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 13C/12C 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 (δ13C), 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 δ13C within each species and between δ13C and growth indices, a principal component analysis (PCA) was performed. δ13C 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 δ13C 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 δ13C 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.

INTRODUCTION

Carbon isotope variability, recorded as 13C/12C 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 (δ13C) in tree rings (Tans et al. 1978). δ13C depends on stomatal conductance and photosynthesis; 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 δ13C in each tree ring as a result of variable climate conditions (temperature
Carbon isotopes in tree rings of Pinus pinaster and Pinus sylvestris

and precipitation); therefore, δ13C 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 (Barbé 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 δ13C in tree rings of Pinus nigra Arnold forests was affected by climate (Granda et al. 2014), whereas δ13C 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 Horwath 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 δ13C 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 δ13C, and b) to establish the relationship between climate variables (temperature, rainfall and air moisture) and δ13C in tree ring of Pinus pinaster and Pinus sylvestris growing in Mediterranean environments. Our hypothesis is that δ13C is significantly influenced by climatic variables; hence a high variability of δ13C 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 δ13C values (McCarroll and Pawellek 1998).

Tree ring isotopes and iWUE analyses. The δ13C 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 (Finningan MAT Delta plus, Bremen, Germany).

The δ13C was determined with the formula [1]:

\[
\delta^{13}C = \left(\frac{(\delta^{13}C_{sample})}{(\delta^{13}C_{VPDB}) - 1}\right) \times 10^3,
\]

[1]

where δ13C (‰) is the proportional deviation from the international Vienna PeeDee Belemnitte (VPDB) carbonate standard (Craig 1957).

Intrinsic water use efficiency (iWUE) was used instead of δ13C wood (‰) as the isotopic carbon ratios (13C/12C) in atmospheric CO2 has steadily decreased since the 1960s due to human activities and their impact on isotope records (Saurer and Siegwolf 2007, Silva and Horwath 2013).

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

<table>
<thead>
<tr>
<th>Location</th>
<th>Pinus pinaster</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude</td>
<td>39° 48' 56&quot; S</td>
<td>42° 04' 36&quot; S</td>
</tr>
<tr>
<td>Longitude</td>
<td>01° 15' 36&quot; W</td>
<td>02° 30' 18&quot; W</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>920</td>
<td>1,676</td>
</tr>
<tr>
<td>Time span</td>
<td>1947-2005</td>
<td>1945-2005</td>
</tr>
</tbody>
</table>

Time span: Chronology longitude (Bogino and Bravo 2008).
Carbon isotopes in tree rings of *Pinus pinaster* and *Pinus sylvestris*

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

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

$$\Delta = \frac{(\delta^{13}C_{\text{atm}} - \delta^{13}C_{\text{wood}})}{(1+\delta^{13}C_{\text{wood}}/1000)}$$

[2]

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

The relations between tree-ring $\delta^{13}C$ and tree net carbon assimilation to stomatal conductance of water vapor and the iWUE strong relation with atmospheric 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 CO$_2$ increase by using the linear relation between $\Delta$ and the ratio of intercellular ($C_i$) to atmospheric ($C_a$) CO$_2$ mole fractions (Farquhar et al. 1982) [3]:

$$\Delta = a + (b-a)C_i/C_a$$

[3]

where $a$ is the fractionation during 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 $\delta^{13}C_{\text{atm}}$ 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 CO$_2$ ($g_c$) according to Fick’s law: $A = g_c(C_a - C_i)$. Stomatal conductance for 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, WUEi = $Ag_w$ [4]

$$WUEi = c(b-\Delta)/[1.6(b-a)].$$

[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 co-
mon patterns in $\delta^{13}C$ and between $\delta^{13}C$ and the residual tree-ring chronologies (data from Bogino and Bravo 2008, Bogino et al. 2009) to detect a significant association between growth and $\delta^{13}C$. 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 $\delta^{13}C$ 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]

$$Y_{k}^{(t)} = \beta_0 + \sum_{i} \beta_{i} X_{i}^{(t)} + \epsilon_{k}^{(t)},$$

where $Y_{k}^{(t)}$ is the logarithm of -1* iWUE for the tree $k$ in the year $t$, $\beta_0$ is the independent term; $\beta_{i}$ 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 $t$, $\gamma_{k}$ is the tree random effect and $\epsilon_{k}^{(t)}$ is the random error of the tree $k$ in the year $t$.

