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## Carbon stable isotope-climate association in tree rings of *Pinus pinaster* and *Pinus sylvestris* in Mediterranean environments

Asociación entre el clima y los isótopos estables de carbono en los anillos de crecimiento de *Pinus pinaster* y *Pinus sylvestris* en ambientes Mediterráneos

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### SUMMARY

Carbon isotope ratios, recorded as  $^{13}\text{C}/^{12}\text{C}$  variations in tree rings of woody species, are the result of physiological changes related to environmental conditions. The objective of this work was to analyze the association among carbon thirteen variability ( $\delta^{13}\text{C}$ ), climate variables and tree-ring growth of *Pinus pinaster* and *Pinus sylvestris* in central Spain. Pulverized woody material from the period 1975-1999 from four trees for each pine species was analyzed. To detect common patterns in  $\delta^{13}\text{C}$  within each species and between  $\delta^{13}\text{C}$  and growth indices, a principal component analysis (PCA) was performed.  $\delta^{13}\text{C}$  of trees and the residual tree-ring chronologies were used at the PCA. Multilevel mixed linear models were applied between intrinsic water use efficiency (iWUE) and climate variables. Our results show an inverse significant correlation between  $\delta^{13}\text{C}$  and tree-ring growth of both species. Winter and spring air moisture was negatively correlated with iWUE of *Pinus pinaster*. July maximum temperature was positively correlated with iWUE of *Pinus sylvestris*. As  $\delta^{13}\text{C}$  is significantly related to climate and growth and it may be recommended as a valuable tool for tree growth dynamic analysis to withstand increasingly stressful climate conditions.

**Key words:** intrinsic water-use efficiency, Mediterranean pines, carbon-isotope, tree ring.

### RESUMEN

La relación entre los isótopos de carbono, almacenada como la variación de  $^{13}\text{C}/^{12}\text{C}$  en los anillos de crecimiento de las especies leñosas, es el resultado de cambios fisiológicos relacionados con las condiciones ambientales. El objetivo de este trabajo fue analizar la asociación entre la variabilidad del isótopo de carbono trece ( $\delta^{13}\text{C}$ ), las variables climáticas y el ancho de los anillos de crecimiento de *Pinus pinaster* y *Pinus sylvestris* que crecen en el centro de España. Se analizó polvo de madera obtenido a partir de los anillos de crecimiento del período 1975-1999 de cuatro árboles de cada especie. Para determinar patrones de variabilidad comunes de  $\delta^{13}\text{C}$  y la asociación entre  $\delta^{13}\text{C}$  con el crecimiento se aplicó un análisis de componentes principales (ACP) entre la  $\delta^{13}\text{C}$  de todos los árboles y las cronologías residuales para cada especie. Se aplicaron modelos lineares mixtos entre la eficiencia intrínseca del uso de agua (EIUA) y las variables climáticas. Los resultados muestran una correlación inversa altamente significativa entre  $\delta^{13}\text{C}$  y el ancho de los anillos de crecimiento de ambas especies. La humedad del invierno y de la primavera se correlacionan negativamente con la EIUA de *Pinus pinaster*. La temperatura media máxima de Julio se correlaciona positivamente con EIUA de *Pinus sylvestris*. Debido a que la  $\delta^{13}\text{C}$  está significativamente relacionada con el clima y el crecimiento se puede recomendar como una herramienta de valor para el análisis de la dinámica forestal ante las condiciones climáticas cada vez más estresantes.

**Palabras clave:** eficiencia intrínseca del uso de agua, pinos mediterráneos, isótopos de carbono, anillo de crecimiento.

### INTRODUCTION

Carbon isotope variability, recorded as  $^{13}\text{C}/^{12}\text{C}$  variations in tree rings of woody species, may represent an annual record of physiological tree responses to environment (Francey and Farquhar 1982). Since cellulose is not transferred between annual growth rings, intra and interannual seasonal events are recorded permanently as carbon thirteen variability ( $\delta^{13}\text{C}$ ) in tree rings (Tans *et al.* 1978).  $\delta^{13}\text{C}$  depends on stomatal conductance and pho-

tosynthesis; when stomatal conductance predominates, relative humidity and soil air moisture are the determining factors. When photosynthetic rate predominates, the main determining factors are solar radiation and temperature (McCarroll and Loader 2004).

The Mediterranean climate is characterized by summer drought and high interannual variability of precipitation and temperature. Species growing in Mediterranean environment show a yearly changing ratio of  $\delta^{13}\text{C}$  in each tree ring as a result of variable climate conditions (temperature

and precipitation); therefore,  $\delta^{13}\text{C}$  may provide a strong indicator of climate severity (McCarroll and Loader 2004), effects on gas exchange, water use and secondary growth of tree species (Granda *et al.* 2014) as well as the long term association among growth, climate and carbon exchange and the tree capacity for coping with severe drought events (Voltas *et al.* 2013).

Maritime pine (*Pinus pinaster* Ait.) is a dominant species in Mediterranean forests, mainly located in the Iberian Peninsula (Blanco *et al.* 1997). This species shows a wide ecological range (Nicolas and Gandullo 1967), as it survives at high or low temperatures, regular or variable rainfall as well as severe droughts. It is adapted to the extremely cold winters of the continental climate in central Spain and to the mild, temperate winters of the Atlantic coast in the western Iberian Peninsula (Alía *et al.* 1996, Blanco *et al.* 1997).

Scots pine (*Pinus sylvestris* L.) is the most widely distributed species of pine in the world (Schweingruber 1996), and in the Iberian Peninsula it occupies drier areas than in the other parts of the world (Barbéro *et al.* 1998). The primary locations to investigate the effects of increased aridity are drier areas of the distribution of *Pinus sylvestris* rather than the humid environment where the species more commonly grows (Martínez-Vilalta and Piñol 2002).

