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Competition and drought limit the response of water-use efficiency to rising atmospheric carbon dioxide in the Mediterranean fir *Abies pinsapo*

Juan-Carlos Linares · Antonio Delgado-Huertas · J. Julio Camarero · José Merino · José A. Carreira

Abstract The gas-exchange and radial growth responses of conifer forests to climatic warming and increasing atmospheric CO_2 have been widely studied. However, the modulating effects of variables related to stand structure (e.g., tree-to-tree competition) on those responses are poorly explored. The basal-area increment (BAI) and C isotope discrimination (C stable isotope ratio; $\delta^{13}C$) in the Mediterranean fir *Abies pinsapo* were investigated to elucidate the influences of stand competition, atmospheric CO_2 concentrations and climate on intrinsic water-use efficiency (WUEi). We assessed the variation in $\delta^{13}C$ of tree-rings from dominant or co-dominant trees subjected to different degrees of competition. A high- (H) and a low-elevation (L) population with contrasting climatic constraints were studied in southern Spain. Both populations showed an increase in long-term WUEi. However, this increase occurred more slowly at the L site, where a decline of BAI was also observed. Local warming and severe droughts have occurred in the study area over the past 30 years, which have reduced water availability more at lower elevations. Contrastingly, trees from the H site were able to maintain high BAI values at a lower cost in terms of water consumption. In each population, trees subjected to a higher degree of competition by neighboring trees showed lower BAI and WUEi than those subjected to less competition, although the slopes of the temporal trends in WUEi were independent of the competitive micro-environment experienced by the trees. The results are consistent with an increasing drought-induced limitation of BAI and a decreasing rate of WUEi improvement in low-elevation *A. pinsapo* forests. This relict species might not be able to mitigate the negative effects of a decrease in water availability through a reduction in stomatal conductance, thus leading to a growth decline in the more xeric sites. An intense and poorly asymmetric competitive environment at the stand level may also act as an important constraint on the adaptive capacity of these drought-sensitive forests to climatic warming.

Keywords Carbon isotope discrimination · Global change · Stand structure · Drought · *Abies pinsapo*

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J.-C. Linares (✉) · J. Merino
Área de Ecología, Universidad Pablo de Olavide,
Ctra. Utrera km. 1, 41002 Sevilla, Spain
e-mail: jclincal@upo.es

A. Delgado-Huertas
Estación Experimental del Zaidín (CSIC),
C/Profesor Albareda 1, 18008 Granada, Spain

J. Julio Camarero
ARAID, Instituto Pirenaico de Ecología (CSIC),
Avda. Montañana 1005, 50192 Zaragoza, Spain

J. A. Carreira
Área de Ecología, Universidad de Jaén,
Campus Las Lagunillas, 23071 Jaén, Spain

Introduction

Mediterranean mountain forests are mainly constrained by water availability, and these ecosystems are expected to be very sensitive to components of global change, such as climate warming and rising C availability (IPCC 2007). Forest growth and C uptake may change as a consequence of rising temperatures and CO_2 concentrations in the

atmosphere, because they are among the primary environmental variables that elicit tree responses (Körner 2000). Over the past two centuries, many anthropogenic influences on the C biogeochemical cycle have been reported (Francey et al. 1999). The rising atmospheric CO_2 concentration is affecting the gas-exchange metabolism of trees in several ways (Körner 2003). For instance, in controlled experiments, C assimilation was generally stimulated and stomatal conductance was reduced by increased CO_2 (Picon et al. 1996), suggesting that plants are able to increase their intrinsic water-use efficiency (WUEi; the ratio of C assimilated to water transpired) as CO_2 levels rise (Ceulemans et al. 1999; Drake et al. 1997).

Physical factors such as atmospheric CO_2 concentration and climatic variables have been commonly studied as the main agents controlling tree growth and WUEi (Boisvenue and Running 2006). However, variables related to stand structure like tree-to-tree competition have seldom been considered as concurrent influences when interpreting changes in tree growth and the inferred changes in WUEi. Although radial growth in trees is influenced by several factors, most studies have focused on the relationships between growth (e.g., tree-ring width) and climatic variables (Zweifel et al. 2006). Moreover, the analysis of C isotope discrimination in tree-rings has been widely applied to study how climatic conditions affect C-water balance during the growth period because WUEi can be estimated from the values of C stable isotope ratios ($^{13}C/^{12}C$; $\delta^{13}C$) obtained from tree-ring wood (McCarroll and Loader 2004). In addition to these widely studied factors, the response of tree growth to other environmental conditions must also be considered in order to comprehend the range of possible responses of forests to global change under natural conditions.

