 2016). Manual editing was performed in case the cells were not recognized automatically or wrongly defined.

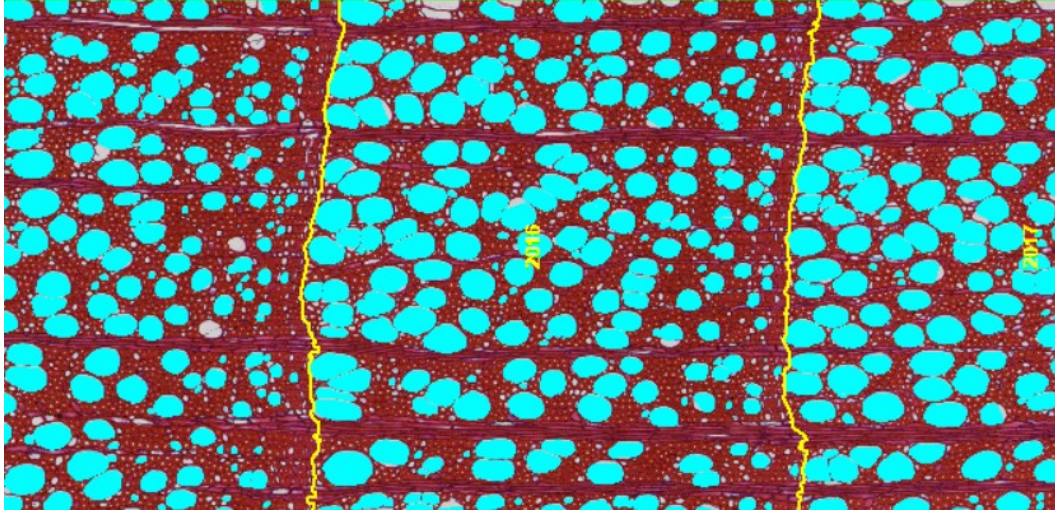


Figure 5 Example of an image obtained with the process described above through the ROXAS software

After dating and editing all the images, Roxas created an Excel sheet with several anatomical parameters for each ring, I took into account mean ring width (Mrw), mean lumen area (Mla), number of cells (Cno) and theoretical hydraulic conductivity (Kh).

For each parameter a mean chronology was built computing a robust mean from the corresponding values computed for all the samples for each year.

3.3 Statistical analyses

Since all the mean chronologies of every parameter showed a typical age/size trends (Carrer et al., 2014a), which can confuse the association with climate, a standardization procedure was necessary. The intent of standardization is manifold: (i) to remove non-stationary processes related to the mostly size-related and, to a lesser extent age-related, trend or disturbance pulses (in the case of ring width, the generally decreasing trend with age is due substantially to the geometric constraint of adding an annual wood layer over an expanding surface represented by the stem cross-section); (ii) to stabilize the variance of tree ring series, which are commonly heteroscedastic; and (iii) to homogenize the growth rate among samples (despite living in the same location, some trees grow faster and others

slower; therefore, it is better to assign the same weight to all samples before they enter the mean growth curve) (Cook & Shiyatov, 1990).

To remove such trends, raw individual series were standardized using Arstan software (Cook, E.R., Krusic, P.J., Peters, K., Holmes, R.L., 2017) through two different standardization procedures, the classical cubic-smoothing spline and the age dependent spline.

Spline function with a 50% frequency response of 20 years are flexible cubic spline curves very efficient in removing both the long-term trend and the effect of localized disturbance events, but at the risk of removing possible low-frequency climatic information (Cook & Shiyatov, 1990).

The age-dependent smoothing does not feature a constant stiffness, in fact, the resulting spline is flexible in the early years and becomes progressively stiffer in later years to satisfactorily retain the initial growth rise while having less sensitivity to the increased local variability in the later years (Time-varying-response smoothing).

3.4 Climatic data and climate-growth associations definition

Datasets of monthly climatological normal (Brunetti et al., 2012) of meteorological variables at a high spatial resolution are crucial in order to have more accurate results. The climate information provided by global datasets often lack representativeness at a local scale. This is even more true when remote sites in mountain terrain are considered. For this reason, I used a reconstructed climate record for the sampling site built by exploiting the high instrumental data availability of Italy and the Alpine region that is one to two orders of magnitude higher than the number of stations entering in the international global datasets (Osborn & Jones, 2014). To obtain temporal series of meteorological variables highly representative at the very local scale it was applied the anomaly method technique (Mitchell and Jones, 2005; New et al., 2000), as described in Brunetti et al. (2012). Climatologies, characterized by remarkable spatial gradients, were reconstructed with an interpolation technique that takes into account the local dependence of the meteorological variable on elevation (Brunetti et al., 2014).

Daily values were reconstructed by following the method described in di Luzio et al., 2008 and using the previously reconstructed monthly series as a constraint. Then, using the

climate dataset available from 1814 to 2019, a Walter-Lieth diagram was created through the R software (Fig 6).

The climate of Monte Giovarello is subcontinental, much similar to the one found in the interior of Europe, but mitigated by Mediterranean influences. Snowfalls are frequent with a distinct warm and cool season. Mean annual temperature is 4.4° C with daily extremes ranging from -5.7° C to 17° C. Average annual precipitation 1864 mm. Relationships between climate parameters and each xylem anatomical traits were assessed using Pearson's correlation coefficient (CC) over the 1938–2020 period (H. C. Fritts et al., 1990).

In these correlation analyses, we included monthly mean of temperature (minimum, and maximum) and precipitation sums over a 18-month window from the previous June to October of the current year. In addition, we considered daily windows of 20 days and we moved the window with a daily step. In this way, each daily value was the result of the mean (for temperatures) or the sum (for precipitation) of the previous 20 days (Castagneri et al., 2017). Moving time windows correlation analysis was performed with Microsoft Excel.

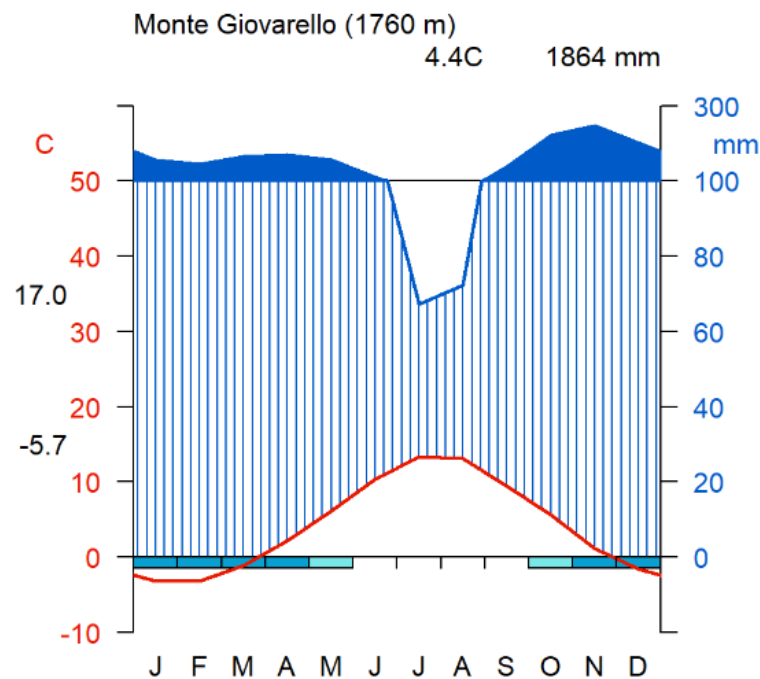


Figure 6 Walter-Lieth diagram for the study area from the 1814-2019 time period.

3.5 NDVI data elaboration and SPEI

The highest resolution satellite images were downloaded using the Earth Explorer website (U.S. Geological Survey).

The images were selected in the period between August and the beginning of September, in order to have quite similar phenological response from the vegetation and avoiding anomalous values due to different solar radiation intensity that occurs throughout the year.

The NDVI was computed through the “Raster calculator “ function of the QGIS software as $NDVI = \frac{(NIR - RED)}{(NIR + RED)}(NIR + RED)$, where RED and NIR stand for the spectral reflectance measurements acquired in the red and near-infrared regions respectively, (Camarero et al., 2021) for the periods 2002-2004 and 2017-2019, for an amount of 6 satellite images (Tab.1).

The result of the ratio yield is a measure of photosynthetic activity and ranges within values between -1 and 1. Low NDVI values indicate moisture-stressed vegetation and higher values indicate a higher density of green-healthy vegetation (Javadnia et al., 2009). Indeed, healthy vegetation (chlorophyll) reflects more near-infrared (NIR) and green light compared to other wavelengths. But it absorbs more red and blue light (GISGeography, 2022). Vice versa occurred for vegetation in stressful conditions.

Table 1 Sensors and date of the satellite images downloaded

	2002	2003	2004	2017	2018	2019
sensors	Landsat 7	Landsat 5	Landsat 5	Landsat 8	Landsat 8	Landsat 8
data	12-Aug	31-Aug	01-Aug	05-Aug	17-Aug	12-Sep

We decided to analyse the years characterized by the most extreme drought events of the last 20 years, that is 2003 and 2018. Furthermore, we also considered the year before and the year after the drought event. The first one were considered as a reference, to be able to detect potential differences with the year in which the drought occurred, and the second one in order to identify any carry over effect in the vegetation's response those climate extremes.

To better track the NDVI values over the considered years, three polygons were created. Then the averaged NDVI values pixels within the polygons were calculated using the “*zonal statistic function*” and 30 NDVI values were extracted for each polygon (using the functions “*random point in polygons*” and “*sample raster values*”) to conduct the T-test in the two periods.

Wood anatomical traits of the same years considered in the NDVI analysis were analysed to compare them with the response of the vegetation detected by remote sensing process.

The Standardized Precipitation Evapotranspiration Index (SPEI; Vicente-Serrano et al., 2010) was also used to assess the sensitivity of the xylem anatomical series to moisture deficit, for the timeframe 1938-2018, and in order to quantify the drought events of 2003 and 2018. The SPEI for 3-month time intervals at monthly scales was downloaded using the “KN climate explorer” tool for the study site

(https://climexp.knmi.nl/select.cgi?id=someone@somewhere&field=spei_03).

The SPEI is a multivariate meteorological drought index, which is based on the original SPI (Standardized Precipitation Index) calculation procedure. The SPI is calculated using monthly (or weekly) precipitation as the input data. The SPEI uses the monthly (or weekly) difference between precipitation and the potential evapotranspiration (PET) (Vicente-Serrano et al., 2010).