In Spain, *Pinus pinaster* and *Pinus sylvestris* occupy 1.6 and 1.21 million ha, respectively, as pure or mixed woodlands, and are two of the main species used in operational forestry (DGCN 2002). Previous studies in the Iberian Peninsula determined that  $\delta^{13}\text{C}$  in tree rings of *Pinus nigra* Arnold forests was affected by climate (Granda *et al.* 2014), whereas  $\delta^{13}\text{C}$  in *Pinus uncinata* Miller, *Pinus sylvestris* and *Pinus nigra* was affected by summer rainfall (Andreu *et al.* 2008). Furthermore, the isotope analysis revealed the vulnerability of Scots pine to winter-drought in its southernmost distribution limit (Voltas *et al.* 2013). Presently, new studies point out the link between atmospheric changes related to human activities and their impact on isotope records (Saurer and Siegwolf 2007, Silva and Horwarth 2013).

Presently, there is no information about stable carbon isotopes in tree rings of *Pinus pinaster* growing under Mediterranean climate conditions. Given the high vulnerability of Iberian plant communities to climate change (Bakkenes *et al.* 2002) and the importance of *Pinus pinaster* and *Pinus sylvestris* forests in this region, stable carbon isotope ratios may provide valuable information about both, climate variables that affect growth variability and how  $\delta^{13}\text{C}$  is correlated with tree-ring growth and climate. These results may help us to explore how populations behaved in the past and how they will behave under the climatic shifts expected in the future (Andreu *et al.* 2008).

The objectives of this study are: a) to determine the link between tree-ring growth and  $\delta^{13}\text{C}$ , and b) to establish the relationship between climate variables (temperature, rainfall and air moisture) and  $\delta^{13}\text{C}$  in tree ring of *Pinus pinaster* and *Pinus sylvestris* growing in Mediterranean environments. Our hypothesis is that  $\delta^{13}\text{C}$  is significantly

influenced by climatic variables; hence a high variability of  $\delta^{13}\text{C}$  can be expected for both species growing in two sites showing contrasting climates in Mediterranean environments.

## METHODS

**Study area and sampled species.** Four dated trees of *Pinus pinaster* from one sampling site (Cuenca) and four dated trees of *Pinus sylvestris* from another one (Soria) were selected. Samples belonged to two previous dendroclimatological studies (Bogino and Bravo 2008, Bogino *et al.* 2009; see figure 1, table 1). Detailed explanation of the dendrochronological methods can be found in both studies. Four trees were used as an adequate number of samples providing acceptable average absolute  $\delta^{13}\text{C}$  values (McCarroll and Pawellek 1998).

**Tree ring isotopes and iWUE analyses.** The  $\delta^{13}\text{C}$  isotope was determined on whole wood (Schleser *et al.* 1999, Barbour *et al.* 2001). Pulverized woody material was obtained for each tree ring with a Micromot 40E instrument with a 0.5 mm thick needle. The samples were obtained after identification of the previously dated cores (Bogino and Bravo 2008, Bogino *et al.* 2009). For each tree ring, 0.2-0.3 mg of pulverized woody material was placed into tin capsules. The stable carbon isotope ratio was determined by combusting the samples with an NA 2500 elemental analyzer (CE Instruments, Rodano, Italy), which was coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta plus, Bremen, Germany).

The  $\delta^{13}\text{C}$  was determined with the formula [1]:

$$\delta^{13}\text{C} (\text{‰}) = [({}^{13}\text{C}/{}^{12}\text{C} \text{ sample}) / ({}^{13}\text{C}/{}^{12}\text{C} \text{ VPDB} - 1)] \times 10^3, \quad [1]$$

where  $\delta^{13}\text{C}$  (‰) is the proportional deviation from the international Vienna PeeDee Belemnite (VPDB) carbonate standard (Craig 1957).

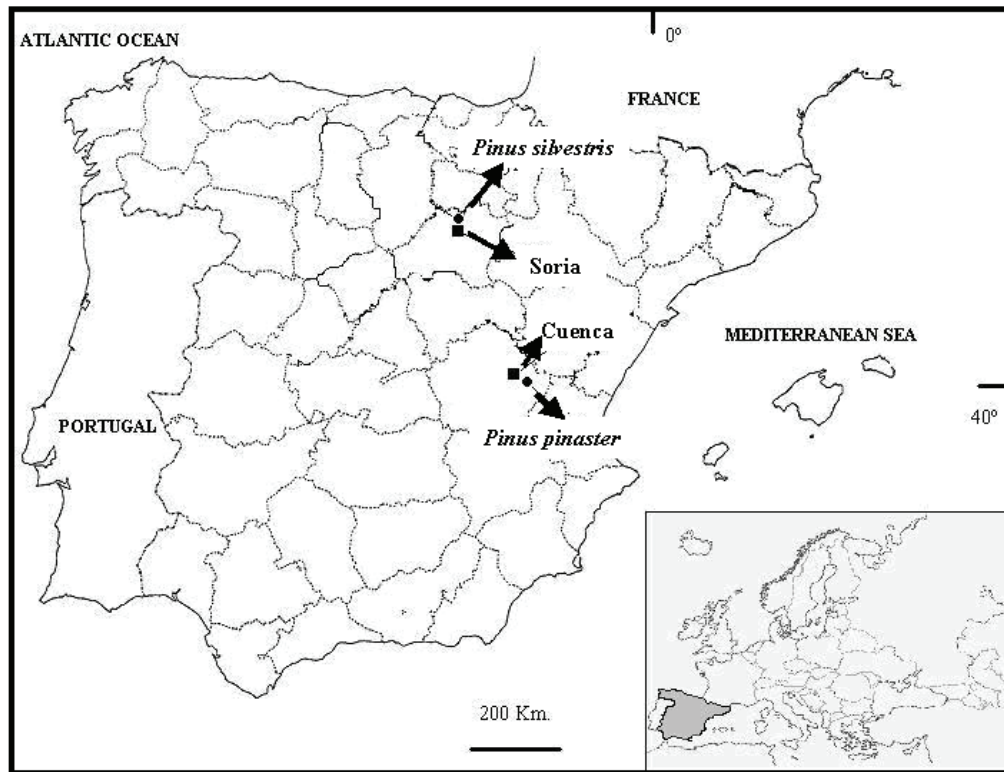
Intrinsic water use efficiency (iWUE) was used instead of  $\delta^{13}\text{C}_{\text{wood}}$  (‰) as the isotopic carbon ratios ( ${}^{13}\text{C}/{}^{12}\text{C}$ ) in atmospheric  $\text{CO}_2$  has steadily decreased since the 1960s due