Some studies undertaken to elucidate tree-growth responses to climate warming and rising atmospheric CO_2 concentrations have been carried out on natural stands, but these studies have mostly considered freely growing, dominant or isolated trees, and often pooled samples from a large number of tree-ring cores by local provenance prior to ^{13}C analyses (see for instance Bert et al. 1997). It is assumed that these pooled or mean values can give insight into how naturally growing trees respond to increasing atmospheric CO_2 concentrations and climate, but little attention has been paid to within-population variability that may bias overall trends. Field studies performed in the USA (Marshall and Monserud 1996; Feng 1998) and in northern Europe (Saurer et al. 2004; Waterhouse et al. 2004) have revealed that there is a wide variance among trees, both in recent growth and in WUEi. In most places, WUEi has been increasing; however, there is a lack of understanding of why this variable has improved more in some sites than in others. In addition, little is known about

WUEi and tree growth in water-limited ecosystems, as is the case for Mediterranean mountain forests. Some argue that the hypothesis that the increase in CO_2 can offset the impact of a higher degree of stomatal closure due to lower water availability, resulting in no substantial reduction of C gain despite increased dryness, is particularly unlikely in Mediterranean systems. Recent findings for temperate trees growing near the ecotone with regions of the Mediterranean biome point in that direction (Peñuelas et al. 2008), but the hypothesis has not been properly evaluated in Mediterranean systems.

We propose that the variability in both tree growth and WUEi in response to rising temperature and CO_2 could be partially related to stand structure. Age effects on isotopic discrimination, for instance, have been well described in mature *Abies alba* trees, and they have been shown to be negligible in the long-term assessment of the $\delta^{13}C$ of tree-rings from dominant, mature trees (Bert et al. 1997). However, the degree of canopy competition has rarely been considered, despite the fact that mature dominant and co-dominant trees compete for light, water, and other resources; the effects of this competition on growth may be as important as climatic factors such as drought (Orwig and Abrams 1997). We hypothesize that contrasting levels of canopy crowding and shading contribute to the occurrence of differing tree-growth responses to global-change components. Thus, an assessment of the ecological relevance of competition as a potential constraint on water and C availability may be essential to evaluate current patterns in tree growth and WUE in natural forests, and such an assessment may also be necessary to predict their future response to climate change (Boisvenue and Running 2006).

This study uses radial-growth data (basal-area increment; BAI), tree-ring C isotope analysis and plant C discrimination (Δ) to investigate the changes in WUEi throughout the second half of the 20th century in *Abies pinsapo*, a drought-sensitive Mediterranean fir species (Linares 2008). We assessed climatic conditions and compared radial growth among mature trees in two stands with contrasting elevations and climatic stresses. Our aims were:

1. To describe the recent radial growth and wood $\delta^{13}C$ patterns in *A. pinsapo* trees.
2. To quantify the basal area increment and the WUEi responses to contrasting elevations, regional climatic trends, and the global increase in CO_2 for *A. pinsapo* trees subjected to different degrees of competition.
3. To evaluate the importance of the modulating effect of tree-to-tree competition on the tree responses to climate dryness and increasing CO_2 concentrations.

We expected to detect a lower radial growth and a higher WUEi in the drier low-elevation (L) stand compared

with the more mesic high-elevation (H) stand. We also hypothesized that *A. pinsapo* should behave as other temperate conifers and show a long-term WUEi increase in response to rising atmospheric CO_2 concentrations. We also expected that the WUEi increase would be greater in trees subjected to higher competition. The results are interpreted in terms of the three different gas-exchange scenarios described by Saurer et al. (2004), representing different physiological responses to the atmospheric increase in CO_2.

Materials and methods

Study species and site

Abies pinsapo Boiss. is a circum-Mediterranean fir species endemic to southern Spain. Pinsapo forests typically occur above 1,000 m a.s.l. At lower elevations, the vegetation is Mediterranean, dominated by *Quercus ilex* L. subsp. *ballota* (Desf.) Samp., *Quercus faginea* Lam. and *Pinus halepensis* Mill. At higher elevations, *A. pinsapo* forms pure stands up to ca. 1,700 m a.s.l., at which point it co-occurs with other relict mesic tree species such as *Quercus faginea* subsp. *alpestris* (Boiss.) Maire, *Acer granatense* Boiss. *Sorbus aria* (L.) Crantz. and *Taxus baccata* L. The soils of the study area are typically mesic, calcic Haploxerepts (low-elevation sites) and mesic Calcixerolls (upper sites) established over limestone and dolomite rocks. The mean annual temperature is $\sim 11°C$, and the annual precipitation ranges from 1,100 mm to approximately 1,600 mm. The seasonal pattern of rainfall is distinctly Mediterranean, with ca. 80% of all the precipitation falling between October and May, followed by a long summer drought.

Climatic data

Twenty-five nearby meteorological stations were used to estimate how mean annual precipitation changed along the altitudinal gradient of *A. pinsapo* in the study area. Fifteen of these stations also had reliable temperature data, and this subset was used to calculate an altitudinal gradient of mean annual temperature. Finally, to estimate robust and long-term regional climatic records, we used a low-elevation regional mean for the period of 1920–2005 from five of the meteorological stations with the longest and most reliable records (see also Linares et al. 2009). The annual water budget was obtained from the sum of the monthly differences between precipitation data and potential evapotranspiration estimated by a modified version of the Thornthwaite method (Willmott et al. 1985).