4. RESULTS

4.1 Chronology description

The selected samples present an average of 75 rings. Sample GIOFS004 is the oldest (1912-2019) with 106 rings, while GIOFS009 is the youngest sample measured with only 42 rings (1961-2003).

An average of 1448 μ width, 1027 vessels, 584 theoretical hydraulic and 804 μ^2 lumen area per ring was measured, with sample GIOFS002 showing minimum and sample GIOFS007 the maximum of width; sample GIOFS004 showing the minimum, and sample GIOFS003 the maximum amount of vessels and theoretical hydraulic conductivity and GIOFS004 and GIOF003 showing respectively the minimum and the maximum of lumen are (*Tab.2*).

Table 2 Descriptive statistics of the xylem anatomical traits considered for each tree: Number of rings (Rings) and the mean ring width (Mean MRW), vessel number (Mean Cno), lumen are (Mean MLA), theoretical hydraulic (Mean KH) with their respective standard deviation. In blue and in red is highlighted respectively the minimum and the maximum values for each variable.

ID	RINGS(n)	Mean MRW	Mean Cno	Mean MLA	Mean KH
GIOFS002	83	1013±600	505±463	930±496	375±290
GIOFS003	68	1687±639	2961±1524	251±68	821±446
GIOFS004	106	1034±537	273±151	1176±427	313±221
GIOFS007	66	2093±894	821±441	905±429	765±557
GIOFS008	77	1082±514	444±184	908±221	333±180
GIOFS009	42	1758±694	1003±489	745±379	762±524
GIOFS011	78	1610±612	1249±1114	853±477	659±367
GIOFS013	89	1307±841	957±531	664±297	643±634

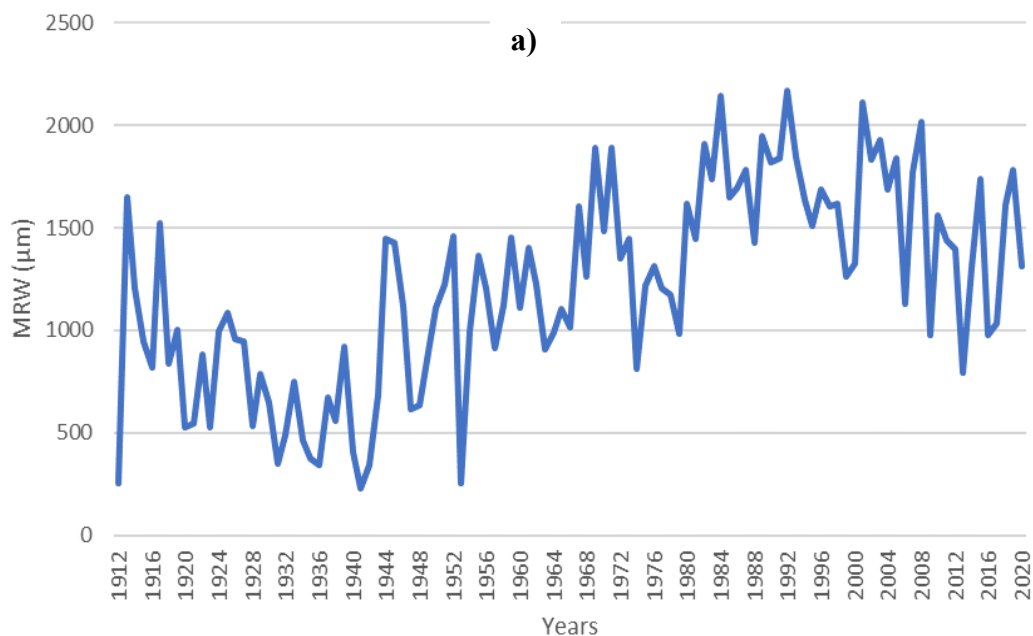
Subsequently, the wood anatomical parameters were correlated with each other through Pearson's linear correlation. *Tab.3* showed that all the parameters were significantly correlated with each other, except for the correlation between Cell number (Cno) and Mean Lumen Area (MLA) that is still positive but not significant.

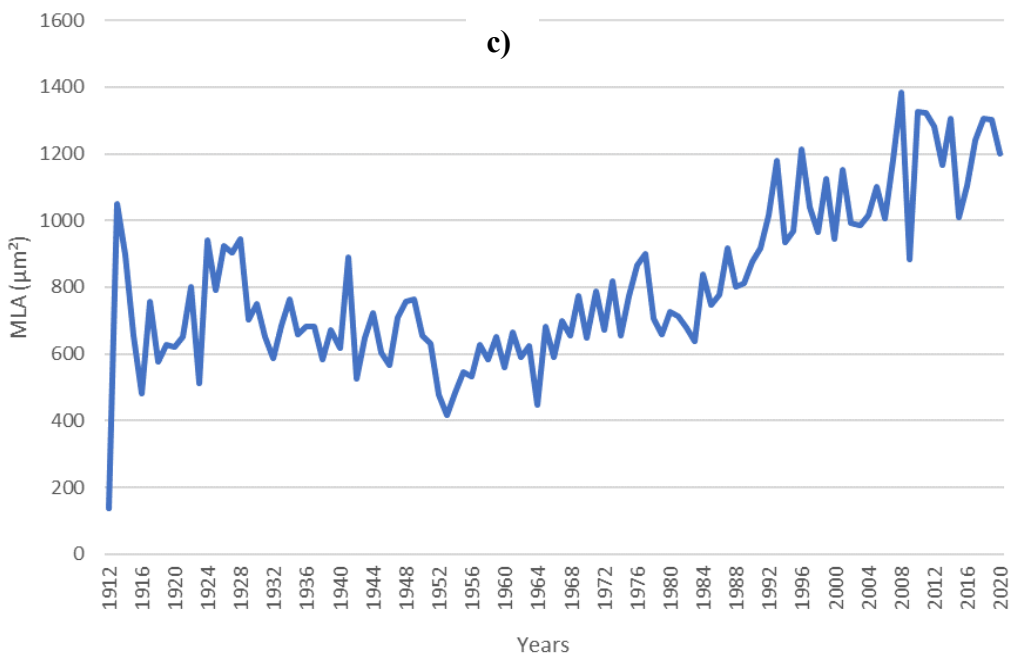
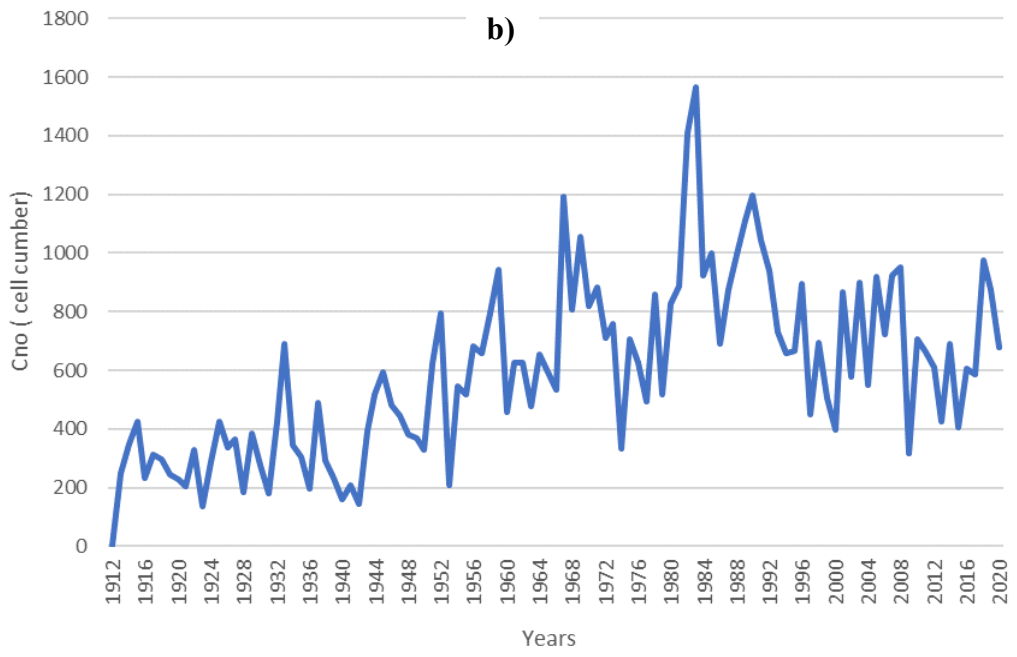
Table 3 Pearson Correlations of the Mean ring width (MRW), number of cells (Cno), Mean lumen area (MLA), theoretical hydraulic conductivity (KH). In green the significant positive correlations ($p < 0.05$).

	<i>MRW</i>	<i>Cno</i>	<i>MLA</i>	<i>KH</i>
MRW	1			
Cno	0.839465	1		
MLA	0.565118	0.309681	1	
KH	0.863498	0.785867	0.735239	1

All the parameters showed an increasing ontogenetic trend since the 1940-1950 period, which is maintained only by MLA almost until the end of the chronology: 2004-2008 (Fig. 7).

The MRW (Mean Ring Width) and Kh (theoretical hydraulic conductivity) showed a decreasing trend which started in the year 2000 and 2009 respectively, while the Cno (number of cells) showed a decreasing trend from the 1980s to the 1990s which became more constant then.





