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<https://doi.org/10.1071/FP13067>

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1 Drought-induced mortality selectively affects Scots pine trees that show limited intrinsic  
2 water-use efficiency responsiveness to raising atmospheric CO<sub>2</sub>

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## Abstract

Widespread drought-induced tree mortality has been documented around the world and might increase in frequency and intensity under warmer and drier conditions. Nevertheless, ecophysiological differences between dying and surviving trees, which might underlie predispositions to mortality, are still poorly documented. Here we study Scots pines (*Pinus sylvestris* L.) from two sites located in north-eastern Iberian Peninsula where drought-associated mortality episodes were registered during the last decades. Time trends of discrimination against  $^{13}\text{C}$  ( $\Delta^{13}\text{C}$ ) and intrinsic water-use efficiency ( $\text{WUE}_i$ ) in tree rings at annual resolution and for a 34 years period are used to compare co-occurring now-dead and surviving pines. Results indicate that both surviving and now-dead pines significantly increased their  $\text{WUE}_i$  over time, although this increase was significantly lower for now-dead individuals. These differential  $\text{WUE}_i$  trends corresponded to different scenarios describing how plant gas exchange responds to increasing atmospheric  $\text{CO}_2$  ( $C_a$ ): the estimated intercellular  $\text{CO}_2$  concentration was nearly constant in surviving pines but tended to increase proportionally to  $C_a$  in now-dead trees. Concurrently, the  $\text{WUE}_i$  increase was not paralleled by a growth enhancement, regardless of tree state, suggesting that in water-limited areas like the Mediterranean, it cannot overcome the impact of an increasingly warmer and drier climate on tree growth.

**Additional keywords:** Western Mediterranean, water stress, die-off, tree rings,  $\delta^{13}\text{C}$

## Introduction

Drought-related episodes of tree mortality have been reported in many areas of the globe (Allen *et al.* 2010), and they are expected to become more frequent as climate gets warmer and drier (IPCC 2007; Williams *et al.* 2013). Western Mediterranean forests are ecosystems subjected to chronic water shortage. They are also likely to be especially vulnerable to an increase in the timing and severity of drought events (Bakkenes *et al.* 2002), since in this region temperature is estimated to raise about 3-4°C (Christensen *et al.* 2007) and precipitation might decrease up to 20% (Bates *et al.* 2008) during the 21<sup>st</sup> century. In particular, species reaching their southern distribution limit in the Mediterranean basin may be especially sensitive to the projected increases in drought frequency and intensity (Castro *et al.* 2004; Hampe and Petit 2005; Matías and Jump 2012).

The mechanisms that underlie drought-induced tree mortality are yet to be completely understood, although they are thought to be tightly linked to the tree water and carbon economy (Manion 1991; McDowell *et al.* 2008; Sala *et al.* 2010; McDowell 2011). Photosynthesis and metabolic sink activities can be substantially reduced or even ceased during severe drought, affecting the allocation of carbon to wood formation to varying degrees (McDowell *et al.* 2010). Thus, dying trees usually show characteristic growth patterns, including reduced growth (Pedersen 1998; Bigler *et al.* 2006; Hereş *et al.* 2012), high growth variability (Ogle *et al.* 2000) or greater growth sensitivity to climate (McDowell *et al.* 2010; Hereş *et al.* 2012).

Wood records climatic and physiological information traceable back in time (Fritts 2001; Vaganov *et al.* 2006) through features such as tree-ring width and carbon isotope composition ( $\delta^{13}\text{C}$ ) (McCarroll and Loader 2004). In seasonally-dry climates, tree-ring  $\delta^{13}\text{C}$  depends largely on tree water availability owing to the influence exerted by drought on the stomatal regulation of gas exchange (Warren *et al.* 2001; Ferrio *et al.* 2003). Thus, it reflects variation in intrinsic water-use efficiency ( $\text{WUE}_i$ ; **the ratio of assimilation to water loss through the stomata**) (Farquhar *et al.* 1982). Under a warmer and drier climate with higher  $\text{CO}_2$  concentrations (IPCC 2007), trees are expected to improve their  $\text{WUE}_i$  (Eamus 1991; Beerling 1997), as higher temperatures and increased  $\text{CO}_2$  concentrations

stimulate photosynthesis (Long 1991; Sage *et al.* 1995) and stomata reduce water losses in response to drought. An enhancement of WUE<sub>i</sub> in trees has already been observed for the 20<sup>th</sup> century, although it has not been paralleled by an expected stimulation of growth (Peñuelas *et al.* 2008; Peñuelas *et al.* 2011; Andreu-Hayles *et al.* 2011). However, the association between WUE<sub>i</sub> and growth appears to be species-dependent in Mediterranean ecosystems (Peñuelas *et al.* 2008; Maseyk *et al.* 2011; Linares and Camarero 2012), which suggests that the impact of global change drivers on Mediterranean forests in the short-term and, hence, future vegetation shifts, will be strongly determined by the extent of acclimation responses of individuals (Pías *et al.* 2010).

Recent investigations reveal that dying hardwoods (Levanič *et al.* 2011) and declining conifer trees (Linares and Camarero 2012) also increase their WUE<sub>i</sub> over time, although at different rates than surviving individuals. Other  $\delta^{13}\text{C}$ -related characteristics of dead trees include no apparent climatic sensitivity of their gas exchange traits and steeper negative relationships between gas exchange and growth (McDowell *et al.* 2010). However, retrospective analyses of tree-ring  $\delta^{13}\text{C}$  on dead, dying or declining trees are still scarce. In this context, direct comparisons of living and dying trees growing together in a particular stand should provide insight into the physiological mechanisms underlying their differential responses to raising atmospheric CO<sub>2</sub> concentrations, including the observed decoupling between growth and WUE<sub>i</sub>.

In this study, we focused on Scots pine (*Pinus sylvestris* L.), a widely distributed boreal tree species that reaches its southwestern (and dry) limit in the Iberian Peninsula (Barbéro *et al.* 1998; Matías and Jump 2012). During the last two decades, Scots pine has suffered important mortality episodes in the western part of the Mediterranean basin, following severe drought events (Martínez-Vilalta and Piñol 2002; Galiano *et al.* 2010; Hereş *et al.* 2012). This species presents a tight coupling between newly produced assimilates and wood formation for individuals undergoing severe drought conditions (Eilmann *et al.* 2010), which is consistent with a low availability of reserve carbohydrates and its possible role in drought-induced mortality (Galiano *et al.* 2011).

Here, we examined, with an annual resolution and at the level of individual trees (i.e., without pooling samples, cf. Dorado Liñán *et al.* 2011), carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) and  $\text{WUE}_i$  derived from  $\delta^{13}\text{C}$  records in tree rings of co-occurring living and now-dead Scots pines. These individuals were sampled at two sites where we recently found a direct association between tree mortality and severe drought episodes characterized by low summer water availability (Hereş *et al.* 2012). Our main objective was to investigate differences in ecophysiological performance between now-dead and surviving Scots pines so as to understand a possible long-term predisposition to mortality in a context of climate change. Also, we were interested in assessing the relationship between  $\text{WUE}_i$  and tree growth (in terms of basal area increment, BAI), as the growth of trees that died started to decline 15-40 years before death compared with that of surviving neighbours (Hereş *et al.* 2012). We hypothesized that now-dead trees would be intrinsically more vulnerable to drought and, therefore, would show a lower rate of  $\text{WUE}_i$  increase in response to rising atmospheric  $\text{CO}_2$  concentrations, a more pronounced climatic sensitivity of  $\text{WUE}_i$  and a steeper negative relationship between  $\text{WUE}_i$  and growth (BAI), compared to their surviving neighbors.