**Table 1.** Location, altitude and time span of *Pinus pinaster* and *Pinus sylvestris* series used in the isotope analyses.

Ubicación geográfica, altitud y período analizado para las series de *Pinus pinaster* y *Pinus sylvestris* usadas en el estudio de isótopos.

	<i>Pinus pinaster</i>	<i>Pinus sylvestris</i>
Latitude	39° 48' 56"S	42° 04' 36"S
Longitude	01° 15' 36"W	02° 30' 18"W
Altitude (m)	920	1,676
Time span	1947-2005	1945-2005

Time span: Chronology longitude (Bogino and Bravo 2008).



**Figure 1.** Sampling sites (circles) of *Pinus pinaster* and *Pinus sylvestris* and the meteorological stations (squares) Soria and Cuenca in the Iberian Peninsula.

Sitios de muestreo (círculos) de *Pinus pinaster* y *Pinus sylvestris* y estaciones meteorológicas (cuadros) Soria y Cuenca en la península Ibérica.

to fossil fuel emissions. To take into account the atmospheric  $\delta^{13}\text{C}$  reduction, the isotopic discrimination between atmospheric carbon and plant carbon ( $\Delta$ ), instead of raw series of  $\delta^{13}\text{C}$  in wood, has been used following the methodology proposed by McCarroll and Loader (2004) [2].

$$\Delta = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{wood}}) / (1 + \delta^{13}\text{C}_{\text{wood}} / 1000) \quad [2]$$

where  $\delta^{13}\text{C}_{\text{atm}}$  and  $\delta^{13}\text{C}_{\text{wood}}$  are the isotopic ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) in atmospheric  $\text{CO}_2$  and plant material, respectively, expressed in parts per thousand (‰) relative to the standard VPDB.

The relations between tree-ring  $\delta^{13}\text{C}$  and tree net carbon assimilation to stomatal conductance of water vapor and the iWUE strong relation with atmospheric  $\text{CO}_2$  partial pressure and long-term trends in the internal regulation of carbon uptake and water loss in plants led to remove the effect of global atmospheric  $\text{CO}_2$  increase by using the linear relation between  $\Delta$  and the ratio of intercellular ( $C_i$ ) to atmospheric ( $C_a$ )  $\text{CO}_2$  mole fractions (Farquhar *et al.* 1982) [3]:

$$\Delta = a + (b - a)C_i/C_a \quad [3]$$

where  $a$  is the fractionation during  $\text{CO}_2$  diffusion through the stomata equal to 4.4 ‰ (O'Leary 1981), and  $b$  is the

fractionation associated with reactions by Rubisco and PEP carboxylase equal to 27 ‰ (Farquhar and Richards 1984). Values for  $C_a$  and  $\delta^{13}\text{C}_{\text{atm}}$  were obtained from McCarroll and Loader (2004).

Finally, the  $C_i/C_a$  ratio reflects the balance between net assimilation ( $A$ ) and stomatal conductance for  $\text{CO}_2$  ( $g_c$ ) according to Fick's law:  $A = g_c(C_a - C_i)$ . Stomatal conductance for  $\text{CO}_2$  and water vapor ( $g_w$ ) is related by a constant factor ( $g_w = 1.6g_c$ ), thus linking the leaf gas exchange of carbon and water. The linear relationship between  $C_i/C_a$  and  $\Delta$  can be used to calculate the intrinsic water use efficiency,  $\text{WUE}_i = A/g_w$  [4]

$$\text{WUE}_i = c_a(b - \Delta) / [1.6(b - a)]. \quad [4]$$

The period 1975-1999 was analyzed. This period was selected as previous studies showed both, a changing relationship between climate variables and tree-ring growth of *Pinus pinaster* from the 1980s onwards (Bogino and Bravo 2008) as well as phenological changes in both species over the last 25 years (Peñuelas *et al.* 2002, Andreu *et al.* 2008, Granda *et al.* 2014).

**Statistical analyses.** Principal component analysis (PCA) was applied among all trees of both species to detect com-

mon patterns in  $\delta^{13}\text{C}$  and between  $\delta^{13}\text{C}$  and the residual tree-ring chronologies (data from Bogino and Bravo 2008, Bogino *et al.* 2009) to detect a significant association between growth and  $\delta^{13}\text{C}$ . Residual tree-ring chronology is the difference between predicted and estimate values obtained by applying a given function to the data set (Fritts 1976). Pearson's correlation coefficient was used between  $\delta^{13}\text{C}$  and monthly climatic variables (monthly air moisture, monthly precipitation and mean monthly maximum temperature) to estimate which environmental variables were statistically significant.