Field sampling

Field sampling was carried out in natural, uneven-aged stands that had not been managed in the past 50 years (Linares and Carreira 2009). Plots were located in the Sierra de las Nieves Natural Park, near the lower (L) and upper (H) elevation limits of the species distribution range (H site, 36°43′19″N, 4°57′54″W, 1,226 m a.s.l; L site, 36°41′34″N, 5°01′10″W, 1,749 m a.s.l.). At each site, all of the trees found within a randomly located plot (ca. 0.25 ha) that had a diameter at breast height (dbh; diameter at 1.3 m) of more than 3 cm and showed no symptoms of decline or pathogenic infection were tagged, mapped, measured for dbh and identified. From this set, 13 trees in the L site and 11 trees in the H site were randomly selected and then bored at breast height. At least three cores per tree were extracted, and the pith was reached in at least one of the three.

Dendrochronological methods

Standard dendrochronological methods were used to estimate the tree age at 1.3 m and to assess changes in radial growth. Cores were sanded until tree-rings were clearly visible under a binocular microscope. All samples were visually cross-dated. Tree-ring widths were measured to the nearest 0.01 mm using a binocular scope and a LIN-TAB measuring device (Rinntech, Germany). Cross-dating quality was checked using COFECHA (Holmes 1983). The trend of decreasing ring width with increasing tree size was removed by converting radial increment into BAI using the formula $BAI = \pi \left(r_t^2 - r_{t-1}^2 \right)$, where r is the tree radius and t is the year of tree-ring formation.

Wood isotopic analyses

In order to avoid the juvenile effect, which produces more negative values of $\delta^{13}C$ in wood generated during the first few decades of growth (Heaton 1999), we excluded the first 15–20 tree-rings of each core. Then, wood segments containing five contiguous annual tree-rings were carefully separated with a razor blade with the help of a binocular microscope (MZ 125; Leica, Wetzlar, Germany), pooled by tree, and ground in a ball mill (MM400; Retsch, Germany). We analyzed five-ring instead of one-ring wood segments in this study in order to account for a larger number of tree individuals while maintaining mid to low frequency temporal variability. A 5-year window is adequate to smooth high-frequency climate variability in Mediterranean-type ecosystems (Sumner et al. 2001; Feidas et al. 2007; Sarris et al. 2007). An aliquot of 0.5–0.7 mg of each wood sample was weighed into a tin capsule for isotopic analyses. Cellulose was not extracted as both whole wood and cellulose isotope time-series show similar long-term trends related to

atmospheric CO_2 and climate (Saurer et al. 2004; Taylor et al. 2008; McNulty and Swank 1995). Furthermore, a carryover effect from year to year would be negligible because we analyzed 5-year periods. The isotopic ratio of $^{13}C/^{12}C$ was determined on a mass spectrometer (Thermo Finnigan Delta plus XP). Two standards were included for analysis after every ten wood samples: cellulose ($\delta^{13}C = -24.72‰$) and phthalic acid ($\delta^{13}C = -30.63‰$). The repeated analysis of these two internal standards yielded a SD of less than 0.1‰.

Tree-age effect on isotopic discrimination

To test for the existence of $\delta^{13}C$ variations related to age, the $\delta^{13}C$ of tree-rings were correlated to their cambial ages (the age of the tree when the ring was formed). To avoid statistical confusion due to long-term environmental changes in $\delta^{13}C$ signals, this test was only carried out on wood samples formed in the 2001–2005 period, when climatic conditions were similar to the mean values of the past 30 years (see also Bert et al. 1997).

Competition index

The degree of competition around each sampled (focal) tree was calculated using a distance-dependent competition index (Hegyi 1974; Lorimer 1983). The degree of competition (competition index; CI) experienced by tree i was calculated as the ratio dbh_j/dbh_i divided by the distance between tree i and tree j ($dist_{ij}$), summed over all j neighboring trees within a radius R of tree i: $CI = \sum_{j=1}^{N(R)} (dbh_j/dbh_i)/dist_{ij}$. The R-value corresponding to the effective neighborhood of competition was tested by linear regression analyses as follows: the CI values obtained using radii from 1 to 10 m (in 1 m increments) were fit against the mean $\delta^{13}C_{wood}$ of each focal tree. Then, the distance R with the highest coefficient of determination was chosen, and the corresponding CI was used in further analyses. Finally, each tree was assigned to one of two classes, low- or high-competition neighborhood (c^- and c^+, respectively), using recursive partitioning and regression-tree analyses (see statistical analyses below).

Temporal trends for C discrimination and intrinsic water use efficiency

Isotopic discrimination between the C of atmospheric CO_2 and plant C (Δ; Farquhar and Richards 1984) in C_3 plants is a result of preferential use of ^{12}C over ^{13}C during photosynthesis, and it is defined in Eq. 1:

$$\Delta = \left(\delta^{13}C_{atm} - \delta^{13}C_{wood}\right)/\left(1 + \delta^{13}C_{wood}/1000\right) \quad (1)$$

where $\delta^{13}C_{atm}$ and $\delta^{13}C_{wood}$ are the $^{13}C/^{12}C$ ratios in atmospheric CO_2 and plant material, respectively, expressed in parts per thousand (‰) relative to the standard Vienna-Pee Dee belemnite. The Δ is linearly related to the ratio of intercellular (C_i) to atmospheric (C_a) CO_2 mole fractions by Eq. 2 (Farquhar et al. 1982):

$$\Delta_{lin} = a + (b' - a)C_i/C_a, \quad (2)$$

where a is the fractionation during CO_2 diffusion through the stomata (4.4‰; O'Leary 1981), and b' is the fractionation associated with reactions by Rubisco and phosphoenolpyruvate carboxylase carboxylase (27‰; Farquhar and Richards 1984). The values for C_a and $\delta^{13}C_{atm}$ were obtained from McCarroll and Loader (2004).