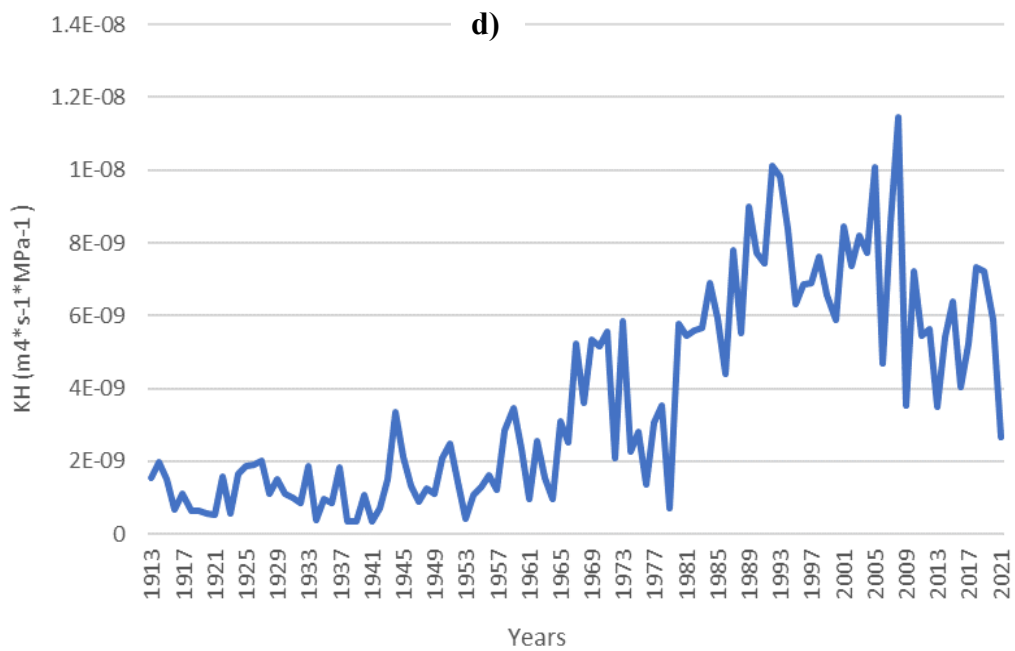


Figure 7 Anatomical traits raw chronologies:(a) mean ring width (MRW), (b) mean lumen area (MLA), (c) theoretical hydraulic conductivity (KH) and (d) number of cells (Cn). The lines represent the site chronologies (average of all the analysed samples)

4.2 Standardization

Two kind of standardization process were utilised to smooth the trend of the mean ring width and wood anatomical traits: the smoothing spline 20 and the age dependent smoothing (*Fig.8*).

In time series of wood-anatomical ring properties, defining the appropriate standardization approach for the specific information of interest is particularly critical because knowledge from several research fields, including tree physiology, dendrochronology and wood anatomy, is required (Carrer et al., 2014).

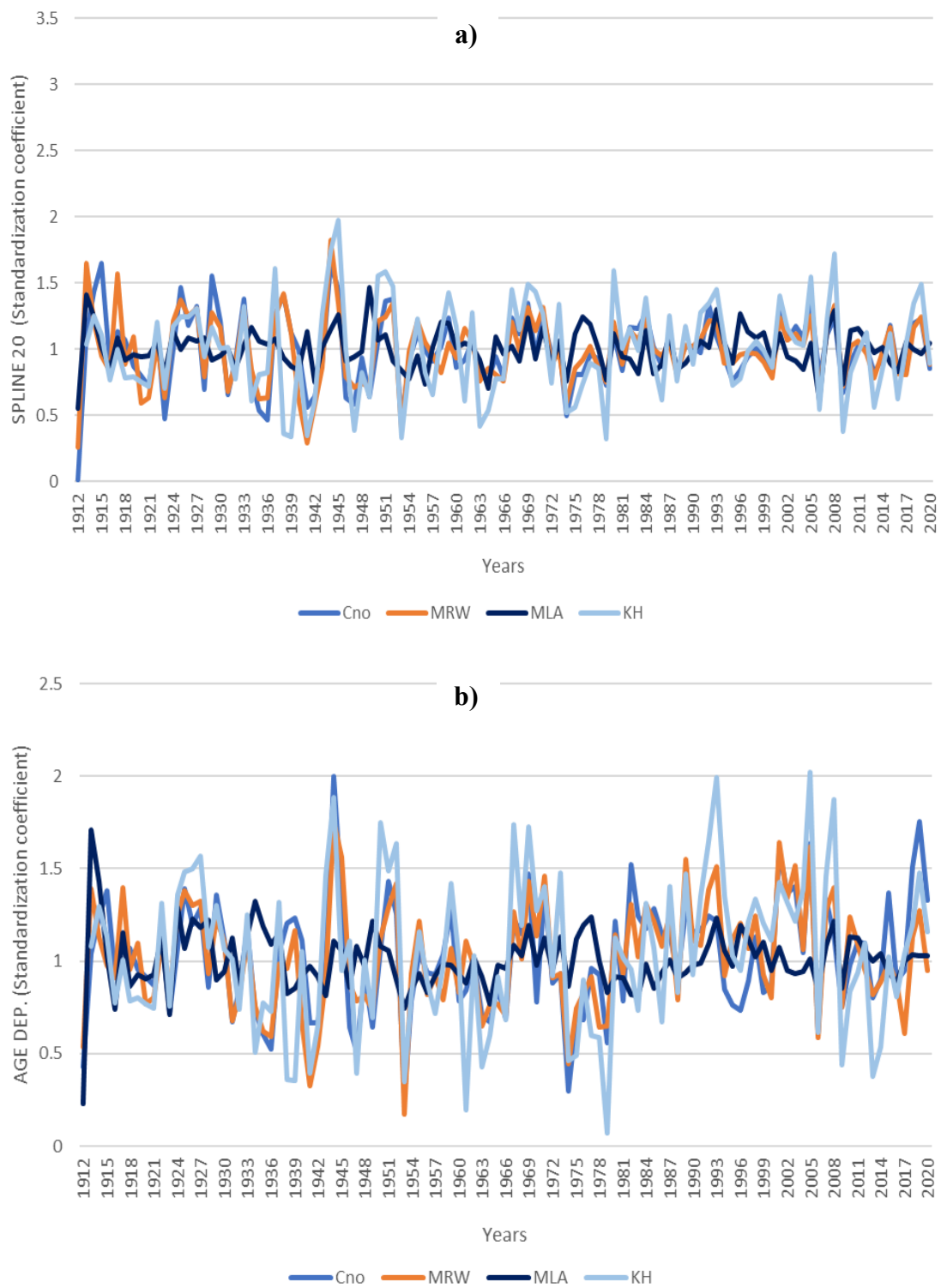


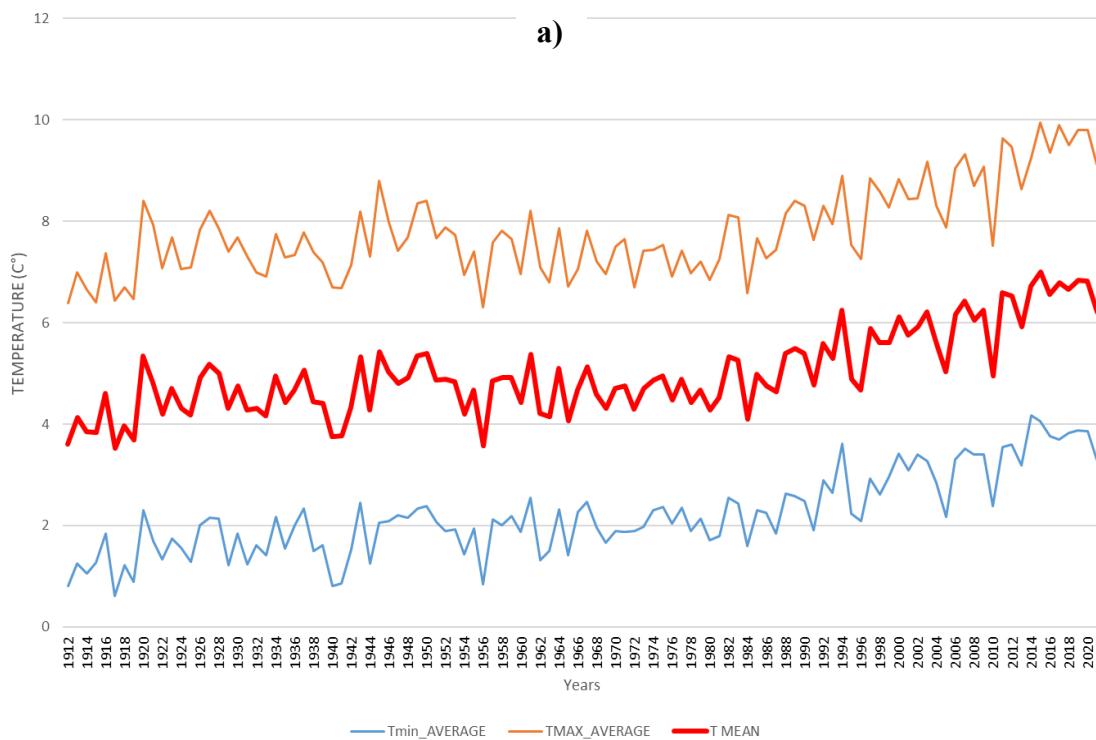
Figure 8 Anatomical traits standardised chronologies: (a) smoothing spline 20 (b) age dependent smoothing spline.

4.3 Climatic data

The Climatic data of the study area, showed a stronger increasing trend in temperature since the 1980s, reported in both minimum and maximum (*Fig.9*).

While the annual precipitation has fluctuated in the past decades without a clear trend.

In addition, the Walter-Lieth diagram (*Fig.6*), didn't show any dry months, but only wet months (part filled in blue) and December, January and February with frost days. While March, April, May, October and November are month with likelihood frost days.



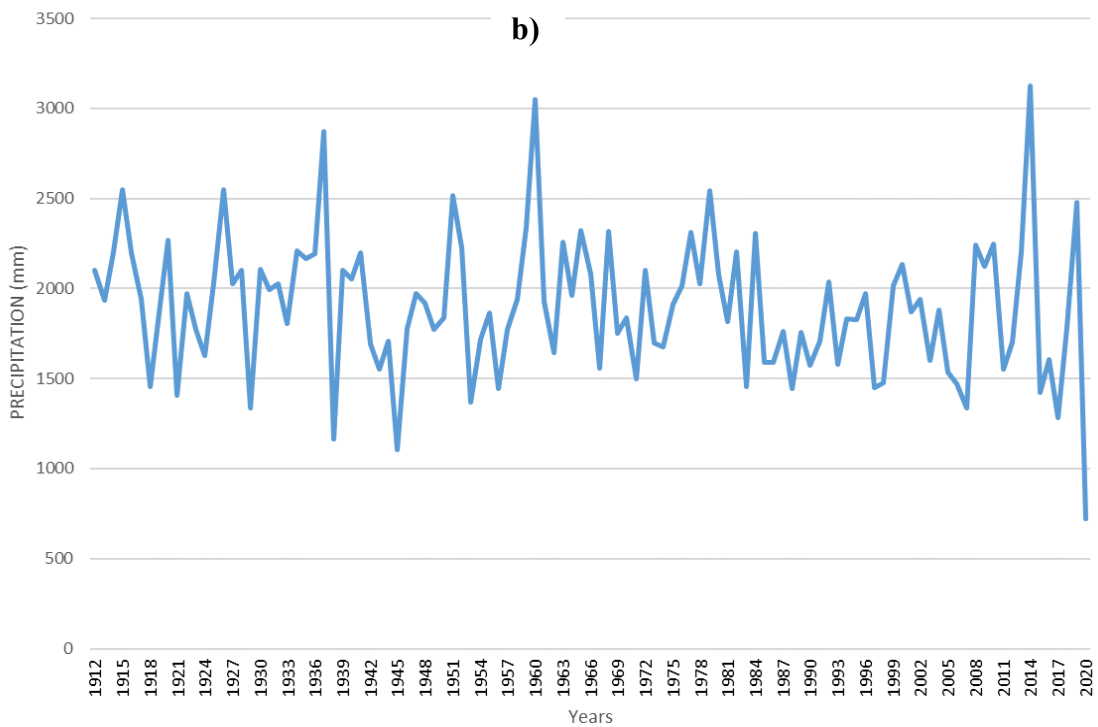


Figure 9 Climatic data trend: (a) annual average of the maximum, mean and minimum temperature (b) sum of annual precipitation.