Climate data from Cuenca and Soria meteorological stations of the Agencia Estatal de Meteorología, Spain were used (table 2). Climate diagrams are included (figure 2). The software used in the statistical analyses was Infostat version 2008 (Di Rienzo *et al.* 2008).

Radial increments of tree rings are a set of longitudinal growth data, which imply that observations from the same tree could be largely correlated. Consequently, to avoid estimation problems a multilevel linear mixed model was used to determine the climate variables that influence iWUE in each tree ring. Each species was analyzed independently, iWUE in tree ring was considered as a random

variable, while climate was considered as a fixed variable. The formula was [5]

$$Y_k^{(t)} = \beta_0 + \sum_i \beta_{ki} * (X_i)_k^{(t)} + \chi_k + \varepsilon_k^{(t)}, \quad [5]$$

where  $Y_k^{(t)}$  is the logarithm of  $-1 * \text{iWUE}$  for the tree  $k$  in the year  $t$ ;  $\beta_0$  is the independent term;  $\beta_k$  is the parameter associate to tree  $k$  and climate variable  $i$  and  $(X_i)_k^{(t)}$  are the climate variables for each  $k$  tree in the year  $t$ ;  $\chi_k$  is the tree random effect and  $\varepsilon_k^{(t)}$  is the random error of the tree  $k$  in the year  $t$ .

Based on an exploratory analysis using Pearson's correlation coefficient between  $\delta^{13}\text{C}$  and monthly climate variables, from January to December of the growing season, six additive combinations of independent variables ( $X_i$ ) were used for both species (Models 1 to 6) and two additional models only for *P. sylvestris* (Models 7 and 8):

Model 1: Winter Precipitation + Spring Precipitation + Summer Precipitation + Fall Precipitation

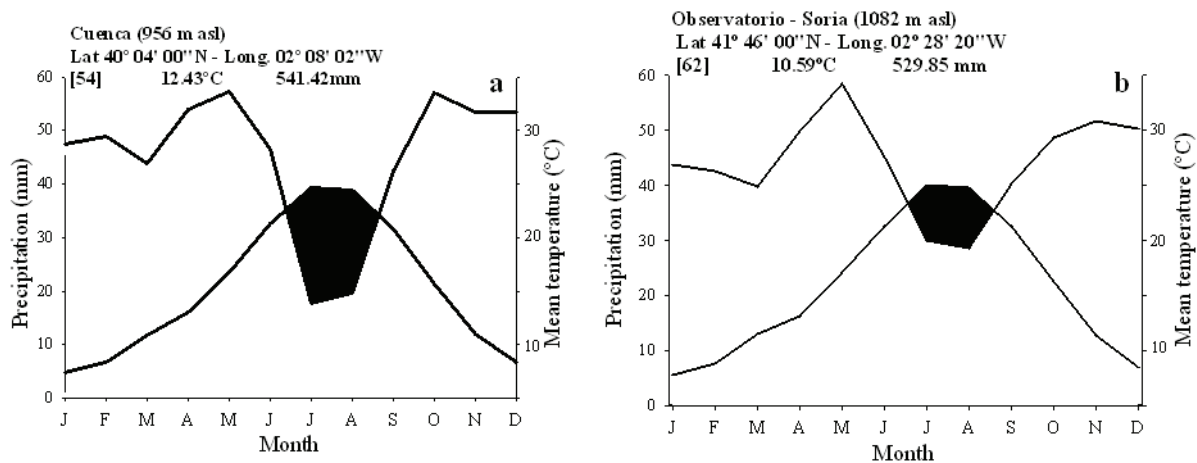
Model 2: Winter Precipitation + Spring Precipitation

Model 3: Spring Precipitation + Fall Precipitation

**Table 2.** Descriptive statistic of climate variables of Cuenca and Soria Meteorological Stations (1975-1999 period).

Estadística descriptiva de las variables climáticas de las estaciones meteorológicas de Cuenca y Soria (período 1975-1999).

Variable	Mean	SD	Min	Max
Mean annual rainfall , Cuenca (mm)	500.3	119.4	266.2	700.6
Mean annual maximum temperature, Cuenca (°C)	18.8	0.7	17.7	20.1
Mean annual relative humidity, Cuenca (%)	62.7	3.0	57.2	69.5
Mean annual rainfall , Soria (mm)	505.8	102.9	370.4	859.6
Mean annual maximum temperature, Soria (°C)	16.8	0.7	15.6	18.0
Mean annual relative humidity, Soria (%)	67.7	3.4	61.0	73.8



**Figure 2.** Climate diagram of Cuenca (a) and Observatorio Soria (b) meteorological stations.

Diagrama climático de las estaciones meteorológicas de Cuenca (a) y Soria (b).



Model 4: Winter Air Moisture + Spring Air Moisture + Summer Air Moisture + Fall Air Moisture  
Model 5: Winter Air Moisture + Spring Air Moisture  
Model 6: Spring Air Moisture + Fall Air Moisture  
Model 7: July maximum mean temperature  
Model 8: January maximum mean temperature

The tree random effect  $\delta_k \rightarrow N(0, \sigma_\delta^2)$  and the random error  $\mathcal{E}_k$  were considered as independently distributed  $N(0, \Sigma_k)$ , where  $\Sigma_k$  is an autoregressive error type 1 variance-covariance matrix (with multiple observations on individual autocorrelated trees over time). The significance of the variables was determined using the  $P$ -value parameter ( $P < 0.05$ ) and their biological behavior. Models were selected using the Akaike's information criterion (AIC), the Bayesian information criteria (BIC) and the statistic  $-2$  times logarithm of the likelihood function ( $-2LL$ ). AIC was corrected considering the low number of samples (100 samples for each tree species). PROC MIXED in SAS (SAS Institute Inc. 2004) was used to fit the models.