## Materials and methods

### *Study sites*

Scots pine trees were sampled at two sites located in the North East of the Iberian Peninsula (Catalonia): Prades (Bosc de Poblet, Prades Mountains, 41°33'N, 1°01'E) and Arcalís (Soriguera, Central Pyrenees, 42°34'N, 1°09'E), where high mortality rates have been observed in the last two decades (Martínez-Vilalta and Piñol 2002; Galiano *et al.* 2010; Hereş *et al.* 2012). The climate in Prades is typically Mediterranean while in Arcalís it is characterized by cool-summer Mediterranean conditions (Köppen 1936). The mean annual temperature is higher in Prades (11.2°C) than in Arcalís (9.7°C), while the mean annual rainfall is slightly lower in Prades (611 mm) than in Arcalís (653 mm). July is the warmest month with an average of 21.3°C for Prades and 19.9°C for Arcalís, while January is the coolest month with temperature averages of 3.1°C and 1.85°C in Prades and Arcalís, respectively (Climatic Digital Atlas of Catalonia, period 1951-2006) (Pons 1996; Ninyerola *et al.* 2000). Scots pine forests appear above 800 m a.s.l. in Prades, with an average density

of about 350 trees ha<sup>-1</sup> (Martínez-Vilalta *et al.* 2009), while at lower altitudes Scots pine is replaced by *Quercus ilex* (L.) and other typical Mediterranean species. At this site, the maximum tree age is ≈150 years (Hereş *et al.* 2012). In Arcalís, Scots pine grows between 600 and 1500 m a.s.l., with an average density of about 1070 trees ha<sup>-1</sup>, while other species are dominant at lower (*Quercus humilis* Mill., *Quercus ilex* L.) or at higher altitudes (*Betula pendula* Roth) (Galiano *et al.* 2010). At Arcalís, the maximum tree age for Scots pine is ≈100 years (Hereş *et al.* 2012). Forest management has been minimal during the last decades at both study sites (Martínez-Vilalta *et al.* 2009; Galiano *et al.* 2010).

### Sampling

The sampling campaigns were conducted in late autumn 2008 (Prades) and in early spring 2009 (Arcalís), and consisted in coring living and dead Scots pine trees along two transects per each site. The last complete annual ring for the living trees was 2008. The two transects were located on north-facing slopes and differed between them in altitude (800-1300 m a.s.l.) and humidity conditions (wet, dry) (Piñol *et al.* 1991; Galiano *et al.* 2010) (Table 1). Transects were linear and perpendicular to the slope. They started at random points and ended when all needed trees had been sampled, having thus a length that varied between 240 and 400 m. Trees were sampled within a 5 m distance from the line-track, taking care to keep a distance of at least 5 m between them (for more details see Hereş *et al.* 2012). At the wet transects, only living trees (wetL) were sampled, as mortality was very low, while at the dry transects, both living and dead individuals (dryL, dryD) were cored. Selected trees had similar diameter (around 30 cm) at breast high (DBH) (Table 1). Two wood cores per tree were extracted at breast height in a direction perpendicular to the slope, using 5 and 12 mm Pressler borers (Suunto®, Vantaa, Finland; Haglöf® AB, Långsele, Sweden). The 5 mm cores were used in a companion dendroecological study, from where BAI data is available (Table 1) (Hereş *et al.* 2012). Thirty of the 12 mm wood cores (representing a total of 30 living and dead trees) were selected here to analyze tree-ring  $\delta^{13}\text{C}$  (Table 1) with annual resolution, after checking their cross-dating consistency with the previously published chronology (Hereş *et al.* 2012). One of the dead trees from Prades was removed from the final dataset as it gave very deviating isotopic values for some tree rings, most

likely as a result of contamination during the  $\alpha$ -cellulose extraction (see next section). The final dataset comprised 20 living (wetL and dryL) and nine dead trees (dryD) (Table 1).

#### *Carbon isotope analyses*

To analyze  $\delta^{13}\text{C}$  with annual resolution, the available wood cores were first separated with a scalpel into individual annual rings under a binocular microscope (Leica EZ4, Leica Microsystems, Germany) for a period that started at the outermost ring of each sample and went back to the year 1975. By doing this, at least the first 25 years of growth were excluded from the analyses, avoiding the juvenile imprinting of the carbon isotope signature (Loader *et al.* 2007). The total number of years for the living trees was 34, while for the dead trees it varied depending on the year of death of each individual, which ranged from 2001 to 2008. In order to optimize the recovery of climate signals,  $\alpha$ -cellulose was extracted following a standard laboratory procedure (Modified Brendel and Water-Modified Brendel) (Brendel *et al.* 2000; Gaudinski *et al.* 2005). No treatment that could have altered the isotopic signal was applied to the sampled cores previous to the  $\alpha$ -cellulose extractions. After  $\alpha$ -cellulose was extracted from each whole annual ring, a homogenization with ultrasounds (Sonifier 250, Branson Ultrasonics, CT, USA) was applied in order to have a representative material of each annual ring (Laumer *et al.* 2009).  $^{13}\text{C}/^{12}\text{C}$  ratios were determined by mass spectrometry analysis at the Stable Isotope Facility of the University of California, Davis (USA) and expressed relative to the international standard Vienna PeeDee Belemnite (VPDB). A total of 943 samples were processed and the accuracy of analyses (standard deviation of working standards) was 0.06‰.

The atmospheric decline in  $\delta^{13}\text{C}$ , caused by fossil fuel emissions (Keeling *et al.* 1989), was removed by calculating  $\Delta^{13}\text{C}$  (Farquhar and Richards 1984) for the period 1975-2008:

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



where  $\delta^{13}\text{C}_{\text{air}}$  and  $\delta^{13}\text{C}_{\text{p}}$  are the carbon isotope ratios of the air (derived from ice-core records) and tree rings, respectively.  $\delta^{13}\text{C}_{\text{air}}$  values were obtained from published datasets (Ferrio *et al.* 2005b).

Using  $\Delta^{13}\text{C}$  data,  $\text{WUE}_i$  and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) values were estimated according to:

$$\text{WUE}_i = C_a \times \frac{(b - a)}{[1.6 \times (b - a)]} \quad (2)$$

$$C_i = \frac{(a - a) \times C_a}{(b - a)} \quad (3)$$

where  $C_a$  represents the atmospheric  $\text{CO}_2$  concentration,  $a$  is the fractionation during diffusion through stomata (4.4‰; O’Leary 1981), and  $b$  is the fractionation during carboxylation by Rubisco and PEP carboxylase (27‰; Farquhar and Richards 1984).  $C_a$  values from 1975 to 2003 were taken from the literature (Robertson *et al.* 2001a; McCarroll and Loader 2004) while for the period 2004 - 2008 they were estimated by means of linear regressions, based on the above mentioned datasets.

Theoretical  $\text{WUE}_i$  values were also calculated according to the three scenarios proposed by Saurer *et al.* (2004). Those scenarios describe how the  $C_i$  might follow the  $C_a$  increase over time: (1) either not at all, when  $C_i$  is maintained constant (**referred to also as “ct” throughout the text**); (2) in a proportional way, when  $C_i/C_a$  is maintained constant; or (3) at the same rate, when  $C_a - C_i$  is maintained constant. Initial  $C_i$  values were obtained for each individual tree by applying equation (3) to the average  $\Delta^{13}\text{C}$  and  $C_a$  values of the first five years of the study period (1975-1979). We used these three scenarios to calculate theoretical  $\text{WUE}_i$  values that were compared to the  $\text{WUE}_i$  data obtained from measured  $\delta^{13}\text{C}$ .

*Climatic data*

Monthly temperature ( $T$ , °C) and precipitation ( $P$ , mm) values (until 2006) were modelled at a spatial resolution of 180 m from discrete climatic data provided by the Spanish Weather-Monitoring System ([www.aemet.es](http://www.aemet.es)) (Ninyerola *et al.* 2007a, b). Data for 2007 and 2008 were estimated by means of linear regression models using a second climatic dataset that was available from the Catalan Meteorological Service (SMC) ([www.meteo.cat](http://www.meteo.cat)).

From the available climatic datasets, we calculated the average  $T$ , the accumulated  $P$  and the accumulated  $P$  over potential evapotranspiration ( $P/PET$ , used as a drought index), corresponding to annual (12 months from January to December of the same year) and to a 13-month period (from October previous to growth year to October current year of growth). The 12-month time period was chosen to represent the time trends (from 1975 to 2008) for the climatic variables. The 13-month time interval was used for statistical modelling to take into account that pines may use reserve carbohydrates assimilated during the previous year for earlywood formation (Saurer *et al.* 1995; Weber *et al.* 2007; Planells *et al.* 2009), and was selected based on the extent of simple correlations between  $WUE_i$  and monthly  $T$  and  $P$  data (see Supplementary Fig.). In all cases,  $PET$  was estimated using the Hargreaves method (Hargreaves and Samani 1982).