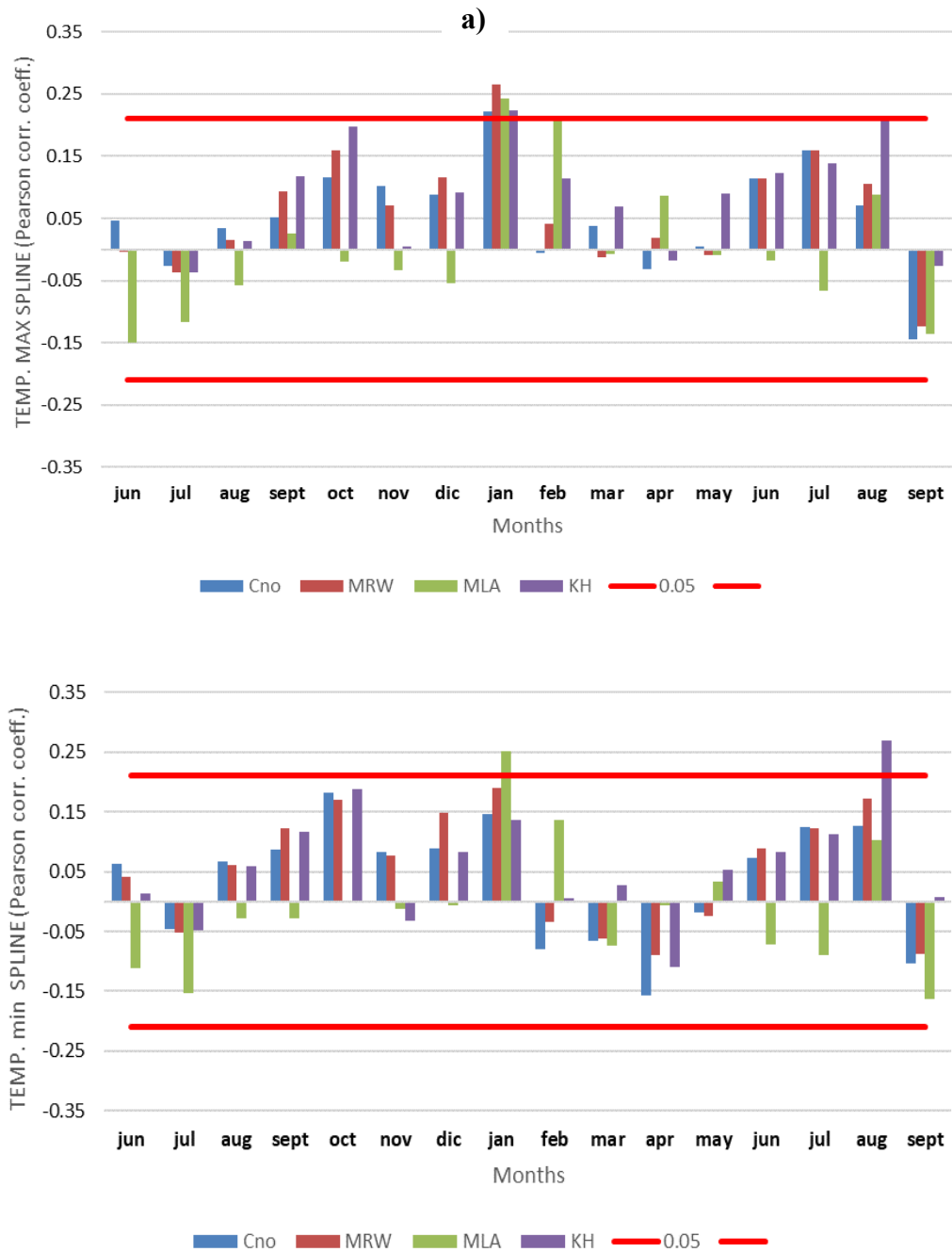
4.4 Monthly-based climate-growth relationships

The standardized chronologies of the mean ring width (MRW) and the anatomical parameters were correlated with the monthly minimum and maximum temperature and sum of monthly precipitation values from 1938 to 2020 and with the 3-months SPEI values from 1938 to 2018, focusing on a timeframe of 15 months, from June of the previous year up to September of the current year.

All the four chronologies, standardised with the smoothing spline 20 process, generally revealed a poor climate association, displaying significant correlations only with maximum and minimum temperature of the current year.

Spline 20 parameters, showed a significant positive correlation with the maximum temperature of January (*Fig.10*). Correlations with minimum temperature evidenced a significant monthly positive correlation for MLA in January and with KH in August of the current year.

Similarly to the spline 20 correlations, all the traits standardized with the age dependent spline revealed similar results with a significant positive correlation in all the traits with January maximum temperature and a significant positive correlation of MLA with the minimum temperature of January. On the other hand, Cno showed significantly monthly positive correlations with the maximum and minimum temperature of the previous October and current July and August.



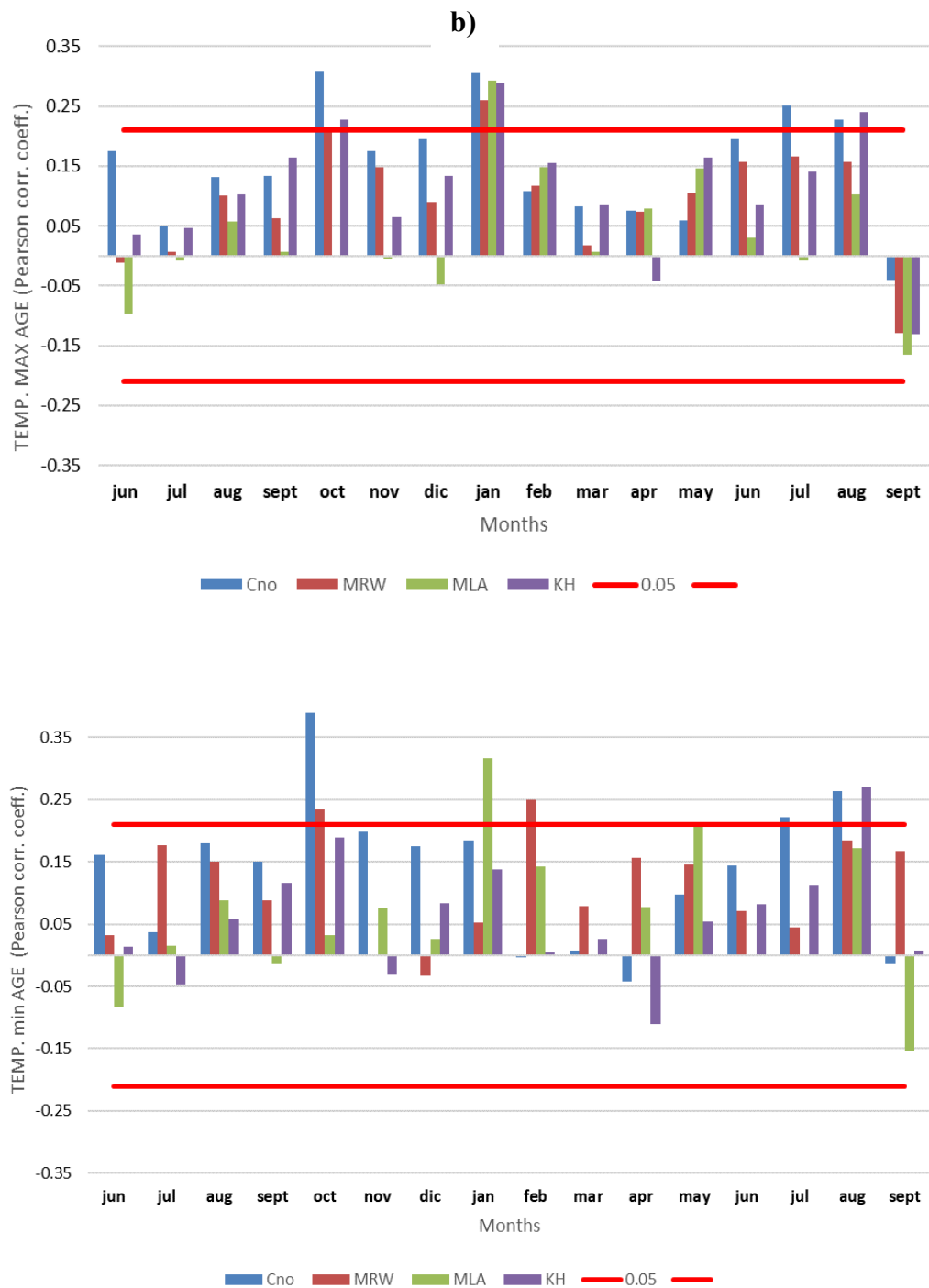


Figure 10 Comparison of the standardisation: (a) smoothing spline20, (b) age dependent smoothing spline. Monthly correlation between the anatomical traits and the monthly mean maximum and minimum temperature for the previous (June to December) and current (January to September) growth year. The red lines of each plot indicate the $p < 0.05$ significant level.

No significant association were found with precipitation for the anatomical parameters standarsied with the smothing spline 20. While for the age-depent standardasied paramaters, the only significant relationship with monthly precipitation, was observed in the negative correlation of the hydraulic theoretical conductivity with current year January precipitation (Fig.11).

