

Dendroecology of *Pinus halepensis* in the green barrier of Algeria: Quarantining the Sahara desert



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Master thesis:

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Preface

This research dissertation was conducted in the University of Lleida- School of Agrifood and Forestry Science and Engineering and is considered as prerequisite to graduate from the European Erasmus Mundus Master (Medfor) offered by a Consortium that includes seven Universities in which Lleida University is a leading part. The research depicted herein was conducted under supervision of Professor Jordi Voltas Velasco, between February and July 2015, and we have done our best to fulfill the objectives of this study.

This thesis is to the best of my knowledge original, except references that were made from previous works.

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Abstract

This investigation aimed to assess the main climatic factors driving the radial growth and ecophysiological performance of *Pinus halepensis* Mill. growing in the green barrier of Algeria (Djelfa province, Sahara) at three sampling sites differing in exposure over the twentieth century. To accomplish this purpose, we applied a dendroecological approach using ring-width chronologies comprising the last 112 years and annual time series of carbon isotope discrimination ($\Delta^{13}\text{C}$) for the last 90 years, and we characterized climate-growth and climate- $\Delta^{13}\text{C}$ relationships. The relationships between climate factors and either radial growth (tree-ring width indices, TRWi) or carbon isotope discrimination were evaluated by bootstrap correlations. Inter-series comparisons showed a good agreement among trees growing at each site and, thus, strong common signals in both tree-ring proxies, suggesting that they hold relevant environmental information. The results revealed negative correlations between TRWi and temperatures in autumn of the previous year, spring and summer. Conversely, a rainy October of the preceding year and a rainy late winter-spring showed a strong positive correlation with tree growth. Thus, we can confirm that low precipitation and high temperatures were limiting factors for most growing season. SPEI drought index depicted similar relationships with TRWi as rainfall, indicating that SPEI variability is dominated by precipitation. Interestingly, $\Delta^{13}\text{C}$ was mainly influenced by the increasing temperature trend over the study period at the site and inter-site levels for almost all months of the year, and especially in summer, as shown by significant negative correlations between both factors. This is interpreted as an effect of temperature increasing drought stress through increased evapotranspiration, which means an increase of water-use efficiency over time. After detrending of $\Delta^{13}\text{C}$ chronologies (i.e. elimination of the low-frequency temperature trend), precipitation and SPEI appeared to also influence the isotope carbon discrimination records, as inferred by positive correlations with October of the previous year and spring rainfall. The results of both proxies demonstrate that the main common signal recorded in tree rings is drought, but $\Delta^{13}\text{C}$ values were extremely sensitive to temperature, which translated into important increases in water-use efficiency. As expected, *P.halepensis* growth and carbon isotope discrimination were strongly dependent on water availability in Djelfa, but the important decrease in $\Delta^{13}\text{C}$ values over time suggests a forced physiological response of the trees that may indicate limited responsiveness to future global warming.

Key words: Dendroecology, tree-ring width, carbon isotope discrimination ($\Delta^{13}\text{C}$), *Pinus halepensis*.

1. Introduction

1.1. Climate change and Mediterranean areas

Current and predicted patterns of global climate change are an important concern in many socio-economic activities, such as agriculture, forestry, food and energy security, etc., and also a major threat for biodiversity and ecosystem function. The effects of climate change have become particularly evident over the last 30 years in natural environments and are affecting all life forms: from the individual, population, species, community and ecosystem to the eco-regional level (Lepetz et al., 2009).

Climate change is especially rapid and extreme in the Mediterranean basin (Thuiller et al., 2005; Giorgi and Lionell., 2008; Lindner et al., 2010). This is especially problematic because of its importance as biodiversity hot-spot and its geo-strategic position in the world. Climate models project an elevation of air temperatures and a decrease in annual mean precipitation over the Mediterranean Basin for the twenty-first century, and therefore a decrease in water availability due to increased evapotranspiration (Parry et al., 2007). Results of some projections indicate that temperatures over the region could rise by about 3.5°C between now and the latter half of the 21st century in response to a doubling of carbon dioxide (or its equivalent) (Wigley, 1992). In addition to a precipitation decrease, annual rainfall patterns have also changed or are expected to change, including episodes rain intensity (De Luis et al., 2001). Altogether, this scenario may have a strong effect on forest growth, because trees are adapted to thrive in a given climate and water regime. But climate change is also decreasing the availability of water resources in the Mediterranean region, leading to plant water stress, as it is projected in figure 1.

The North African countries, with semiarid and arid desert climates, are especially vulnerable to climate change. Model projections available for the region indicate a clear increase in temperature over the next 20 years that is expected to continue throughout the 21st century, probably at a rate higher than the estimated global average (Agoumi, 2003). A new reconstruction of the past climate of the Maghreb has inferred that 20th century was the driest in North Africa since the severe droughts occurring in the 13th and 16th centuries (Touchan et al.,

2010). The projections also point to a decrease of 4% to 27% in annual rainfall (PNUE, 2008). Therefore, water deficit, which represents a major risk for the region, will be worsened by an increase in evaporation rates. Indeed, water scarcity will be one of the most critical problems to be faced by North African countries in the future. For instance, it is predicted that Morocco and Algeria's water resources will decline by 10-15% in 2020, Tunisia's water resources will drop by 28% in 2030, and most Egyptian people (75%) will have less than adequate fresh water by the same year (Paeth et al, 2009).

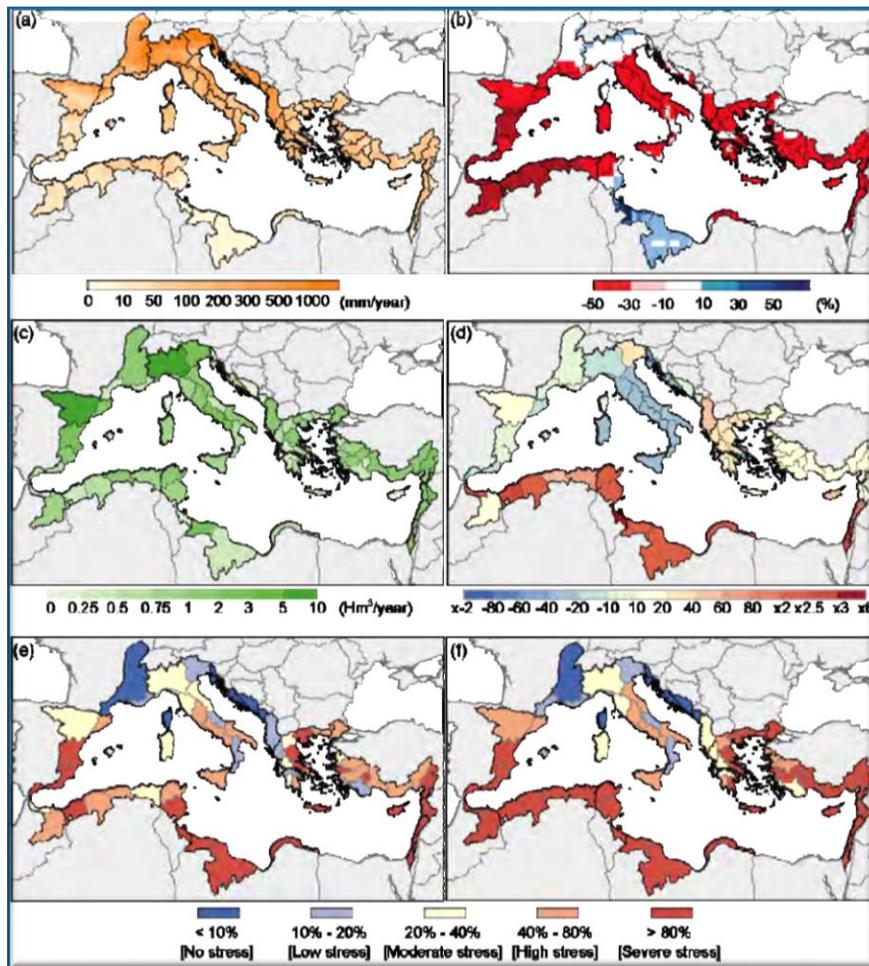


Figure 1. Changes in water use characteristics in the Mediterranean Basin in the context of global change. a) Available water resources for the period 1971–1990; b) Projected available water resources in 2050; c) State of groundwater extraction for the period 2001–2009; d) Projected state of groundwater extraction in 2050; e) Current water stress; f) Projected water stress in 2050 due to climatic and anthropogenic changes; <10% = no stress; 10–20% = low stress; 20–40% = moderate stress; 40–80% = high stress; >80% = severe stress.

However, some recent studies on vulnerability of terrestrial ecosystems to climate changes show that most vulnerable regions have also the potential capacity for adaptation (Gładysz and Claudia, 2009). The responses of forest systems to extreme climate events and longer-term changes in climate patterns have been increasingly studied in order to (among others) develop strategies for adaptive management. Moreover, the investigation of the consequences of past climate on forest species represents one of the most important ways to validate models of the evolutionary consequences of future changes in climate (Petit et al., 2005).

1.2. Ecological and climatological features of the study area

The Algerian forests are divided into three great geographical areas: chains of the Tellian Atlas in the North, Saharan Atlas in the South, and the zone of the Higher-plateaus that separate them. Algerian forests are distributed especially in the northern part of the country where the climate conditions are more favorable for growth. In contrast, the study area of this work is situated in the Saharan Atlas, characterized by an arid, Saharan bioclimate marked by long periods of aridity with great thermal variations. Although the total Algerian forests account for only 2% of the country's land, it is the only ecological and floristic barrier that protects from the advance of the Sahara desert (EFI, 1992).

In fact, in the early 1970's the reforestation project known as 'green barrier' was launched to prevent the northward spread of the Sahara, being perhaps the most ambitious agro-ecological project of Algeria in the seventies following the post-independence euphoria. This "green Dam" was aimed at protecting against desertification by creating a true barrier of greenness, which would protect the steppe-Mediterranean vegetation from the Moroccan border to the Tunisian one.

1.3. Dendroecology and dendroclimatology

In 1963, Vinš suggested for the first time the concept of dendroecology to extend the science of dendrochronology and dendroclimatology to the field of forest ecology (Serre-Bachet and Tessier, 1989). This definition was used to apply the information gained from natural forest and derived from tree rings to assess the forest responses to common climatic or environmental factors.

At the end of 1980s, Fritts and Swetnam (1986) redefined this concept with a slight difference, as “a tool for evaluating variations in past and present forest environments”.

Closely related to dendroecology, Kaennel and Schweingruber (1995) defined dendroclimatology as the use of annual tree rings properties to study and reconstruct past and present climate. Dendroclimatology starts with site and tree selection and continues with dating,, measuring, data quality control, and chronology construction. Tree rings are related to climate using statistical models that are then assessed for their full length to retrospect past climate (Sheppard, 2010).

Generally, dendroclimatology assumes that relationships between tree growth and climate are age and size independent. However, there is obviousness that climate responses can be unstable across different species, sites and age (Tardif et al., 2003; Campelo et al., 2013). Also, the effect of climatic factors on tree growth can change over time, as it has been shown in previous studies performed in Mediterranean area (Andreu et al., 2007).

1.4. Study background (North Africa)

Mediterranean ecosystems have been rarely studied for dendroecology and dendroclimatology (Bachet, 1985; Cherubini et al., 2003), and most of works have been conducted in high elevation areas when the climatic signal is clear and depending mainly on temperatures variations (Chbouki et al., 1995). In North Africa, dendroclimatic studies are also rare, except for Morocco, which has a rich history of dendroclimatic studies going back 40 years (e.g. Berger et al., 1979; Chbouki et al., 1995; De Corte,1979; Esper et al., 2007; Glueck and Stockton, 2001; Helleputte, 1976; Meko, 1985; Munaut, 1978; Stockton, 1985; Till, 1997; Till and Guiot, 1990).