## RESULTS

**Average isotope values.** Mean values that characterized each tree-ring  $\delta^{13}C$  of both species in each analyzed year showed that *Pinus pinaster* had the highest and the lowest

$\delta^{13}C$  values ( $-22.62$  ‰ and  $-25.87$  ‰), whereas for *Pinus sylvestris* these values varied between  $-23.85$  ‰ and  $-25.80$  ‰ (figure 3).

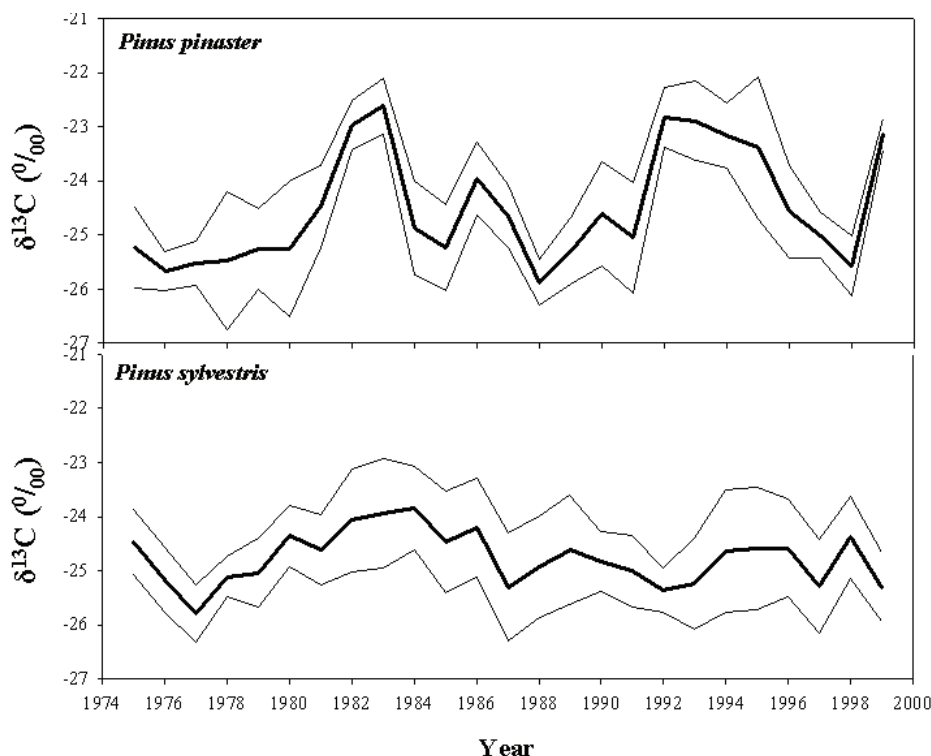
Mean values that characterized the eight trees are shown in table 3. *Pinus sylvestris* showed S.D. lower than 1 ‰, whereas *Pinus pinaster* showed higher variability throughout the 25 year period (SD = 1.41, tree 48).

**Relationships among  $\delta^{13}C$ , growth and climate.** PCA shows an opposite distribution of residual tree-ring chronologies and  $\delta^{13}C$ . Conversely,  $\delta^{13}C$  of the same species is grouped suggesting a common  $\delta^{13}C$  variability pattern. The 72 % of the variability was explained by the two first eigenvalues (figure 4).

Pearson's correlation coefficient from the PCA between trees of the same species showed a positive significant correlation in *Pinus pinaster* and *Pinus sylvestris*. Only trees 4 and 8 of *Pinus sylvestris* did not show a significant correlation (table 4).

A negative significant correlation was detected between  $\delta^{13}C$  and tree-ring growth. Pearson's correlation coefficient between the residual tree-ring chronology and the mean  $\delta^{13}C$  was  $r = -0.83$  ( $*** = P < 0.001$ ) for *Pinus pinaster* and  $r = -0.41$  ( $* = P < 0.05$ ) for *Pinus sylvestris*.

Pearson's correlation coefficient between climate variables and  $\delta^{13}C$  of *Pinus pinaster* showed that air moisture



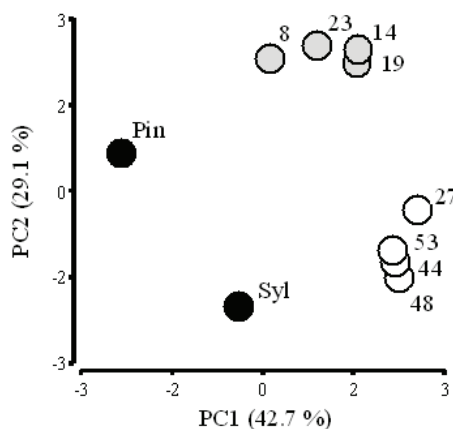
**Figure 3.** Mean values (thick lines) and confident interval at 95 % (thin lines) of isotope analyses for *Pinus pinaster* and *Pinus sylvestris* in central Spain.

Valores medios (líneas gruesas) e intervalos de confianza al 95 % (líneas finas) del análisis de isótopos de *Pinus pinaster* y *Pinus sylvestris* en el centro de España.

**Table 3.** Descriptive statistic of the four individuals of *Pinus pinaster* and *Pinus sylvestris* used in the isotope analyses in central Spain.  
Estadística descriptiva de los cuatro individuos de *Pinus pinaster* y *Pinus sylvestris* usados en el análisis de isótopos en el centro de España.