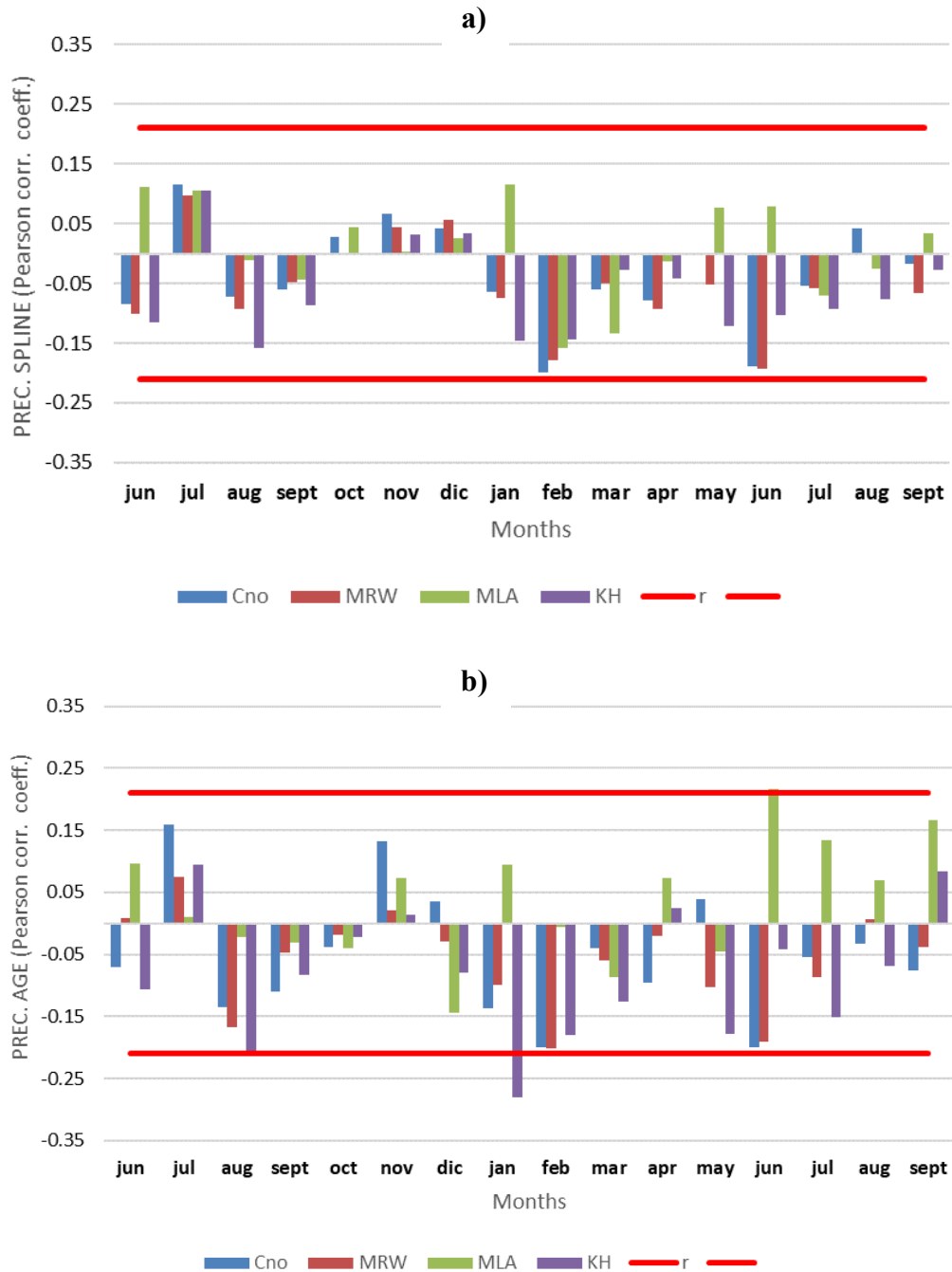


Figure 11 Comparison of the standardisation: (a) smoothing spline20, (b) age dependent smoothing spline. Monthly correlation between the anatomical traits and the monthly precipitation sum for the previous (June to December) and current (January to September) growth year. The red lines of each plot indicate the $p < 0.05$ significant level.

By comparing the monthly correlation of the two standardisation processes, no significant difference was observed. Thus, the decision was based on the method most widely used in the past studies. The variables standardized with spline 20 were used to perform the moving average windows correlation between climate and anatomical traits.

Pearson correlations were performed between the anatomical parameters chronologies and the monthly SPEI. The anatomical traits revealed a really poor sensitivity towards the moisture deficit, with only MLA displaying a significant negative correlation with the SPEI value of April (*Fig.12*).

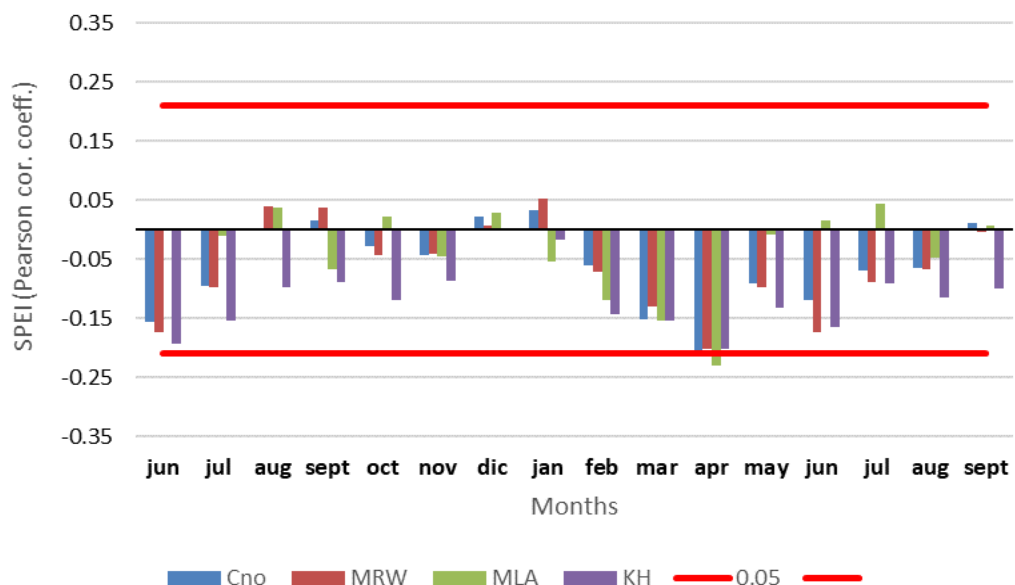


Figure 12 Monthly correlation between the anatomical traits and the monthly SPEI for the previous (June to December) and current (January to September) growth year. The red lines of each plot indicate the $p < 0.05$ significant level.

4.5 Intra-annual sensitivity

The climate-growth associations were quantified by calculating Pearson correlations between the detrended wood-anatomical chronologies and the different time windows of climatic data for the period (1938-2020).

The 20 days moving average windows showed an overall more significant correlations of the selected anatomical traits with the climatic variables than the monthly correlations.

As for the monthly correlation, all the parameters displayed a mainly significant positive correlation with the maximum temperature of January.

In addition both MRW and Cno showed a positive correlation with previous September, October, November and December and current April.

Cno was the highest sensitive parameter showing a positive correlation with current early-mid July and mid- August as well (*Fig.13*).

Others significant positive correlations emerged for MLA and KH respectively in February and previous September, current August.

An interesting significant negative correlation resulted for Mean lumen area (MLA) with the previous mid-June.

MAX. TEM. 20 DAYS MOV. AVER WINDOWS

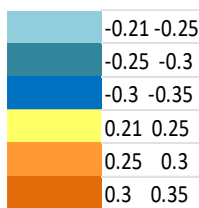
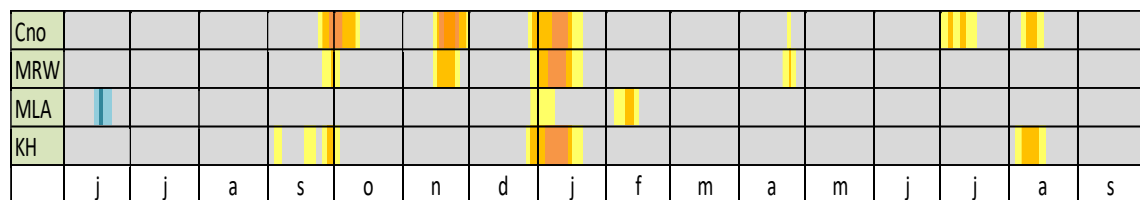


Figure 13 20 days moving average windows of the maximum temperature correlation with the anatomical traits for the previous (June to December) and current (January to September) growth year. Coloured parts indicate the significant correlations for $p < 0.05$, according the legend.

The minimum temperature-selected parameters correlations were generally weaker than the maximum temperature correlations, with mainly positive correlations displayed in November for both Cno and MRW, in January for MRW and MLA, and in August for KH. (*Fig.14*). While significant negative correlations were displayed in the current March and previous June respectively for Cno and MLA.

Min. TEMP. 20 DAYS MOV. AVERAGE WINDOWS

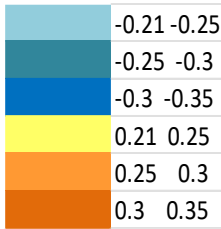
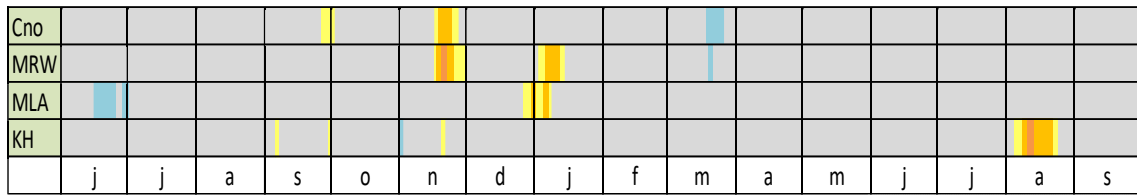


Figure 14 20 days moving average windows of the minimum temperature correlation with the anatomical traits for the previous (June to December) and current (January to September) growth year. Coloured parts indicate the significant correlations for $p < 0.05$, according to the legend.

A generally higher sensitivity of the selected parameters with the precipitation was revealed with the 20 days moving windows than with the monthly correlations.

All the parameters, except for the MLA, revealed a significant negative correlation mainly with the precipitation of the previous August, late-January, early-February and April.