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) are related by a constant factor ($g_w = 1.6g_c$), thus linking the leaf gas exchange of C and water. The linear relationship between C_i/C_a and Δ (Eq. 2) can be used to calculate the $WUEi_{lin} = A/g_w$.

$$WUEi_{lin} = c_a(b' - \Delta_{lin})/[1.6(b' - a)]. \quad (3)$$

This ratio of net assimilation to stomatal conductance of water vapor has been widely related to long-term trends in the internal regulation of C uptake and water loss in plants. However, the internal CO_2 transfer and photorespiration are important components of the isotopic budget of net photosynthesis (Farquhar et al. 1982) that are not directly accounted for by Eq. 3 (Seibt et al. 2008). To include these processes, Δ is calculated as:

$$\Delta_c = a\frac{C_a - C_i}{C_a} + a_m\frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - f\frac{\Gamma^*}{C_a}, \quad (4)$$

where C_c is the CO_2 mol fraction at sites of carboxylation (µmol mol^{-1}), a_m is the fractionation during the internal (mesophyll) CO_2 transfer (1.8‰), b is the fractionation during carboxylation (29‰), f is the fractionation during photorespiration (approximately 8‰), and Γ^* is the CO_2 compensation point in the absence of dark respiration (30 µmol mol^{-1}). By using Fick's law [$A = g_i (C_i - C_c)$] for the gradient between C_i and C_c (where g_i is the mesophyll conductance; 0.2 mol m^{-2} s^{-1}, see Seibt et al. 2008) and replacing A by the ratio g_c/g_i, the WUEi can be written as:

$$WUEi_c = \frac{C_a}{1.6}\left(\frac{b - \Delta - f\frac{\Gamma^*}{C_a}}{b - a + (b - a_m)\frac{g_c}{1.6g_i}}\right). \quad (5)$$

The results of the linear models of Δ and WUEi (Eqs. 2, 3) were compared with those of the classic models (Eqs. 4, 5) as follows: we calculated the value of Δ by Eq. 1 for the first sample of each tree (the oldest tree-ring segment analyzed in each individual tree), and then we calculated initial values of

C_i, g_c, and C_c by assuming that in this sample $\Delta = \Delta_{lin} = \Delta_c$. This assumption was made to constrain the unknown parameters in Eqs. 4 and 5. Then, from this starting point, the temporal trends of Δ_{lin}, Δ_c, $WUEi_{lin}$ and $WUEi_c$ were calculated under three scenarios (see Saurer et al. 2004) for the theoretical regulation of plant gas exchange at increased C_a to achieve the following: (1) constant C_i, (2) constant C_i/C_a, and (3) constant $(C_a - C_i)$. In scenario 1, C_i is kept constant, so $\delta^{13}C_{wood}$ must increase, whereas C_i/C_a and Δ decrease, and $WUEi$ increases strongly. In scenario 2, the $\delta^{13}C_{wood}$ decreases in parallel with $\delta^{13}C_{atm}$ because C_i/C_a and Δ are constant; accordingly, $WUEi$ is still improved, but not as strongly as in scenario 1. In scenario 3, C_i follows the increase in C_a, and $WUEi$ is not improved.

Statistical analyses

Temporal trends in BAI, $\delta^{13}C_{wood}$, Δ and $WUEi$, and cambial age vs. $\delta^{13}C_{wood}$ were tested by simple linear regression. We also applied a two-slope comparison test (analysis of covariance) in order to detect differences in the rates of change among elevation and tree competition classes. Climatic variables and C_a were transformed to smooth average values over 5 years, which also reduced possible interference due to temporal autocorrelation, and were then related to radial growth and $\delta^{13}C_{wood}$ by correlation analyses. The significance level used in all of these tests was $P = 0.05$. We defined tree competition classes by recursive partitioning and regression-tree analyses (rpart package in the R software), using $\delta^{13}C_{wood}$ as the dependent variable and CI as a predictor (Breiman et al. 1984). To assess how Δ (Eq. 1) was related to Δlin (Eq. 2) and Δc (Eq. 4) under the three scenarios described above, we applied a linear mixed-effects model (nlme package in the R software) that included individuals as a random factor. We used an information-theoretic approach to multi-model selection, based on the Akaike information criterion corrected for small sample size (AICc), and the model with the lowest AIC was selected (Burnham and Anderson 2002). We considered the models with substantial support to be those in which the ΔAICc (i.e., the difference of AICc between models) was <2. All statistical analyses were performed using R software (R Development Core Team 2008).