### Data analysis

All variables were first checked for normality (Kolmogorov-Smirnov test) and logarithm-transformed whenever necessary (BAI). Pearson correlation coefficients were used as a measure of association between  $WUE_i$  and monthly  $T$  and  $P$ , while linear regressions were conducted to assess temporal trends of annual climatic variables ( $T$ ,  $P$  and  $P/PET$ ). In order to check for differences in the relative strength of the common  $\Delta^{13}C$  variance signal for different within- and between-sites series combinations, we used the concept of fractional common variance (**hereafter referred to as  $a_{fcv}$  throughout the text**) as defined for dendroclimatology (e.g. Wigley *et al.* 1984):

$$a_{fcv} = \frac{\hat{\sigma}_y^2}{\hat{\sigma}_y^2 + \hat{\sigma}_e^2} \quad (4)$$

where  $\hat{\sigma}_y^2$  and  $\hat{\sigma}_e^2$  represent the population between-year (or within-series) and the population error estimates of variance component, respectively, that appear in a two-way mixed model analysis of variance in which the tree identity effect is considered as fixed factor and the year effect as random factor. The term  $a_{fcv}$  is directly related to the average inter-series correlation, which indicates how closely the various time series are related (Wigley *et al.* 1984). Closely related series are therefore expected to have a high  $a_{fcv}$  and a strong common signal. Being  $a_{fcv}$  a function of variance components, its standard error was approximated from the variance and covariance of between-year and error variance as reported elsewhere (Fischer *et al.* 2004). The expressed population signal statistic (EPS) was estimated as follows (Wigley *et al.* 1984):

$$EPS = \frac{Na}{1+(N-1)a} \quad (5)$$

where  $N$  is the number of  $\Delta^{13}\text{C}$  tree-ring series. The number of trees ( $t$ ) needed to achieve  $EPS=0.85$  (the consensus acceptable threshold for signal strength; Wigley *et al.* 1984) was also calculated.

The similarity of  $\Delta^{13}\text{C}$ -based  $\text{WUE}_i$  data with the three theoretical scenarios was quantified by means of linear regressions and root mean square error (RMSError) statistics. In order to analyze the time trends of  $\Delta^{13}\text{C}$  and  $\text{WUE}_i$ , the influence of the climatic variables on  $\text{WUE}_i$  and the relationships between  $\text{WUE}_i$  and growth ( $\log\text{BAI}$ ), three different sets of mixed linear models testing the assumption of constant responses among tree groups to selected covariates (i.e. heterogeneity of slopes ANOVA) were fitted to the data. The first model explained  $\Delta^{13}\text{C}$  or  $\text{WUE}_i$  as a function of: Condition, Site (Prades, Arcalís), the interaction Condition  $\times$  Site, the covariate Year (from 1975 to 2008), and the interactions Condition  $\times$  Year, Site  $\times$  Year, and Condition  $\times$  Site  $\times$  Year, which tested for heterogeneity of responses over time due to differences between conditions, sites or their interaction. Condition was defined as a categorical variable with three levels (wetL, dryL, dryD) coding for transect humidity (wet or dry) and tree state (Living or Dead). The second model was fitted to test

for the joint impact of atmospheric CO<sub>2</sub> rise and climate on WUE<sub>i</sub>. To this end, WUE<sub>i</sub> was modelled by sequentially introducing the following terms: Condition, Site, the interaction Condition × Site, the covariate C<sub>a</sub> and its interactions Condition × C<sub>a</sub>, Site × C<sub>a</sub>, and Condition × Site × C<sub>a</sub>, and the covariate P/PET and its interactions Condition × P/PET, Site × P/PET, Condition × Site × P/PET. The covariate P/PET was introduced after correcting for the effect of C<sub>a</sub> as it seemed sensible to test for the potential effect of climate warming on WUE<sub>i</sub> once the impact of rising atmospheric CO<sub>2</sub> had been removed from the data. Other climatic variables, such as *T* or *P*, were also tested but not included in the final models because they were highly correlated with each other and with P/PET and C<sub>a</sub> over the study period (*r* > 0.4 in all cases). P/PET was preferred over *T* or *P* because it can be interpreted as an integrative measure of the severity of the annual dry season in Mediterranean-like bioclimates (UNESCO 1979; Le Houerou 2004). In the third mixed linear model, logBAI was fitted according to the following model: Condition, Site, the interaction Condition × Site, the covariate WUE<sub>i</sub>, and the interactions Condition × WUE<sub>i</sub>, Site × WUE<sub>i</sub>, and Condition × Site × WUE<sub>i</sub>. Explanatory variables were introduced into the models following the ordering stated above for each of them (i.e. using type I sum of squares). In all three models, tree identity was introduced as subject (random effect), while Year was introduced as repeated effect at the tree level with a first-order autoregressive covariance structure to account for temporal autocorrelation. Significant differences in the response (slopes) of tree groups to the selected covariates were further examined by means of the following set of orthogonal contrasts comparing: a) dead (dryD) versus living (wetL and dryL) trees, and b) living trees between them (wetL versus dryL). If second order interactions were significant, independent contrasts for every site were considered. Model parameters were estimated using restricted maximum likelihood methods (REML).

**Relationships were considered significant at *P* < 0.05.** Statistical analyses were carried out with SAS (version 9.3, SAS Inc., Cary, NC) and SPSS (version 19.0, SPSS Inc., Chicago, IL).

## Results

### *Time trends*

Mean annual temperature increased significantly both in Prades ( $R^2=0.39$ ,  $P<0.001$ ) and Arcalís ( $R^2=0.54$ ,  $P<0.001$ ) from 1975 to 2008 (Fig. 1), at a similar rate of  $\approx 0.05^\circ\text{C year}^{-1}$ . For the same time period, mean annual precipitation decreased significantly in Arcalís ( $R^2=0.16$ ,  $P<0.05$ ) with an average rate of  $-5.87 \text{ mm year}^{-1}$ , while in Prades it showed no significant trend ( $R^2=0.005$ ,  $P=0.696$ ) (Fig. 1). P/PET also showed a significant decrease in Arcalís, at a rate of  $-0.007 \text{ year}^{-1}$  ( $R^2=0.22$ ,  $P<0.01$ ) and no time trend in Prades ( $R^2=0.001$ ,  $P=0.884$ ) (Fig. 1).

Overall,  $\Delta^{13}\text{C}$  showed a negative tendency for the 34-year period of this study (Fig. 1). The comparison of slope responses **suggested** that changes in  $\Delta^{13}\text{C}$  over time **were probably** not homogeneous among tree conditions ( $P=0.056$  for the term Condition  $\times$  Year, Table 2a). **In fact, the contrast** between dryD and living (wetL and dryL) trees **was found to be significant** irrespective of site (the slope was  $0.017\text{‰ year}^{-1}$  higher (less negative) in dryD trees than in living trees,  $P<0.05$ ). On the other hand, no statistical difference was found between the wetL and dryL groups ( $P=0.345$ ). Although  $\Delta^{13}\text{C}$  values were higher at Arcalís than at Prades, there were no significant changes in  $\Delta^{13}\text{C}$  over time due to either site or site by condition interaction (Table 2a).

Overall,  $\text{WUE}_i$  registered a positive tendency between 1975 and 2008 for all Scots pine groups from both sites (Fig. 2). The comparison of slope responses indicated that changes in  $\text{WUE}_i$  over time differed among tree conditions, regardless of site ( $P<0.05$  for the term Condition  $\times$  Year, Table 2b). The contrast between dryD and living (wetL and dryL) trees was statistically significant (the slope was  $0.172 \mu\text{mol mol}^{-1} \text{ year}^{-1}$  smaller (less positive) in dryD than in living trees,  $P<0.05$ ), whereas the slope comparison between living individuals (wetL versus dryL) showed no statistical significance ( $P=0.374$ ). As for  $\Delta^{13}\text{C}$ , there were no significant changes in  $\text{WUE}_i$  over time due to either site or site by condition interaction (Table 2b).

### *Signal strength of $\Delta^{13}\text{C}$ series*

The strength of the common  $\Delta^{13}\text{C}$  signal for different series combinations is summarised in Table 3. At the within-site level, the six tree groups were initially considered as separate

(wetL, dryL and dryD for the two sites). For this primary grouping, the mean inter-series correlation ( $a_{fcv}$ ) took values around 0.5 or less, and no obvious differences in signal strength were evident when comparing these six groups (as indicated also by the magnitudes of SE ( $a_{fcv}$ )). EPS did not reach the 0.85 threshold value, although in most cases it was close to it (0.84). Therefore, the estimated number of trees required to achieve EPS=0.85 exceeded the amount of sampled trees for each group ( $t>5$  in most cases).

When combining series of living trees (wetL and dryL) at either site,  $a_{fcv}$  values did not depart substantially from the averaged  $a_{fcv}$ 's of the original tree groups, indicating that there was not a transect-specific chronology signal for the surviving individuals. EPS values were now  $>0.85$ , as this parameter is strongly dependent on the number of series. In Prades, when now-dead individuals were also added, a decrease of  $a_{fcv}$  coupled with an increase of  $t$  was registered, indicating that those trees did not have a good synchronicity with their surviving neighbours. This wasn't the case for Arcalís, where  $a_{fcv}$  value slightly increased and  $t$  value slightly decreased when now-dead trees were considered together with the surviving ones. EPS values remained higher than 0.85 at both sites.

