

# Long tree-ring chronologies reveal 20th century increases in water-use efficiency but no enhancement of tree growth at five Iberian pine forests

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## Abstract

We investigated the tree growth and physiological response of five pine forest stands in relation to changes in atmospheric CO<sub>2</sub> concentration ( $c_a$ ) and climate in the Iberian Peninsula using annually resolved width and  $\delta^{13}\text{C}$  tree-ring chronologies since AD 1600.  $^{13}\text{C}$  discrimination ( $\Delta \approx c_i/c_a$ ), leaf intercellular CO<sub>2</sub> concentration ( $c_i$ ) and intrinsic water-use efficiency (iWUE) were inferred from  $\delta^{13}\text{C}$  values. The most pronounced changes were observed during the second half of the 20th century, and differed between stands. Three sites kept a constant  $c_i/c_a$  ratio, leading to significant  $c_i$  and iWUE increases (active response to  $c_a$ ); whereas a significant increase in  $c_i/c_a$  resulted in the lowest iWUE increase of all stands at a relict *Pinus uncinata* forest site (passive response to  $c_a$ ). A significant decrease in  $c_i/c_a$  led to the greatest iWUE improvement at the northwestern site. We tested the climatic signal strength registered in the  $\delta^{13}\text{C}$  series after removing the low-frequency trends due to the physiological responses to increasing  $c_a$ . We found stronger correlations with temperature during the growing season, demonstrating that the physiological response to  $c_a$  changes modulated  $\delta^{13}\text{C}$  and masked the climate signal. Since 1970 higher  $\delta^{13}\text{C}$  values revealed iWUE improvements at all the sites exceeding values expected by an active response to the  $c_a$  increase alone. These patterns were related to upward trends in temperatures, indicating that other factors are reinforcing stomatal closure in these forests. Narrower rings during the second half of the 20th century than in previous centuries were observed at four sites and after 1970 at all sites, providing no evidence for a possible CO<sub>2</sub> 'fertilization' effect on growth. The iWUE improvements found for all the forests, reflecting both a  $c_a$  rise and warmer conditions, seem to be insufficient to compensate for the negative effects of the increasing water limitation on growth.

**Keywords:**  $\delta^{13}\text{C}$  ratios, global warming, intrinsic water-use efficiency (iWUE), Mediterranean region, ring width, rise of atmospheric CO<sub>2</sub> concentration, tree rings

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## Introduction

Mediterranean forests are extremely vulnerable to climate change (Lindner *et al.*, 2010). Patterns of terrestrial carbon exchange may already be changing as a consequence of land-use modifications and climatic changes (Schimel *et al.*, 2001). Presently, the temperature increase is widespread over the globe, and in addition precipitation declined in the Mediterranean region from 1900 to 2005 (Trenberth *et al.*, 2007). Beyond the direct radiative effect of CO<sub>2</sub> concentration as a key driver of climate change, the increase in atmospheric CO<sub>2</sub> concentration

plays an important role in tree physiology. Higher CO<sub>2</sub> concentrations might theoretically produce a 'fertilization' effect during photosynthesis leading to higher growth rates. Enhanced forest growth and wood production are projected to occur due to rising atmospheric CO<sub>2</sub> concentrations and warming in northern and western Europe, whereas in southern and eastern regions decreased productivity can occur because of increased frequency of droughts and fire risks (Lindner *et al.*, 2010). In Mediterranean climates, the increase in atmospheric CO<sub>2</sub> might enhance tree growth if the 'fertilizing' effect is able to compensate for possible constraints on growth due to changes in temperature and rainfall. In southern Europe, a very sensitive region to climate variability, climate change is projected to result in warmer and drier conditions with longer droughts, as well as a decrease in water availability (Alcama *et al.*,

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2007). In this context, the response of trees to environmental changes (drier conditions and a CO<sub>2</sub>-enriched atmosphere), in terms of growth and intrinsic water-use efficiency (iWUE), is a relevant issue, especially in Mediterranean regions.

Tree rings have been extensively used to assess the effects of climatic and environmental changes on forest ecosystems (Fritts, 1976). More complete knowledge about how the terrestrial carbon reservoir is responding to rising atmospheric CO<sub>2</sub> concentrations ( $c_a$ ), requires more detailed studies on plant physiological adaptations (Feng, 1998). High  $c_a$  can lead to a reduction in stomatal conductance and transpiration and result in improved water-use efficiency, while it can also simultaneously enhance photosynthesis rates and light-use efficiency (Drake *et al.*, 1997). Even though this has been previously demonstrated in controlled experiments (Ceulemans & Mousseau, 1994; Ainsworth & Long, 2005), extrapolations to natural ecosystems are complicated. By using stable carbon isotopes ( $\delta^{13}\text{C}$ ) of tree-ring cellulose, discrimination ( $\Delta$ ) can be evaluated in natural forests through time (Saurer *et al.*, 2004). This provides the possibility of analyzing changes in iWUE because discrimination ( $\Delta \approx c_i/c_a$ ) is linked to iWUE through the effects of assimilation and stomatal conductance.

This approach allows the assessment of potential changes in tree physiology through time and has been used in several regions of the World (i.e. Marshall & Monserud, 1996; Williams & Ehleringer, 1996; Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Feng, 1998; Saurer *et al.*, 2004; Klein *et al.*, 2005; Silva *et al.*, 2009; Gagen *et al.*, 2011; Nock *et al.*, 2011). Although the results varied among sites and regions, most of these investigations show an enhancement in iWUE for the 20th century. In the Mediterranean area, an increasing number of studies have related  $\delta^{13}\text{C}$  ratios to meteorological variables (Ferrio & Voltas, 2005; Ferrio *et al.*, 2006; Andreu *et al.*, 2008; Correia *et al.*, 2008; Voltas *et al.*, 2008; Battipaglia *et al.*, 2009; Planells *et al.*, 2009). Others have estimated the iWUE via the extrapolations proposed above using leaves (Martínez-Vilalta & Piñol, 2002), wood samples (Ferrio *et al.*, 2003), 3-year (Peñuelas *et al.*, 2008) and 5-year blocks from tree-ring wood (Linares *et al.*, 2009), as well as annual tree-ring holocellulose over short periods (Martin-Benito *et al.*, 2010). Here, we present annually resolved tree-ring width and  $\delta^{13}\text{C}$  chronologies (of  $\alpha$ -cellulose), from five Iberian pine forests, three of them covering the last four centuries. The study deals with their responses to rising  $c_a$  and changing climate in terms of tree physiology and growth. Since real  $\Delta$  measurements are only available for recent decades (Saurer *et al.*, 2004), the use of this approach on these long tree-ring chronologies provides a long-term per-

spective on changes in mature natural pine forests since AD 1600 in the Iberian Peninsula.

The climatic signal in these tree-ring chronologies of three pine species (*Pinus sylvestris* L., *P. uncinata* Ramond ex. DC and *P. nigra* Arnold subsp. *salzmannii*) has already been assessed in previous studies (Andreu *et al.*, 2008; Planells *et al.*, 2009). All  $\delta^{13}\text{C}$  chronologies recorded summer precipitation and temperature variability, whereas a wide range of responses to climate were found for the corresponding tree-ring width chronologies at the same five sites (Andreu *et al.*, 2008). Each ring-width chronology showed its own relationship with climate depending on varied stand features and local climatic conditions, with only two of them apparently constrained directly by water availability. Our present study extends the data set provided by Andreu *et al.* (2008) back to the 17th century and focuses primarily on distinguishing between the effects of changes in  $c_a$  and climate modulating  $\delta^{13}\text{C}$  of tree-ring cellulose. High  $\delta^{13}\text{C}$  ratios are the result of a reduction in stomatal conductance, related to air relative humidity and antecedent rainfall, and/or high photosynthetic rates, related to temperature and photon flux (McCarroll & Pawellek, 2001). Previously, we explored the influence of climatic variables on  $\delta^{13}\text{C}$  at all the study sites (Andreu *et al.*, 2008), while here we assess the effect of the recent environmental changes. Recently, an objective correction (the 'preindustrial' or 'PIN' correction) has been proposed to remove the low-frequency trends from tree-ring  $\delta^{13}\text{C}$  chronologies caused by tree physiological responses to changes in  $c_a$  (McCarroll *et al.*, 2009). Here, we use the PIN correction to evaluate whether the trends observed in the  $\delta^{13}\text{C}$  series can be explained by the physiological responses to changes in  $c_a$  also described by the three theoretical scenarios suggested in Saurer *et al.* (2004), and/or by changes in climate. Since the iWUE rates depend on carbon assimilation ( $A$ ) and stomatal conductance ( $g$ ), an enhancement in the iWUE could be due to either an increase in  $A$ , a decrease in  $g$ , or both. The assessment of changes in tree growth will provide indirect information regarding  $A$ , aiding us in elucidating the causes of the observed patterns.

The aims of this work are: (1) to evaluate changes in the  $\delta^{13}\text{C}$  values,  $\Delta$ ,  $c_i$  and iWUE of the studied forests; (2) to test the physiological response of trees to the increase of  $c_a$  by the use of the PIN correction; (3) to assess changes in growth during the studied periods; and (4) to determine whether changes in climate have also had a role in modulating the observed trends in  $\delta^{13}\text{C}$  ratios and growth. Answering these questions will allow us to conclude whether there has been an increase in the iWUE at the studied stands, caused by increasing  $c_a$  and/or changes in climate, which compensates, in

terms of tree growth, for the negative effects of the increasing water limitation already reported for the Mediterranean region.

## Material and methods

### Study sites and sampling

Five forest stands were sampled on the Iberian Peninsula (Fig. 1; Table 1). One *P. nigra* stand is located in the south-east (*pnCaz*), whereas the rest, two forests of *P. sylvestris* (*psLil* and *psUrb*) and two of *P. uncinata* (*puPed* and *puUrb*), are in the north. *psUrb* and *puUrb* are located only 10 km apart from each other, permitting the assessment of the effects of different site conditions and species-specific responses under similar climatic conditions (Planells *et al.*, 2009). Sampling focused on the oldest natural forest stands in the area when possible. At each site, more than fifteen trees were cored with an increment borer at around 1.30 m stem height. At least four cores were taken from each tree: two for ring-width chronology building and the rest for isotope analyses.

### Ring-width chronologies

Cores were sanded until wood cells were clearly visible under the microscope (Stokes & Smiley, 1968). All samples were visually crossdated following the procedures described by Yamaguchi (1991). After crossdating, the ring widths were measured at an accuracy of 0.01 mm using an ANIOL semi-automatic device (Aniol, 1983). The resulting series underwent a crossdating quality control using the statistical program COFECHA (Holmes, 1983). The characteristics of the tree-ring width chronologies are shown in Table 2.