In addition, KH resulted significantly negatively correlated with the precipitation of the previous mid-June and current late-December.

While Cno showed a significant positive correlation in the previous July.

MLA revealed a poor climate sensitivity with the precipitation, displaying significant negative correlations only in late-November and early February (Fig. 15).

PRECIPITATION 20 DAYS MOV. AVERAGE

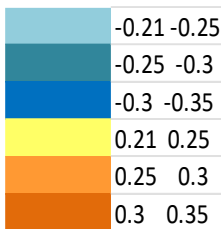
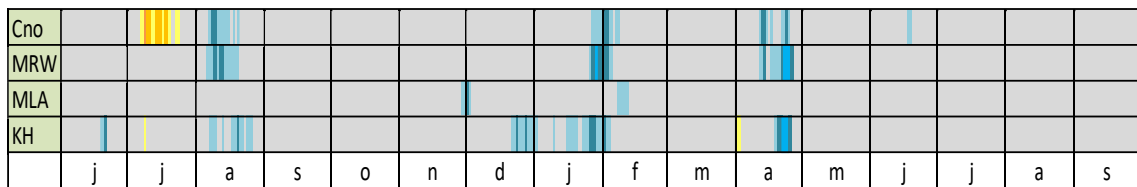


Figure 15 20 days moving average windows of the precipitation correlation with the anatomical traits for the previous (June to December) and current (January to September) growth year. Coloured parts indicate the significant correlations for $p < 0.05$, according to the legend.

4.6 Focus on drought sensitivity

Given that frequency and intensity of extreme drought events have increased worldwide, particularly over the past couple of decades, we decided to investigate the response of the studied forest to the extreme drought events of 2003 and 2018.

The drought occurred during the growing season of both years were analysed from a large scale method (NDVI) to a detailed scale (radial growth and wood anatomical traits).

NDVI index was used to assess forest health in the timeframe 2002-2004 and 2017-2019.

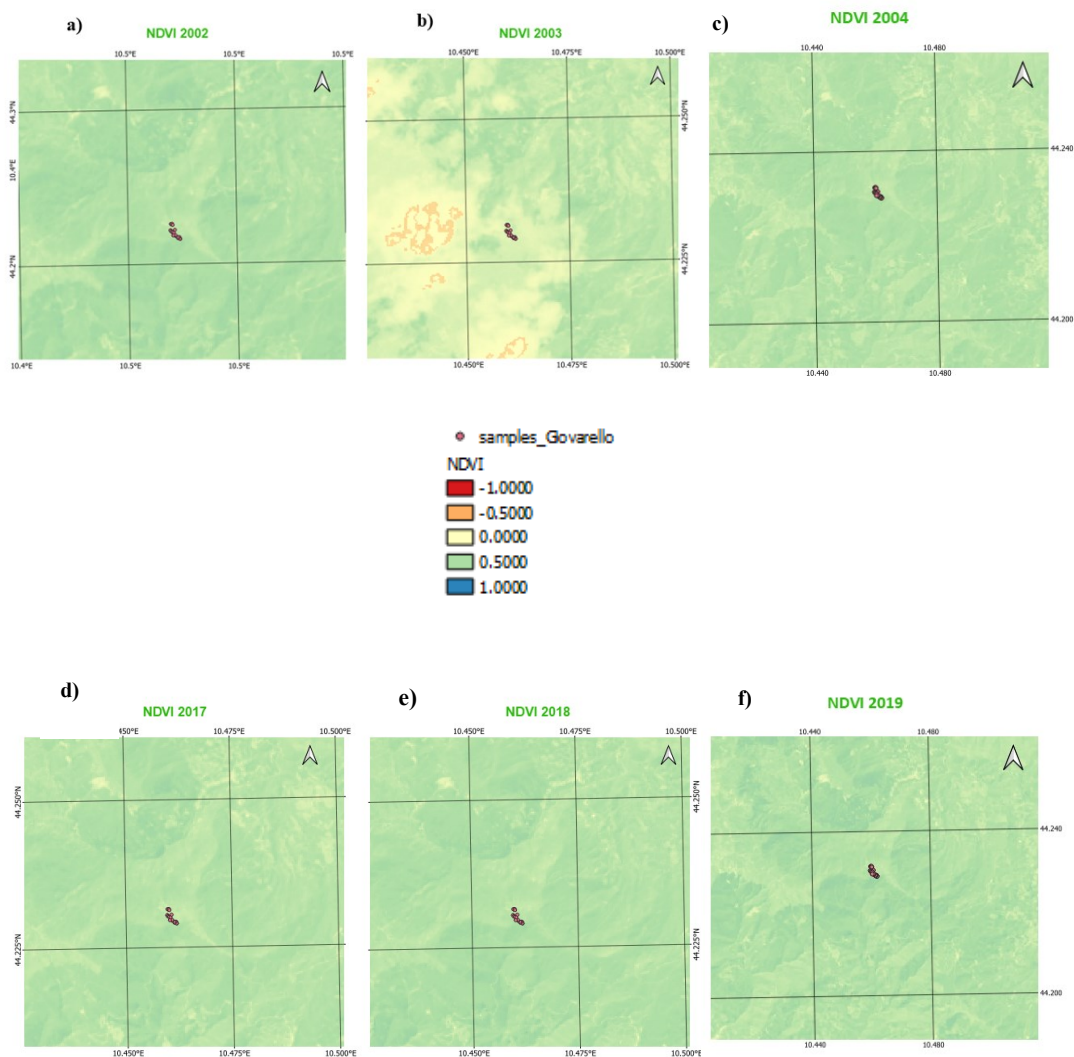


Figure 16 NDVI maps of the years: (a) 2002; (b) 2003; (c) 2004; (d) 2017; (e) 2018; (f) 2019

The thematic maps obtained computing the NDVI didn't revealed any large decrease of the index over the considered timeframes, but only a slight difference was observed in 2003 (Fig.16).

To better track the NDVI values over the considered years, three polygons were created and the mean index values were extracted (Fig.17).

An halving of the NDVI values was displayed for all three polygons in 2003 even if this difference wasn't significant, and no large significant difference was revealed for the 2018 drought in all three polygons (Tab. 4).

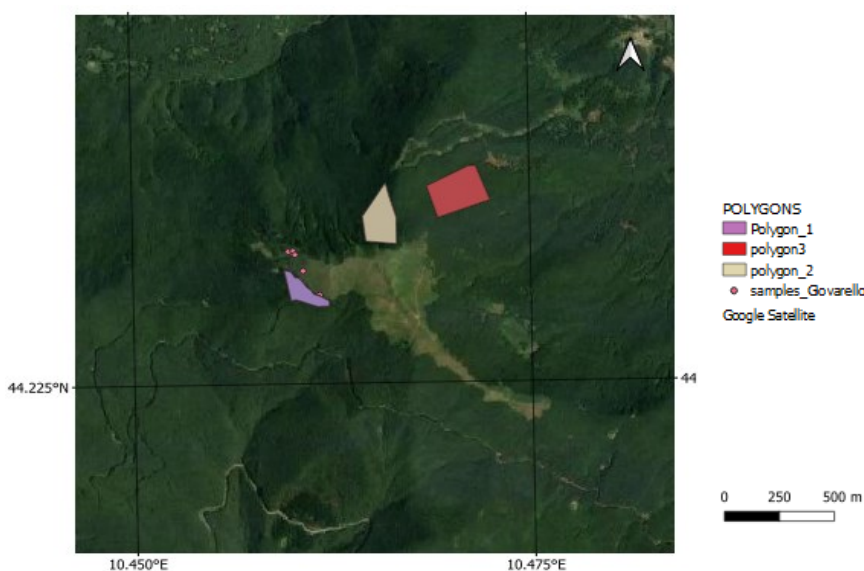


Figure 17 Polygons created to extract mean NDVI numeric values

Table 4 Mean NDVI values extracted from the 3 polygons for the years: 2002, 2003, 2004, 2017, 2018, 2019.

NDVI	2002	2003	2004	2017	2018	2019
Pol1	0.4817664	0.233574	0.448848	0.44346	0.481175	0.499142
Pol2	0.4042429	0.285568	0.387589	0.393338	0.393338	0.424613
Pol3	0.478246	0.195911	0.482114	0.478246	0.479852	0.517129
Average	0.4547518	0.238351	0.439517	0.438348	0.451455	0.480295

The mean chronologies of the xylem anatomical traits were analysed for the selected years (Tab. 5).

No significant decrease in Cno, MRW occurred in 2003, but both parameters significantly decreased in 2004, it might be a late respond to the drought of the year before.

While MLA and KH revealed a slight decrease already in 2003 which continued in 2004 and became significant.

Table 5 Anatomical traits values for the selected

	2002	2003	2004	2017	2018	2019
Cno	1.065	1.17	1.083*	0.83	1.192	1.226
MRW	1.067	1.114	1.03*	0.81	1.161	1.243
MLA	0.944	0.908	0.847*	1.056	0.999	0.963
KH	1.158	1.049	1.025*	1.045	1.348	1.49

On the contrary in 2018 all parameters, except the MLA, displayed a slight not significant increase of the mean values which continued also in 2019.

A slight decrease, but not significant, occurred in 2018 and in 2019 for MLA values.

Indeed, while the mean NDVI values showed a decrease of the index in 2003, such difference didn't occur in the anatomical traits. Contrarily, the values were really similar over 2002 and 2003, slightly lower only for MLA and KH (*Tab.5*).

Analysing the radial growth and anatomical traits, some differences were observed in the years 2017-2019 (an increase in Cno, MRW and KH values and a slight decrease in MLA), but they weren't significant. NDVI values remained virtually unchanged in all three years, showing no significant differences.