Tree	n	Mean (‰)	SD (‰)	Min. (‰)	Max. (‰)
<i>Pinus pinaster</i>					
Tree 48*	25	-24.52	1.41	-27.07	-22.2
Tree 44	25	-25.03	1.23	-27.10	-23.07
Tree 27	25	-23.89	0.96	-25.61	-22.07
Tree 53	25	-24.58	1.06	-26.05	-22.40
<i>Pinus sylvestris</i>					
Tree 23	25	-25.79	0.48	-26.65	-24.91
Tree 19	25	-24.23	0.78	-25.75	-22.85
Tree 8	25	-24.45	0.55	-25.51	-23.57
Tree 4	25	-24.6	0.58	-25.79	-23.2

\*For more information about selected trees, see Bogino and Bravo (2008); n: number of analyzed tree rings; Mean: Average  $\delta^{13}\text{C}$  of each tree; SD: Standard deviation of  $\delta^{13}\text{C}$  in each tree; Min and Max values of  $\delta^{13}\text{C}$  in each tree



**Figure 4.** Principal component analysis (PCA) of residual tree-ring chronologies of *Pinus pinaster* (Pin) and *Pinus sylvestris* (Syl) and  $\delta^{13}\text{C}$  in each tree. White circles belong to *Pinus pinaster*  $\delta^{13}\text{C}$ , grey circles to *Pinus sylvestris*  $\delta^{13}\text{C}$  and black circles to the residual tree-ring chronologies of both species. The numbers identify each tree.

Análisis de componentes principales (ACP) de las cronologías residuales de ancho de anillos de *Pinus pinaster* (Pin) y *Pinus sylvestris* (Syl) y  $\delta^{13}\text{C}$  de cada árbol. Los círculos blancos pertenecen a la  $\delta^{13}\text{C}$  de *Pinus pinaster*, los círculos grises a la  $\delta^{13}\text{C}$  de *Pinus sylvestris* y los círculos negros a las cronologías residuales de ambas especies. Los números identifican a cada árbol.

negatively affects  $^{13}\text{C}/^{12}\text{C}$  variability from winter to summer (January to July) (figure 5A). Also, rainfall showed a negative effect on  $\delta^{13}\text{C}$  from winter to springtime but was statistically significant only in April and May (figure 5B). Monthly mean maximum temperature in March showed a positive significant effect on  $\delta^{13}\text{C}$  (figure 5C).

Pearson's correlation coefficient between climate variables and  $\delta^{13}\text{C}$  of *Pinus sylvestris* showed that air mois-

**Table 4.** Pearson's correlation coefficient involving carbon thirteen variability ( $\delta^{13}\text{C}$ ) in trees of *Pinus pinaster* and *Pinus sylvestris*.

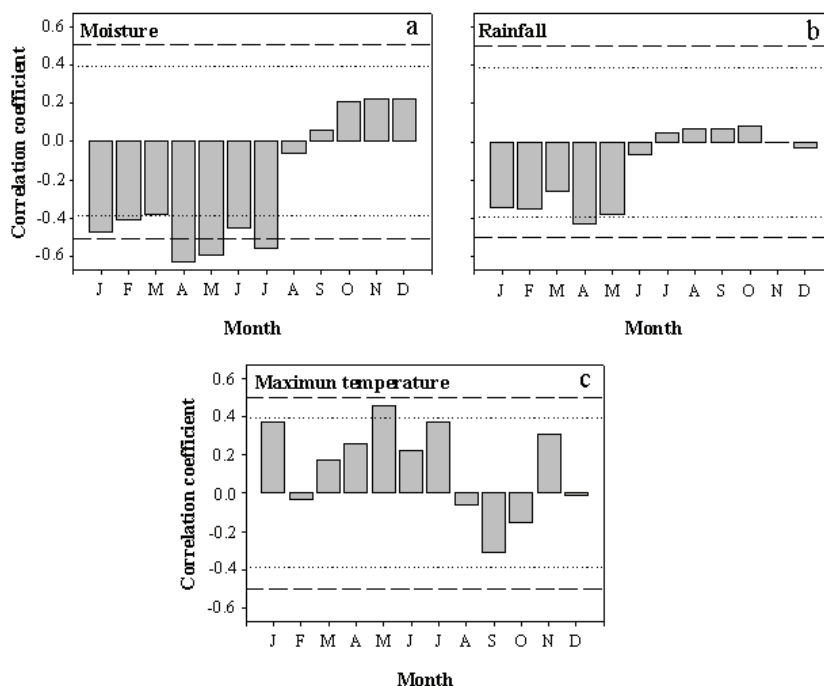
Coefficiente de correlación de Pearson entre la variabilidad del carbono trece ( $\delta^{13}\text{C}$ ) de los árboles de *Pinus pinaster* y *Pinus sylvestris*.

<i>Pinus pinaster</i>			
	Tree 48	Tree 44	Tree 27
Tree 44	0.82***	-	-
Tree 27	0.8***	0.73***	-
Tree 53	0.68***	0.7***	0.73***
<i>Pinus sylvestris</i>			
	Tree 23	Tree 9	Tree 8
Tree 19	0.59***	-	-
Tree 8	0.41*	0.5**	-
Tree 4	0.72***	0.78***	0.37

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ , correlation with asterisks are significant.