When living trees from Prades and Arcalís were pooled together, the quality of the signal strength remained unaffected (i.e.  $a_{fcv}$  and  $t$  exhibited similar values to those observed for either site separately). In contrast, now-dead trees from Prades and Arcalís behaved quite differently, as  $a_{fcv}$  and EPS decreased when they were pooled together (yielding the lowest  $a_{fcv}$  value of all possible combinations), and  $t$  increased, as compared with the values of both sites separately. When all trees of both sites were combined to provide an estimate of signal strength at a broad geographical scale, the  $a_{fcv}$  and  $t$  estimates were similar to those of Prades alone.

#### *WUE<sub>i</sub> scenarios and climate influence on WUE<sub>i</sub>*

The results obtained comparing the time trends of  $\Delta^{13}\text{C}$ -based WUE<sub>i</sub> records against the three theoretical scenarios of WUE<sub>i</sub> change ( $C_i=\text{ct}$ ,  $C_i/C_a=\text{ct}$ ,  $C_a-C_i=\text{ct}$ ) indicated that the surviving trees from Prades and Arcalís (wetL and dryL) had a behaviour similar to the  $C_i=\text{ct}$  scenario. **By contrast, the behaviour of now-dead trees from Prades and Arcalís**

was closer to the  $C_i/C_a=ct$  scenario, particularly at the former site (see Supplementary Table, Fig. 2). These results remained qualitatively similar if year 1996, which showed unusually low values for all trees at the Arcalís site (Fig. 2), was not included in the analyses (data not shown).

Both  $C_a$  and P/PET significantly influenced  $WUE_i$  at the two study sites (Table 4).  $C_a$  had an overall positive effect, while P/PET determined an overall negative influence on  $WUE_i$  during the 34 years considered here (Fig. 3). The comparison of slope responses indicated that changes in  $WUE_i$  due to raising atmospheric  $CO_2$  were heterogeneous among tree conditions, regardless of site ( $P<0.05$  for the term Condition  $\times C_a$ , Table 4). The contrast between dryD and living (wetL and dryL) trees was statistically significant (the slope was  $0.125 \mu mol mol^{-1} ppm^{-1}$  smaller (less positive) in dryD than in living trees,  $P<0.01$ ), whereas the slope comparison between living individuals (wetL versus dryL) showed no statistical significance ( $P=0.369$ ). There were no significant changes in the response of  $WUE_i$  to raising atmospheric  $CO_2$  due to either site or site by condition interaction. On the other hand, changes in  $WUE_i$  driven by P/PET fluctuations did not depend on condition, site or condition by site interaction (Table 4).

#### *Relationships between $WUE_i$ and BAI*

The positive trend that  $WUE_i$  showed over the 34 years study period did not translate into an enhancement of BAI. In fact, a negative relationship was found between these two variables, indicating that while Scots pines experienced an increase in  $WUE_i$ , their BAI decreased (Fig. 4). The comparison of slopes indicated that the response of BAI to  $WUE_i$  depended simultaneously on condition and site (Table 5). In Prades the contrast between dryD and living (wetL and dryL) trees was statistically significant (the slope was  $0.009 cm^2 mol \mu mol^{-1}$  smaller (more negative) for surviving trees,  $P<0.05$ ). In Arcalís, however, the contrast was statistically significant between dryL and wetL individuals (the slope was  $0.008 cm^2 mol \mu mol^{-1}$  smaller (more negative) for living trees from the dry transect,  $P<0.01$ ), but not between now-dead and surviving individuals.

## **Discussion**

396 *Structure of the  $\Delta^{13}\text{C}$  signal in now-dead and living trees*

397 Overall, mean inter-series correlations ( $a_{fcv}$ ) for  $\Delta^{13}\text{C}$  at the within-site level were in the  
398 lower range of values reported in the literature, e.g. 0.62-0.80 for *Pinus sylvestris* (L.) in  
399 Finland (McCarroll and Pawellek 1998) or 0.57 for *Pinus edulis* (Engelm.) in Arizona  
400 (Leavitt 2010). Accordingly, EPS values below the consensus threshold value of 0.85  
401 (Wigley *et al.* 1984) were observed at the group level, regardless of site, transect and tree  
402 condition. This threshold value is considered particularly relevant for paleoclimatic  
403 reconstructions (McCarroll and Pawellek 1998), while in dendroecological studies it is just  
404 an indicator of signal strength useful to compare different series combinations. Our EPS  
405 values, however, were similar to those reported in mountain dry environments for  $^{13}\text{C}$   
406 chronologies made up of 4-6 trees, either for conifers (0.80-0.90; Gagen *et al.* 2004) or  
407 hardwoods (0.84; Aguilera *et al.* 2011), which suggests that under such conditions it may  
408 be advisable to increase the number of sampled trees to at least 7-8 individuals in order to  
409 strengthen the combined chronology  $^{13}\text{C}$  signal.

410  
411 At the within-site level, our results suggest that now-dead trees were slightly more  
412 synchronous than their surviving neighbours. However, it must be stressed that the  
413 associated SE's were high enough as to prevent strong conclusions on this point. The clear  
414 reduction in synchronicity observed after pooling series of now-dead and living individuals  
415 from Prades (the most xeric of the two sites) points to the presence of a differential  
416 physiological reaction in dying and surviving Scots pine trees to the climatic fluctuations of  
417 the last decades under very limiting conditions. Indeed, such an outcome is related to the  
418 differential response to factors underlying  $\text{WUE}_i$  variation at the tree groups level (see  
419 below), and may be associated to differences in their ability to cope with different local  
420 factors (e.g. drought, microecological conditions) (Andreu *et al.* 2008). On the other hand,  
421 the realisation that living trees from a particular site were about equally correlated between  
422 them regardless of ecological condition (dry or wet transect) might indicate that  
423 microenvironmental heterogeneity (e.g. edaphic, age or competition effects) tends to blur  
424 larger-scale ecological constraints of tree performance (e.g. water availability) registered in  
425 tree rings, at least in this Mediterranean system.



At a larger geographical scale, the relatively high  $a_{fcv}$  (unrelated to sample size) and EPS (highly dependent on sample size) values found when living individuals from Prades and Arcalís were combined, suggests that climate could be the principal cause underlying synchronicity among trees. Prades and Arcalís are >100 km away from each other and no other factors besides the climatic ones are likely to act at such a wide spatial scale (Hughes *et al.* 1982; Andreu *et al.* 2007). Previous studies have shown that  $\delta^{13}\text{C}$  series are less sensitive to local factors than growth patterns and therefore they reflect better the climatic signal at larger scales (Gagen *et al.* 2004; Andreu *et al.* 2008).

#### *Temporal dynamics of $\text{WUE}_i$*

Now-dead and living Scots pines from Prades and Arcalís significantly increased their  $\text{WUE}_i$  over the 34 years considered for this study, **consistent with recent findings for temperate and boreal forests of the Northern Hemisphere (Keenan *et al.* 2013).** Nevertheless, the rate of increase of  $\text{WUE}_i$  was lower in now-dead individuals, implying that, compared to surviving individuals, those pines were not able to take full advantage (e.g. controlling water losses while maintaining photosynthetic rates) of the increasing  $C_a$  over time (Waterhouse *et al.* 2004). Contrary to our hypothesis that now-dead trees would show a more pronounced climatic sensitivity of  $\text{WUE}_i$  (i.e., a steeper response to P/PET), no such differences were found with surviving individuals. In contrast, McDowell *et al.* (2010) found that *Pinus ponderosa* (Doug.) trees that died showed no climatic sensitivity of their gas exchange traits, although this sensitivity was strong in surviving individuals. The lower rates of  $\text{WUE}_i$  increase shown by the now-dead Scots pine trees **from Prades and Arcalís** were the result of a behaviour that **was consistent with the constant  $C_i/C_a$  scenario over time (Prades) or in between the  $C_i/C_a=\text{ct}$  and  $C_i=\text{ct}$  scenarios (Arcalís), in contrast to the constant  $C_i$  behaviour observed for the surviving trees at both sites.** These results are in agreement with those reported by Linares and Camarero (2012) showing that declining *Abies alba* (Mill.) individuals also behaved closer to the constant  $C_i/C_a$  scenario. Nevertheless, this behaviour seems not to be limited to trees affected by drought-induced decline, as it has also been reported for *Pinus* and *Larix* species growing at high northern latitudes in Eurasia, where it has been attributed to a regulative response to

the rising CO<sub>2</sub> concentrations in which  $C_i/C_a$  is used as a set point for the gas exchange (Saurer *et al.* 2004).