The main reason of this deficiency is related to the inability, in many cases, to identify and date tree annual rings unequivocally (Cherubini et al., 2003). Moreover, in Mediterranean regions the cambial activity stops on winter with low temperatures, but also in hot, dry summer, coincident with the period of maximum drought stress. This effect is called Mediterranean ‘double-stress’, which induces the occurrence of false or double rings (Susmel et al., 1976; Mitrakos, 1980; Terradas and Savel, 1992).

Recently, however, a tree-ring chronology of *Pinus halepensis* from northwestern Tunisia has been studied to develop the first dendroclimatic reconstruction of precipitation in Tunisia

(spanning 230 years), which is a valuable tool for water resources planning (Touchan et al., 2008). Furthermore, a systematic dendroclimatic sampling campaign has been conducted for the first time in western North Africa (Morocco, Algeria, and Tunisia) comprising 39 sites, resulting in a completely new tree-ring network for drought reconstruction (PDSI drought index). The result of this reconstruction has revealed large-scale regional droughts previous to the sixteenth century and more heterogeneous drought patterns in the sixteenth, eighteenth and twentieth centuries (Touchan et al., 2010).

1.5. Pines in the Mediterranean area

Pines are native, widespread and long-lived tree species of the Mediterranean basin (Barbero et al., 1998) where they have keystone ecological and economical roles.

Several studies on Mediterranean pines have demonstrated clear relationships between climatic trends and tree growth. In southern Portugal, *Pinus pinea* radial growth is positively correlated with precipitation (Campelo et al., 2007). The same result has been stated for *Pinus pinaster* in central Portugal (Vieira et al., 2009) and in central Spain (Bogino and Bravo, 2008). However, *Pinus halepensis* growth is sensitive mainly to temperature variations during the wet season and to soil humidity variations during the dry season in southern Italy (Attolini et al., 1990). In France, *Pinus halepensis* growth is regulated by soil water availability during the growing season (Rathgeber et al., 2005), while in Greece it is positively related with winter and spring precipitations and negatively with the temperatures of the spring months (Papadopoulos et al., 2008).

Most pines are isohydric species, able to reduce stomatal conductance during low water availability periods, hence reducing carbon uptake (growth) and maintaining relatively constant leaf water potential regardless of drought intensity (McDowell et al., 2008). *Pinus halepensis* (the species studied in the present work) is a clear example of this general performance, being a drought-avoiding species as confirmed by the analysis of carbon isotope discrimination in tree rings (Ferrio et al., 2003).

1.6. Tree rings and climate

A cross section of tree stems reveals an alternation of lighter and darker bands, which correspond to seasonal growth increments produced by meristematic tissues in the cambium. Each increment

consists of earlywood (a light band of spring season) and latewood (a dark band produced in autumn); jointly, they form the tree ring. The different types of wood formed during the early and late growing seasons have very different hydraulic properties. Early wood in conifers is specialized for conducting water, whereas latewood provides mechanical stability and a large amount of stored water; therefore, a higher latewood proportion may increase resistance to drought (Domec and Gartner, 2002; Martinez-Meier et al., 2008).

Tree rings have been widely used to study climate-growth relations based on the fact that each ring records information of past environmental factors patterns that influence tree growth on an annual basis, assuming that trees growing in the same area are influenced in a similar way by climatic variability (Fritts, 2001; Vaganov et al., 2006). Particularly, tree rings have been used to retrace past climate variations (e.g. Grudd et al., 2002; Helama et al., 2002, 2009).

1.7. Carbon isotopes theory

In the biosphere there are two stable isotopes of carbon which are present in natural abundances of about 98.9% (^{12}C) and 1.1% (^{13}C). Tree-ring stable carbon isotope composition (that is, the ratio of ^{13}C to ^{12}C expressed relative to the PDB standard) is used to infer water-use efficiency and reconstruct past ecophysiological performance of trees in relation to their carbon and water budgets. The carbon isotope composition ($\delta^{13}\text{C}$) of plant tissues is lower as compared to $\delta^{13}\text{C}$ in the atmosphere (in which carbon is present in the form of CO_2), indicating that plants discriminate against ^{13}C during photosynthesis. Variation in discrimination against ^{13}C during photosynthesis in C3 plants is due to both diffusion (through stomata) and enzymatic processes (RuBisCO) (Cregg and Zhang, 2000). Indeed, if RuBisCO is not limited by intercellular CO_2 discrimination occurs mostly during the carboxylation step. In contrast, when stomatal conductance is reduced, CO_2 flux is limited and intercellular CO_2 pressure is significantly lower than that of air CO_2 . Therefore, photosynthesis is strongly limited by stomatal conductance.

To estimate carbon isotope discrimination ($\Delta^{13}\text{C}$), we should eliminate the effect of the general atmospheric decline in the heavier isotope ^{13}C due to land use change and the effects of burning fossil fuel, which is reflected in the carbon isotope composition of tree rings (Farquhar et al., 1982). $\Delta^{13}\text{C}$ has the advantage to directly express the consequences of biological processes, while $\delta^{13}\text{C}$ of the plant is the result of both source isotopic composition and carbon isotope discrimination. In fact, carbon isotope discrimination has become a useful tool to understand

photosynthesis and its coordination with water use in physiological studies of C3 species (Farquhar et al., 1989).

1.8. Objective and research questions

The main objective of this master thesis is to identify the main climatic factors driving the radial growth and ecophysiological performance of *Pinus halepensis*.Mill. and characterize climate-growth relationship over the twentieth century for this species in the green barrier of Algeria (Sahara), using a dendroecological approach (ring-width and carbon isotope composition data).

To this end we attempt to answer the following research questions:

- i) What is the impact of climate conditions on the growth dynamics of *Pinus halepensis* thriving in the southernmost edge of its distribution range (Sahara north border in Algeria)?
- ii) How climate factors influence the carbon isotope discrimination (a surrogate for water-use efficiency) of tree rings for this species?
- iii) What is the change in carbon isotope discrimination over the last century and its relationship with radial growth?
- iv) What is the expected performance of *Pinus halepensis* in the future context of climate change?

2. Material and methods

2.1. Ethics Statement

Sampling sites were located in national forests estate (Senalba forest), and the authorization for field sampling was delivered by the Directorate General Forests of Djelfa province (Algeria).

2.2. Study area

The study area (Senalba forest) is located in the Djelfa province, part of Algerian highlands (Figure2), about 300 km south of Alger (north border of the Sahara).

According to the Forests Conservation Department, the total forest area in the region is estimated at 214,117 ha, which represents 6.63% of the total land area (Derouech and Gahdab, 2014).

The area is characterized by natural forest and reforestation of Aleppo pine, without any management practice.

The soil type in Senalba forest is marl limestone, (Pouget, 1980) with a dominant sandy texture, once deprived of herbaceous cover, is subject to severe erosion (in addition to overgrazing as second cause of land degradation) (Zegrar, 2015).

The climate of Djelfa area is Mediterranean continental of xerothermic trend with a hot dry summer and a variably wet and cold winter, with an annual mean temperature of 14.6 °C and an annual mean rainfall of 360 mm for the period 1901-2013(CRU database (Harris et al., 2014)). Three *P. halepensis* stands were selected based on their different exposure (Figure 2, Table 1). The dominant trees at all sampling sites include *Pinus halepensis*, *Quercus ilex*, *Juniperus oxycedrus* and *Juniperus phoenicea*.

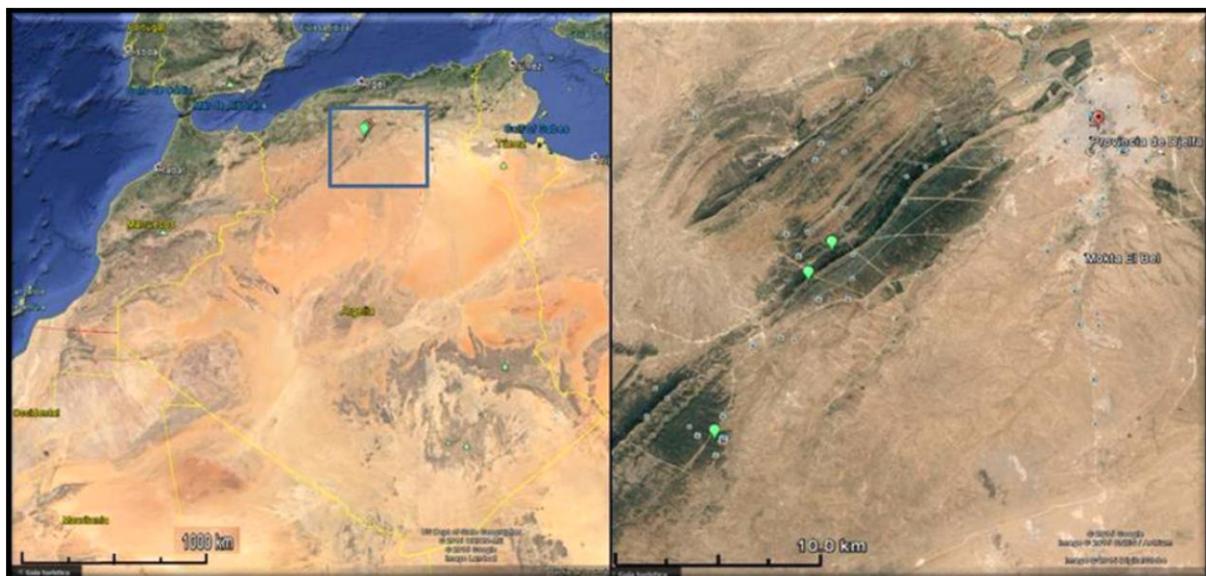


Figure 2. Location of sampling sites (Google earth Pro).

Table 1. Sampling sites description

Site N°	Site	Altitude(m)	Latitude(N)	Longitude(E)	Aspect	No.trees	Chronology time span	Diameter at 1.30m±SD	EPS≥0.85 since	Mean sensitivity
1	Toughersan	1359	34°32'0.93"	3° 02'14.56"	Flat without clear aspect	11	1850-2013	0.24±0.04	1928	0.3240
2	Theniat Enser	1379	34° 36' 21.79"	3° 05'37.89"	South	5	1870-2013	0.23±0.037	1921	0.3873
3	Theniat Enser	1360	34° 37' 10.79"	3° 06'27.19"	North	5	1846-2013	0.22±0.019	1906	0.3312

2.3. Sampling and sample preparation for tree-ring chronology construction

Tree cores were sampled with an increment borer (5 mm diameter) at 1.30 m from 5 to 11 dominant trees per site (Table 2). Two increment cores were extracted from each selected tree at a height distance of about 5 cm within the trunk following the same direction. A total of 21 cores of *Pinus halepensis* were used for tree-ring width analysis (Table 2) The surface of the cores was prepared using a microtome to enhance the visibility of the ring boundaries and to obtain a smooth cross-section, and then we scanned each core, identified the tree rings and dated them with WinDENDRO (image analysis system EPSON Perfection V700). Tree rings were also identified under a binocular microscope coupled to a computer (LINTAB) and dated using the cross dating method following classical procedures (Fritts, 1976; Schweingruber, 1996).

Afterwards, we used the COFECHA program (Holms, 1983), to test the quality of cross-dating and measurement accuracy of the tree ring series. This program calculates the Pearson correlation coefficients between the indexed tree-ring series and a master reference chronology, ensuring high degree of confidence that tree rings have been correctly cross-dated (Grissino-Mayer, 2001).