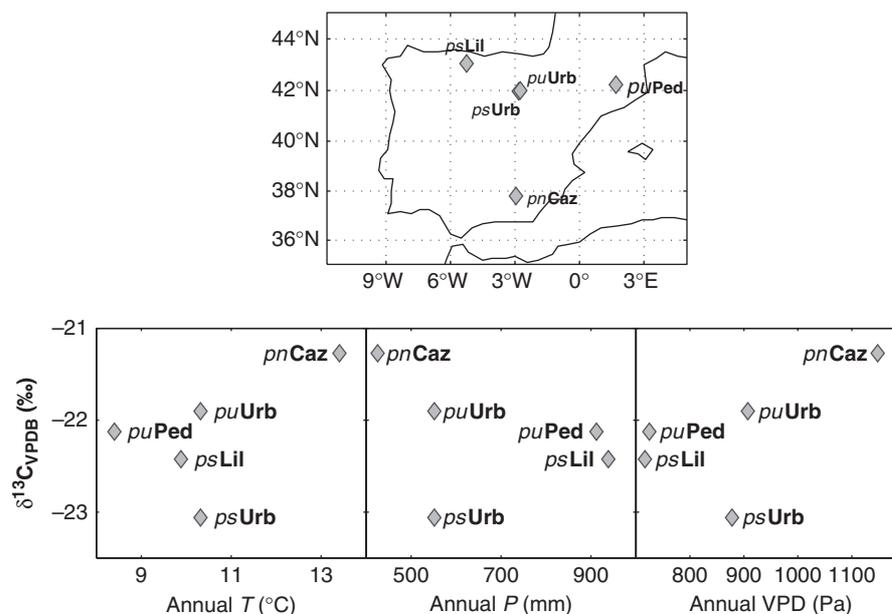
### Isotope chronologies

For *pnCaz*, *psLil* and *puPed*, eight to ten crossdated cores from four different trees were selected, whereas for *psUrb* and *puUrb*, eight cores from eight trees were chosen (Table 3). These cores were dated with an absolute precision using the ring-width chronologies established at each site (Table 2). For each location, the annual tree rings were split using a scalpel and pooled year by year (Leavitt & Long, 1984; Treydte *et al.*, 2001; Leavitt, 2008).  $\alpha$ -cellulose was extracted to avoid isotope variations caused by varying contents of other wood fractions. Sodium hydroxide, sodium chlorite and acetic acid were used to remove the extractives (Loader *et al.*, 1997). The  $\alpha$ -cellulose was homogenized in two different ways: *psUrb* and *puUrb* samples were ground with an ultra centrifugation mill (Retsch ZM1, mesh size of 0.5 mm); samples from *pnCaz*, *psLil* and *puPed* were homogenized with an ultrasonic device (Laumer *et al.*, 2009).  $^{13}\text{C}/^{12}\text{C}$  ratios were measured as  $\text{CO}_2$  by combusting the  $\alpha$ -cellulose samples in an elemental analyzer (Fisons NA 1500 NC, Fisons Instruments, Milan, Italy) interfaced with an IRMS (Micromass Optima isotope ratio mass-spectrometer, VG Instruments, Manchester, UK) operating in continuous flow mode. The reproducibility was better than 0.1‰. The isotope signature is expressed in the delta notation relative to the standard VPDB (IAEA, 1995):

$$\delta^{13}\text{C}_{\text{sample}} = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}} - 1 \right] 1000(\text{‰}). \quad (1)$$

### Calculations for $c_i/c_{a,i}$ , $c_i$ and $i\text{WUE}$

The  $\delta^{13}\text{C}$  raw data were used to calculate carbon isotope discrimination against  $^{13}\text{C}$  ( $\Delta$ ), which expresses the isotope shift between air ( $\delta^{13}\text{C}_{\text{air}}$ ) and plant organic matter



**Fig. 1** Above: Geographical location of the studied sites in the Iberian Peninsula. Below:  $\delta^{13}\text{C}_{\text{raw}}$  data vs. annual temperature ( $T$ ), precipitation ( $P$ ) and vapor pressure deficit (VPD) from 1901 to 1999.

**Table 1** Location and characteristics of the sites, as well as annual mean temperature (*T*), annual total precipitation (*P*) and annual vapor pressure deficit (VPD) obtained using the meteorological CRU 2.1 data set (resolution of 0.5°) from 1901 to 2002, annually and from June to August (JJA)

Site ID	<i>pnCaz</i>	<i>psLil</i>	<i>puPed</i>	<i>psUrb</i>	<i>puUrb</i>
Site	Cazorla	Pinar de Lillo	Pedraforca	Urbión	Urbión
Species	<i>Pinus nigra</i>	<i>P. sylvestris</i>	<i>P. uncinata</i>	<i>P. sylvestris</i>	<i>P. uncinata</i>
Range	Baetic	Cantabrian	Pre-Pyrenees	Urbión	Urbión
Latitude (°)	37.80	43.05	42.23	41.97	42.00
Longitude (°)	-2.95	-5.25	1.70	-2.82	-2.75
Altitude (m asl)	1800	1600	2100	1750	1950
Aspect	SW	NW	E	NE	SW
Slope (°)	15	28	36	23	10
Stand structure	Open forest	Mainly open forest	Open forest	Closed forest	Open forest with closed areas
CRU TS 2.1 grid point					
Latitude (°)	37.75	43.25	42.25		41.75
Longitude (°)	-2.75	-5.25	1.75		-2.75
Mean annual <i>T</i> (°C)	13.4 ± 0.7	9.9 ± 0.6	8.4 ± 0.6		10.4 ± 0.7
Total annual <i>P</i> (mm)	424.2 ± 102.9	944.4 ± 183.6	902.2 ± 186.4		552.5 ± 110.1
Mean annual VPD (Pa)	1151.7 ± 66	717.6 ± 37	726.5 ± 44.8	880.6 ± 52.5	910 ± 53.4
Mean JJA <i>T</i> (°C)	22.4 ± 1	15.9 ± 0.9	15.8 ± 1		18.5 ± 1
Total JJA <i>P</i> (mm)	38.6 ± 25.2	156.8 ± 75	223.8 ± 92.8		101.5 ± 43.8
Mean JJA VPD (Pa)	2079.6 ± 143	1150.2 ± 83.1	1223.2 ± 103.9	1571.1 ± 115.8	1612.1 ± 117.5

**Table 2** Characteristics of the tree-ring width chronologies

Site ID	<i>pnCaz</i>	<i>psLil</i>	<i>puPed</i>	<i>psUrb</i>	<i>puUrb</i>
Trees/cores	33/68	21/45	23/57	15/25	17/32
Time span (years)	1125–2002	1511–2002	1269–2002	1612–1999	1752–1999
Mean intercorrelation	0.676	0.595	0.565	0.560	0.562
Mean sensitivity	0.253	0.258	0.175	0.207	0.175
Mean length (years)	389.8	310.2	435	204	187.7

Numbers of trees and cores; time span; mean correlation of all the series with the master chronology (Mean intercorrelation); measure of the interannual variability (Mean sensitivity) and average of the number of years measured in each core (Mean length).

**Table 3** Characteristics of the raw  $\delta^{13}\text{C}$  tree-ring chronologies

Site ID	<i>pnCaz</i>	<i>psLil</i>	<i>puPed</i>	<i>psUrb</i>	<i>puUrb</i>
Trees/cores	4/9	4/10	4/8	8/8	8/8
Time span (years)	1600–2002	1600–2002	1600–2002	1900–1999	1800–1999
Mean ± SD (‰)	-21.05 ± 0.60	-22.13 ± 0.69	-22.01 ± 0.61	-23.55 ± 0.58	-21.91 ± 0.82
Min (‰)	-22.9	-24.2	-24.3	-24.9	-24.5
Max (‰)	-19.9	-20.6	-20.5	-22.5	-20.3
Range (‰)	2.99	3.54	3.82	2.36	4.27

Numbers of trees and cores pooled at each site for performing the isotopic analyses. Time span, mean, standard deviation (SD), minimum (Min), maximum (Max) and range of the raw  $\delta^{13}\text{C}$  series obtained.

( $\delta^{13}\text{C}_{\text{tree}}$ ) in ‰:

$$\Delta = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{tree}}}{1 + \delta^{13}\text{C}_{\text{tree}}/1000} \quad (2)$$

For C3 plants, the relationship between carbon isotope discrimination and leaf gas exchange can be described by

the following equation (Farquhar *et al.*, 1982, 1989):

$$\Delta \approx \delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{tree}} = a + (b-a) \frac{c_i}{c_a} \quad (3)$$

where *a* is the fractionation between  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  during diffusion of  $\text{CO}_2$  through the stomata (4.4‰; O'Leary, 1981); *b* is the discrimination against  $^{13}\text{CO}_2$  due to RuBP carboxylase

(~ 27‰: Farquhar & Richards, 1984); and  $c_i$  and  $c_a$ , are the needle intercellular spaces and ambient concentrations ( $\mu\text{mol mol}^{-1}$ ) of  $\text{CO}_2$ , respectively.

Records of  $\delta^{13}\text{C}_{\text{atm}}$  obtained from Antarctic ice core data and direct measurements (Francey *et al.*, 1999), summarized by McCarroll & Loader (2004), were used to calculate  $\Delta$  using Eqn (3). It was assumed that the  $\delta^{13}\text{C}_{\text{air}}$  at the experimental site equalled the  $\delta^{13}\text{C}_{\text{air}}$  of the ambient atmosphere. Knowing  $\Delta$ ,  $c_i/c_a$  can be calculated by rearranging Eqn (3):

$$\frac{c_i}{c_a} = \frac{\Delta - a}{b - a}, \quad (4)$$

$c_i$  was determined using the data of the atmospheric  $\text{CO}_2$  concentration from Robertson *et al.* (2001) until 1993 and the mean annual values from Mauna Loa from 1994 to 2002 in the composite series provided by McCarroll *et al.* (2009). iWUE is the ratio of the fluxes of net photosynthesis ( $A$ ) and conductance for water vapor ( $g_{\text{H}_2\text{O}}$ ) (Feng, 1999), citing Ehleringer *et al.* (1993):

$$\text{iWUE} = \frac{A}{g_{\text{H}_2\text{O}}} = (c_a - c_i) \frac{1}{1.6}. \quad (5)$$

Although the Francey and Farquhar model (1982) was developed for whole-leaf tissue, which differs isotopically from stem cellulose, many authors (e.g. Bert *et al.*, 1997) have used  $\delta^{13}\text{C}$  tree-ring cellulose to calculate this set of equations considering a constant isotopic offset between wood and leaves (Feng, 1998). In addition, intraseasonal investigations revealed identical trends in cellulose of leaf and tree ring, where the latter was found to be generally enriched in  $^{13}\text{C}$  when compared with the former (Helle & Schleser, 2004). Furthermore, the use of cellulose from tree rings instead of needle organic matter allows precise annual dating and assessment of long-term variation through time depending on the time span of the tree-ring chronology. Simple linear regression analysis was used to assess temporal trends in  $\Delta$  ( $\approx c_i/c_a$ ),  $c_i$  and iWUE, as well as to assess their significance. The corresponding linear regression slopes are the rates of change per year for each variable for a chosen period of time.