The constant  $C_i/C_a$  scenario implies a progressive increase of the WUE<sub>i</sub> due to the proportional regulation of stomatal conductance and photosynthesis (Wong *et al.* 1979; Saurer *et al.* 2004). Water stress conditions are known to reduce stomatal conductance and photosynthesis, although stomatal conductance is normally more affected (Farquhar *et al.* 1989). A possible explanation of the result that trees suffering drought-induced decline are not able to react to changes in  $C_a$  over time as efficiently (i.e. maintaining a constant  $C_i$ ) as their living counterparts, is the presence of non-stomatal limitations to photosynthesis. Under drought conditions, these limitations might involve decreases in mesophyll conductance to CO<sub>2</sub>, as has been reported in experimental (e.g. Galmés *et al.* 2007) and modelling studies (Keenan *et al.* 2010) for Mediterranean species. Differences in whole plant structure and tolerance to hydraulic failure, either environmentally- or genetically-induced, have been reported to determine different rates of recovery of mesophyll conductance after recurrent drought events (Flexas *et al.* 2012), which may have conditioned the capacity to survive drought in declining trees by hindering photosynthetic activity and WUE<sub>i</sub> in the long-term.

Since respiration rate increases with temperature, an alternative explanation of the shallower response of WUE<sub>i</sub> to  $C_a$  in now-dead trees could be the higher sensitivity of respiration to rising temperature in these trees. Both higher non-stomatal limitations to photosynthesis and increased respiration affect negatively the tree carbon balance and, in combination with drought-induced defoliation, may lead to depleted reserves and carbon starvation, as has been observed at the study sites (Galiano *et al.* 2011; **Poyatos *et al.* 2013**). In any case, the partial decoupling between rising CO<sub>2</sub> concentrations and WUE<sub>i</sub> in now-dead trees suggests that a critical point in raising WUE<sub>i</sub> under increasing CO<sub>2</sub> concentrations may have been reached, and that drought can counterbalance the stimulating effect of increasing CO<sub>2</sub> concentrations on the plant carbon budget (Duquesnay *et al.* 1998; Saurer *et al.* 2004; Waterhouse *et al.* 2004; Linares *et al.* 2009; Linares and Camarero 2012).

*Relationship between  $WUE_i$  and radial growth*

Overall, Scots pine trees from Prades and Arcalís showed a negative relationship between  $WUE_i$  and BAI, indicating that increasing  $WUE_i$  over time may not translate into growth enhancements, particularly in drought-prone areas (Peñuelas *et al.* 2008; Peñuelas *et al.* 2011; Andreu-Hayles *et al.* 2011). Contrary to McDowell *et al.* (2010) in *Pinus ponderosa* (Doug.) or Voltas *et al.* (2013) in *Pinus sylvestris* (L.) affected by a winter-drought induced die-back, we did not observe a steeper negative relationship between  $WUE_i$  and BAI in now-dead trees. This might be due, at least in part, to the fact that measured BAI values were low and showed low variability over the study period considered in this study (Hereş *et al.* 2012). **In addition, it remains unclear how changes in volumetric growth would translate into biomass growth rates, as wood density might have also changed. Although we did not measure wood density, our own wood anatomy data for these same trees shows that most of the radial growth difference between now-dead and surviving trees was explained by differences in tracheid cell production per year (and not by tracheid dimensions) (Hereş *et al.* unpublished data) which suggests that wood density changes did not play a major role in this case.**

Our results show that increasing  $WUE_i$  might not be sufficient to overcome the impacts of a warmer and drier climate on growth, as those conditions might actually overcome the stimulating effect of rising  $CO_2$  in water-limited areas such as the Mediterranean (Peñuelas *et al.* 2008; Linares *et al.* 2009). This indicates that water availability, when limiting, tips the balance towards low growth rates due either to low carbon gain per se (Linares *et al.* 2009), either to the direct effect of drought on cell formation and development (Hsiao 1973), or to a combination of both factors.

A direct effect of drought on growth, not necessarily mediated by lowered photosynthesis, is supported by the notion that growth is normally more sensitive to moderate drought than assimilation (Sala *et al.* 2012 and references therein). Previous studies show that now-dead pines from the same sites studied here started to reduce their growth (compared to surviving neighbours) decades before death (Hereş *et al.* 2012) and that decaying pines have low leaf

area and extremely low levels of carbohydrate reserves (Galiano *et al.* 2011). As it is unclear whether under extreme drought the growth of decaying trees relies increasingly on stored carbon pools (cf. Eilmann *et al.* 2010), an alternative (or complementary) hypothesis would be that stored carbon is preferentially allocated to other metabolic uses (e.g., defence, rooting).

## Conclusions

Our study shows that now-dead Scots pine trees had a distinct time trend of  $WUE_i$ , with lower rates of increase in response to rising  $C_a$  than surviving individuals. This result adds to previous studies at the same sites showing that tree mortality is the last stage of a long declining process marked by characteristic growth patterns (Hereş *et al.* 2012) and ecophysiology (Galiano *et al.* 2011), and suggests fundamental differences in photosynthetic limitations and, perhaps, carbon allocation or respiration costs between dying and surviving trees. Considering that all studied trees at each site were growing in the same valley and thus exposed to reasonably similar environmental conditions, our results also suggest that Scots pine trees from Prades and Arcalís may be living close to an abrupt survival threshold that, once exceeded, leads to tree mortality (Peñuelas *et al.* 2008; Linares *et al.* 2009; Linares and Camarero 2012; Williams *et al.* 2013). If the projections of increased drought in the studied region are correct (IPCC 2007), episodes of Scots pine die-off are likely to continue, leading eventually to a shift in the dominant vegetation (Galiano *et al.* 2010; Matías and Jump 2012).

## Acknowledgments

The authors would like to thank JP Ferrio, M Aguilera, P Sopeña and M Lucà for laboratory assistance and interesting discussions related to the study. We are indebted to M Mencuccini for field work and valuable discussion on the research topic. The authors also thank M Ninyerola and the Catalan Meteorological Service (SMC) for providing the two climatic datasets used in this study. **Two anonymous referees contributed to the improvement of the original version of the article.** This research was funded by the Spanish Ministry of Science and Innovation via competitive projects (“CGL2007-60120”,

549 “CSD2008-0040”, “CGL2010-16373”), a FPU PhD scholarship and a short study stay at  
550 the University of Lleida, Department of Crop and Forest Sciences.

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**Table 1** – Sites characteristics, BAI,  $\delta^{13}\text{C}$  and  $\text{WUE}_i$  data

*BAI* values are calculated for the 1975-2008 period for the living trees and for the 1975-  
year of death interval for the now-dead ones. Abbreviations: *DBH*, diameter at breast high,  
*s.d.*, standard deviation

	Altitude (m a.s.l.)	Number of trees	Mean DBH $\pm$ s.d. (mm)	Mean BAI $\pm$ s.d. ( $\text{cm}^2$ )	Mean $\delta^{13}\text{C}$ $\pm$ s.d. (‰)	Mean $\text{WUE}_i$ $\pm$ s.d. ( $\mu\text{mol}$ $\text{mol}^{-1}$ )
<b>Prades wet</b>						
living (wetL)	800	5	28.64 $\pm$ 3.14	6.92 $\pm$ 4.39	<b>-23.38<math>\pm</math>0.65</b>	108.59 $\pm$ 9.87
<b>Prades dry</b>						
living (dryL)	1000	5	31.18 $\pm$ 3.19	10.25 $\pm$ 5.85	<b>-22.98<math>\pm</math>0.82</b>	112.70 $\pm$ 10.59
Prades dry dead (dryD)	1000	4	32.25 $\pm$ 6.84	3.46 $\pm$ 3.79	<b>-23.37<math>\pm</math>0.84</b>	107.93 $\pm$ 9.05
<b>Arcalís wet</b>						
living (wetL)	1300	5	31.58 $\pm$ 1.71	9.81 $\pm$ 5.94	<b>-23.55<math>\pm</math>0.85</b>	106.83 $\pm$ 10.95
<b>Arcalís dry</b>						
living (dryL)	1000	5	31.66 $\pm$ 3.40	10.28 $\pm$ 4.78	<b>-23.82<math>\pm</math>0.64</b>	104.13 $\pm$ 8.90
Arcalís dry dead (dryD)	1000	5	33.76 $\pm$ 6.36	9.39 $\pm$ 6.61	<b>-23.89<math>\pm</math>0.93</b>	102.16 $\pm$ 10.52