For the standardization of the tree ring series we applied the ARSTAN program to remove the long-term trend from each time series of ring-width measurements. We fitted a spline curve and calculated the tree-ring width index chronology for each site defined as the actual ring-width for each year divided by the curve-fit value (detrending by a 67% cubic smoothing spline with a 50% cutoff frequency). This procedure allows eliminating non-climatic trends, reducing the

growth variations that are not common to most trees and also the effects of stand dynamics (Cook and Kairiukstis, 1990). Additionally, an autoregressive model was applied to each detrended series to remove the persistence of non-climatic trends. From the chronology indices of each stand, a mean time-series of indices was constructed from 1850-2013 (Site 1), 1870-2013 (Site 2), and 1846-2013 (Site 3). The master chronologies were examined separately to analyze differences in tree growth between sites.

2.4. Carbon isotopes analysis ($^{13}\text{C}/^{12}\text{C}$)

The stable isotope analysis was performed on tree rings with annual resolution for the period 1925–2013 in order to avoid a possible juvenile imprint on the isotope values. Cores corresponding to five different trees per site were pooled together on an annual basis and milled to a fine powder to have enough material for the isotopic analysis. However, five particular years (1999, 1983, 1971, 1951 and 1940) were milled and analyzed separately to have an estimate of inter-tree variability of the carbon isotope signal.

The isotopic analyses were conducted in the Stable Isotope Facility Lab (Davis Campus, University of California). For carrying out carbon isotope composition ($\delta^{13}\text{C}$) analysis, an aliquot of 0.9-1.1 mg of the processed material (grinded wood), was weighed and encapsulated into tin (Sn) capsules for mass spectrometry analysis; the samples underwent combustion in an oxygen atmosphere (EA-IRMS: elemental analyzer isotope ratio mass spectrometer). The $\delta^{13}\text{C}$ values are defined as:

$$\delta^{13}\text{C} = [(\text{R}_{\text{sample}} / \text{R}_{\text{standard}}) - 1] \times 1000 \text{ (in ‰)}$$

where $\text{R} = ^{13}\text{C}/^{12}\text{C}$.

To eliminate the effect of the general atmospheric decline in the heavier isotope ^{13}C due to land use change and the effects of burning fossil fuel, which translates into the carbon isotope composition of tree rings, we calculated carbon isotopic discrimination ($\Delta^{13}\text{C}$) from the following equation (Farquhar et al., 1982):

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{TR}}) / (1 + \delta^{13}\text{C}_{\text{TR}}/1000)$$

where $\delta^{13}\text{C}_a$ and $\delta^{13}\text{C}_{\text{TR}}$ are the isotopic compositions of atmospheric CO_2 and tree-ring, respectively.

Tree-ring $\Delta^{13}\text{C}$ is inversely proportional to water-use efficiency according to the following expression (Farquhar and Richards, 1984; Farquhar et al., 1989b):

$$\text{WUE}_i = \frac{Pa(b - \Delta)}{1.6(b - a)}$$

Where a is the fractionation during binary diffusion in air (4.4 ‰); b is fractionation during carboxylation in C3 plants; and Pa is the atmospheric CO_2 partial pressures. The factor 1.6 denotes the ratio of diffusivities of water vapour and CO_2 in air.

The following statistical parameters were used to compare and evaluate the chronology quality of tree-ring $\Delta^{13}\text{C}$ and ring-width series using mixed models (JMP Pro 11 software) to estimate variance components:

- Mean inter-series correlation (r): it reflects the signal strength of a set of series of tree rings for a particular site
- Mean inter-chronology correlation (\hat{a}): it reflects the signal strength (or synchrony) of a set of chronologies of tree rings:

$$\hat{a} = \frac{\sigma^2_y}{\sigma^2_y + \sigma^2_e}$$

where σ^2_y is the variance of year effects and σ^2_e is the error variance.

- Expressed population signal (EPS): the degree to which the particular sample chronology portrays a hypothetically perfect chronology. Values higher than 0.85 imply that the chronology is representative of tree growth dynamics at the stand level (Wigley et al., 1984):

$$\text{EPS} \approx \frac{N\hat{a}}{1 + (N - 1) \times \hat{a}}$$

where N is the number of chronologies (trees) and \hat{a} is the mean interseries correlation.

- Signal-to-noise ratio (SNR): the proportion of the variability explained by climate or other causal factors divided by the residual or unexplained variability (Fritts and Swetnam, 1989):

$$\text{EPS}(1 + \text{SNR}) = \text{SNR}$$

2.5. Climatic data

The main climatic dataset used in this study included monthly mean temperature (T_{mean}), precipitation and drought index SPEI values (Standardized Precipitation Evapotranspiration Index). The dataset covered the period 1901-2013 and was obtained from high-resolution 0.5° gridded climate records (CRU TS3.22; Harris et al., 2014) for the region defined by grid coordinates 34.5°-35°N, 3-3.5°E.

2.6. Data analysis

The relationships between monthly climatic variables (T_{mean} , SPEI, and precipitation) and ring-width indices were investigated from September of the previous year to October of the current year for the period 1901-2013. These relationships were first assessed separately for each of the three sites by bootstrap correlations using the program DendroClim2002. A moving correlation analysis using 30 years intervals was applied for the months that showed significant climate correlations for the whole study period ($P < 0.05$) in order to test for consistency of climate-growth relationship through time. The statistical significance of correlations and moving correlations was evaluated with a bootstrap procedure.

Similarly to ring-width indices, the carbon isotope discrimination chronologies were constructed and for each of three sites, and then the climate - $\Delta^{13}\text{C}$ relationship we investigated for both annual mean and monthly climatic variable, applying respectively linear regression and bootstrap correlation ($p < 0.05$).

3. Results

3.1. Local climate data

The climate of the area shows a warming trend for the period 1901–2013 which can be quantified as an increase in 0.16 °C per decade (Figure 3a). The temperature increase is coupled with a reduction in precipitation for the last 30 years approximately (Figure 3b). Altogether, this translates into an increased frequency and intensity of dry seasons in recent years. Values for the Standardised Precipitation-Evapotranspiration Index (SPEI) are shown in Figure 3c. These are derived from values of precipitation and temperature, both of which can contribute to available soil moisture and, thus, to the water stress experienced by plants. SPEI values close to zero are considered “normal” moisture conditions, while more negative values mean the occurrence of drought conditions. In the study area we observe a long drought period at around the turn of the century but, in contrast, relatively wet conditions during the last decade.

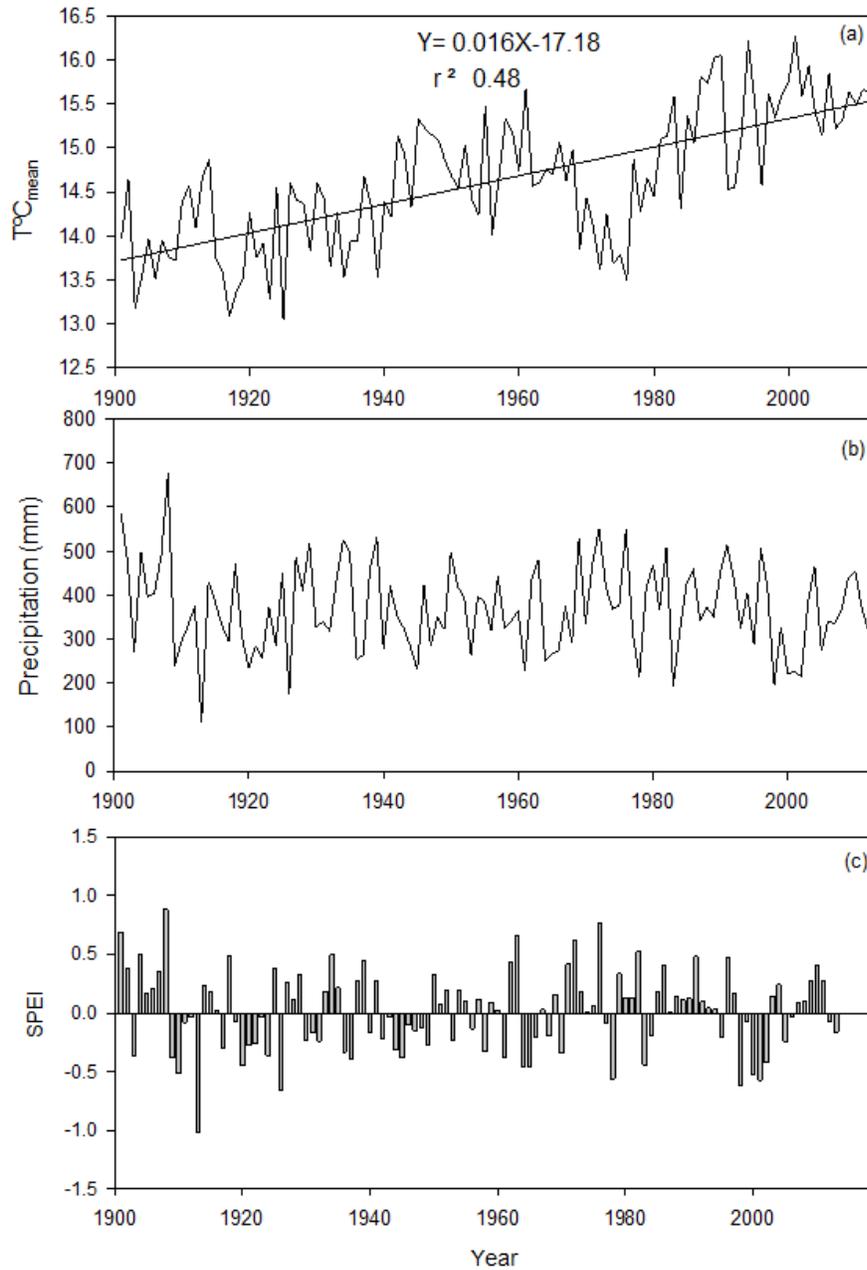


Figure 3. Temporal evolution of climate factors during the period 1901-2013. a) Mean annual temperature ($^{\circ}C$), b) Mean annual precipitation (mm) and c) Drought index, SPEI.

The climograph of Figure 4 summarizes the climate in Djelfa, depicting monthly precipitation (graph bar) and mean monthly temperature (graph line). The total annual precipitation is 360 mm

mm, while the mean annual temperature is 14.6 °C. The maximum mean temperature is reached in July and August (about 25.8 °C), while the lowest temperature is about 5°C in January. Concerning precipitation, it is mainly present in winter and spring, while summer is very dry; the highest rainfall is found in January (about 40 mm). Therefore, we can conclude that Djelfa climate is characterized by cold, wet winters and long, hot and dry summers, with drought stress spanning four months, from June to September.

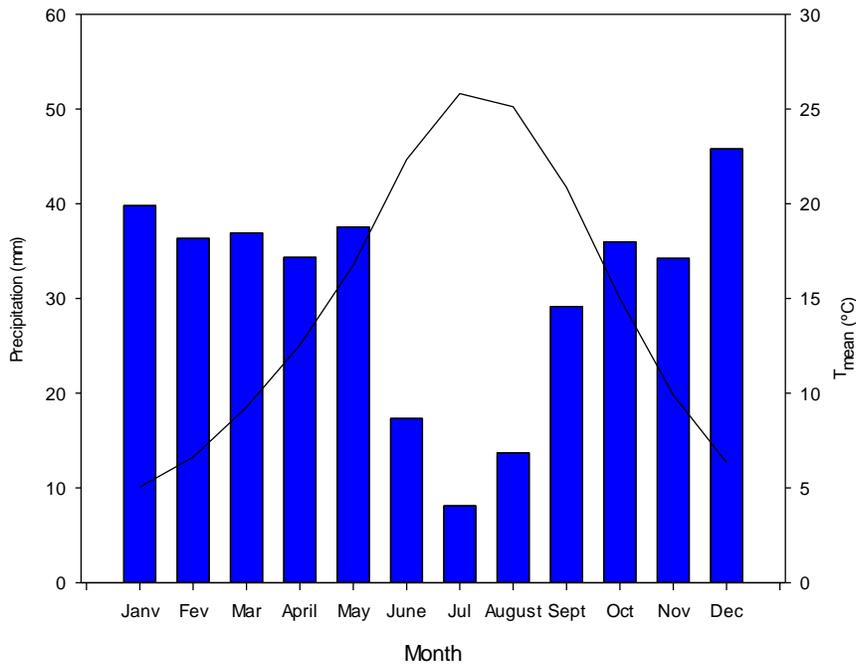


Figure 4. Climograph of Djelfa (average records for the period 1901-2013). Source: CRU dataset (Harris et al., 2014).