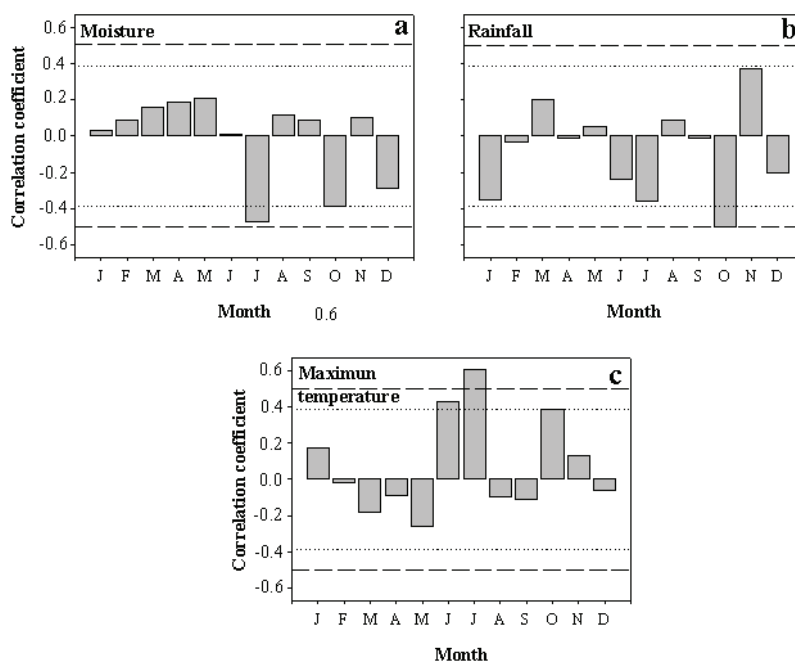
ture negatively affected  $^{13}\text{C}/^{12}\text{C}$  variability in July and in October (figure 6A). Also, rainfall in October showed a negative effect on  $\delta^{13}\text{C}$  (Figure 6B). Monthly mean maximum temperature in summer (June and July) showed a positive significant effect on  $\delta^{13}\text{C}$  (figure 6C).

*Relationships among iWUE and climate.* Following the criteria model selection, model 5 was selected for *Pinus pinaster* and model 7 was selected for *Pinus sylvestris* (table 5). Most of the *Pinus pinaster* trees show significant relationship between iWUE and winter and spring air moisture. July maximum temperature has a significant influence on iWUE in all *Pinus sylvestris* trees (table 6).



**Figure 5.** Pearson's correlation coefficients between  $\delta^{13}\text{C}$  of *Pinus pinaster* and mean monthly air moisture (A), monthly rainfall (B) and maximum mean monthly temperature. Bars outside dashed lines show significant coefficients at  $** = P < 0.01$ . Bars outside dotted lines show significant coefficients at  $* = P < 0.05$ .

Coefficiente de correlación de Pearson entre la  $\delta^{13}\text{C}$  de *Pinus pinaster* y la humedad mensual media del aire (A), la lluvia mensual (B) y la temperatura máxima media mensual. Las barras superiores a las líneas cortadas muestran coeficientes significativos a  $** = P < 0,01$ . Las barras superiores a las líneas de puntos muestran coeficientes significativos a  $* = P < 0,05$ .



**Figure 6.** Pearson's correlation coefficients between  $\delta^{13}\text{C}$  isotopes of *Pinus sylvestris* and mean monthly air moisture (A), monthly rainfall (B) and maximum mean monthly temperature (C). Bars outside dashed lines show significant coefficients at  $** = P < 0.01$ . Bars outside dotted lines show significant coefficients at  $* = P < 0.05$ .

Coefficiente de correlación de Pearson entre la  $\delta^{13}\text{C}$  de *Pinus sylvestris* y la humedad media mensual del aire (A), la lluvia mensual (B) y la temperatura máxima media mensual. Las barras superiores a las líneas cortadas muestran coeficientes significativos a  $** = P < 0,01$ . Las barras superiores a las líneas de puntos muestran coeficientes significativos a  $* = P < 0,05$ .



**Table 5.** Model selection procedure for intrinsic water use efficiency (iWUE) of trees of *Pinus pinaster* and *Pinus sylvestris* in central Spain. Modelos seleccionados para eficiencia en el uso intrínseco del agua (EUIA) de los árboles de *Pinus pinaster* y *Pinus sylvestris* en el centro de España.

Model	AR(1) Estimator (residual)	-2LL	AICc	BIC	Mixed model Pr < chi-squared
<i>Pinus pinaster</i>					
Model 1	0.3551 (0.0412)	219.6	223.8	222.4	0.0103
Model 2	0.3560 (0.0389)	87.4	91.5	90.2	0.0008
Model 3	0.4099 (0.0438)	95.6	99.7	98.4	<0.0001
Model 4	0.3276 (0.0333)	98.7	102.9	101.5	0.0047
Model 5	0.3861 (0.0341)	23.8	27.9	26.6	0.0002
Model 6	0.3553 (0.0349)	30.1	34.2	32.9	0.0009
<i>Pinus sylvestris</i>					
Model 1	0.5782 (0.0187)	135.9	140.0	138.7	<0.0001
Model 2	0.5039 (0.0212)	17.0	21.2	19.8	<0.0010
Model 3	0.5090 (0.0170)	-2.4	1.7	0.4	<0.0010
Model 4	0.5074 (0.0170)	23.5	27.7	26.3	<0.0001
Model 5	0.4299 (0.0154)	-54.8	-50.7	-52.1	<0.0001
Model 6	0.4260 (0.0164)	-46.3	-42.2	-43.6	0.0001
Model 7	0.6318 (0.0194)	-119.7	-115.5	-116.9	<0.0001
Model 8	0.5069 (0.0191)	-107.5	-103.3	-104.7	<0.0001

-2LL: -2 times logarithm of the likelihood function, AIC: Akaike's information criterion and BIC: Bayesian information criteria.