807 **Table 2a** – Results of mixed linear models (ANOVA table) with  $\Delta^{13}\text{C}$  as a function of  
808 Condition, Site and Year. **Significant relationships ( $P < 0.05$ ) are marked in bold.**  
809 Abbreviations: *DF*, degrees of freedom

<b>Fixed effects</b>	<b>DF</b> <b>(numerator)</b>	<b>DF</b> <b>(denominator)</b>	<b><i>F</i> value</b>	<b><i>P</i> value</b>
Condition	2	905	0.61	0.546
<b>Site</b>	<b>1</b>	<b>905</b>	<b>5.78</b>	<b>0.016</b>
Condition × Site	2	905	0.78	0.460
<b>Year</b>	<b>1</b>	<b>905</b>	<b>33.02</b>	<b>&lt;0.001</b>
Condition × Year	2	905	2.89	0.056
Site × Year	1	905	0.17	0.682
Condition × Site × Year	2	905	0.17	0.841



810 **Table 2b** – Results of mixed linear models (ANOVA table) with WUE<sub>i</sub> as a function of  
811 Condition, Site and Year. **Significant relationships ( $P < 0.05$ ) are marked in bold.**  
812 Abbreviations: *DF*, degrees of freedom

Fixed effects	DF (numerator)	DF (denominator)	<i>F</i> value	<i>P</i> value
Condition	2	905	0.89	0.411
<b>Site</b>	<b>1</b>	<b>905</b>	<b>5.94</b>	<b>0.015</b>
Condition × Site	2	905	0.79	0.453
<b>Year</b>	<b>1</b>	<b>905</b>	<b>414.30</b>	<b>&lt;0.001</b>
<b>Condition × Year</b>	<b>2</b>	<b>905</b>	<b>3.12</b>	<b>0.045</b>
Site × Year	1	905	0.00	0.981
Condition × Site × Year	2	905	0.08	0.924

813 **Table 3** – Strength of the common  $\Delta^{13}\text{C}$  signal. Abbreviations:  $n$ , number of trees  
814 combined;  $a_{fcv}$ , fractional common variance;  $SE(a_{fcv})$ , standard error associated to the mean  
815 inter-correlation  $a_{fcv}$ ;  $EPS$ , expressed population signal;  $t$ , number of trees needed to  
816 achieve an  $EPS=0.85$

Site	Condition	$n$	$a_{fcv}$	$SE(a_{fcv})$	$EPS$	$t$
Prades	wetL	5	0.51	0.134	0.84	6
	dryL	5	0.34	0.151	0.72	11
	dryD	4	0.57	0.301	0.84	5
	wetL + dryL + dryD	14	0.36	0.135	0.89	10
	wetL + dryL	10	0.41	0.122	0.88	8
Arcalís	wetL	5	0.38	0.146	0.76	9
	dryL	5	0.51	0.101	0.84	6
	dryD	5	0.52	0.122	0.84	6
	wetL + dryL + dryD	15	0.45	0.095	0.92	7
	wetL + dryL	10	0.43	0.105	0.88	8
Prades and Arcalís	wetL + dryL + dryD	29	0.36	0.101	0.94	10
	wetL + dryL	20	0.41	0.112	0.93	8
	dryD	9	0.32	0.210	0.81	12

817 **Table 4** – Results of mixed linear models (ANOVA table) with WUE<sub>i</sub> as a function of  
818 Condition, Site, C<sub>a</sub> and P/PET. **Significant relationships ( $P < 0.05$ ) are marked in bold.**  
819 Abbreviations: *DF*, degrees of freedom

Fixed effects	DF (numerator)	DF (denominator)	<i>F</i> value	<i>P</i> value
Condition	2	899	0.91	0.415
<b>Site</b>	<b>1</b>	<b>899</b>	<b>6.09</b>	<b>0.022</b>
Condition × Site	2	899	0.80	0.463
<b>C<sub>a</sub></b>	<b>1</b>	<b>899</b>	<b>456.89</b>	<b>&lt;0.001</b>
<b>Condition × C<sub>a</sub></b>	<b>2</b>	<b>899</b>	<b>3.55</b>	<b>0.031</b>
Site × C <sub>a</sub>	1	899	0.00	0.954
Condition × Site × C <sub>a</sub>	2	899	0.08	0.926
<b>P/PET</b>	<b>1</b>	<b>899</b>	<b>159.70</b>	<b>&lt;0.001</b>
Condition × P/PET	2	899	0.08	0.927
Site × P/PET	1	899	1.61	0.206
Condition × Site × P/PET	2	899	2.09	0.125

**Table 5** – Results of mixed linear models (ANOVA table) with logBAI as a function of Condition, Site and WUE<sub>i</sub>. **Significant relationships ( $P < 0.05$ ) are marked in bold.**  
Abbreviations: *DF*, degrees of freedom

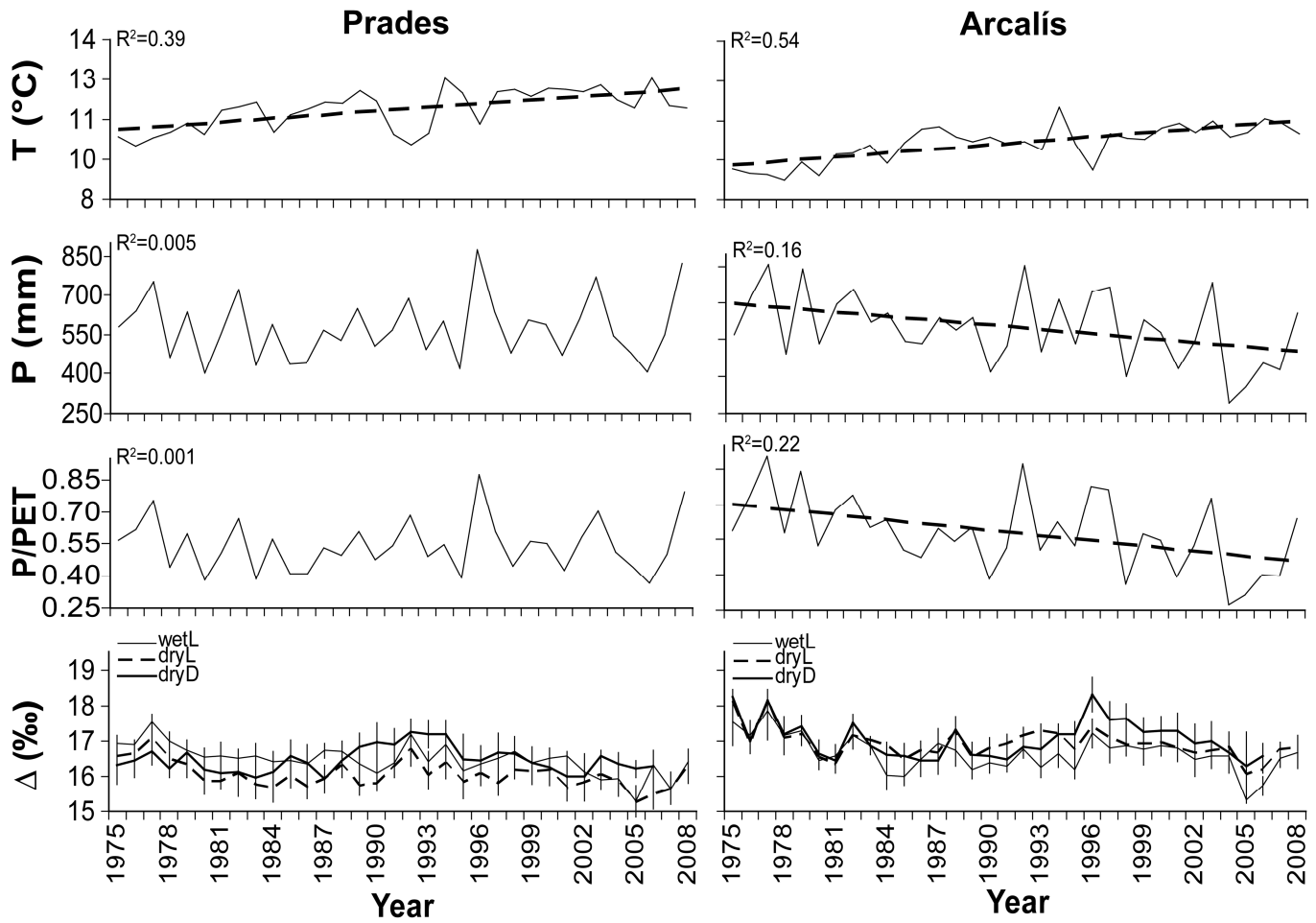
Fixed effects	DF (numerator)	DF (denominator)	<i>F</i> value	<i>P</i> value
Condition	2	905	5.28	0.005
Site	1	905	4.65	0.031
Condition × Site	2	905	3.24	0.040
WUE <sub>i</sub>	1	905	49.71	<0.001
Condition × WUE <sub>i</sub>	2	905	2.91	0.055
Site × WUE <sub>i</sub>	1	905	4.18	0.041
Condition × Site × WUE <sub>i</sub>	2	905	4.59	0.010