### Meteorological data

The meteorological data used was from the CRU TS 2.1 0.5° gridded product (Mitchell & Jones, 2005). Monthly minimum, mean and maximum temperatures, as well as total monthly precipitation from 1901 to 1999 were used. The coordinates of the closest grid point selected at each site are described in Table 1. Vapor pressure deficit (VPD) is the difference between the actual and the maximum amount of moisture in the air when it is saturated. VPD was computed by subtracting the vapor pressure (VP) from the daytime saturation vapor pressure (VP<sub>sat</sub>), obtained using the equations proposed by Ferrio & Voltas (2005) for the Mediterranean region:

$$\ln(\text{VP}) = 6.34 + 0.047T_m + 0.93 \left( \frac{P}{1000} \right) - 0.22 \left( \frac{Z}{1000} \right),$$

$$\text{VP}_{\text{sat}} = 613.75 \exp \left( 17.502 \frac{T_{\text{day}}}{240.97 + T_{\text{day}}} \right),$$

where  $T_m$  is the monthly mean temperature ( $^{\circ}\text{C}$ ),  $P$  is monthly precipitation (mm) and  $Z$  is altitude (masl).  $T_{\text{day}}$  (daytime temperature) is calculated summing one-third of average minimum temperatures and two-thirds of average maximum temperatures. Simple linear regressions were used to assess the significance of temporal trends in these meteorological variables. Where appropriate, one-way analysis of covariance (ANCOVA) was used to test for significant differences between two regressions. Monthly mean temperature and total monthly precipitation, from 29.75 to 50.25°N (latitude) and from 11.75°W to 19.75°E (longitude), were used to perform spatial correlation analyses by computing the Pearson correlation coefficients between the grid points and the  $\delta^{13}\text{C}$  chronologies from 1901 to 1999.

### Corrections of nonclimatic trends

All  $\delta^{13}\text{C}$  chronologies (raw data) showed a decreasing trend attributed to the rise of  $^{13}\text{C}$ -depleted atmospheric  $\text{CO}_2$  due to fossil fuel burning and deforestation since industrialization (the Suess effect). Therefore, before performing the field correlation analyses the raw series had to be corrected. The corrected  $\delta^{13}\text{C}$  chronologies ( $\delta^{13}\text{C}_c$ ) were obtained by adding the correction values, tabulated by McCarroll & Loader (2004), given as the differences between the actual atmospheric  $\delta^{13}\text{C}$  value (for each year) and the preindustrial isotope signature (-6.4‰, AD 1850). The PIN correction (McCarroll *et al.*, 2009), which tries to estimate the  $\delta^{13}\text{C}$  values that would be expected under the  $\text{CO}_2$  concentrations prior to industrialization, was also applied to the series after the classical one described above. The PIN correction is based on two logical constraints delimited by the likely physiological response of trees: first, 'that a unit increase in  $c_a$  cannot result in more than the same unit increase in  $c_i$ ' (passive response); and second, 'that increases in iWUE as a result of an increase in  $c_a$  are limited to maintaining a constant  $c_i/c_a$  ratio' (active response). The  $\delta^{13}\text{C}_c$  chronologies and the series after applying both corrections ( $\delta^{13}\text{C}_{\text{pin}}$ ) were used to perform spatial correlations. The results were used to test the effectiveness of the PIN correction. Simple linear regressions were used to assess the significance of changes in  $\delta^{13}\text{C}_c$  and  $\delta^{13}\text{C}_{\text{pin}}$  series vs.  $c_a$  and time. ANCOVA was performed where necessary to test whether two regressions differed significantly.

### Tree growth

We assessed the modern tree growth by comparing the ring-width (RW) raw measurements of the last 50 years with those of the previous centuries. For each site, RW raw data were used to calculate the frequency of the RWs for different time intervals. This approach was inspired by Salzer *et al.* (2009), who used raw RW data directly to assess bristlecone pine growth trends in the Great Basin (USA) in order to avoid possible artefacts due to dendrochronological standardization methods. Here, before performing the histograms, the first 150 years from all the series were removed to avoid the age/size trend. The nonparametric Kolmogorov–Smirnov test, based on the empirical cumulative distribution function (CDF), was

used to test whether the empirical distributions before and after 1950 were significantly different at each site. Additionally, trends in these selected RW raw data sets against  $c_a$  and time were assessed by means of simple linear regressions and tested for significance between regressions using ANCOVA.

## Results

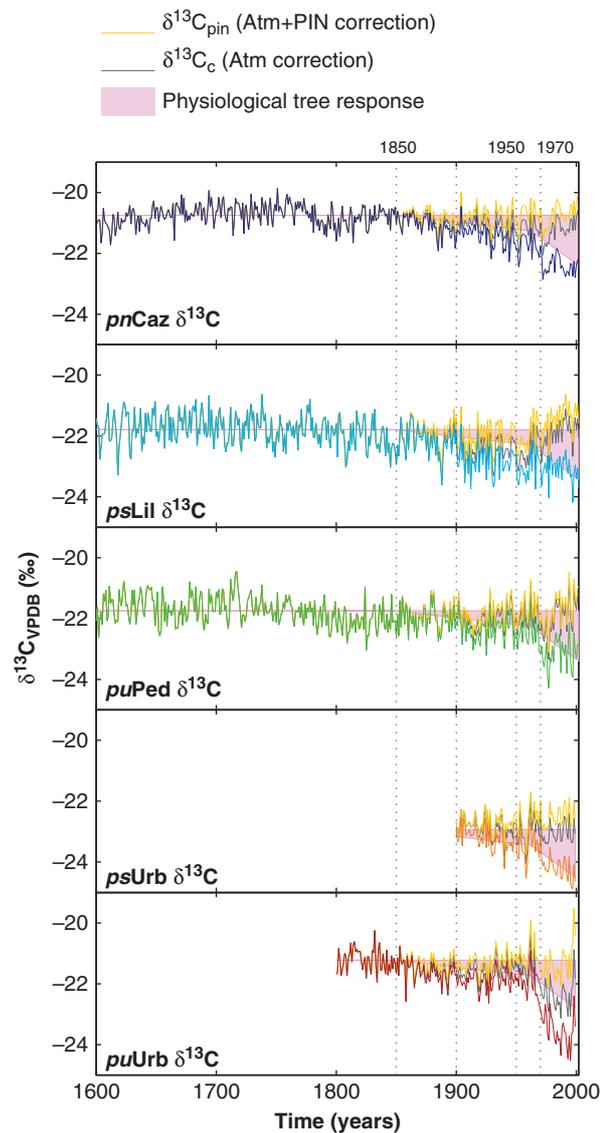
### Site locations and climatic regimes

Due to the location of the sites in the Iberian Peninsula (Fig. 1), different climatic regimes affect the studied stands (Table 1). The southernmost forest (*pnCaz*) showed the highest  $\delta^{13}\text{C}$  values reflecting the driest annual conditions of all the sites: the highest mean temperature, the lowest precipitation and the highest VPD annually (Fig. 1), as well as during the summer season (Fig. S1). The highest amounts of precipitation during the year are observed at the *psLil* and *puPed* stands (Fig. 1), which have very similar mean temperatures during summer (Fig. S1). These factors result in the lowest annual and summer VPDs and thus lead to lower  $\delta^{13}\text{C}$  values in comparison to *pnCaz* and *puUrb*. The *puUrb*  $\delta^{13}\text{C}$  values are in an intermediate range. However, although *psUrb* experiences similar climatic conditions to *puUrb*, it appears as an outlier from the water availability gradient defined by VPD apparently shown by the rest of the sites (Figs 1 and S1).

### Temporal variations in $\delta^{13}\text{C}$ , $\Delta$ ( $\approx c_i/c_a$ ), $c_i$ and $i\text{WUE}$

All the  $\delta^{13}\text{C}_{\text{raw}}$  chronologies decreased from the second half of the 19th century onwards, similar to the declining  $\delta^{13}\text{C}_{\text{atm}}$  trend (Fig. 2), and at four of the sites showed the largest decreases from 1950 to 1999 (Table 4). However, the trends for the most recent period, from 1970 to 1999, showed nonsignificant declines in three of the sites, and in the rest, lower rates than expected due to the steeper decline of  $\delta^{13}\text{C}_{\text{atm}}$  since 1970 (Table 4). Nevertheless, the mean of the tree-ring  $\delta^{13}\text{C}$  rates calculated for different periods since industrialization tracks very close to the decreasing rate of  $\delta^{13}\text{C}_{\text{atm}}$  (in  $\text{‰ yr}^{-1}$  since 1850:  $-0.010$  vs.  $-0.008$ ; since 1900:  $-0.013$  vs.  $-0.012$ ; since 1950:  $-0.023$  vs.  $-0.025$ , respectively). In addition, the  $\delta^{13}\text{C}_{\text{atm}}$  values fall within the range defined by the standard deviation (SD) of the tree-ring  $\delta^{13}\text{C}$  rates, except for the period from 1970 to 1999. In this latter period, the decreasing rate of  $\delta^{13}\text{C}_{\text{atm}}$  ( $-0.028\text{‰ yr}^{-1}$ ) was higher than the mean rate for tree-ring  $\delta^{13}\text{C}$  ( $-0.010\text{‰ yr}^{-1}$ ) and outside the range defined by the SD of the tree-ring rates ( $\pm 0.014$ ).