3.2. Ring-width chronologies

The master chronologies for ring-width of *Pinus halepensis* are shown in Figure 5 for each sampling site.

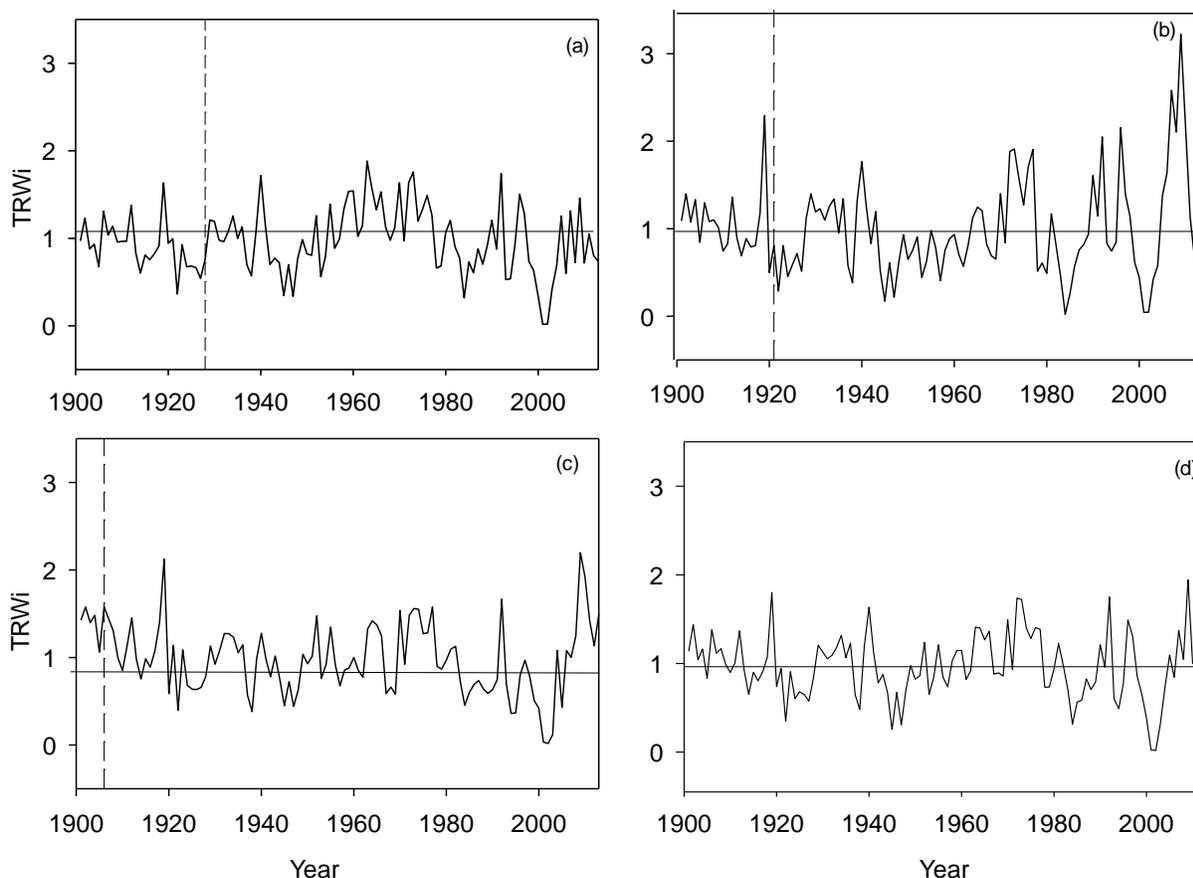


Figure 5. Standardized site chronologies for tree-ring width indices (TRWi) of *Pinus halepensis* spanning the period 1901-2013. a) Site 1; b) Site 2; c) Site 3 and d) Master regional chronology common to all sites. Dashed vertical lines indicate the first year corresponding to $EPS \geq 0.85$.

The length of the master chronologies spans for the last 112 years for the three sites, although the exact year with EPS (“expressed population signal”) reaching 0.85 differs between sites (Figure 5). A visual inspection of the tree-ring chronologies suggest a clear dependence on annual precipitation (Figure 3b); for instance, in the period 1999-2002, a clearly reduced tree growth occurred in all three sites, which is related to a decrease in precipitation in this period (from 326 mm in 1999 to 214 mm in 2002).

Based on the statistics commonly used to assess chronology quality, the three ring-width chronologies are of good quality for most of the twentieth century, having EPS values equal or above 0.85 (Table 2), the critical value for inter-series agreement proposed by Wigley et al (1984). This implies that the site chronologies presumably bear a strong climate signal. The

mean correlation at each site represents the strength of the common signal among trees and ranged from 0.46 to 0.60 (Table 2).

Table 1. Descriptive statistics of chronologies for ring-width indices (TRWi) and carbon isotope discrimination ($\Delta^{13}\text{C}$) of *Pinus halepensis* at three sites in Djelfa. Results for the combined (regional) master chronology are also included. Abbreviations: EPS, expressed population signal; Rbar, mean interseries correlation; SNR, signal.

	TRWi				N	$\Delta^{13}\text{C}$		
	No. cores	<i>Rbar</i>	<i>SNR</i>	<i>EPS</i>		<i>Rbar</i>	<i>SNR</i>	<i>EPS</i> (1925-2013)
Site 1	11	0.47	4.55	0.91	5	0.49	4.88	0.83
Site 2	5	0.60	7.33	0.88	5	0.57	6.70	0.87
Site 3	5	0.53	5.66	0.85	5	0.90	49.00	0.98
Combined	21	0.46	19.00	0.95	3	0.72	8.08	0.89

Figure 6 depicts long term radial growth (mm) fluctuations for individual trees and the mean radial growth for each site. If the first years of cambial age are omitted to minimize juvenile effects on growth patterns (i.e. considering the period with $\text{EPS} \geq 0.85$), the trees showed a tendency to decrease their radial growth; significant (95%) for site1 and site 3 with a quite consistent pattern of fluctuations over time.

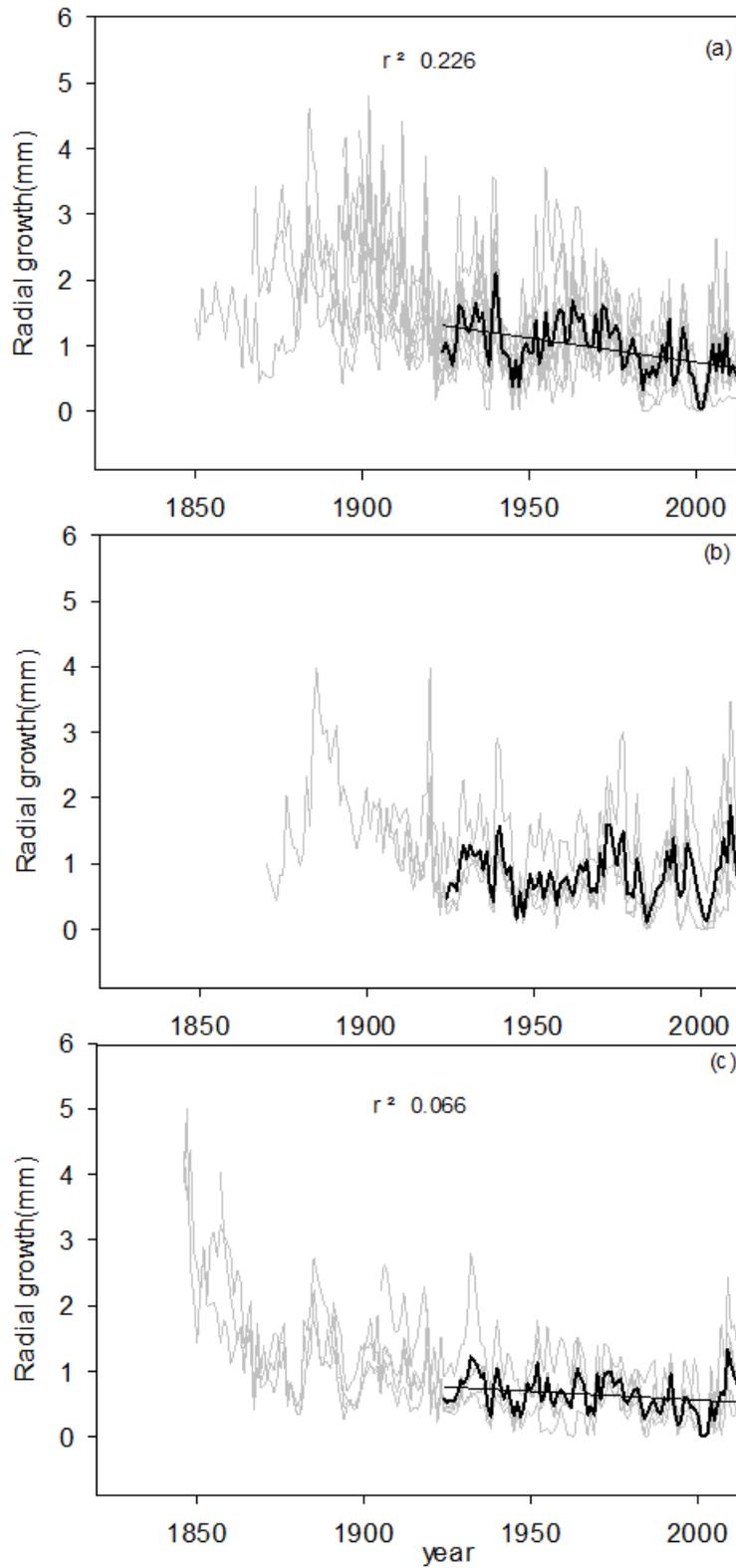


Figure 6. Raw tree-ring width series. The black lines show mean values for the period in which $EPS \geq 0.85$ and grey lines shows individual trees at the site level. a) Site 1; b) Site 2; c) Site 3.

Similarly to ring-width chronologies, the raw $\Delta^{13}\text{C}$ chronologies of the three sites are of overall good quality (Table 2), although the number of replicates was lower (5 replicates for selected years) with a shorter time span (starting in 1925). In particular, we observed high values of EPS and a consistent strong common signal as indicated by high mean inter-series correlations (R_{bar}) ranging from 0.49 (Site 1) to 0.90 (Site 3).

The three $\Delta^{13}\text{C}$ series, corresponding to each of the three study sites, showed a pronounced decreasing trend through time, suggesting a common external driver controlling the balance between carbon uptake and water losses (Figure 7). Indeed, the decreasing trend over time suggests enhanced water-use efficiency. This long-term trend is beyond the actual effect of increasing atmospheric CO_2 on improved water-use efficiency through higher photosynthetic rates and is likely related to the impact of increasing temperatures over time (Figure 3), reducing water availability, promoting stomatal closure and, finally, increasing water-use efficiency. To assess changes in $\Delta^{13}\text{C}$ in the high-frequency domain (i.e. similarly to TRWi), such long-term linear trend was removed from the raw $\Delta^{13}\text{C}$ chronologies (Figure 8).