**Table 6.** Selected models for intrinsic water use efficiency (iWUE) of trees of *Pinus pinaster* and *Pinus sylvestris* in central Spain.

Modelos seleccionados para eficiencia en el uso intrínseco del agua (EUIA) de los árboles de *Pinus pinaster* y *Pinus sylvestris* en el centro de España.

	Parameter estimator	P-value
<i>Pinus pinaster</i>		
Intercept	-1.2821	0.0169
Winter moisture		
Tree 27	0.001914	0.2830
Tree 44	0.003226	0.0721
Tree 48	0.002707	0.1301
Tree 53	0.003588	0.0459
Spring moisture		
Tree 27	0.005565	0.0069
Tree 44	0.005138	0.0123
Tree 48	0.005288	0.0101
Tree 53	0.004304	0.0351
<i>Pinus sylvestris</i>		
Intercept	1.0284	0.0070
July max temp		
Tree 4	-0.02813	<0.0001
Tree 18	-0.02828	<0.0001
Tree 19	-0.03096	<0.0001
Tree 23	-0.02152	0.0002

## DISCUSSION

Contrasting climate and growth signals on  $\delta^{13}\text{C}$  in tree rings of *Pinus pinaster* and *Pinus sylvestris* were found over time among the analyzed Mediterranean forests of central Spain.

The individual tree response in relation to fractionation (the ratio of carbon isotope ratios in reactant and products) (Farquhar *et al.* 1989) was clear in this study, where different trees in the same calendar year showed a high variability of  $\delta^{13}\text{C}$ , which varied from -24.11‰ to -27.10‰ (year 1978) and from -23.43‰ to -26.12‰ (year 1994) in *Pinus pinaster* and *Pinus sylvestris*, respectively. The highest values of  $\delta^{13}\text{C}$  of *Pinus sylvestris* highlight previous studies that concluded that species growing at higher altitudinal positions have less  $\delta^{13}\text{C}$  discrimination than that presented by trees growing at lower sites (Fernandez *et al.* 2005). The variability in  $\delta^{13}\text{C}$  between species growing in the same area in Mediterranean environments showed opposite iWUE under severe drought events (Granda *et al.* 2014) and our results emphasized that this variability was detected between and within the species.

The inverse significant association between  $\delta^{13}\text{C}$  and tree-ring width of *Pinus pinaster* and *Pinus sylvestris* emphasises that both variables are affected by the similar driving environmental factors, thus,  $\delta^{13}\text{C}$  can be used to predict residual tree-ring width and vice versa (Andreu *et al.* 2008). Results showed a highly significant correlation between  $\delta^{13}\text{C}$  signal of *Pinus pinaster* trees and a significant correlation between  $\delta^{13}\text{C}$  signal of *Pinus sylvestris* trees, ex-

cept for two individuals, which underline the previous statements suggesting that species growing in variable environments show a changing ratio of  $^{13}\text{C}/^{12}\text{C}$  in each tree ring as a result of the variable climate conditions (high temperature and low precipitation) (Porté and Loustau 2001).  $\delta^{13}\text{C}$  provides a strong indicator of the intensity of these climate variables in this site (McCarroll and Loader 2004) being the long term tendency among iWUE, growth and climate a clear signal of drought causing death in trees growing in Mediterranean environments (Voltas *et al.* 2013).

Positive air moisture association with iWUE in *Pinus pinaster* and negative maximum temperature association with iWUE in *Pinus sylvestris* confirm the hypothesis that  $\delta^{13}\text{C}$  of conifers worldwide is an indicator of drought stress in arid and semiarid environments (Warren *et al.* 2001), which include the Iberian Peninsula (Andreu *et al.* 2008, Sanchez-Salguero *et al.* 2012).

De Micco *et al.* (2007) suggest that *Pinus pinaster* growing under maritime climatic conditions did not show any strong  $\delta^{13}\text{C}$  differences among different years, although a severe drought occurred during the summer of 2001; consequently, they concluded that a stable carbon analysis may not be useful to assess the severity of drought in those environments characterized by seasonal aridity, being site conditions a limiting factor that determines  $\delta^{13}\text{C}$  (Candel-Pérez *et al.* 2012). Even though we analyzed the same species, our results showed that *Pinus pinaster* is an accurate tool for studying climatic conditions recorded as  $\delta^{13}\text{C}$  (Porté and Loustau 2001) supporting previous studies which concluded that the same species may have different isotopic response growing in different environments (Sternberg and DeNiro 1983, Leavitt and Long 1984).

The association between climatic and different dendrochronological variables (tree-ring width, intraannual density fluctuation (IADFs) and carbon isotopes) of *Pinus pinaster* and *Pinus sylvestris* may provide an excellent instrument to understand the growth dynamics of the species under changing climatic conditions (Bogino and Bravo 2008, 2009, Bogino *et al.* 2009). In some cases isotopes may have a better correlation with climatic variables than it has with tree-ring width (Andreu *et al.* 2008), although one proxy does not limit the use of others. For example in sub-fossil chronologies of *Pinus sylvestris* in Finland, the use of  $\delta^{13}\text{C}$  could be limited for climatic reconstruction if it is not used with other proxies (McCarroll and Pawellek 2001).

To summarize, *Pinus pinaster* and *Pinus sylvestris* growing in Mediterranean environments show a strong  $\delta^{13}\text{C}$  signal, which allows us to detect a) an individual tree response to the local environment conditions, b) a physiological inverse association between  $\delta^{13}\text{C}$  and growth, and c) a significant association with climate variables.

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