Figure S2 illustrates  $^{13}\text{C}$  discrimination ratios ( $\Delta \approx c_i/c_a$ ), the leaf intercellular  $\text{CO}_2$  concentration ( $c_i$ ) and  $i\text{WUE}$  inferred from  $\delta^{13}\text{C}$  tree-ring data. Figure 3 shows



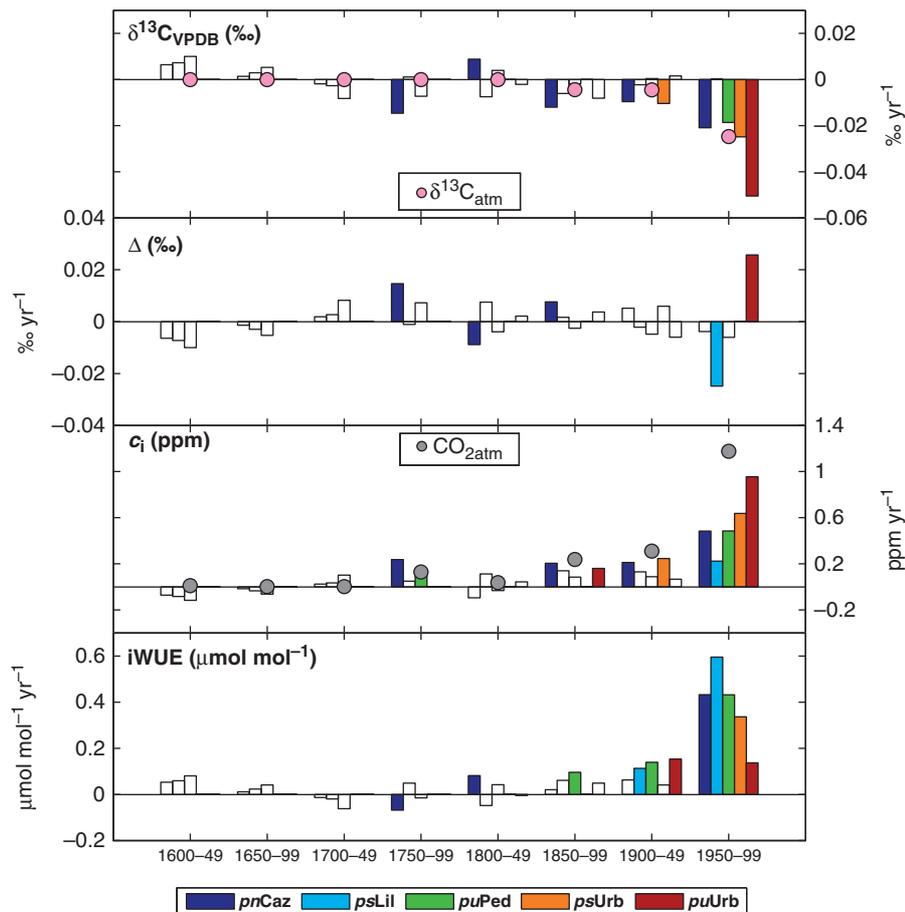
**Fig. 2** The raw  $\delta^{13}\text{C}$  chronologies, the corrected  $\delta^{13}\text{C}$  chronologies ( $\delta^{13}\text{C}_c$  and  $\delta^{13}\text{C}_{\text{pin}}$ ) and the physiological tree response range at the five studied stands. The  $\delta^{13}\text{C}_c$  chronologies (grey line) were obtained after applying the well-known atmospheric correction and the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies (yellow line) after using both the atmospheric correction and the 'preindustrial' or PIN correction. The limits defined by the two physiological tree response constrains are shaded: upper boundary (constant  $c_i/c_a$ , active response to  $c_a$ ) and lower boundary (constant  $c_a - c_i$ , passive response to  $c_a$ ).

that the most pronounced changes, when computing trends using 50-year intervals, were observed from 1950 to 1999. Thus, during the second half of the 20th century, three of the stands (*pnCaz*, *puPed* and *psUrb*) kept constant  $c_i/c_a$ , which led to significant increases in  $c_i$  and improvements in  $i\text{WUE}$ . However, *puUrb* showed a significant increase in  $\Delta$ , resulting in the

**Table 4** Rates of changes per year in  $\delta^{13}\text{C}_{\text{raw}}$ ,  $^{13}\text{C}$  discrimination ratios ( $\Delta$ ), leaf intercellular  $\text{CO}_2$  concentration ( $c_i$ ) and intrinsic water-use efficiency (iWUE) calculated using simple linear regression

Period of time	<i>psLil</i>		<i>psUrb</i>		<i>psUrb</i>		5 sites average		atmospheric changes
	Rates	$R^2$	Rates	$R^2$	Rates	$R^2$	Mean $\pm$ SD		
1850–1899	$\Delta\delta^{13}\text{C}_{\text{raw}}/\Delta t$ (‰ yr <sup>-1</sup> )	0.67	$\Delta\delta^{13}\text{C}_{\text{raw}}/\Delta t$ (‰ yr <sup>-1</sup> )	0.37	$\Delta\delta^{13}\text{C}_{\text{raw}}/\Delta t$ (‰ yr <sup>-1</sup> )	0.54	$\Delta\delta^{13}\text{C}_{\text{raw}}/\Delta t$ (‰ yr <sup>-1</sup> )	$\Delta\delta^{13}\text{C}_{\text{atm}}/\Delta t$ (‰ yr <sup>-1</sup> )	-0.008
1900–1999	-0.011	**	-0.008	**	-0.014	**	-0.010 $\pm$ 0.003	-0.012	-0.012
1950–1999	-0.015	**	-0.010	**	-0.021	**	-0.013 $\pm$ 0.006	-0.025	-0.025
1970–1999	-0.021	**	-0.019	**	-0.051	**	-0.023 $\pm$ 0.018	-0.028	-0.028
	$\Delta\text{Discr.}/\Delta t$ (‰ yr <sup>-1</sup> )	0.07	$\Delta\text{Discr.}/\Delta t$ (‰ yr <sup>-1</sup> )	0.02	$\Delta\text{Discr.}/\Delta t$ (‰ yr <sup>-1</sup> )	0.15	$\Delta\text{Discr.}/\Delta t$ (‰ yr <sup>-1</sup> )		
1850–1999	0.003	**	0.000	ns	0.005	**	0.002 $\pm$ 0.003		
1900–1999	0.003	*	-0.008	**	0.003	*	0.001 $\pm$ 0.006		
1950–1999	-0.004	ns	-0.025	**	0.002	ns	-0.002 $\pm$ 0.018		
1970–1999	-0.019	**	-0.026	**	-0.005	ns	-0.018 $\pm$ 0.014		
	$\Delta c_i/\Delta t$ (ppm yr <sup>-1</sup> )	0.23	$\Delta c_i/\Delta t$ (ppm yr <sup>-1</sup> )	0.29	$\Delta c_i/\Delta t$ (ppm yr <sup>-1</sup> )	0.01	$\Delta c_i/\Delta t$ (ppm yr <sup>-1</sup> )	$\Delta\text{CO}_{2\text{atm}}/\Delta t$ (ppm yr <sup>-1</sup> )	0.46
1850–1999	0.25	**	0.22	**	0.30	**	0.25 $\pm$ 0.04	0.46	0.46
1900–1999	0.33	**	0.21	**	0.44	**	0.33 $\pm$ 0.09	0.63	0.63
1950–1999	0.48	**	0.22	**	0.96	**	0.56 $\pm$ 0.27	1.18	1.18
1970–1999	0.40	**	0.34	*	0.67	**	0.46 $\pm$ 0.23	1.48	1.48
	$\Delta\text{iWUE}/\Delta t$ ( $\mu\text{mol mol}^{-1}\text{ yr}^{-1}$ )	0.67	$\Delta\text{iWUE}/\Delta t$ ( $\mu\text{mol mol}^{-1}\text{ yr}^{-1}$ )	0.68	$\Delta\text{iWUE}/\Delta t$ ( $\mu\text{mol mol}^{-1}\text{ yr}^{-1}$ )	0.46	$\Delta\text{iWUE}/\Delta t$ ( $\mu\text{mol mol}^{-1}\text{ yr}^{-1}$ )		
1850–1999	0.13	**	0.15	**	0.10	**	0.13 $\pm$ 0.02		
1900–1999	0.19	**	0.26	**	0.12	**	0.19 $\pm$ 0.06		
1950–1999	0.43	**	0.60	**	0.14	**	0.39 $\pm$ 0.17		
1970–1999	0.67	**	0.71	**	0.51	**	0.63 $\pm$ 0.14		

The slopes of the linear regressions are the rates of change per year (Rates),  $R^2$  is the coefficient of determination and the significance levels are indicated by \*\* $P < 0.01$  and \* $P < 0.05$ .



**Fig. 3** Temporal changes for 50-year time intervals in  $\delta^{13}\text{C}_{\text{raw}}$   $^{13}\text{C}$  discrimination ratios ( $\Delta \approx c_i/c_a$ ), leaf intercellular  $\text{CO}_2$  concentration ( $c_i$ ) and intrinsic water-use efficiency (iWUE) at the studied stands. All the rates of change per year (slopes of the linear regression fitted to the data) are plotted, but only colored when significant ( $P < 0.01$ ).

lowest iWUE increase observed, whereas *psLil* showed a significant decrease in  $\Delta$  that lead to the greatest increase in iWUE for this time period.

Table 4 shows that all sites experienced significant increases in  $c_i$  and iWUE since the preindustrial period, either since 1850 or 1900, although larger increments were observed since 1950, and even more since 1970. The largest increment in iWUE for all sites, from 1970 to 1999, derived from the significant lowest  $\Delta$  rates at three of the sites and constant  $\Delta$  rates at the Urbión sites (*psUrb* and *puUrb*). The mean over all sites for this period for  $\Delta$  was  $-0.018 \pm 0.014 \text{‰ yr}^{-1}$ , for  $c_i$   $0.46 \pm 0.23 \text{ ppm yr}^{-1}$  and for iWUE  $0.63 \pm 0.14 \text{ μmol mol}^{-1} \text{ yr}^{-1}$ .