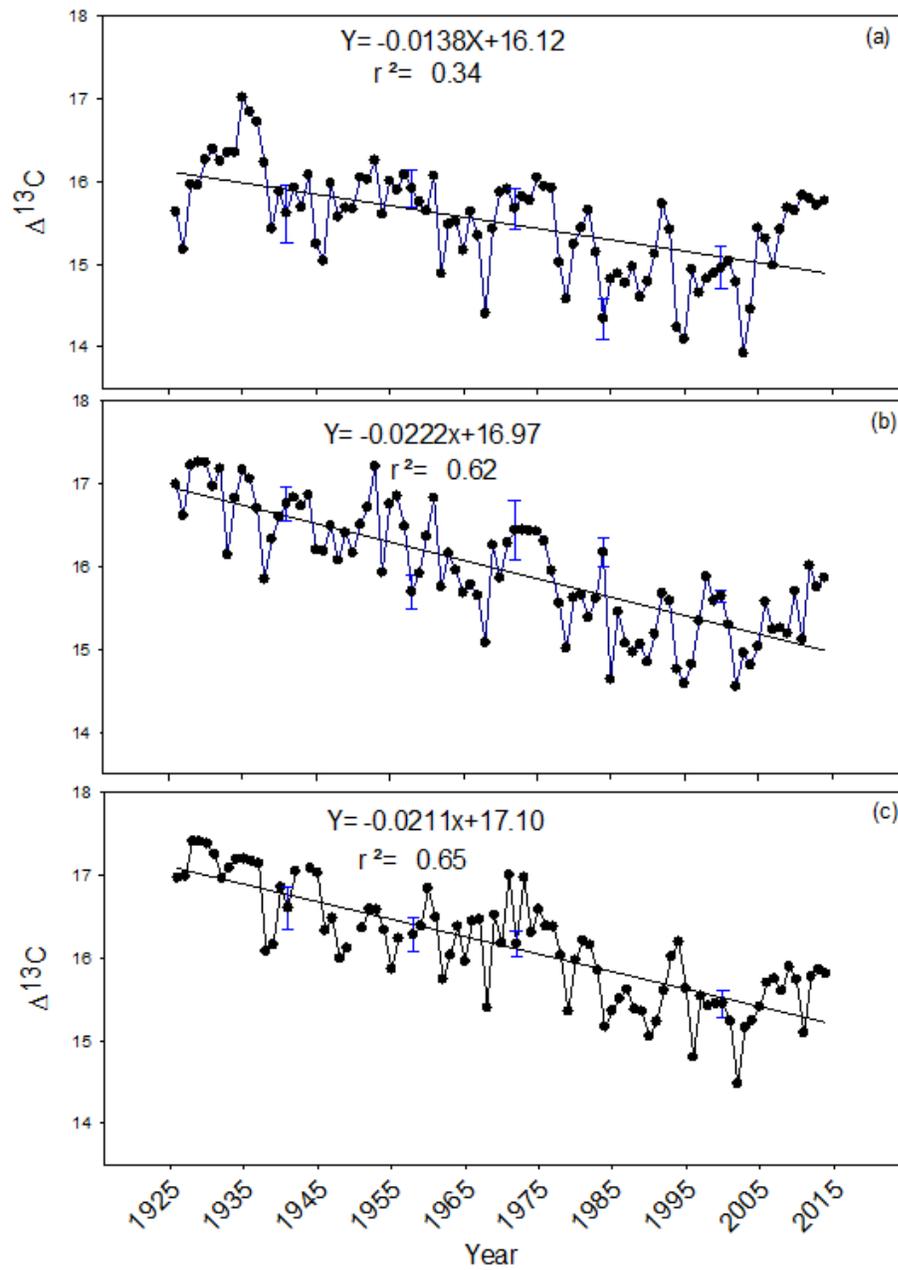


Figure 7. Tree-ring carbon isotope discrimination ($\Delta^{13}\text{C}$) chronologies of the three study sites. a) Site 1; b) Site 2; c) Site 3. Error bars represent the standard error of mean values for particular years.

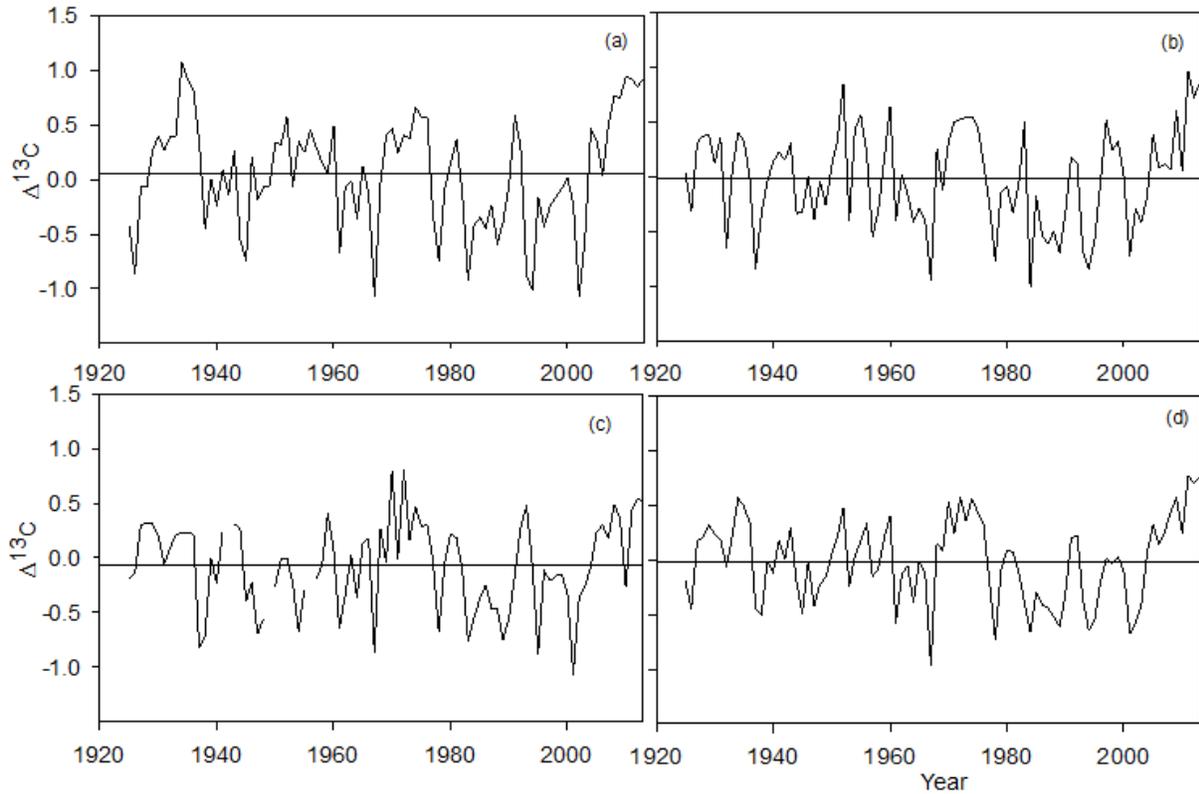


Figure 8. Detrended isotope carbon discrimination ($\Delta^{13}\text{C}$) time series of the study sites and mean isotopic values of the three sites combined. a) Site1; b) Site2; c) Site3; d) Average isotopic series.

3.3. Tree-ring width – climate relationships

The relationship between site chronologies for ring-width and monthly climatic variables is summarized at the site level in Figure 9. The tree-ring width *versus* climate relationships showed overall similar responses for the three sites (i.e. without clearly distinct patterns among sites). Radial growth at the three sites correlated negatively with spring and summer mean temperature (Figure 9a). Significant positive correlations were also observed between tree growth and spring precipitation (March, April, June), and also with October precipitation of the previous year (Figure 9b). Similar relationships were found for drought index (Figure 9c). In order to summarize these results the combined response of the three sites was also examined (Figure 9, right panels). Therefore, we observed a significant positive correlation between tree growth and precipitation in October previous to the growing season and also during late winter, spring and early summer (February, March, April and June). The relationship between temperature and tree-

ring width was negative especially during the summer season, while drought index (SPEI) followed the same pattern as precipitation.

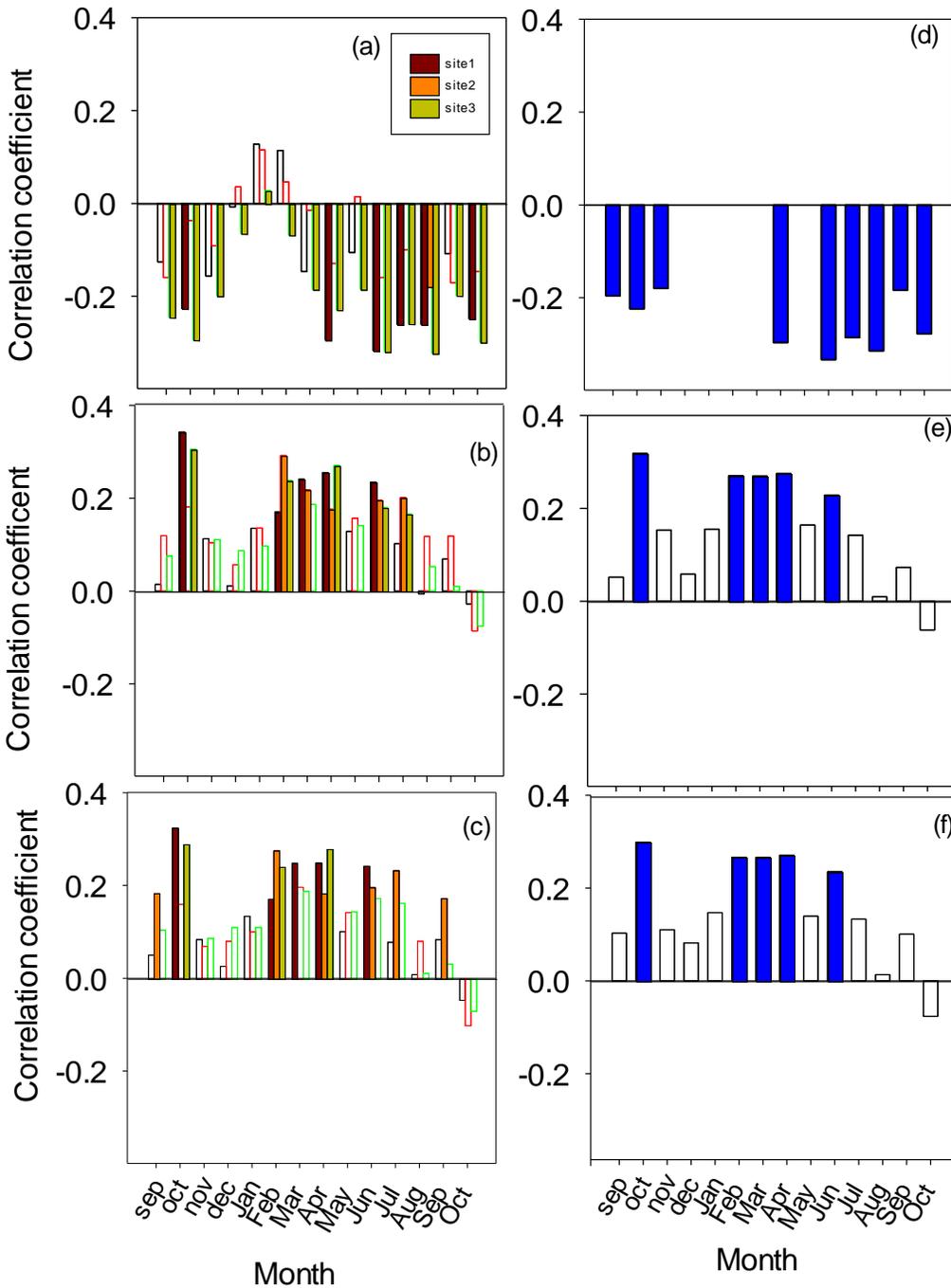


Figure 9. Correlation coefficients between tree-ring width indices and mean monthly climatic factors. Left panels depict relationships at the site level, right panels for the three sites combined. The filled bars refer to significant bootstrapped correlation coefficients (a-d: mean temperature, b-e: precipitation, c-f: drought index (SPEI)).