Results

Climatic trends

Annual precipitation in the study area increases with elevation following a curvilinear shape [$y = 0.0005x^2 - 0.2633x + 695.86$; R^2(adj) = 0.83, $P < 0.0001$; $n = 25$ accounting for a range of 200–1,715 m a.s.l. in the predictor variable]. Data from meteorological stations below ~ 900 m a.s.l. ($n = 18$) did not show an altitudinal effect on precipitation, and the average rainfall was ~ 700 mm. Above 1,000 m a.s.l ($n = 7$), mean annual precipitation increased at a rate of 90 mm for every 100-m rise in elevation. The estimated annual precipitation was 1,089 mm for the L site and 1,631 mm for the H site. Mean annual temperature decreased linearly by 0.67°C for every 100 m of elevation [$y = -0.0067x + 19.65$; R^2(adj) = 0.73, $P < 0.0001$; $n = 15$, range of 209–1,283 m a.s.l]. The estimated mean annual temperatures were 11.6 and 8.3°C in the L and H sites, respectively.

The low-elevation regional annual series showed a significant increase in mean annual temperature (+0.65°C over the past half century; Fig. 1a) and a decrease in water surplus (precipitation minus evapotranspiration; Fig. 1c). The relative contributions of atmospheric CO_2 and mean air temperature to the pattern of long-term change are summarized in Fig. 1b. During the past 100 years, the global CO_2 mole fraction in the atmosphere increased from 300 to 380 μmol mol^{-1}, but this increase only overlapped with the regional warming of the study area since the early 1980s.

Most of the studied trees started released growth between the 1950s and 1960s (Fig. 2). The BAI for the L site showed an increasing trend from 1950 to the 1980s, when it reached values of ~ 5 cm^2 year^{-1}, and decreased thereafter. A sharp BAI reduction was observed in 1995, at the end of a period of severe drought (Fig. 1c). The H site showed a steady mean radial growth of ~ 3 mm year^{-1} (Table 1) and a rising BAI up to the current mean values of 19.1 cm^2 year^{-1}. The strong divergence in BAI between the L and H sites started around 1985, and the difference reached a maximum in the second half of the 1990s. Currently, the mean radial growth is about 4 times lower in the L than in the H site. Tree-ring width was positively correlated with spring precipitation in the L site, whereas it was correlated with spring temperature in the H site (data not shown).

Despite the fact that the studied trees were not very old (40–100 years), the $\delta^{13}C_{wood}$ was not significantly related to age in any of the sites [L site, $\delta^{13}C_{wood} = -0.0011$ age-26.07; R^2(adj) = 0.002, $P = 0.89$; H site, $\delta^{13}C_{wood} = -0.0042$ age-26.44; R^2(adj) = 0.005, $P = 0.82$]. Thus, the effect of tree-ring cambial age on $\delta^{13}C_{wood}$ values can be regarded as negligible.

The maximum correlation between $\delta^{13}C_{wood}$ and competition (CI) was reached in the L site for the neighborhood of trees within a 2-m radius from the focal tree, whereas in the H site, the highest correlation was found for a radius of 5 m (Fig. 3). In both populations, the mean $\delta^{13}C_{wood}$ of the trees decreases as the degree of competition they experience increases (Fig. 3). Recursive partitioning and regression-tree analyses between $\delta^{13}C_{wood}$ and the corresponding CI for a radius of 2 m (L site) and 5 m (H site) discriminated

A

$y = -8.71 + 0.013x; R^2(Adj) = 0.23; P < 0.001$

B

C

$y = 9332.29 - 4.47x; R^2(Adj) = 0.31; P < 0.001$
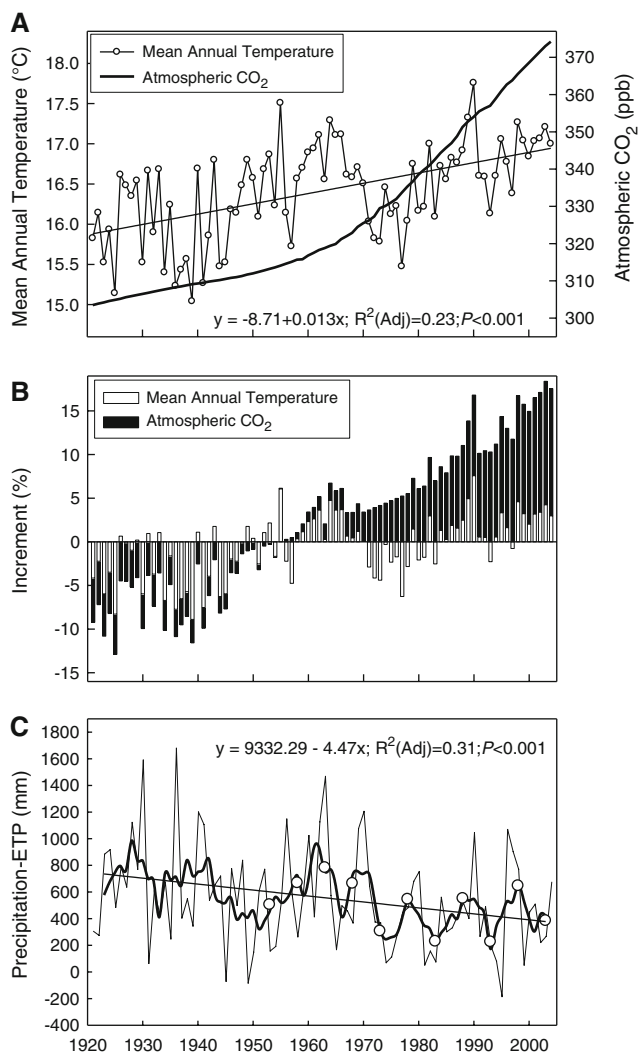
Fig. 1 **a** Mean annual temperature for the study area and changes in the global atmospheric CO_2 concentration (data from McCarroll and Loader 2004). **b** Trends of mean temperature and CO_2 concentration, expressed as the percentage change with respect to the average value during the 1950s. **c** Differences between annual precipitation and evapotranspiration (*ETP*) in the study area. The *thick line* shows a 5-year running average. *Points* indicate the median of time periods where tree-ring segments were sampled for C isotope analyses