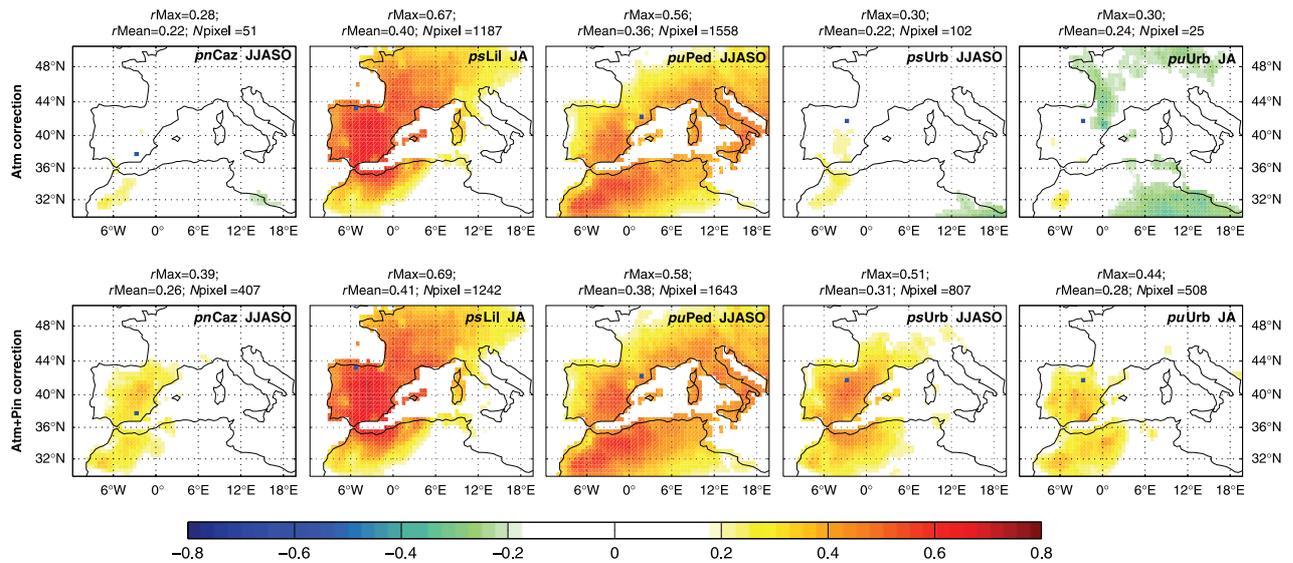
#### The $\delta^{13}\text{C}_c$ series temporal variations

The temporal variations in  $\delta^{13}\text{C}_c$  chronologies can be used to assess if trees are responding in an active or passive way to  $c_a$ . Figure 2 shows the  $\delta^{13}\text{C}_c$  chronologies, obtained after applying the atmospheric  $\delta^{13}\text{C}$

correction, and the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies, obtained after applying first the atmospheric  $\delta^{13}\text{C}$  correction and then the PIN correction, which theoretically removes physiological changes due to  $c_a$  increases. The boundaries defined by the two logical constraints based on the physiological response of trees according to the PIN correction are also depicted in this figure. Most of the  $\delta^{13}\text{C}_c$  chronologies appeared to be close to the upper boundary (constant  $c_i/c_a$ ), showing an active response to  $c_a$ , with the exception of *puUrb*, which was close to the lower boundary (constant  $c_a - c_i$ ) and displayed a passive response to  $c_a$ .

#### Spatial correlations with the $\delta^{13}\text{C}$ corrected series

Both kinds of corrected  $\delta^{13}\text{C}$  tree-ring chronologies were used to perform spatial correlations with monthly mean temperature and total precipitation data from the CRU TS 2.1 gridded climate data from 29.75 to 50.25°N in latitude and from 11.75°W to 19.75°E in longitude.



**Fig. 4** Significant spatial correlations ( $P < 0.05$ ) between the corrected  $\delta^{13}\text{C}$  chronologies ( $\delta^{13}\text{C}_c$  and  $\delta^{13}\text{C}_{\text{pin}}$ ) and monthly mean temperature ( $T$ ) from the CRU TS 2.1 data set from 29.75 to 50.25°N in latitude and from 11.75°W to 19.75°E in longitude. The analyzed period (1901–1999) was from June to October (JJASO) for *pnCaz*, *puPed* and *psUrb* and from July to August (JA) for *psLil* and *puUrb*.

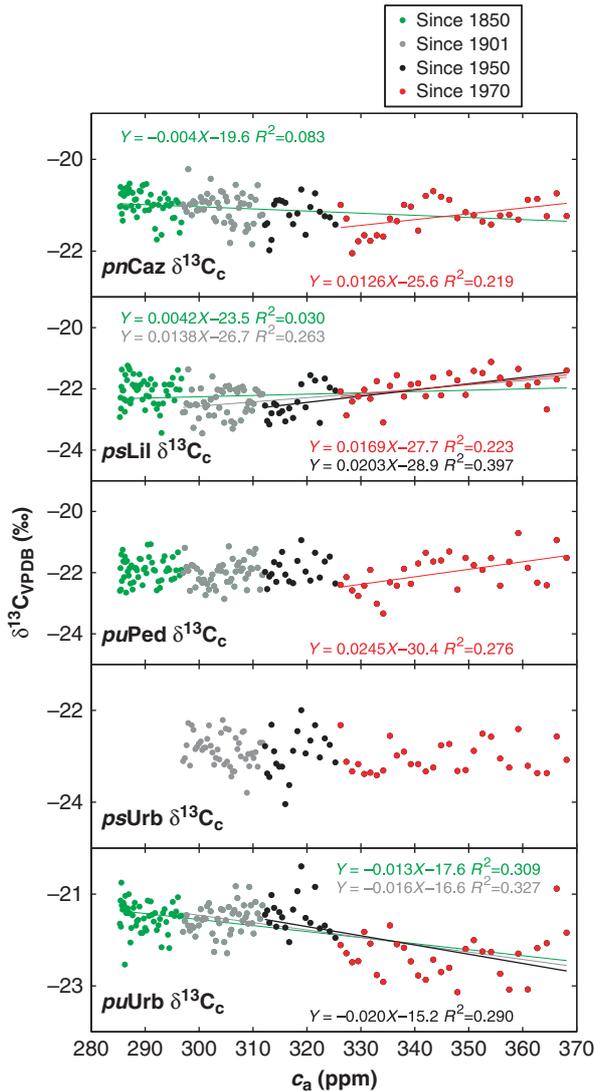
Significant relationships were found predominantly during summer and early autumn, although the strongest individual monthly correlations varied slightly at each location (results not shown). Figure 4 summarizes these results and illustrates the months with the highest spatial correlations between temperature and the corrected  $\delta^{13}\text{C}$  chronologies ( $\delta^{13}\text{C}_c$  and  $\delta^{13}\text{C}_{\text{pin}}$ ) for each site. These months were from June to October (JJASO) for *pnCaz*, *puPed* and *psUrb*; and from July to August for *psLil* and *puUrb*. In all the cases, after applying the PIN correction, the maximum correlation coefficient ( $r_{\text{Max}}$ ), the mean correlation coefficient ( $r_{\text{Mean}}$ ) and the extension of the areas showing significant positive correlations with temperature represented by the number of grid points or pixels ( $N_{\text{pixel}}$ ) were higher. It is remarkable that *pnCaz*, *psUrb* and *puUrb*, the driest sites, presented nonsignificant or very low correlation in the vicinity of the sampling area when only the atmospheric correction was used ( $\delta^{13}\text{C}_c$ ). However, significant correlations appeared over the Iberian Peninsula after applying the PIN correction ( $\delta^{13}\text{C}_{\text{pin}}$ ) at those sites. Additionally, Fig. S1 shows that the water availability gradient characterized by summer VPD at four of the sites is better defined when using  $\delta^{13}\text{C}_{\text{pin}}$  chronologies instead of the  $\delta^{13}\text{C}_{\text{raw}}$  or  $\delta^{13}\text{C}_c$  series. The spatial correlations with precipitation showed predominantly the highest negative correlations with July at all sites (results not shown). However, all metrics were lower in comparison with the results obtained using temperature, and after applying the PIN correction correlations did not change significantly.

#### The effect of the 'preindustrial' (PIN) correction

Figure S3 shows the expected negative relationships between the  $\delta^{13}\text{C}_{\text{raw}}$  chronologies and  $c_a$  increase since 1850. After applying the atmospheric correction (Fig. 5), two of the  $\delta^{13}\text{C}_c$  chronologies (*pnCaz* and *puUrb*) still kept a significant negative relationship with  $c_a$ , whereas some series presented no significant trends (*psUrb*) or significant positive relationships (*psLil*). Since 1970 positive trends were observed in three of the sites (*pnCaz*, *psLil* and *puPed*). Figure S4 illustrates that after using the PIN correction none of the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies showed a significant negative trend against  $c_a$ , presenting positive relationships at all the sites. The most dramatic changes were observed in *puUrb*, where the significant negative trends obtained using the  $\delta^{13}\text{C}_c$  series were replaced by significant positive ones, particularly since 1970. Figure 6 plots the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies vs. time, showing the same significant trends as observed vs.  $c_a$ .

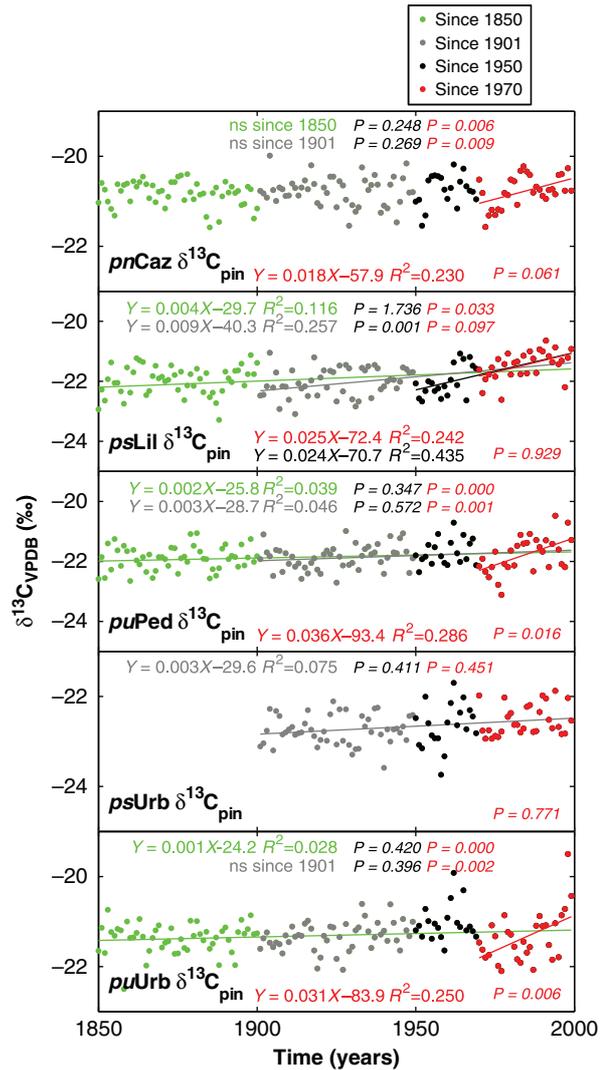
#### Trends in $\delta^{13}\text{C}_{\text{pin}}$ chronologies and mean temperatures

Figure 7 shows the mean temperature trends from the closest grid point at each site (Table 1) for the months for which the most significant relationships were found with the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies. These temperature trends are in agreement with the trends reported in the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies (Fig. 6). The *pnCaz*  $\delta^{13}\text{C}_{\text{pin}}$  series displayed a significant positive trend since 1970 in agreement with the significant 1970 upward JJASO temperature trend,



**Fig. 5** Trends in the  $\delta^{13}\text{C}_c$  chronologies vs. the atmospheric  $\text{CO}_2$  concentration ( $c_a$ ). Linear regressions plotted since 1850 (green), 1901 (grey), 1950 (black) and 1970 (red) only when significant ( $P < 0.05$ ).