To investigate the consistency and stability of ring width - climate associations over time, we calculated moving correlations for thirty year periods lagged by five years from 1901 to 2013. All monthly climate variables were inspected (temperature, precipitation and drought index SPEI), but only graphs involving a significant change in the dependency of radial growth from climate factors are shown in Figure 10.

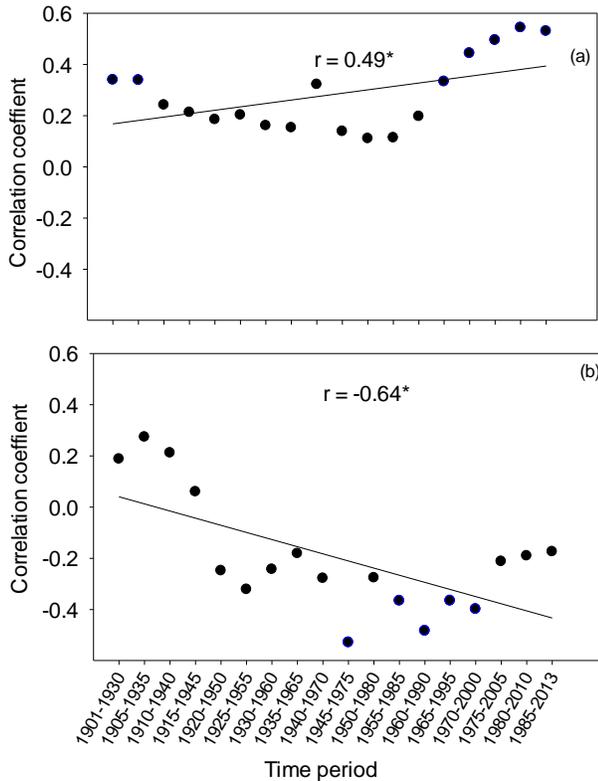


Figure 10. Moving correlations for the relationship between indexed ring-width and relevant climate factors for the period 1901-2013. a) April precipitation; b) September temperature of the previous year. Black circles represent non-significant associations and blue circles show 30-year periods having a significant association between ring-width and climate ($p < 0.05$).

The changing association over time between precipitation and growth is highly significant for April ($r = 0.49$), indicating that radial growth is increasingly dependent on April precipitation during the last decades. Conversely, the mean temperature of the September of the previous year is decreasing importance over time in explaining radial growth ($r = -0.64$) (Figure 10).

3.4. $\Delta^{13}\text{C}$ - climate relationships

Figure 11 shows the correlations between carbon isotope discrimination and climate factors. $\Delta^{13}\text{C}$ displayed significant negative associations with mean temperature throughout all the year at the three sites (left panels), with the highest correlations present in summer: site 1 (July: $r = -0.43$), site 2 (August: $r = -0.46$), site 3 (July: $r = -0.50$). However, there is not any clear association between either precipitation or drought index (SPEI) and carbon isotope discrimination at any of

the three sites, indicating that the dominant climatic signal in the original $\Delta^{13}\text{C}$ chronologies appears to be related to temperature, not rainfall. Hence, we linearly detrended the carbon isotope discrimination series (Figure 8), which are dominated by the long-term effect of increasing mean temperature due to global warming, and used the resulting values for correlation analysis with climate (Figure 11, right panels). Accordingly, mean precipitation and $\Delta^{13}\text{C}$ correlated positively for October of the previous year and also for the spring season (February, March and April). Similarly to precipitation patterns, spring SPEI was significantly positively associated to detrended $\Delta^{13}\text{C}$, with highest correlations observed for April (site 1, $r=0.31$; site 2, $r=0.23$) and February (site 3, $r=0.36$). Overall, the three sites responded similarly, showing similar patterns of reaction to climate; thus, the three $\Delta^{13}\text{C}$ series (for both raw and residual data) were combined, and the climate- carbon isotope discrimination relationships were investigated in the resulting time series (Figure 12).

The results obtained at the inter-site level (Figure 12) mirrored those observed at the site level: mean temperature seems to be the main factor driving changes in carbon isotope discrimination, displaying negative correlations throughout the year. Besides, the detrended $\Delta^{13}\text{C}$ data showed positive associations with precipitation in spring (February: $r=0.26$; March: $r=0.32$ and April: $r=0.25$) (Figure 12e). Also October precipitation of the previous year had a significant positive correlation with detrended $\Delta^{13}\text{C}$ ($r=0.29$). Similarly to rainfall responses, SPEI exhibited a significant positive correlation with spring precipitation and as well with the rainfall of previous October (Figure 12f). This is consistent with the correlations observed between ring-width and October rainfall, and ring-width and October SPEI (Figure 9).

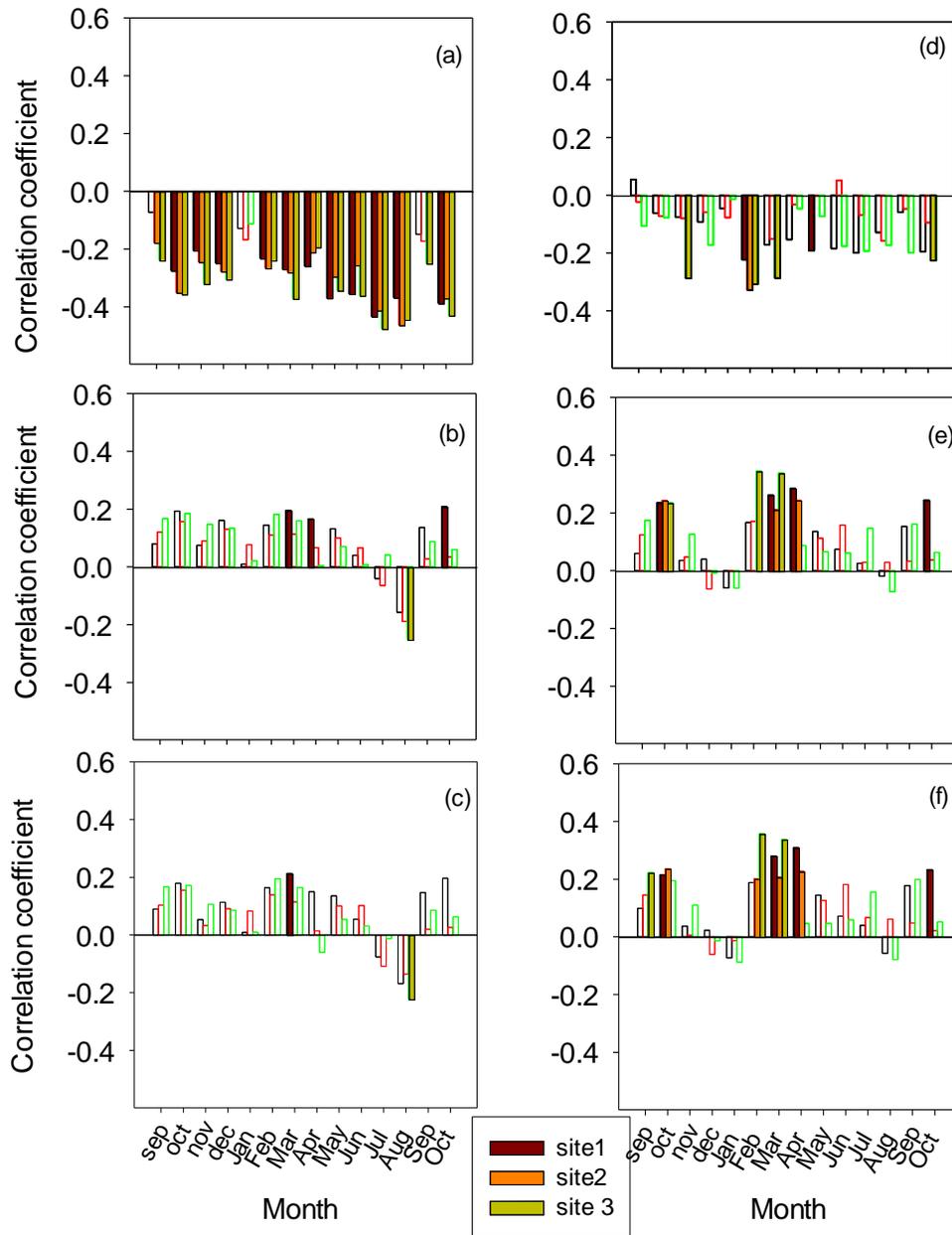


Figure 11. Correlation coefficients (bootstrap correlations) between $\Delta^{13}\text{C}$ and monthly climatic factors. (a,d) temperature; (b,e) precipitation; (c, f) drought index (SPEI). Left panels: raw data; right panels: linearly detrended data. Period 1925-2013. Filled bars indicate significant correlations.

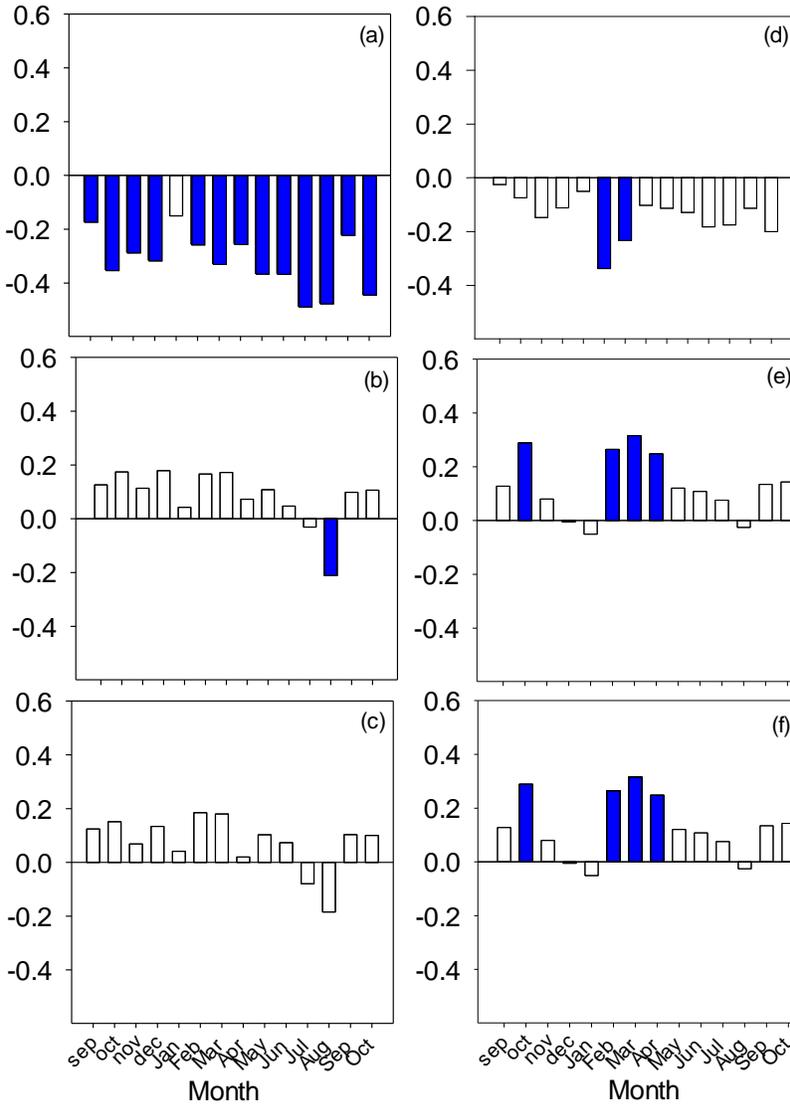


Figure 12. Correlation coefficients between $\Delta^{13}\text{C}$ and monthly climatic factors. (a,d) temperature; (b,e) precipitation; (c, f) drought index (SPEI). Left panels: raw data; right panels: detrended data. Period 1925-2013. Filled bars indicate significant correlations.