824 **Supplementary table** – Results of linear regressions between  $\Delta^{13}\text{C}$ -based and theoretical  $\text{WUE}_i$  values according to the three  
825 scenarios proposed by Saurer *et al.* (2004). RMSError values are also given. Abbreviations:  $\beta_o$ , intercept;  $\beta_1$ , slope;  $\beta_1'$ , slope  
826 when the intercept is set to 0; *RMSError*, root mean square error; **ct, constant**

Site	Condition	Scenario	$\beta_o$	Confidence	$\beta_1$	Confidence	$\beta_1'$	Confidence	RMSError
				interval (95%) for $\beta_o$		interval (95%) for $\beta_1$		interval (95%) for $\beta_1'$	
Prades	wetL	$C_i$ =ct	22.831	9.21 – 36.45	0.777	0.65 - 0.90	0.982	0.97 - 0.99	4.22
	wetL	$C_i/C_a$ =ct	-64.031	(-)91.38 – (-)36.68	1.675	1.41 – 1.94	1.054	1.04 – 1.07	6.98
	wetL	$C_a$ - $C_i$ =ct	-	-	-	-	-	-	14.25
	dryL	$C_i$ =ct	34.178	19.60 – 48.75	0.689	0.56 – 0.82	0.987	0.97 – 1.00	4.56
	dryL	$C_i/C_a$ =ct	-40.445	(-)68.81 - (-)12.09	1.432	1.17 – 1.70	1.054	1.04 – 1.07	6.89
	dryL	$C_a$ - $C_i$ =ct	-	-	-	-	-	-	14.22
	dryD	$C_i$ =ct	53.853	36.73 – 70.98	0.474	0.33 – 0.62	0.941	0.92 – 0.96	8.63
	dryD	$C_i/C_a$ =ct	3.245	(-)29.78 – 36.27	0.970	0.67 – 1.28	1.000	0.99 – 1.01	3.49
	dryD	$C_a$ - $C_i$ =ct	-	-	-	-	-	-	8.24
Arcalís	wetL	$C_i$ =ct	29.471	11.58 – 47.36	0.724	0.56 – 0.89	0.998	0.98 – 1.01	5.01
	wetL	$C_i/C_a$ =ct	-54.084	(-)91.19 - (-)16.98	1.619	1.25 – 1.99	1.076	1.06 – 1.09	8.98
	wetL	$C_a$ - $C_i$ =ct	-	-	-	-	-	-	15.70
	dryL	$C_i$ =ct	34.221	19.04 – 49.40	0.660	0.52 – 0.80	0.980	0.96 – 1.00	5.19
	dryL	$C_i/C_a$ =ct	-42.541	(-)74.28 - (-)10.80	1.490	1.17 – 1.81	1.059	1.04 – 1.07	7.09
	dryL	$C_a$ - $C_i$ =ct	-	-	-	-	-	-	13.73
	dryD	$C_i$ =ct	44.982	22.28 – 67.68	0.553	0.34 – 0.77	0.982	0.96 – 1.00	6.61
	dryD	$C_i/C_a$ =ct	-19.880	(-)68.03 – 28.27	1.262	0.77 – 1.76	1.057	1.04 – 1.08	7.57
	dryD	$C_a$ - $C_i$ =ct	-	-	-	-	-	-	13.09

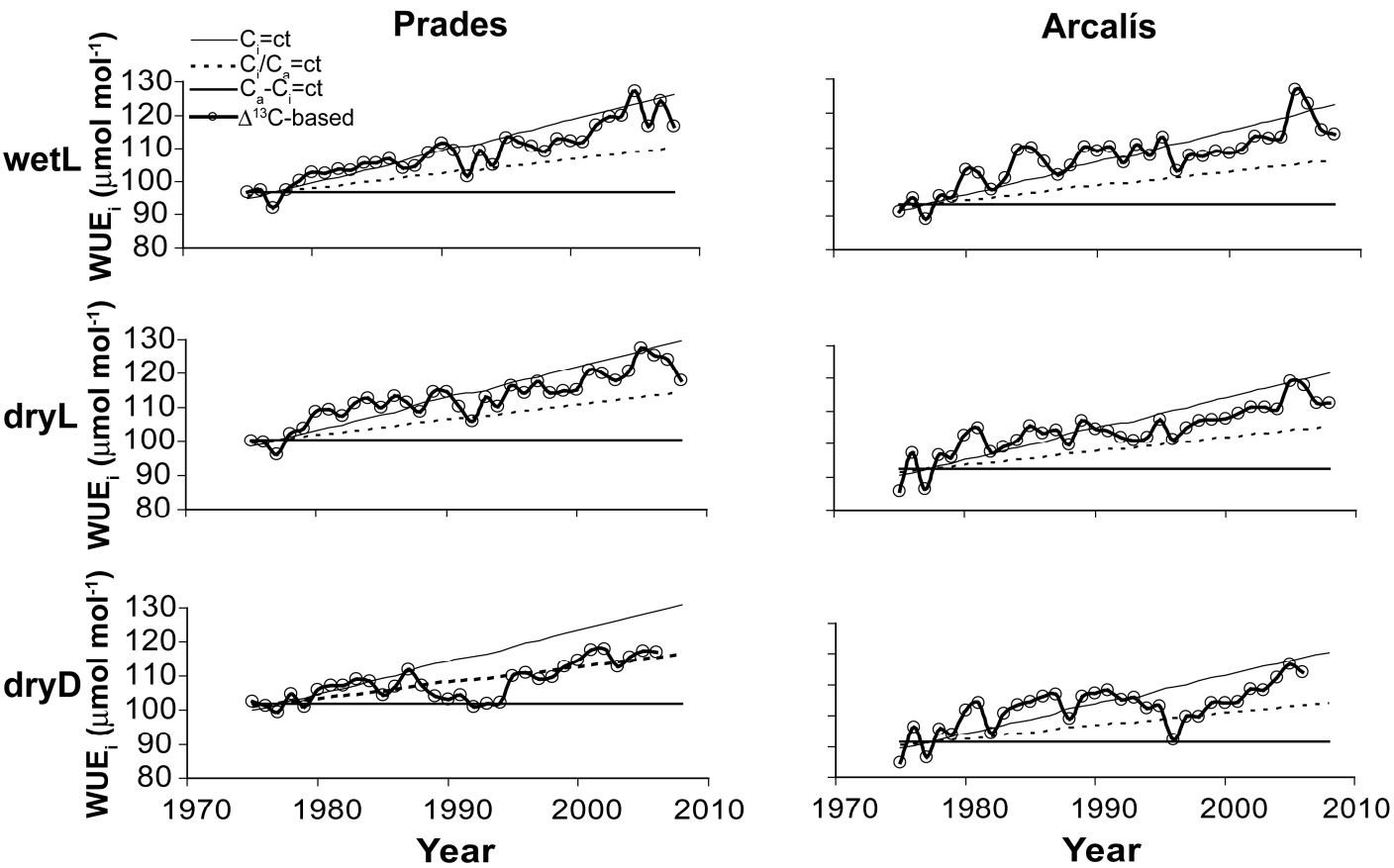
827 **Fig. 1** Temporal trends (1975-2008) of  $T$ ,  $P$ ,  $P/PET$  and  $\Delta^{13}C$  for Prades and Arcalís. The  $\Delta^{13}C$  trend for the  
 828 dryD trees ends in 2006, as this was the last year with a sample size > 2. Error bars indicate standard error.  
 829 Regression lines are represented only if significant.