which showed the largest trends, with both 1970 regressions being significantly different from the previous periods. Significant positive trends (since 1901, 1950 and 1970) were shown by the *psLil*  $\delta^{13}\text{C}_{\text{pin}}$  chronology and the July–August temperatures near this site, but the difference between the 1950 and 1970 trend was not significant in either case. The *puPed*  $\delta^{13}\text{C}_{\text{pin}}$  series and nearby JJASO temperatures also shared significant positive trends after 1901 and 1970, and the difference between the 1950 and 1970 trends was significant in both cases. The *puUrb*  $\delta^{13}\text{C}_{\text{pin}}$  and July–August temperature showed the highest increasing rate since 1970, significantly different from the other periods. At this grid point, the 1970 JJASO temperature trend was also

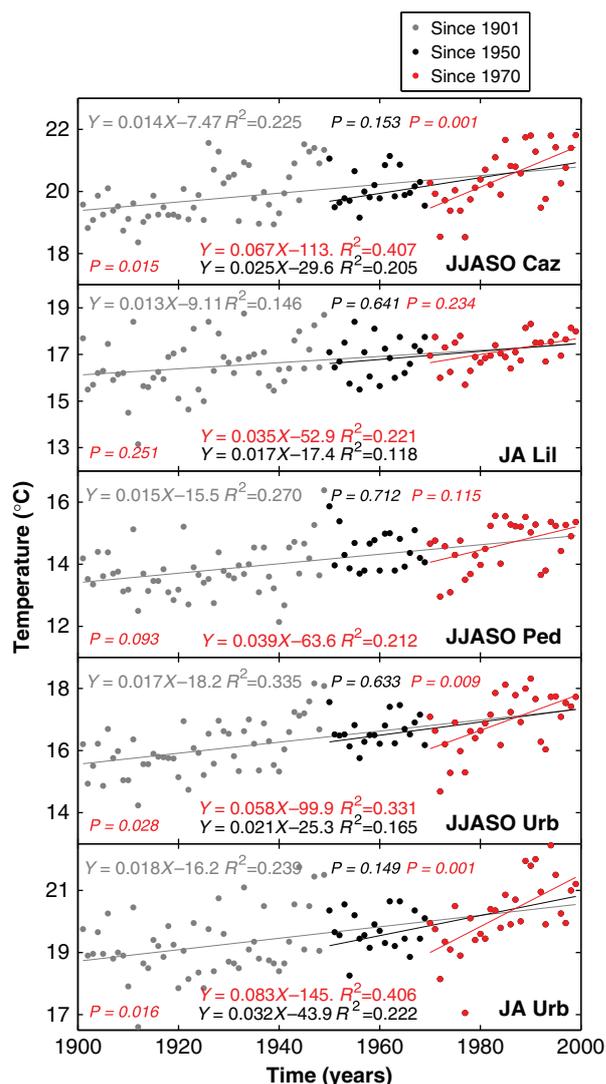


**Fig. 6** Trends in the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies throughout time. Linear regressions plotted since 1850 (green), 1901 (grey) and 1970 (red) only when significant ( $P < 0.05$ ). The  $P$ -value located next to the regression expression indicates significant difference from the regression since 1950 (text in black) and the regression since 1970 (text in red), as a result of one-way analysis of covariance (ANCOVA). The  $P$ -value located in the right bottom corner indicates the significance of the difference between the 1950 and the 1970 regressions.

significantly different from the others, but with lower increasing rate since 1970 than July–August temperature considered alone, as well as similar  $R^2$  values than the 1901 trend. *psUrb*  $\delta^{13}\text{C}_{\text{pin}}$  showed significant trends only after 1901.

*Tree growth*

Figure 8 (left column) shows the ring-width frequencies from 1950 to 1999 in comparison with those prior to



**Fig. 7** Trends in mean temperature from the closest grid point at each site (Table 1) from June to October (JJASO) for *pnCaz*, *puPed* and *psUrb* and from July to August (JA) for *psLil* and *puUrb*. Linear regressions plotted since 1901 (grey), 1950 (black) and 1970 (red) only when significant ( $P < 0.05$ ). The  $P$ -value located next to the regression expression indicates significant difference from the regression since 1950 (text in black) and the regression since 1970 (text in red), as a result of one-way analysis of covariance (ANCOVA). The  $P$ -value located in the left bottom corner indicates the significance of the difference between the 1950 and the 1970 regressions.

1950, since 1600 for *pnCaz*, *psLil*, *puPed*, and since 1800 and 1900 for *psUrb* and *puUrb*, respectively. A higher frequency of narrow rings during the second half of the 20th century was found at all the stands in comparison to the frequencies calculated for the previous centuries, except for *psLil*. The Kolmogorov–Smirnov test (Table S1) and the CDF plots (Fig. 8, right column) demonstrate that RW distribution before and after 1950 are

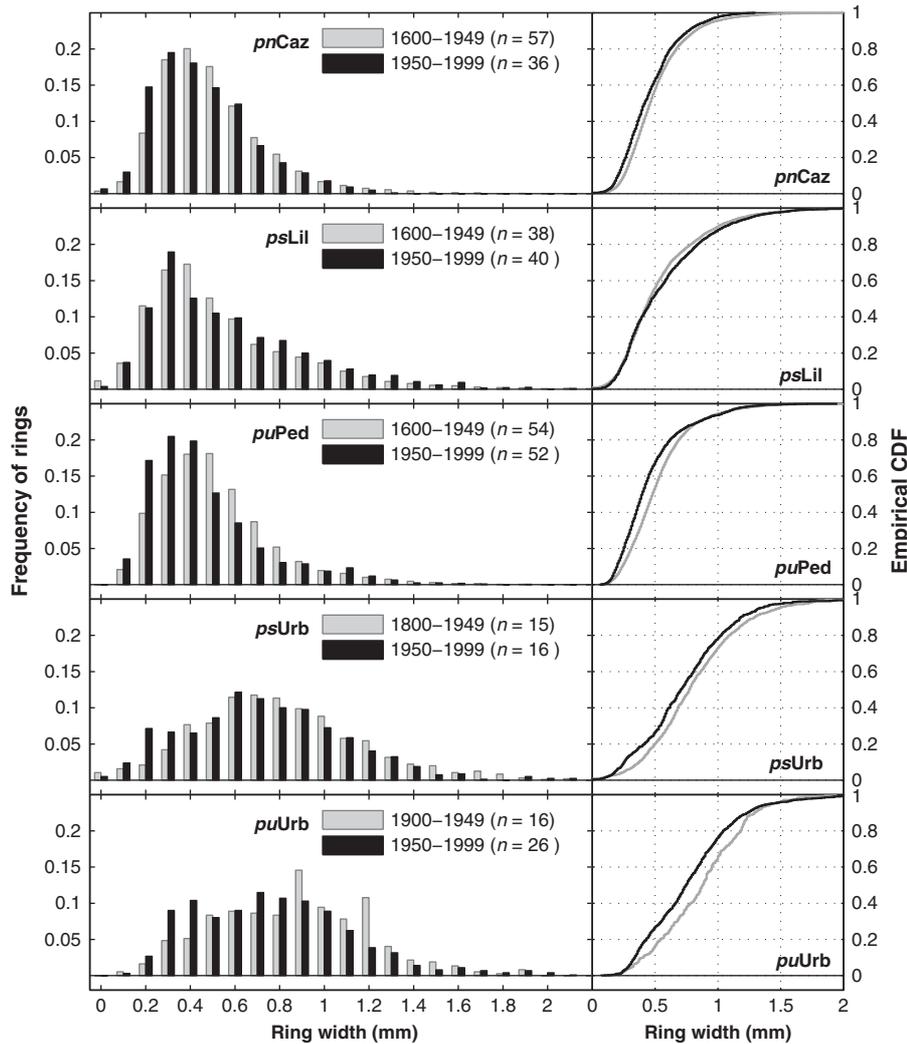
significantly different. All sites show a significant shift toward narrower rings after 1950, with the exception of *psLil*, which has wider rings after 1950. However, all sites show significantly narrower rings after 1970 (Fig. S5; Table S1). Figure S6 shows the means of the raw RW data before and after selection with the criteria described above (see ‘Material and methods’). This figure indicates that the filters applied to select the raw RW data worked well for the long chronologies (*pnCaz*, *psLil* and *puPed*), effectively eliminating the age trends. The youngest chronologies (*psUrb* and *puUrb*) showed clearly higher growth rates during the first years of the selected RW data, and it is therefore difficult to ensure entirely the effectiveness of the filtering method in these shorter series.

Figure 9 illustrates the trends of the selected RW data vs. time. It is noteworthy that almost all of them were negative. *pnCaz* RW showed decreasing rates since 1850 and increasing rates after 1950. *psLil* RW showed significant decreasing trends after 1950 and 1970; and *puPed* RW since 1850, 1901 and 1950, with higher declining rates in the two latter periods. The decreasing trends in *psUrb* RW were significant since 1850, 1901, 1950 and 1970, the latter showing the highest rates of decline. *puUrb* only showed significant negative trends after 1901. Figure S7 shows ring-width frequencies using the same data set for the periods 1850–1900, 1901–1949, 1950–1969 and 1970–1999, excluding the first period for *puUrb* due to the lack of data. *pnCaz* showed a higher frequency of narrow rings during the second half of the century than previously, but the rings since 1970 were not notably narrower than for the period 1950–1969. *psLil* did not show a clear distribution, but wider rings were observed during the brief interval from 1950 to 1969. In contrast, *puPed* clearly shows a higher frequency of narrow rings after 1950, and especially after 1970. *psUrb* and *puUrb* have narrower rings since 1950 in comparison with the preceding periods, although *puUrb* ring widths do not noticeably decline further after 1970.