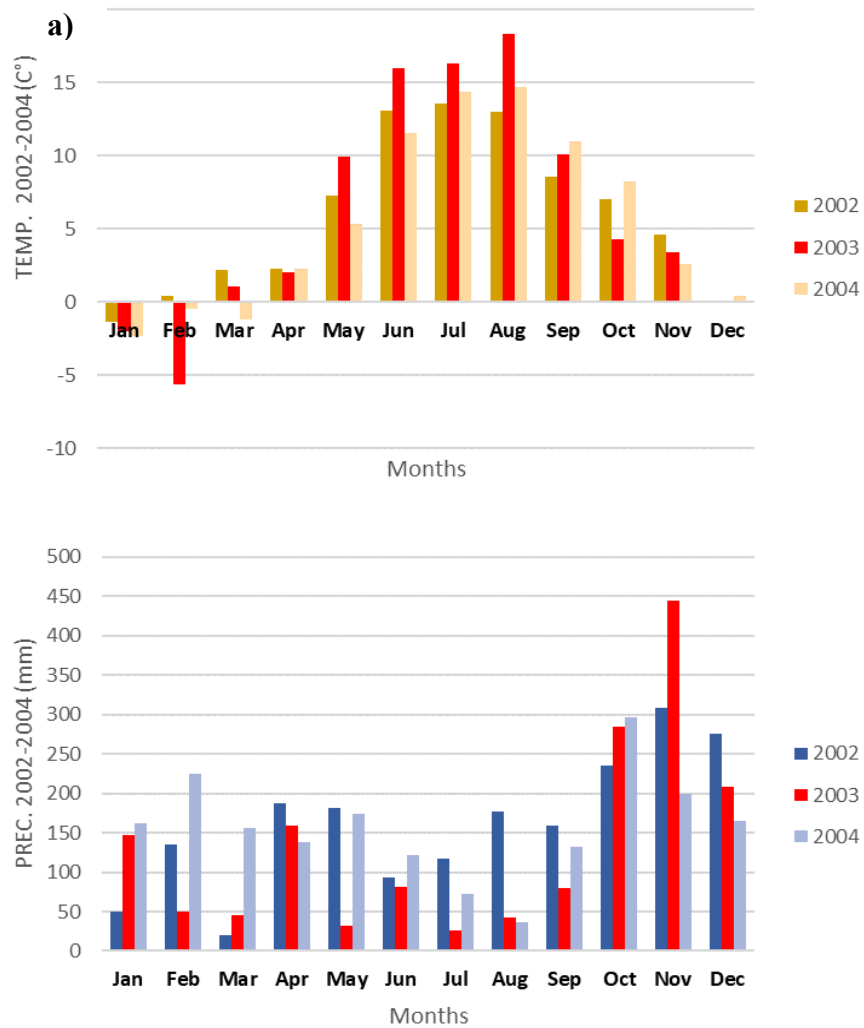
To better understand how the two drought events occurred in the study area temperature, precipitation and SPEI graphs were created for the analysed timeframes 2002-2004 and 2017-2019 for precipitation and temperature while 2002-2004 and 207-2018 for the SPEI, since no monthly data were available for 2019 year (*Fig.18*).

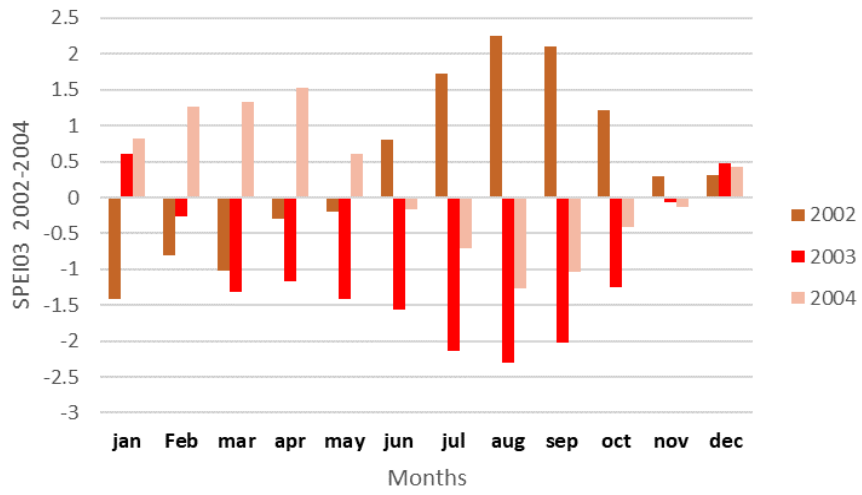
In the timeframe 2002-2003, the 2003 was highlighted with highest temperature and lowest precipitation in summer period, resulting in particularly negative SPEI values. 2004 revealed negative SPEI values in summer period, but much less important than 2003.

While from analysis of the 2018 drought graphs, 2017 revealed higher negative SPEI values than 2018 and low amount of monthly precipitation occurred throughout the year, with particularly low values in the summer period.

In addition, a Walter-Lieth diagram was created for the two characteristic year (2003 and 2018) (*Fig.19*). Indeed, one very useful feature of Walter and Lieth (1960) climate diagrams

is that they immediately allow to identify dry and wet periods in a given year (Strieder & Vospernik, 2021). In 2003 the diagram showed a dry period in July, while in 2018 no dry period occurred. Thus explain the higher reaction of all parameters after the 2003 drought in the same and/or the following year, and the lack of no significant decreases after the 2018 drought.





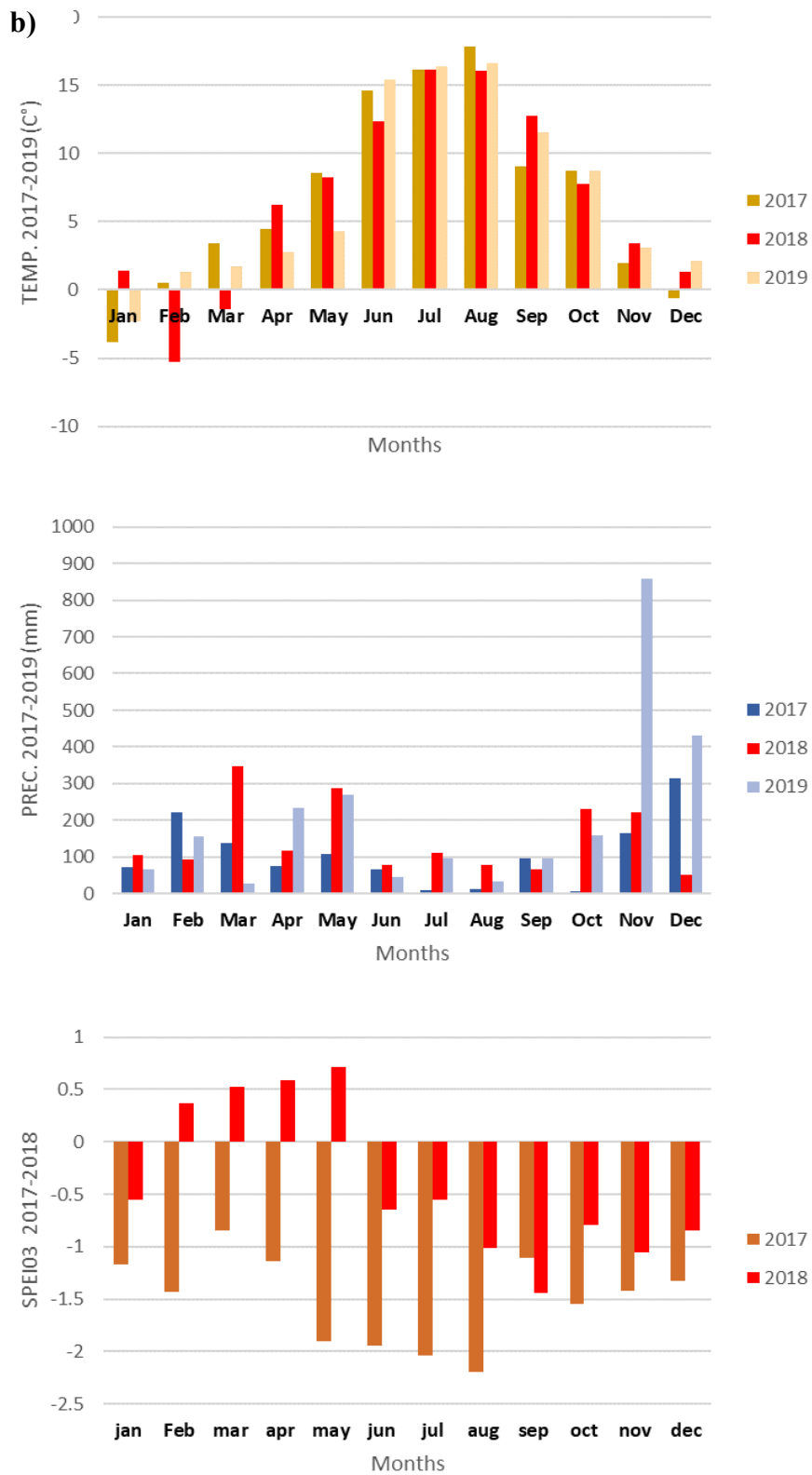


Figure 18 Monthly precipitation, temperature and SPEI graphs for: a) 2002-2004 timeframe b) 2017-2019 for precipitation and temperature graph and 2018-2018 for SPEI graph

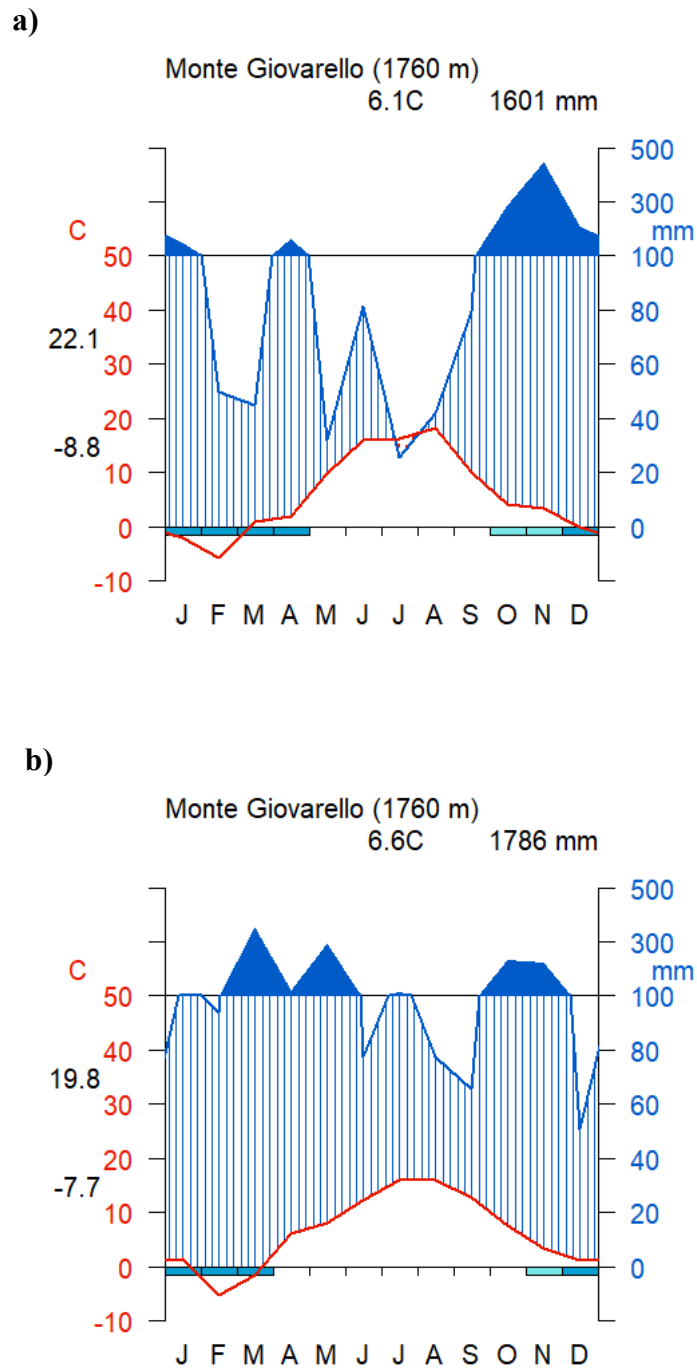


Figure 19 Walter-Lieth diagram for the study area for the years: (a) 2003 and (b) 2018