two groups of trees based on the effect of competition on $\delta^{13}C_{wood}$ values (see Electronic Supplementary Material, Figs. S1 and S2). One group includes trees surrounded by a relatively weak competitive environment (c^-). This means either that the competitive strength of neighboring trees is low (irrespective of the size of the focal tree), or that the balance between the competitive strengths of the focal tree and its neighborhood is favorable to the focal tree (irrespective of the number, sizes and distances of neighboring trees). The other group includes trees that are subject to a relatively higher degree of competition (c^+). The properties of both groups of trees and their neighborhood are summarized in Table 2.
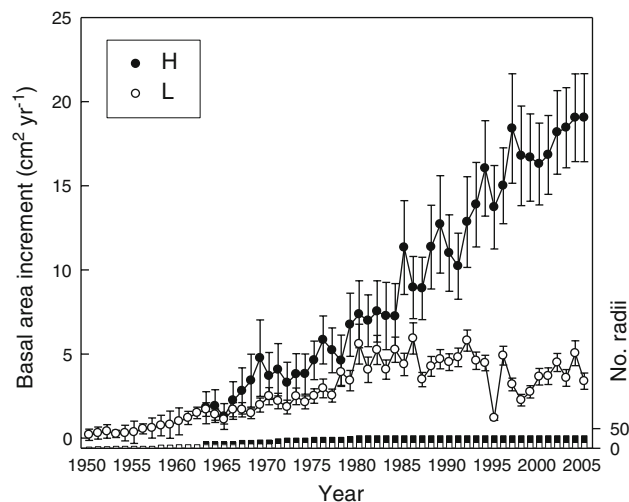
Fig. 2 Variations in basal area increment (BAI) for the high-elevation (*H*) and low-elevation sites (*L*). The *bottom bars* represent the number of radii measured every year. *Error bars* correspond to SEs

The $\delta^{13}C_{wood}$ differed between sites according to their contrasting elevation and climatic constraints (Fig. 4), with mean values of $-25.84 \pm 0.05‰$ and $-26.75 \pm 0.06‰$ in the L and H sites, respectively. Accordingly, mean Δ values (Fig. 4b) were lower in the L ($18.83 \pm 0.06‰$) than in the H site ($19.51 \pm 0.07‰$). In the L site, we observed a slight decrease in the $\delta^{13}C_{wood}$ of $-0.10‰$ per decade, with similar trends in both c^+ and c^- trees. In contrast, we found a rising trend of $+0.14‰$ per decade in the H site. These trends resulted in the convergence in $\delta^{13}C_{wood}$ values between sites, nearing each other in recent years (Fig. 4). Consequently, the temporal trends of Δ were $-0.16‰$ and $-0.43‰$ per decade in the L and H sites, respectively. Within stands, differences in the isotopic signal of the wood were very consistent among tree classes. The $\delta^{13}C_{wood}$ of c^+ trees was a fixed amount lower than that in c^- trees and, therefore, the degree of isotopic discrimination is increased in trees experiencing a highly competitive environment (Fig. 4).

Table 3 shows the linear regression results for the temporal trends in WUEi. Since 1970, the mean WUEi (Eq. 3) of $c-$ trees increased at a rate of $+0.44$ µmol mol^{-1} per year in the L site, and at a rate of $+0.79$ µmol mol^{-1} per year in the H site. For c^+ trees, the rates were $+0.54$ and $+0.67$ µmol mol^{-1} per year in the L and the H site, respectively. The two-slopes comparison tests showed significant differences between sites (L vs. H; Table 3), but the slopes corresponding to c^- or to c^+ trees within each site did not differ significantly.