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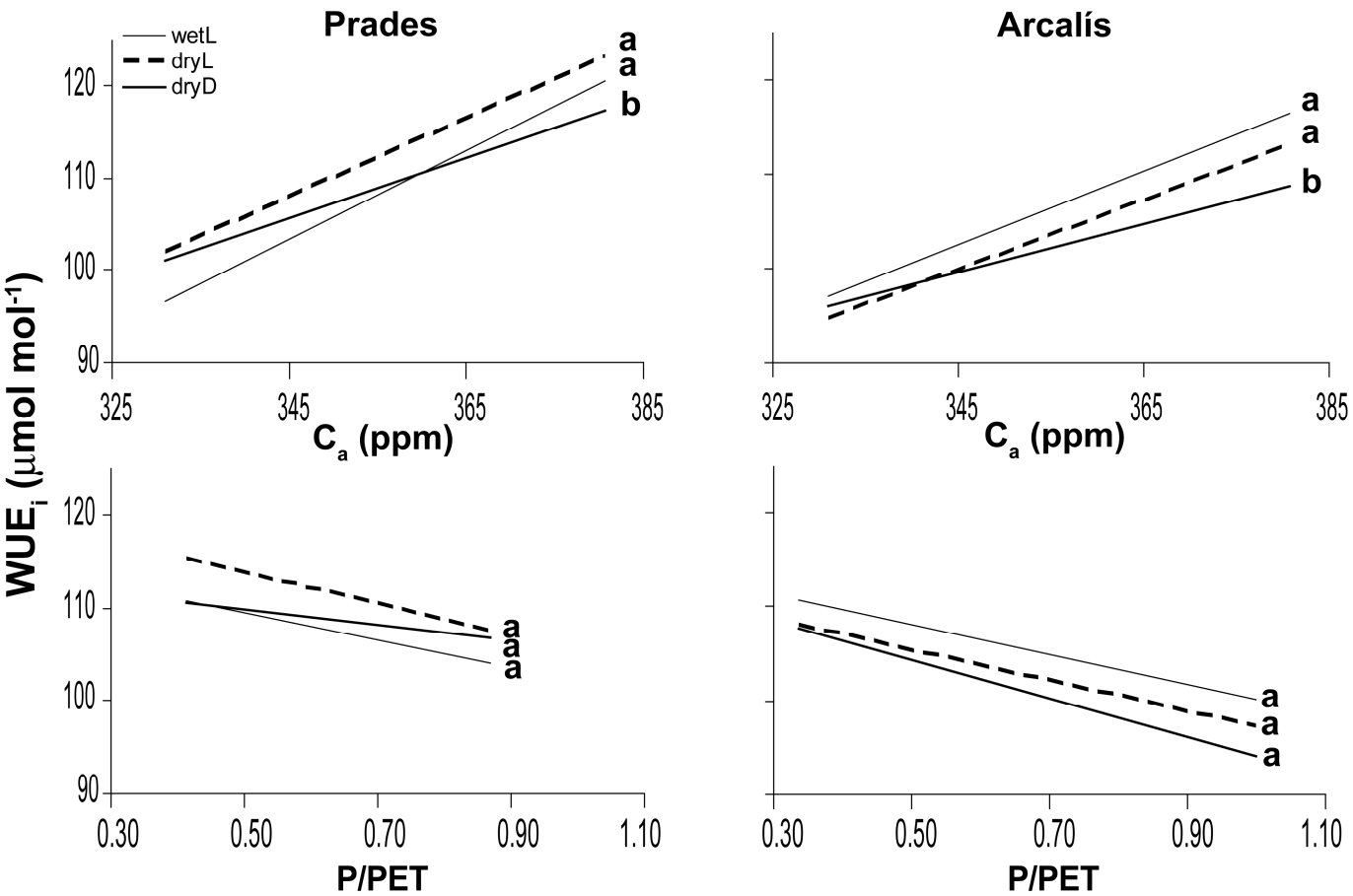
832 **Fig. 2** Time trends of  $\Delta^{13}\text{C}$ -based  $\text{WUE}_i$  data in relation to three theoretical scenarios (described in Materials  
833 and methods) for living and now-dead Scots pines from Prades and Arcalís. The  $\Delta^{13}\text{C}$ -based  $\text{WUE}_i$  trend for  
834 the dryD trees ends in 2006, as this was the last year with a sample size  $> 2$ . **Abbreviation: ct, constant.**  
835



836

837 **Fig. 3** The effects of  $C_a$  and P/PET on  $WUE_i$  as a function of Condition and Site. **Different letters indicate**  
 838 **significant differences between slopes of wetL, dryL and dryD trees according to a Least Significant**  
 839 **Difference test ( $\alpha=0.05$ ).**

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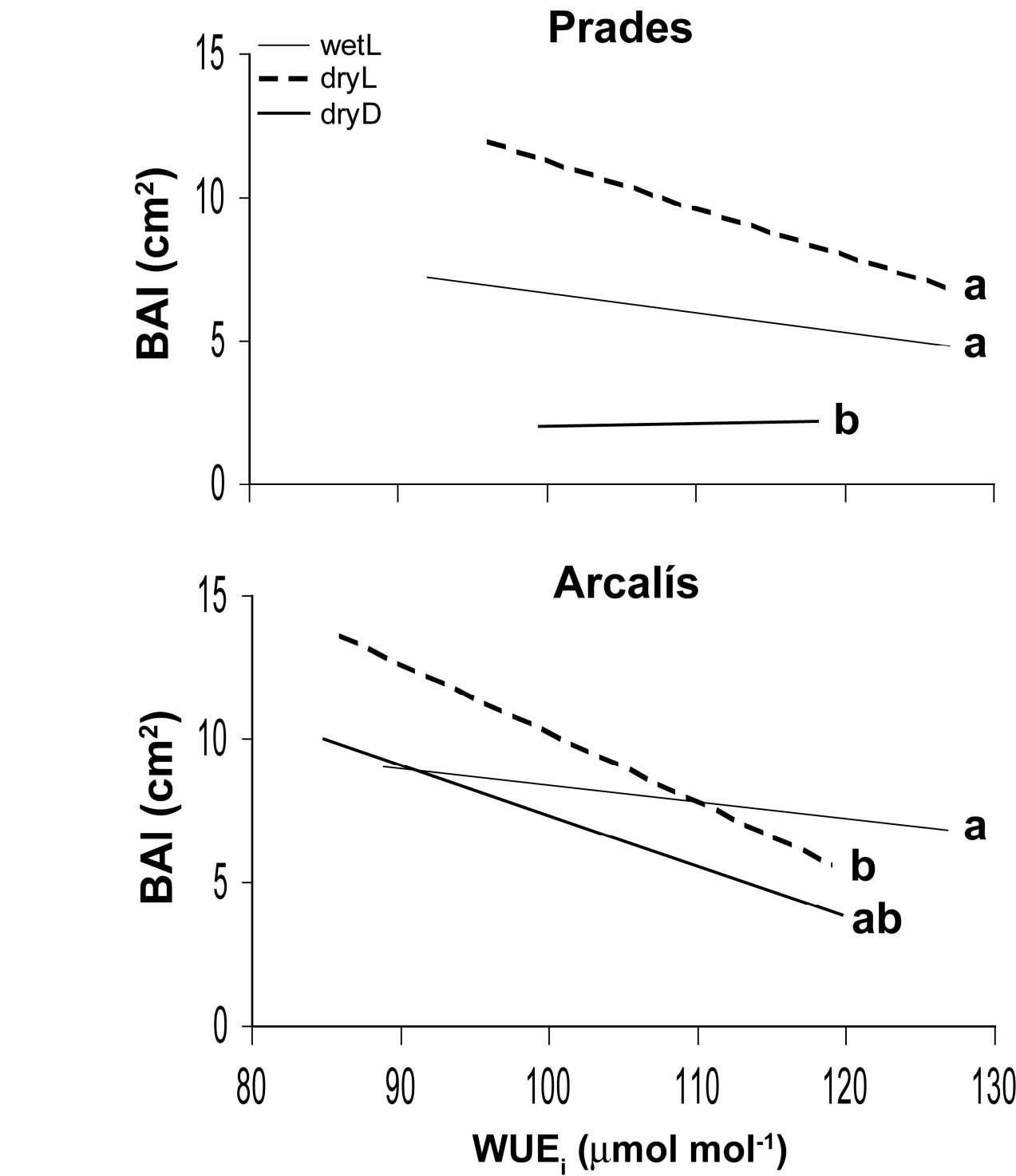


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842 **Fig. 4** Relationships between  $WUE_i$  and BAI as a function of Condition and Site. **Different letters indicate**  
843 **significant differences between slopes of wetL, dryL and dryD trees according to a Least Significant**  
844 **Difference test ( $\alpha=0.05$ ).**

845



846

847 **Supplementary Fig.** Pearson correlations between  $WUE_i$  and monthly climatic variables ( $T$  and  $P$ ). Time  
 848 interval covers months from previous to growth year (jul to dec) and from current year of growth (JAN to  
 849 DEC). Significant correlations are **indicated** with \* ( $P<0.05$ ) and \*\* ( $P<0.01$ ).  
 850