3.5. Relationships between tree-ring width and $\Delta^{13}\text{C}$

To assess the relationship between tree-ring width indices and carbon isotope discrimination, we calculated correlation coefficients at the site and inter-site levels (Table 3). For sites 1 and 3, the correlation between raw $\Delta^{13}\text{C}$ and indexed ring-width chronologies was significant ($r= 0.36$ and $r= 0.36$ for site 1 and site 3 respectively) and stronger than for site 2 ($r= 0.07$). The inter-site

correlation was also positive and significant ($r= 0.38$). In order to remove the decreasing linear trend of $\Delta^{13}\text{C}$ from the relationships, we used residuals (detrended records) of $\Delta^{13}\text{C}$ chronologies instead. The relationships between radial growth and $\Delta^{13}\text{C}$ improved both at the site and inter-site levels (Table 3).

Table 2. Pearson’s correlation coefficients between carbon isotope discrimination ($\Delta^{13}\text{C}$) (raw and detrended data) and indexed tree-ring width (TRWi) for the period 1925-2013 at the site and inter-site level.

Variables	N	Site 1	Site2	Site3	Inter-site
TRWi vs. $\Delta^{13}\text{C}$ (raw values)	89	0.36*	0.07	0.33*	0.38*
TRWi vs. $\Delta^{13}\text{C}$ (detrended values)	89	0.37*	0.37*	0.57*	0.58*

(*) $p < 0.05$

4. Discussion

This study presents results on tree-ring width and $\Delta^{13}\text{C}$ records from the southernmost edge of *Pinus halepensis* distribution for the last 90 years. The situation of the Djelfa forest provides a unique ecological setting for anticipating the consequences of climate warming for forest tree species thriving in the Mediterranean basin.

4.1. The dendroecological potential of *Pinus halepensis*

The present dendroecological study of *P. halepensis* has demonstrated a good temporal coherence among trees growing at each of the three study sites for the traits analysed; despite the limited number of available trees, the ring-width chronologies developed for the three sites were reliable, with high EPS values and, therefore, strong common signals, as it made evident from their mean interseries correlations (Site 1: $r = 0.47$; Site 2: $r = 0.60$; Site 3: $r = 0.53$). The quality of our ring-width chronologies is in the upper range of those obtained for tree species from dry environments elsewhere. For instance, mean correlations among series for chronologies developed from water-limited regions typically range from around 0.30 (oak trees in the western black sea of Turkey and *Sabina przewalskii* in China) (Akkemik et al. 2005; Liu et al. 2006) to ca. 0.60 (white spruce in Canada, and Chinese pine *Pinus tabulaeformis* Carr. trees from the Helan Mountain range in central China) (Chhin et al. 2004; Liu et al. 2005). Also, $\Delta^{13}\text{C}$ demonstrated a good agreement among series which translated into high EPS and stronger common signal than for tree-ring width. Indeed, the mean interseries correlation (R_{bar}) in all sites pointed to stronger signals for $\Delta^{13}\text{C}$ than for ring-width records which indicated that, despite micro-environmental influences, a larger common pattern of year-to-year variation existed across all sites for this ecophysiological trait (Site 1: $r = 0.49$; Site 2: $r = 0.57$; Site 3: $r = 0.90$). A chronology with stronger common signal (interseries correlation; R_{bar}) does not require a larger sample size to maintain reliability, compared to a chronology with a lower interseries correlation, because the EPS parameter is proportional to the number of available series. Nevertheless, the interseries correlation statistic is not commonly used as a measure of a chronology's utility for climate reconstruction; instead, EPS is the accepted statistic in dendrosciences.

Therefore both proxies contain a strong enough common signal (i.e. $EPS \approx 0.85$ or higher), suggesting that they contain a relevant environmental signal. Often, carbon isotopes are more sensitive to climatic variables than tree-ring width (Andreu et al. 2008), but one proxy does not limit the use of the other as they can be regarded as complementary. Indeed, it has been previously reported that ring-width and $\delta^{13}C$ chronologies contain different climatic information in Spanish pine forests (Andreu et al., 2008).

4.2. Tree-ring width responses to climate

Overall, ring-width records were often associated with climate conditions of the year prior to ring formation. Growth of *Pinus halepensis* was constrained by high temperatures in autumn of the previous year, spring and summer, as it was shown by negative correlations between radial growth indices (TRWi) and mean temperature of the corresponding months. A warm autumn could affect the radial growth by depletion of carbon reserves available for the formation of a new ring, resulting in low secondary growth. Indeed, the climatic relationships for evergreen trees are attributed mainly to the influence of environmental factors on both current photosynthesis and the accumulation of carbon reserves (Fritts, 1965).

However, a rainy October of the preceding year and a rainy late winter-spring (February, March, April and June) showed a strong positive correlation with tree growth (Fig. 9e). Conversely, May precipitation was unrelated to growth, although the rainfall in this month is usually large, as it is illustrated in the climograph (Fig. 4). This result suggests that May precipitation is not a limiting factor for tree growth, probably because the soil moisture is already optimal regardless of the amount of precipitation occurring in this month. Remarkably, the trees responded in this same way to fluctuations in the drought index (SPEI), indicating that SPEI is mainly driven by rainfall variation. Some monthly climatic relationships with growth were variable over the last 90 years according to moving correlation analyses, indicating that warmer September months of the previous year and increasing water limitation during April are increasing their importance as drivers of growth over the last decades. We can explain that by the fact that September temperatures increased significantly over time, leading to reduced radial growth perhaps through depletion of carbohydrate reserves available for next year growth. While we could explain the only importance of April precipitation increasing sensitivity of earlywood formation to mid-spring since, probably, late spring and summer are becoming irrelevant to lay down new wood

owing to increasing water stress. Currently, the plant would benefit from the more favourable temperatures in winter and early-mid spring to produce early wood and would stop cambial activity in summer when temperature becomes higher.

4.3. $\Delta^{13}\text{C}$ responses to climate

From 1925 to 2013, a very strong negative linear tendency in $\Delta^{13}\text{C}$ tree-ring values was observed. This trend is unprecedented in the literature to the best of our knowledge and concurs with the steady increase of the mean annual temperature during this period (Fig. 3a).

In addition, we found a negative significant relationship between $\Delta^{13}\text{C}$ values and mean annual temperature ($r = -0.60$, $p < 0.05$ for sites 1 and 2, and $r = -0.70$, $p < 0.05$ for site 3), and as well as with mean monthly temperatures at the site (Fig. 11a) and at inter-site (Fig. 12a) levels for almost all months of the year and, particularly, for summer months (July and August). With regard to precipitation, $\Delta^{13}\text{C}$ didn't show any clear significant response, and the same was found for SPEI. These results suggest that mean temperature is the main factor driving carbon isotope discrimination decreasing trends over the last century. Typically the temperature signal stored in $\Delta^{13}\text{C}$ records is interpreted as an indirect effect through increased evapotranspiration. As temperature increases, the air relative humidity decreases, and there is a simultaneous decrease in soil moisture content (Yin et al., 2008), leading to stomatal closure to minimize water losses through evapotranspiration; therefore, the CO_2 concentration inside the leaves decreases, hence producing a low discrimination against the heavy isotope (^{13}C). Alternatively, as the RuBisCo (Ribulose-1,5-bisphosphate carboxylase/oxygenase) enzyme approaches its optimum temperature photosynthetic rates increase, but above this optimum temperature the rate begins to decrease, as enzymes are denatured. However, the impact of enzyme denaturalization of carbon isotope discrimination values is unclear.

After detrending the $\Delta^{13}\text{C}$ chronologies, we could observe a significant positive correlation with October precipitation of the previous year and spring precipitation, implying high discrimination against ^{13}C in rainy months. Similar results have been reported for $\Delta^{13}\text{C}$ and precipitation in *P. halepensis* (Ferrio et al., 2003) and in other conifer species such as *Pinus sylvestris* (Eilmann et al 2010). Moreover, it has been demonstrated that the correlation between rainfall and $\Delta^{13}\text{C}$ is stronger in dry than in wet sites (Del Castillo et al., 2015). The fact that monthly precipitation

and SPEI records showed significant correlations using detrended data implies that their effects were masked by the effect of mean temperature that was dominant when raw data was used (Fig.12b, 12c).

The dominant environmental factors that control the $\Delta^{13}\text{C}$ values in tree rings should be those that regulate the rate of stomatal conductance and the rate of photosynthesis (Farquhar et al., 1982; McCarroll and Loader, 2004). In our case, an increase of mean temperature seems to promote stomatal closure, and thus an increase in water-use efficiency of the plant, as it is inferred by negative correlations and decreasingly low carbon isotope discriminations values over time. Particularly, high temperatures are usually accompanied with high vapor-pressure deficits, and the resulting stomatal closure therefore leads to an enrichment of ^{13}C (Linares et al., 2009). Indeed, intrinsic water use efficiency (iWUE) increased by 52% across sites from 1925 to 2013 (for Site1:44.82%; Site2: 55.72%; Site3: 54.76%). This substantial increase is related to the rise of atmospheric CO_2 , but also to an active reaction of the trees to increasing temperatures and drought stress, enhancing iWUE. Hence, the increase in iWUE due to CO_2 across sites equals 30.3%, while the remaining 21.7% is due to the downward trend in carbon isotope discrimination for the last 90 years.

Conversely, spring and October of the previous year rainfall appear to promote stomata opening, leading to high discrimination against ^{13}C , as proved by the strong positive correlation between $\Delta^{13}\text{C}$ and precipitation. SPEI exhibited the same relationships as with precipitation, as it was mainly governed by rainfall patterns. Since in the western Mediterranean basin most precipitation occurs in autumn and spring, these relationships demonstrate the dependence of tree performance and, by extension, of Mediterranean forest ecosystems on soil recharge precipitation in drought-prone areas.

Our findings are coherent with studies from other Mediterranean semi-arid sites. Ferrio and Voltas (2005) confirmed that isotope carbon discrimination of Iberian *P. halepensis* was highly water availability-dependent, and this dependence could be used to predict spatial patterns of precipitation with as much accuracy as that achieved from networks of instrumental records (del Castillo et al., 2013).

4.4. Ring-width vs. $\Delta^{13}\text{C}$ relationships

The association between tree-ring width and $\Delta^{13}\text{C}$ (Table 3) is significant and positive for the three sites after detrending of $\Delta^{13}\text{C}$ chronologies (elimination of low-frequency temperature trends), and as well for site means ($r=0.58$). We can explain this result by the fact that tree-ring width index (TRWi) and carbon isotope carbon discrimination are partly driven by similar climatic factors. Indeed, TRWi reflected both precipitation and mean temperature signals, while $\Delta^{13}\text{C}$ was mainly influenced by high temperature at the low-frequency domain and precipitation at the high-frequency domain, which suggests that the common climatic effect recorded in both proxies is drought. Therefore, tree growth and performance is constrained by moisture deficit. Consistently, in southern Oregon (USA), $\Delta^{13}\text{C}$ and TRWi for *Quercus macrocarpa* displayed a strong positive correlation in the drier regions (Steven et al., 2014). In addition, radial growth and $\Delta^{13}\text{C}$ were positively correlated in the case of other conifers such as *Pinus radiata* as it was confirmed by Rowell et al. (2009), suggesting that growth was water-limited.