The model selected as the most likely to explain the variability of Δ in the H site was the constant-C_i scenario estimated by the linear model (Eq. 3), which accounted for 62.6% of the variance, although the constant-C_i scenario estimated by the classic model (Eq. 5) achieved a similar support value ($\Delta AICc = 0.29$; Table 4). At this site, we

Table 1 Characteristics[a] of the study sites and sampled *Abies pinsapo* trees. SEs are in *parentheses*. *n* number of trees analyzed for tree-ring width and C stable isotope ratios of wood ($\delta^{13}C_{wood}$), *dbh* diameter at breast height (diameter at 1.3 m)

Site	Elevation (m a.s.l.)	Annual precipitation (mm)	Mean annual temperature (°C)	Stand density (m² ha⁻¹)	n	dbh (cm)	Age (years at dbh)	Tree-ring width (mm year⁻¹)	Tree-ring width 1984–1994 (mm year⁻¹)	Tree-ring width 1995–2005 (mm year⁻¹)	$\delta^{13}C_{wood}$ (‰)
High-elevation site (H)	1,749	1,631	8.3	8.9	11	23.50 (5.38)	33.70 (7)	3.55 (0.66)	3.57 (0.92)	3.67 (0.64)	−26.75 (0.06)
Low-elevation site (L)	1,226	1,089	11.6	38.9	13	17.91 (8.21)	53.84 (18)	1.63 (0.38)	1.52 (0.42)	0.88 (0.37)	−25.84 (0.05)

[a] Stand density, mean growth and mean $\delta^{13}C_{wood}$ were significantly different, but mean values of dbh and age did not differ between sites (Tukey HSD test, *P*-level = 0.05)

did not find substantial support for a relationship of Δ with the CI or with variables related to precipitation. However, a slight association of Δ with air temperature was detected (data not shown). For the L site, the constant-c_i scenario was also a selected model, but in this case, the classic model was selected ahead of it and accounted for 75.3% of the variance. The linear model also received substantial support (ΔAICc = 0.64). When CI was included in the model, we obtained a slight improvement based on the AIC criteria (data not shown), but the increase in the explained variance was negligible (0.4%). There was not substantial support for an effect on Δ of any climatic variable in the L site, based on our model selection criteria.
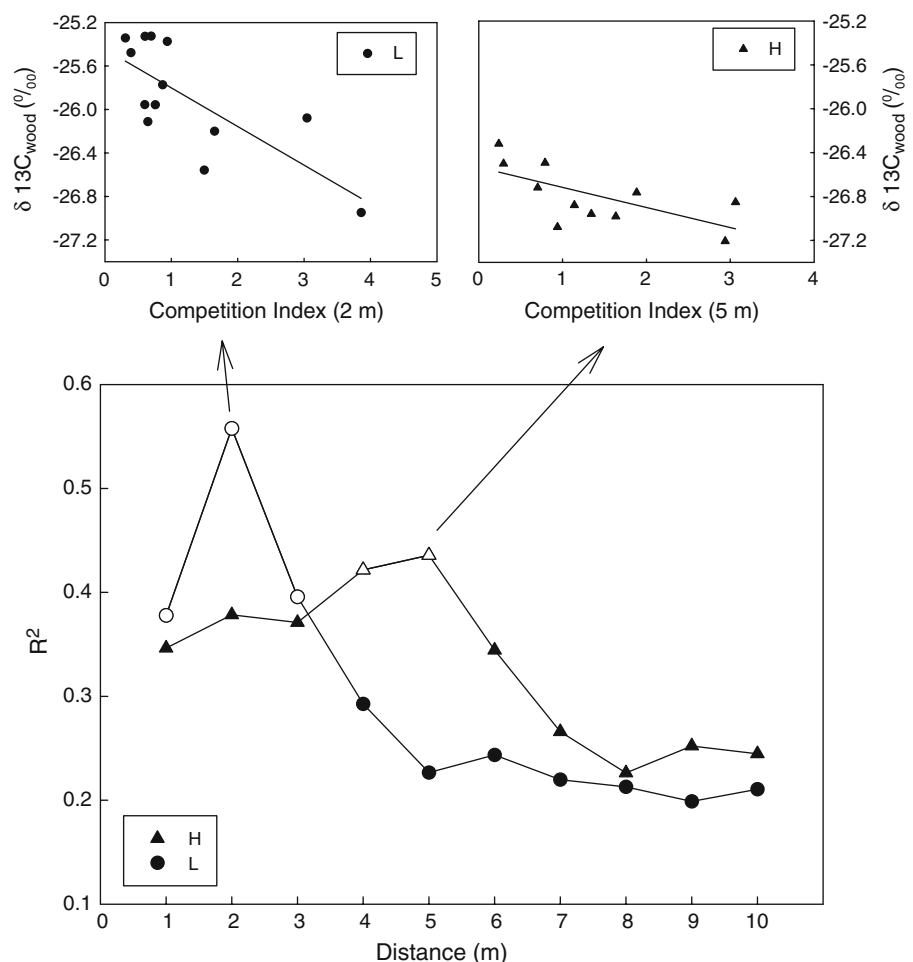
Table 5 summarizes the mean values and trends predicted by the likelihood models compared with those obtained from the raw tree-ring $\delta^{13}C_{wood}$. In the H site, both the linear (Eqs. 2, 3) and the classic (Eqs. 4, 5) models accounted for ∼35% of the variance in the WUEi of c^- trees (Table 5; Fig. 5). In contrast, the observed values for the trees from the L site were always lower than those predicted under a constant-C_i scenario.