## Discussion

### Site locations and climatic regimes

Each site is under a different climatic regime due to their geographical location in the Iberian Peninsula. This results in different combinations of Atlantic and Mediterranean atmospheric circulation patterns, and different degrees of continentality. The location of the stands in mountainous ranges at different altitudes and aspects is also an important factor with a considerable influence on the final water balance at each site. In this context, the  $\delta^{13}\text{C}$  values of the studied stands reflect a



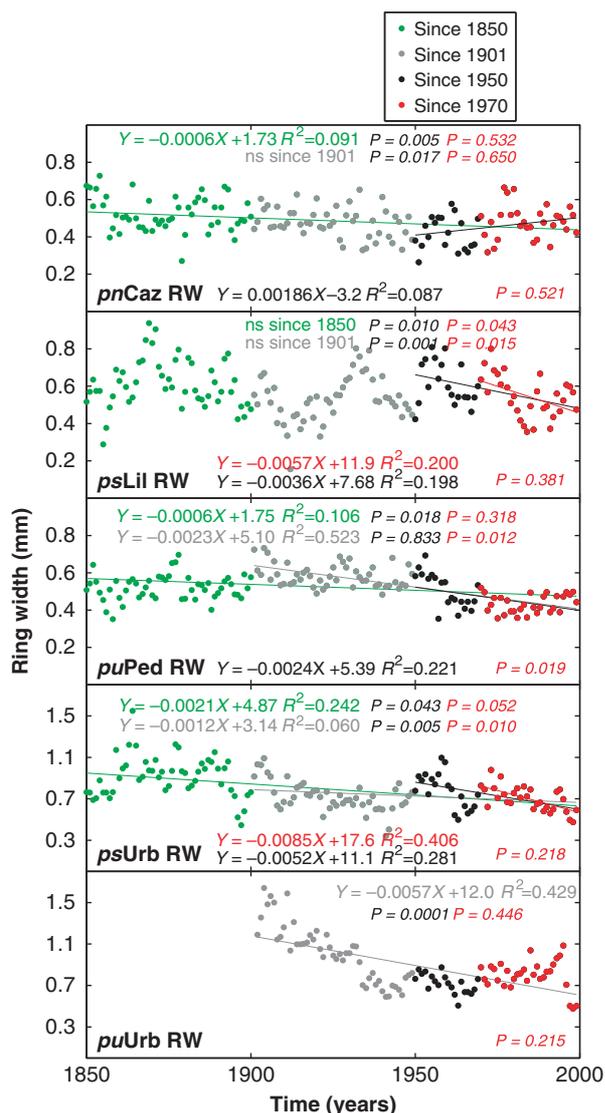
**Fig. 8** Left: Ring-width frequencies from 1950 to 1999 in comparison with the frequencies prior to 1950, since 1600 for *pnCaz*, *psLil*, *puPed* and since 1800 and 1900 for *psUrb* and *puUrb*, respectively (*n* indicates the number of trees for each group). Right: Empirical cumulative distribution function (CDF) for each group of ring-width data at each site.

water availability gradient well defined by the VPD, except for *psUrb*. The differences found in the  $\delta^{13}\text{C}$  ratios between the two sites of the Urbión range indicate the importance of site and stand features. The *puUrb* stand is in an open forest with closed areas over a rocky, shallow and well-drained soil at 1950 m asl facing to the southwest; whereas *psUrb* is a closed forest stand growing in a deep, partially drained soil located at a lower altitude (1750 m asl) facing towards the northeast. Thus, the overall lower *psUrb*  $\delta^{13}\text{C}$  values could be explained by a canopy closure effect on intercepting radiation by shading (Francey & Farquhar, 1982) or to more depleted  $\text{CO}_2$  respired from low growing plants and the soil (Schleser & Jayasekera, 1985). A dampening of the climatic signal due to a deeper soil or a more humid microclimate as a consequence of the northeast

aspect could be reasons for this site deviating from the water availability gradient followed by the rest.

*Physiological implications of the isotope trends*

All  $\delta^{13}\text{C}$  tree-ring series decreased, following the declining trend in the  $\delta^{13}\text{C}$  of the atmospheric  $\text{CO}_2$  related to the Suess effect. The most pronounced changes were observed mainly during the second half of the 20th century and differed between stands. Since 1950, the  $c_i/c_a$  ratios were constant at three sites, while one had a negative and another a positive trend. These observations are in agreement with Feng's (1998) findings in western North America, where  $c_i/c_a$  of trees remained predominantly constant before the 20th century, but changed with varied signs during the 20th century. In



**Fig. 9** Trends in the selected ring-width (RW) raw data over time. Linear regressions plotted since 1850 (green), 1901 (grey), 1950 (black) and 1970 (red) only when significant ( $P < 0.05$ ). The  $P$ -value located next to the regression expression indicates significant difference from the regressions since 1950 (text in black) and the regression since 1970 (text in red), as a result of one-way analysis of covariance (ANCOVA). The  $P$ -value located in the right bottom corner indicates the significance of the difference between the 1950 and the 1970 regressions.

a more theoretical context, Saurer *et al.* (2004) proposed three different scenarios that mainly differ in the degree by which the increase in  $c_i$  follows the increase in  $c_a$ : (1)  $c_i$  constant  $\rightarrow c_i/c_a$  decreases  $\rightarrow$  iWUE increases; (2)  $c_i$  increases proportional to  $c_a \rightarrow c_i/c_a$  constant  $\rightarrow$  iWUE increases; (3)  $c_i$  increases at the same rate as  $c_a \rightarrow c_i/c_a$  increases  $\rightarrow$  iWUE constant.

In this study, the three sites with constant  $c_i/c_a$  ratios (*pnCaz*, *puPed* and *psUrb*) had significant increases in  $c_i$

and improvements in iWUE. According to theory, this illustrates scenario 2, the most common reaction reported for conifers in Eurasia (Saurer *et al.*, 2004), and in agreement  $\delta^{13}\text{C}_c$  chronologies displayed an active response to  $c_a$  changes (McCarroll *et al.*, 2009).  $c_i/c_a$  reflects gas exchange and integrates the response of plants to environmental changes (Ehleringer & Cerling, 1995). Constant  $c_i/c_a$  may indicate tree adaption to  $c_a$  increases and could be attained by a simultaneous decrease of both stomatal conductance and photosynthetic rates (Saurer *et al.*, 2004). A constant  $\Delta$  pattern was also found in *P. edulis* from the southwestern United States along a summer monsoon gradient (Williams & Ehleringer, 1996).

At *psLil*, the northwestern studied stand, a significant decrease in  $c_i/c_a$  resulted in the greatest iWUE improvement, following scenario 1 (Saurer *et al.*, 2004). The  $\delta^{13}\text{C}_c$  chronology exceeded the expected values due to an active tree response to  $c_a$  increase, indicating that other factors are reinforcing the stomatal closure in the trees of this forest. In agreement, several other papers have also reported a decrease in  $\Delta$  with an even stronger enhancement in iWUE (Peñuelas & Azcón-Bieto, 1992; Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Hietz *et al.*, 2005).

The *puUrb* relict forest showed a significant increase in the  $c_i/c_a$  ratio that resulted in the lowest iWUE increase of those observed. This finding illustrates and agrees with scenario 3 discussed by Saurer *et al.* (2004) that, although no iWUE improvements should be expected theoretically, iWUE was often increased on real data. This suggests a very weak stomatal response to  $c_a$  increase and, in agreement, the  $\delta^{13}\text{C}_c$  chronology displayed a passive response to  $c_a$  changes (McCarroll *et al.*, 2009). The reduction in the *puUrb* sensitivity to  $c_a$  could be interpreted as a 'saturation effect' (Waterhouse *et al.*, 2004). This relict forest of *P. uncinata* could be less reactive due to its location at the western limit of its phylogeographical distribution, perhaps more vulnerable to environmental changes than forests growing in the main core of their distribution range. Similarly, a slower increase in iWUE was detected in lower elevation populations of *Abies pinsapo*, a relict species located in southern Spain, than in the higher elevation stands growing in a more favorable environment that might be less susceptible to environmental changes (Linares *et al.*, 2009).

The patterns after 1970 are different and merit further interpretation. The decline of the  $\delta^{13}\text{C}$  rates was lower than the  $\delta^{13}\text{C}_{\text{atm}}$  decline rate. As a consequence, all studied stands showed the highest iWUE of all time periods derived from the lowest  $\Delta$  rates (significant decreases for *pnCaz*, *psLil* and *puPed*; nonsignificant trends in the Urbión sites). It seems that other environmental factors reduced the stomatal conductance since

1970, making all  $\delta^{13}\text{C}$  series more enriched in  $^{13}\text{C}$  than would be expected from merely following the declining trend in  $\delta^{13}\text{C}_{\text{atm}}$ .

#### *Physiological response to the $c_a$ increases*

After removing the low-frequency trends due to the theoretical tree physiological responses to  $c_a$  using the PIN correction (McCarroll *et al.*, 2009), the climatic signal registered in all  $\delta^{13}\text{C}$  series was enhanced. It is noteworthy that *puUrb*, which showed a passive response to  $c_a$ , subsequently increased following the correction and revealed the highest improvements in the temperature signal: it changed from largely insignificant and even negative responses to significant positive relationships with summer temperatures over the Iberian Peninsula. The driest sites (*pnCaz* and *Urbión* stands) showed greater improvement than the wettest (*psLil* and *puPed*), suggesting that the PIN correction is more effective at separating the climatic and physiological trends at drier sites. The PIN correction could be more useful at the driest sites because this method is based on the response of stomatal conductance to increased  $c_a$ .  $\delta^{13}\text{C}$  at the driest sites is supposed to be more regulated by stomatal conductance (related to air relative humidity and antecedent rainfall) than at the wettest sites, where  $\delta^{13}\text{C}$  is expected to be more influenced by photosynthetic rates (related to temperature and photon flux); (McCarroll & Pawellek, 2001). Our results demonstrate that there is a physiological response to  $c_a$  changes at each forest site, modulating the  $\delta^{13}\text{C}$  ratios and contributing to the difference observed in the *iWUE* trends.

Furthermore, the PIN correction is confirmed as an effective tool for extracting climatic information recorded in  $\delta^{13}\text{C}$  tree-ring data, and thus can be very useful in paleoclimatic research for improving the reliability of climate reconstructions using this proxy.

The well-known and extensively used atmospheric correction used to remove the Suess effect from the  $\delta^{13}\text{C}_{\text{raw}}$  chronologies was not sufficient to compensate for the decline in the  $\delta^{13}\text{C}_c$  series vs.  $c_a$  when the trees showed a passive response to  $c_a$  increase. The PIN correction successfully handled these declining trends, revealing  $\delta^{13}\text{C}_{\text{pin}}$  chronologies with positive trends. These positive trends, in some cases indeed shared with the  $\delta^{13}\text{C}_c$  series, cannot be caused by changes in  $c_a$  according to the second logical physiological constraint from McCarroll *et al.* (2009). The theory postulates that the *iWUE* improvements as a product of rising  $c_a$  are limited to keep a steady  $c_i/c_a$  ratio (active response), guaranteeing that any  $\delta^{13}\text{C}$  rise beyond maintaining a constant  $c_i/c_a$  ratio is not eliminated. Therefore, the upward trends observed in the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies

throughout time are very likely to be caused by another environmental factor than  $c_a$ .