Under the scope of climate change and according to our results, increases of temperature and in frequency of drought events are predicted in the study area. Various parts of North Africa have also experienced devastating drought events in the last 30 years (e.g. Nicholson and Wigley 1984; Chbouki 1992; Swearingen 1992; Hoerling and Kumar 2003). Higher temperatures in spring and summer may increase the rate of evapotranspiration leading to water stress and, thus, affecting tree radial growth. However, our study highlights the substantial plasticity of Aleppo pine in response to different climatic variables. This is indicated by the fact that radial growth is not reduced or slightly reduced over the study period, while $\Delta^{13}\text{C}$ records decrease to a large extent, pointing to large increases in water-use efficiency. Species such as Aleppo pine that tolerate a broad range of environmental conditions are expected to adjust to some degree to future climate conditions (Benito-Garzón et al., 2011). Nevertheless, the extent of such plastic responses observed in this study for *Pinus halepensis* growing at its southernmost distribution edge is, from a physiological point of view, remarkable. It is therefore unlikely to expect a similar pace in the degree of physiological adjustment of these trees for the near future, which may suggest that forests of the area may be reaching a physiological threshold, hence compromising the future of the whole ecosystem. The prediction of these thresholds is a current research priority, which means that further and complementary experiments should be developed to face this fundamental issue. In this regard, exploiting the existing genetic variation for drought

tolerance at the intra-specific level may be a tool to mitigate climate change impacts in such delicate ecosystems that are fundamental for quarantining the Sahara desert.

5. Conclusions

The present dendroecological study of *Pinus halepensis* in three sites (different exposures) along the green barrier of Djelfa (Algeria) has given useful insights for understanding the climatic significance of this Mediterranean conifer growing in an extreme habitat and for forecasting its response to future climate conditions.

- 1) Despite the influence of local environmental conditions and the limited number of available trees, we found a good agreement among trees growing at the same site and strong common signals for both proxies (TRWi and $\Delta^{13}\text{C}$). Therefore, this species can be reliable to retrieve climatic information from single trees at the study area.
- 2) Tree-ring width indices and climatic variables showed consistent negative relationships involving the temperature in autumn of the previous year, spring and summer. Conversely, October of the previous year and spring rainfall was related positively with tree-ring growth across sites. Remarkably, the trees responded in this same way to drought index (SPEI).
- 3) $\Delta^{13}\text{C}$ was mainly influenced by mean temperature, as displayed by negative correlations throughout the year at site and inter-site levels, reflecting an indirect effect through increased evapotranspiration. At the high frequency domain, we could observe a significant positive correlation with October precipitation of the previous year and spring precipitation. SPEI exhibited the same relationships as with precipitation, as it was mainly governed by rainfall patterns. Therefore, both proxies (TRWi and $\Delta^{13}\text{C}$) provided complementary climatic information, suggesting that drought is the common signal recorded in tree rings, as it was confirmed by positive correlations between tree growth and carbon isotope discrimination.
- 4) The results confirm the extreme plasticity of *P. halepensis* in response to climate variability, providing clues on its future performance in the context of climate change for the region. Also, we substantiate the joint use of TRWi and $\Delta^{13}\text{C}$ as a powerful tool for

climate inference and reconstruction for this Mediterranean conifer, including temperature, precipitation and drought trends.

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Annexes

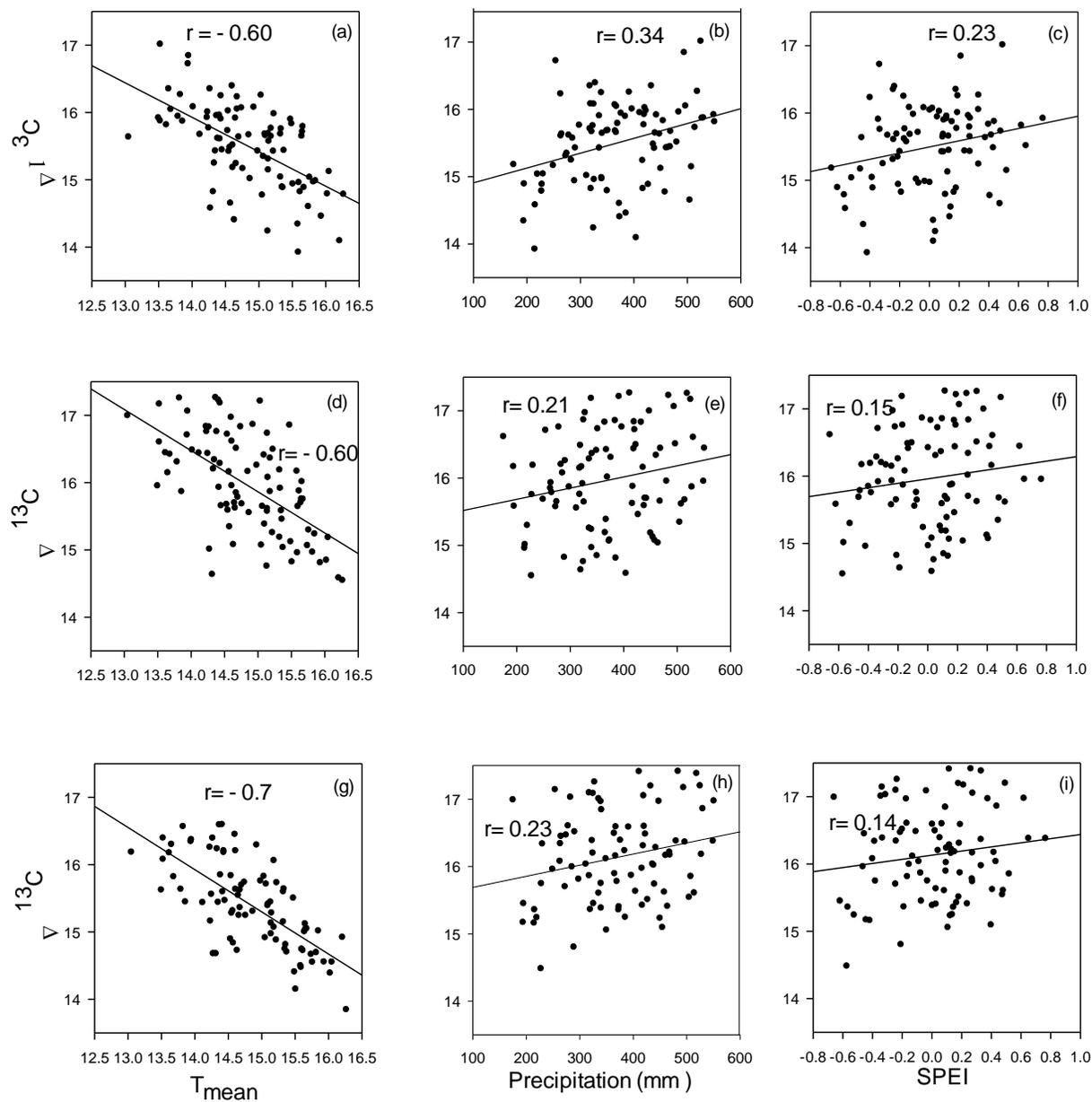


Figure 13. Correlation coefficients (r) between isotope carbon discrimination $\Delta^{13}C$ (raw data) and annual mean climatic factors (T_{mean} , precipitation and SPEI), period (1925-2013): a-c) site1; d-f) site2; g-i) site3.

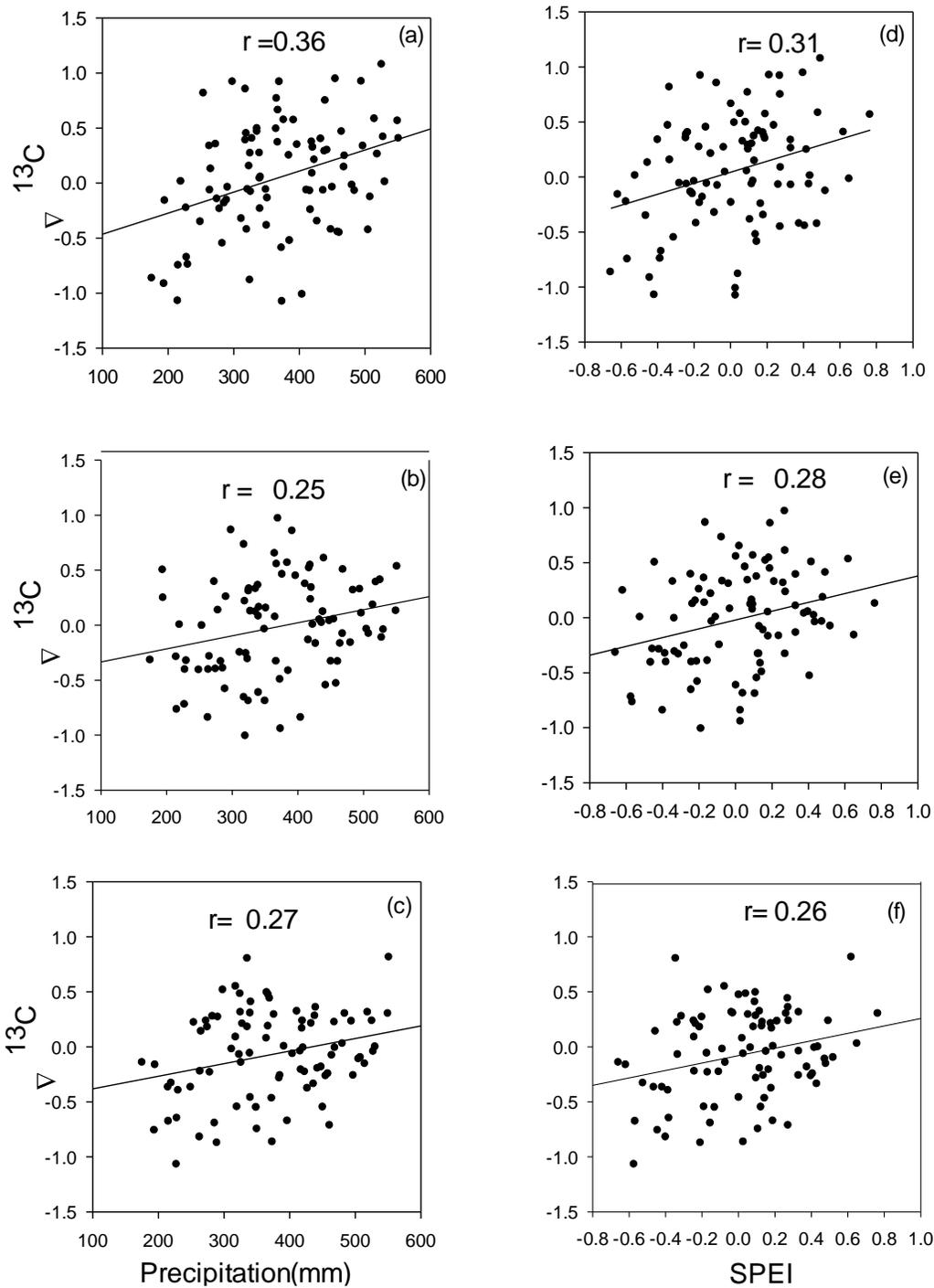


Figure 14. Correlation coefficients (r) between detrended isotope carbon discrimination $\Delta^{13}\text{C}$ and annual climatic factors (precipitation and SPEI): a, d) site1; b, e) site2; c, f) site3.