DISCUSSION

Beech is the most widespread broadleaf tree in Italy and therefore the effects of climate change on this species are of particular interest. I analysed whether the beech stand is sensitive to climate and to change in climate (i.e. the current warming trend) adopting a classic dendrochronological and a new dendroanatomical approach.

Beech samples revealed an overall high sensitivity highlighted by the high variability in their mean ring width and others anatomical parameters. This variability can be explained by the high elevations of the study site (1642-62 m a.s.l.) and the corresponding limiting conditions for the species, where elevational responses of both phenology onset dates and tree-ring increments vary significantly between years. In particular, in years with extreme weather conditions, the effects on growth and xylem anatomical traits could be remarkable (Kraus et al., 2016). A similar study performed in Cansiglio forest (Vanotti, 2021) highlighted similar growth rate with 1300 vessels per ring compared to 1027 vessels per ring of this study.

Strong significant correlations resulted between all the anatomical traits, except for the MLA that features no correlation with Cno and a barely one with MRW. This confirms the independency of the MLA from the number of cells as also found in the study conducted on a beech plantation in Ticino (Visentin, 2020). Nevertheless, MLA revealed a strong correlation with KH, confirming the strong dependency of KH to the MLA as well as to Cno and MRW.

Considering the decadal trend in the raw mean chronologies of anatomical parameters, while MRW, Cno, and KH have a similar increasing trend from 1940-1950 and decrease thereafter, MLA revealed an increasing trend that only becomes stable just in the final years. This peculiar trend, mainly from 1940 to 1950, can be explained considering that only two samples constituted the raw chronologies of the selected parameters from 1912 to 1931.

The subsequent decrease in MRW is due to the geometric constrain of adding an annual tree ring over an increasing stem circumference (Fritts 1976). Therefore, with the increase in diameter due to tree growth, tree ring area tends to increase but its width usually decreases. The similar tendency recorded for Cno and KH can be easily explained by the high correlations that these two traits have with MRW.

On the other side, the MLA increasing trend over time simply represents the yearly imprint of height growth on the xylem anatomical feature. Indeed, the conduit-lumen

diameter in a tree increases with cambial age at rates dependent on the rates of stem elongation to minimize the adverse effect of height growth on path way length resistance (Carrer et al., 2014a). Therefore, the MLA trend stabilization since 2008 can be explained by the achievement of the maximal height in most of the sampled trees. Indeed as claimed by Husch et al. 2003, trees approach their maximal height soon after maturity, and the imprint of this achievement on anatomical characteristics can be identified in the levelling off of the time series of conduit-lumen area (Anfodillo et al., 2006).

The results obtained with the monthly correlations with climatic variables revealed no significant difference between standardization methods, i.e. between the spline 20 and the age dependent standardized chronologies. Both the methods are fairly good in removing most of the variability not related to climate. Nonetheless, monthly climate correlations indicated a reduced sensitivity of the species, thought located at a high-elevation site, respect to the effects of climatic variables.

Despite a lack of strong correlations with precipitations, monthly correlations revealed a positive influence of the maximum temperature in January for all parameters and of the minimum temperature in January on MLA. Contrarily, most of the studies conducted on European beech found a high sensitivity of the species and a negative reaction to summer temperatures and/or a positive reaction to spring temperature and positive reactions to precipitation (di Filippo et al., 2012; Dittmar et al. 2003; Piovesan & Schirone, 2000; Piovesan et al. 2005; Biondi, 2018; Calderaro et al., 2020). These results are likely due to the occurrence of drought period, and therefore to the direct effect of water availability as a limiting factor (Fotelli et al. 2001), or late frosts in spring (Piovesan & Schirone, 2000), to which European beech is susceptible. However, it is important to highlight that such studies are mainly conducted in Central and Southern Apennines that are more susceptible to the occurrence of water deficit (Piovesan et al., 2005) than Northern Apennines, where this study was conducted. Indeed, the lack of negative correlation with summer temperature and positive correlation with precipitation was expected from the annual average precipitation exceeding 1000 mm (*Fig.9*) and the absence of dry periods shown by the Walter-Lieth diagram (*Fig.6*).

The lack of positive correlation with temperature in the spring months can be explained by the occurrence of cool temperatures that protect the bud burst and foliage from late spring frosts keeping the trees in vegetative rest. Furthermore, Piovesan et al., 2005 found that climate influences beech growth to a greater extent going from north to south and from west

to east within the Italian peninsula in line with the poor sensitivity of the selected beech stand being rather on the northern and western side of the Italian distribution.

Intra-annual higher resolution analysis such as the division of each ring into tangential sectors of equal width from early to latewood could provide better information and may explain what I found with the correlations computed at monthly resolution.

In the analysis of the 20-day moving average windows, an overall higher sensitivity emerged, which was generally similar among the anatomical parameters, with the exception of the MLA. The latter seems less influenced by climatic variables and in an opposite or different way respect the other parameters. Furthermore, MLA was negatively influenced by the minimum and maximum temperatures of the previous December and of the current January and the maximum temperature in February. These correlations are complicated to justify and the same applies to all significant correlations resulting in months outside the growing season (November, January and December correlations *Fig. 13-14-15*). On the opposite, the positive correlations between autumn temperature (especially maximum) and Cno, MRW and Kh may be due to the correspondence with the cessation of xylem formation, as found in two beech forests of Panska reka (PA) and Menina planina (ME) by Prislán et al., 2018. Since the studied site is located along the timberline and, as noted by Kraus et al. (2016), beech trees have a negative impact on the length of the growing season due to the delayed onset and early cessation of xylem formation, the more favourable temperatures in the earlier September-October months may extend the growing season by positively influencing xylem formation.

Similarly, to the monthly correlations a lack of significant influenced of the climatic variables in the summer periods was detected.

An overall difficult interpretation of the climate signal resulted from the 20 days moving windows; indeed, even if European beech wood formation dynamics have been monitored in various locations across Europe: in France (Michelot et al., 2012), Slovenia (Čufar et al., 2008), Romania (Semeniuc et al., 2014), Ljubljana, Kamnik– Savinja Alps (Prislán et al., 2018) and the Netherlands (van der Werf et al., 2007), the relationship between weather conditions and intra-annual variation of xylogenesis is still not fully explained, due to its high intra-specific plasticity (e.g., Vitasse et al. 2010; Prislán et al., 2018).

Drought is becoming a major driver of tree growth globally (Babst et al., 2019) revealing a temporal instability in its impacts on forests over the last decades (Peltier and Ogle 2020; Wilmking et al. 2020; Camarero et al., 2021). Along with this, beech is

considered to be a drought sensitive species (Zimmermann et al. 2015, Dulamsuren et al. 2017). Thus, a focus on the two most extreme drought (2003, 2018) occurred in the last decades, was conducted on the study area using both remote sensing and the analysis of wood anatomical traits (*chapter 4.5*).

Regarding the year 2003, the NDVI values reduced by 50%, show that the plants decreased their photosynthetic activity, probably due to the water stress, as confirmed by the corresponding low SPEI values. This stress was not mirrored by the wood anatomical traits, with the exception of MLA, but the decrease occurred one year after, in 2004. This result is likely related to the favourable growth conditions in 2002 and the favourable weather conditions in autumn/winter 2002 and in early summer 2003. Unfavourable starting conditions occur, however, if the conditions during the previous growing season limited growth: the summer drought in 2003 resulted in lower growth rate in 2004 even though weather conditions in 2004 were favourable, in line with van der Werf et al., 2007.

Analysing the 2018 drought, no large difference was revealed by the NDVI values, while wood anatomical traits revealed a decrease mainly in 2017, MLA apart. This is probably due to another drought occurred in summer 2017 (D'Andrea et al., 2020). A plausible explanation for the fact that most of the anatomical traits were more affected by the 2017 drought than the 2018 drought may be the low amount of precipitation throughout the year, as also shown by the lower SPEI values in 2017 compared to 2018.

Usually, MLA displayed the highest sensitivity to drought and this may be explained by the tight link to water availability, in fact vessel size is controlled by water supply, because turgor pressure, as the driving force of cell expansion, is largely affected by water availability (Tyree & Sperry, 1989). Nonetheless, in our study, we could not confirm any significant link between precipitation (or other weather conditions) and conduit characteristics in beech (Prislan et al., 2018).

A low sensitivity to drought emerged, as demonstrated by the absence of large differences in the values of anatomical traits between the years of the two periods considered and the lack of significant influence of the monthly SPEI on anatomical parameters (*Fig. 20*). Indeed, the two Walter-Lieth diagrams of 2003 and 2018 (*Fig. 19*) revealed that a dry period occurred only in July of the 2003 and no dry occurred in 2018. This is in line with Piovesan et al. 2005 and Di Filippo et al. 2007 who claimed that the boundary between the two bioclimatic zones of Apennines and Alps is across the Northern Apennines and corresponds to the limit of summer drought control over growth variability.

These results suggest that beech in the studied area is not yet affected by rising temperature trend and drought, confirming Monte Giovarello as still an optimal area for the growth of the species.

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