#### *Temporal changes in the climatic constraints*

The spatial correlation fields showed a clear relationship between the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies and mean seasonal temperature from the gridded CRU TS 2.1 data. The months showing the highest correlations (JJASO for *pnCaz*, *puPed* and *psUrb*; July–August for *psLil* and *puUrb*) were in agreement with the response functions developed by Andreu *et al.* (2008), using a Spanish gridded data set ( $25 \times 25$  km grid box) created by the ‘Instituto Nacional de Meteorología’ (INM). Mean temperatures play an important role in the  $\delta^{13}\text{C}$  ratios at the studied sites and could therefore plausibly be the driver of the increases in the  $\delta^{13}\text{C}$  values not explained by the rise in  $c_a$ , leading to lower  $\Delta$  rates and the highest improvements in the *iWUE*.

The stomatal conductance is strongly influenced by VPD, which can be considered a driving force for transpiration. Thus, an increase in VPD could be linked to temperature increases and/or precipitation decreases, reducing stomatal conductance. The results of this work did not show any clear precipitation pattern, but a good and logical agreement between the trends in mean temperature and  $\delta^{13}\text{C}$  series was found. This indicates that the upward trends in temperature are linked to the VPD at the studied sites, reinforcing our hypothesis that temperature is significantly influencing stomatal closure (less  $\Delta$ ), increasing the  $\delta^{13}\text{C}$  ratios and, as a consequence, increasing the *iWUE*. However, stomatal closure may constrain photosynthesis and plant production when water availability in the soil is reduced and VPD is elevated (Smith, 1989). This is supported at a larger scale by satellite observations showing that ‘a warming climate does not necessarily lead to higher  $\text{CO}_2$  growing-season uptake’ (Angert *et al.*, 2005).

#### *Tree growth*

For *pnCaz*, narrower rings since 1950 and 1970 (compared to the widths from previous centuries) and a slightly significant decreasing trend since 1850 were found. However, a further decline in growth was not detected after 1970 in agreement with a stable mean growth reported in a nearby stand of the same species (Linares & Tiscar, 2010). Although for *psLil* some wider rings were found after 1950 in comparison to preceding time periods, significantly narrower rings were detected after 1970 and significant declining growth trends observed since 1950 and 1970. This Cantabrian site also presented the highest *iWUE* rates, which might have

briefly ameliorated potential growth declines from 1950 to 1969. However, since 1970 increases in iWUE seem to be insufficient to compensate for environmental stress. On the other hand, *pu*Ped has undoubtedly narrower rings during the second half of the 20th than in the former centuries, particularly after 1970. In agreement, declining growth trends were shown since 1850, 1901 and 1950, demonstrating the high sensitivity to climate changes at this pre-Pyrenees high altitude site. Both Urbión sites had narrower rings since 1950, especially since 1970 for *ps*Urb, and accordingly significant declines in growth trends.

Overall our results reveal declines in growth and narrower rings during the second half of the 20th century at four sites and after 1970 at all sites. However, due to the nature of our data and the approach used, these interpretations require some caution. Although age trends are not very likely to remain in tree-ring data after 150 years, the higher growth rates at the beginning of the shorter series raise some concern on the efficiency of the age trend removal at the youngest stands. In contrast to the trends observed in Bristlecone pines (Salzer *et al.*, 2009), the observed trend in the studied Iberian pine forests were in the same direction as a trend that could reflect tree age. A negative relationship between lifespan of trees and their growth rates reported for other species (Larson, 2001; Black *et al.*, 2008) should also be taken into account. Our results provide no evidence for a fertilization effect on tree growth as a consequence of the  $c_a$  increase. Rather, we observed an overall growth decline. In the context of the widespread growth increase recently reported at temperate deciduous forests without water limitations (McMahon *et al.*, 2010), no evidence for an increase in growth at the more moisture stressed Mediterranean pine forests is a very relevant conclusion.

#### *Ecological consequences of the observed patterns*

Considering that iWUE rates depend on assimilation ( $A$ ) and stomatal conductance ( $g$ ), the observed enhancement in iWUE could be due theoretically to an increase in  $A$ , a decrease in  $g$ , or both. However, no evidence of growth increase is seen at four of the sites during the second half of the 20th century, and not at any site after 1970. This suggests that there is no evidence to support an increase in  $A$ , indicating that a reduction in  $g$  is most likely responsible for the observed iWUE patterns. We demonstrated that among the causes of this reduction in  $g$  are the global rise in  $c_a$ , causing a direct physiological response at each stand, as well as the reported changes in climate. Especially since the last decades of the 20th century, warmer conditions seem to be causing an increase in VPD. This would

reduce  $g$ , leading to higher  $\delta^{13}\text{C}$  values (lower  $\Delta$  rates), and therefore higher iWUE than would be expected solely as a result of changes in  $c_a$ .

Our results agree with previous findings in the Iberian Peninsula. An enhancement in iWUE did not ameliorate the decrease in growth produced by warming at low elevation stands in the *Fagus sylvatica* southern distribution limit (Peñuelas *et al.*, 2008). Additionally, iWUE increases and basal area increment (BAI) declines were observed at low altitude *Abies pinsapo* populations in the south of Spain where water availability decreased during the last 30 years (Linares *et al.*, 2009). Increases in iWUE and BAI declines were also reported at a dense *P. nigra* afforestation in the Iberian Peninsula (Martin-Benito *et al.*, 2010). Ferrio *et al.* (2003) reported that *P. halepensis* and *Quercus ilex* showed iWUE enhancements as a result of a reduction in water availability. The intensification of warming in a longer term could affect the trees more sensitive to drought, as seen during the severe drought that occurred in Catalonia in 1994, causing a canopy dieback in *Quercus ilex* (Lloret *et al.*, 2004) and even mortality in some populations of *P. sylvestris* (Martínez-Vilalta & Piñol, 2002).

The term of 'physiological forcing' defined the influence of a reduction in plant transpiration in climate (Gagen *et al.*, 2011). This reduction in evapotranspiration could affect hydrological budgets by increasing warming near surfaces (Betts *et al.*, 2000). Hence, a vegetation–climate feedback reducing canopy evapotranspiration in the Spanish forests can potentially alter energy and moisture transport between the surface and the atmosphere (Betts *et al.*, 2000). The consequences of lower conductance in the Iberian Peninsula, surrounded by the Mediterranean sea in the East and the Atlantic Ocean in the West, might be climatically less critical than in more continental areas where a high part of precipitation is generated by transpiration (Saurer *et al.*, 2004), but is still relevant for the land surface schemes of general circulation models that demand a precise representation of plant transpiration (Gagen *et al.*, 2011).

#### **Conclusions**

An enhancement in iWUE was observed at all the sites during the last decades of the 20th century, and was related to changes in  $c_a$  and climate. The  $c_a$  rise provoked particular physiological responses at each site. Less water availability due to the upward trends observed in mean temperatures may produce a reduction in the stomatal conductance, leading to higher iWUE than would be expected from changes in  $c_a$  alone. Our findings do not support any sustained increase in tree growth due to a  $\text{CO}_2$  fertilization effect during the

second half of the 20th century. Indeed, iWUE improvements seem to be insufficient to compensate for the negative effects of the reduced water availability on growth. A reduction in forest productivity due to water limitations could have serious implications regarding the degree of carbon sequestration by the Iberian forests, affecting the terrestrial biosphere carbon cycle. More research in natural forests of different regions worldwide is needed for a better understanding of how mature forests react under natural conditions to recent environmental changes. Our work renders new insights regarding the response to changes in  $c_a$  and climate in terms of tree physiology ( $\delta^{13}\text{C}$ ) and growth (ring width) at five sites of three different tree species in the Iberian Peninsula.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Results of the Kolmogorov–Smirnov tests.  $H$  indicates the result of the test:  $H = 0$  (Do not reject the null hypothesis),  $H = 1$  (Reject the null hypothesis at significance level of  $\alpha$ ). The null hypothesis is that empirical cumulative distribution functions (CDF) are equal, while the alternative hypothesis is ‘unequal’ CDFs. Additionally, whether RW data after 1950 or after 1970 has a ‘larger’ or ‘smaller’ CDF than before 1950 were also tested. Note that a ‘larger’ CDF corresponds to a major number of narrow rings, whereas ‘smaller’ corresponds to a high number of wide rings.

**Figure S1.**  $\delta^{13}\text{C}_{\text{raw}}$  (above),  $\delta^{13}\text{C}_c$  (middle),  $\delta^{13}\text{C}_{\text{pin}}$  (below) data vs. June to August (JJA) temperature ( $T$ ), precipitation ( $P$ ) and vapor pressure deficit (VPD) from 1901 to 1999.

**Figure S2.** Yearly values of  $^{13}\text{C}$  discrimination ratios ( $\Delta \approx c_i/c_a$ ), leaf intercellular  $\text{CO}_2$  concentration ( $c_i$ ) and intrinsic water-use efficiency (iWUE) inferred from  $\delta^{13}\text{C}_{\text{raw}}$  tree-ring data at all the studied stands. The  $\delta^{13}\text{C}_c$  chronologies (grey line) and the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies (yellow line) are also depicted. The limits defined by the two physiological tree response constraints are shaded: upper boundary (constant  $c_i/c_a$ , active response to  $c_a$ ) and lower boundary (constant  $c_a - c_i$ , passive response to  $c_a$ ).

**Figure S3.** Trends in the  $\delta^{13}\text{C}_{\text{raw}}$  chronologies vs. the atmospheric  $\text{CO}_2$  concentration ( $c_a$ ). Linear regressions plotted since 1850 (green), 1901 (grey), 1950 (black) and 1970 (red) only when significant ( $P < 0.05$ ).

**Figure S4.** Trends in the  $\delta^{13}\text{C}_{\text{pin}}$  chronologies vs. the atmospheric  $\text{CO}_2$  concentration ( $c_a$ ). Linear regressions plotted since 1850 (green), 1901 (grey), 1950 (black) and 1970 (red) only when significant ( $P < 0.05$ ).

**Figure S5.** Left: Ring-width frequencies from 1970 to 1999 in comparison with the frequencies prior to 1950, since 1600 for *pnCaz*, *psLil*, *puPed*, and since 1800 and 1900 for *psUrb* and *puUrb*, respectively ( $n$  indicates the number of trees for each group). Right: Empirical cumulative distribution function (CDF) for each group of ring-width data at each site.

**Figure S6.** Mean of the ring-width (RW) raw data for all samples and mean after removing the first 150 years from all the series (selected samples). The first number after  $n$  indicates the total amount of samples, whereas the second number shows the amount of the selected samples.

**Figure S7.** Ring-width frequencies for the periods 1850–1900, 1901–49, 1950–69 and 1970–99 at all the studied forests, except for *puUrb* where the former period is not shown ( $n$  indicates the number of trees for each group).

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