Based on an exploratory analysis using Pearson’s correlation coefficient between $\delta^{13}C$ 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
Model 4: Winter Precipitation + Summer Precipitation + Fall Precipitation
Model 5: Winter Precipitation + Summer Precipitation
Model 6: Winter Precipitation + Summer Precipitation + Fall Precipitation
Model 7: Winter Precipitation + Summer Precipitation + Fall Precipitation
Model 8: Winter Precipitation + Summer Precipitation

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean annual rainfall, Cuenca (mm)</td>
<td>500.3</td>
<td>119.4</td>
<td>266.2</td>
<td>700.6</td>
</tr>
<tr>
<td>Mean annual maximum temperature, Cuenca (ºC)</td>
<td>18.8</td>
<td>0.7</td>
<td>17.7</td>
<td>20.1</td>
</tr>
<tr>
<td>Mean annual relative humidity, Cuenca (%)</td>
<td>62.7</td>
<td>3.0</td>
<td>57.2</td>
<td>69.5</td>
</tr>
<tr>
<td>Mean annual rainfall, Soria (mm)</td>
<td>505.8</td>
<td>102.9</td>
<td>370.4</td>
<td>859.6</td>
</tr>
<tr>
<td>Mean annual maximum temperature, Soria (ºC)</td>
<td>16.8</td>
<td>0.7</td>
<td>15.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Mean annual relative humidity, Soria (%)</td>
<td>67.7</td>
<td>3.4</td>
<td>61.0</td>
<td>73.8</td>
</tr>
</tbody>
</table>

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 \( \delta_k \rightarrow N(0, \sigma^2_k) \) and the random error \( \varepsilon_k \) 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.

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

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

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

Figure 4. Análisis de componentes principales (ACP) de las cronologías residuales de ancho de anillos de *Pinus pinaster* (Pin) y *Pinus sylvestris* (Syl) y $\delta^{13}$C en cada árbol. Los círculos blancos pertenecen a la $\delta^{13}$C de *Pinus pinaster*, los círculos grises a la $\delta^{13}$C de *Pinus sylvestris* y los círculos negros a las cronologías residuales de ambas especies. Los números identifican a cada árbol.

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

<table>
<thead>
<tr>
<th></th>
<th>Tree 48</th>
<th>Tree 44</th>
<th>Tree 27</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pinus pinaster</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree 44</td>
<td>0.82***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tree 27</td>
<td>0.8***</td>
<td>0.73***</td>
<td>-</td>
</tr>
<tr>
<td>Tree 53</td>
<td>0.68***</td>
<td>0.7***</td>
<td>0.73***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Tree 23</th>
<th>Tree 9</th>
<th>Tree 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pinus sylvestris</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree 19</td>
<td>0.59***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tree 8</td>
<td>0.41*</td>
<td>0.5**</td>
<td>-</td>
</tr>
<tr>
<td>Tree 4</td>
<td>0.72***</td>
<td>0.78***</td>
<td>0.37</td>
</tr>
</tbody>
</table>

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

negatively affects $^{13}$C/$^{12}$C variability from winter to summer (January to July) (figure 5A). Also, rainfall showed a negative effect on $\delta^{13}$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 $\delta^{13}$C (figure 5C).

Pearson’s correlation coefficient between climate variables and $\delta^{13}$C of *Pinus sylvestris* showed that air moisture negatively affected $^{13}$C/$^{12}$C variability in July and in October (figure 6A). Also, rainfall in October showed a negative effect on $\delta^{13}$C (Figure 6B). Monthly mean maximum temperature in summer (June and July) showed a positive significant effect on $\delta^{13}$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 δ¹³C 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.

Figure 6. Pearson’s correlation coefficients between δ¹³C 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.
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.

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

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

DISCUSSION

Contrasting climate and growth signals on δ¹³C 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 δ¹³C, 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 δ¹³C of Pinus sylvestris highlight previous studies that concluded that species growing at higher altitudinal positions have less δ¹³C discrimination than that presented by trees growing at lower sites (Fernandez et al. 2005). The variability in δ¹³C 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 δ¹³C and tree-ring width of Pinus pinaster and Pinus sylvestris emphasizes that both variables are affected by the similar driving environmental factors, thus, δ¹³C can be used to predict residual tree-ring width and vice versa (Andreu et al. 2008). Results showed a highly significant correlation between δ¹³C signal of Pinus pinaster trees and a significant correlation between δ¹³C 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 $^{13}$C/$^{12}$C in each tree ring as a result of the variable climate conditions (high temperature and low precipitation) (Porté and Loustau 2001). $^{13}$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 (Volta 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 $^{13}$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-Salgueiro et al. 2012).

De Micco et al. (2007) suggest that Pinus pinaster growing under maritime climatic conditions did not show any strong $^{13}$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 $^{13}$C (Candel-Perez 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 $^{13}$C (Porté and Loustau 2001) supporting previous studies which concluded that the same species may have different isotopic response growing in different environments (Sterenberg 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 $^{13}$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 $^{13}$C signal, which allows us to detect a) an individual tree response to the local environment conditions, b) a physiological inverse association between $^{13}$C and growth, and c) a significant association with climate variables.

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