Discussion

During the past 100 years, the CO_2 mole fraction in the atmosphere has increased from 300 to 380 μmol mol⁻¹ (McCarroll and Loader 2004). If rising atmospheric CO_2 levels lead to increased photosynthetic rates (Boisvenue and Running 2006) but transpiration rates remain either constant or reduced, then the ratio of C assimilated to water transpired by plants will increase. The change in climate over the past few decades towards warmer conditions (IPCC 2007) strongly affects plant performance (Peñuelas and Filella 2001). Most of the impacts of a warmer climate on the growth and survival of Mediterranean firs have been negative (Macias et al. 2006; Linares et al. 2009).

The BAI at the *A. pinsapo*, L site first increased until the 1980s, and then it decreased (Fig. 2) in concert with the regional warming trend that led to more xeric conditions in the study area (Fig. 1). In contrast, at the H site, which represents the upper limit of the current distribution of *A. pinsapo* in southern Spain, secondary growth increased during this same period. Higher secondary growth at higher elevations was also found in a more extensive survey of the same study area (Linares et al. 2009). In addition, we recorded similar trends in other Spanish (Grazalema range) as well as northern Moroccan *A. pinsapo* forests (J. C. Linares et al., in preparation). These kinds of changed altitudinal growth patterns have been reported previously at the ecotone of the Mediterranean Biome, in relict *Fagus sylvatica* forests from north-eastern Spain (Jump et al. 2006) and from central Italy (Piovesan et al. 2008).

Despite the fact that the rise in atmospheric CO_2 was likely similar for both the H and the L stands, a decoupling of CO_2 and secondary growth patterns was evident at the lower limit of the *A. pinsapo* altitudinal distribution. Our results support the hypothesis that other factors beside CO_2, such as drought and temperature, might interfere with and prevent any CO_2-induced growth stimulation. Drought stress was probably lower in the more mesic *A. pinsapo* H site, where tree growth was positively related to temperature (Linares et al. 2009). At this upper elevation limit, an increase in the length of the growing season linked to the warming trend might be improving radial growth (Fig. 2). In contrast, tree-ring width at the L site was positively although weakly correlated with precipitation during the spring, the period when resumption of cambial activity and maximum secondary growth rates occur. The observed increase in mean temperature may impact the soil water budget at lower elevations. Additionally, the higher stand density in the L site (Table 1) might enhance drought stress by enhancing tree-to-tree competition for water. If *A. pinsapo* shows a drought-avoidance rather than a drought-tolerance response to water stress, an earlier depletion of soil water resources in spring should result in a reduction of the growing period length

rather than in a decrease in growth rates over a similarly extended period. Thus, low water stress during the early spring period, when most growth occurs, would explain the weak relationship between the C isotopic ratio in *A. pinsapo* and climate data. However, $\delta^{13}C_{wood}$ in less drought-sensitive Mediterranean conifers show a stronger response to precipitation variability, as has been recently reported for Mediterranean pine forests by Andreu et al. (2008). The poor relationship we obtained in this study might also be explained by the fact that we used 5-ring samples for isotopic analysis, which smooths the trends.

The decrease in the mean $\delta^{13}C$ signature of atmospheric CO_2 ($\delta^{13}C_{atm}$) due to fossil fuel combustion and biomass burning during the period encompassing our $\delta^{13}C_{wood}$ data was 1.23‰ (from −6.85‰ in 1946–1950 to −8.07‰ in 2001–2005; Francey et al. 1999), somewhat larger than the change in tree-ring $\delta^{13}C_{wood}$ values (Fig. 4a). Furthermore, the $\delta^{13}C_{wood}$ values were negatively correlated to competition intensity (tree-rings were more depleted in ^{13}C values at the higher level of competition; Fig. 3), and also to BAI (Table 2). The correlation between the isotopic discrimination in wood and competition was stronger than any relationship with climatic variables, which illustrates the

Table 2 Characteristics of the studied focal trees (mean values ± SE) grouped by low (c^-) and high (c^+) competition classes, at the H and L sites

Site	Focal trees				Neighborhood (10 m radius circle around the focal tree)		
	Competition class	dbh (cm)	Age (years at dbh)	BAI (cm² year⁻¹)	Mean distance to neighbors (m)	Mean number of neighbors	Mean dbh of neighbors (cm)
H	c^-	22.3 ± 2.6 (32–17)	35.4 ± 3.4 (44–24)	16.4 ± 5.5a	6.7 ± 0.17a	13.6 ± 2.6a	16.2 ± 1.2a
	c^+	24.7 ± 2.4 (32–17)	32.0 ± 3.0 (40–23)	9.7 ± 2.0a	5.7 ± 0.24b	16.0 ± 1.9a	12.8 ± 1.5ab
L	c^-	22.0 ± 4.5 (34–10)	61.5 ± 9.6 (97–34)	11.2 ± 3.7a	6.8 ± 0.17a	34.5 ± 10.5b	17.3 ± 1.8a
	c^+	14.3 ± 0.8 (18–12)	47.0 ± 1.9 (54–39)	3.9 ± 0.4b	6.1 ± 0.19b	53.3 ± 6.5b	11.7 ± 1.0b

The maximum–minimum range in values for dbh and age are shown in *parentheses*. Different letters denote significant differences between means (one-way ANOVA, $P < 0.05$). *BAI* Basal area increment; for other abbreviations, see Table 1
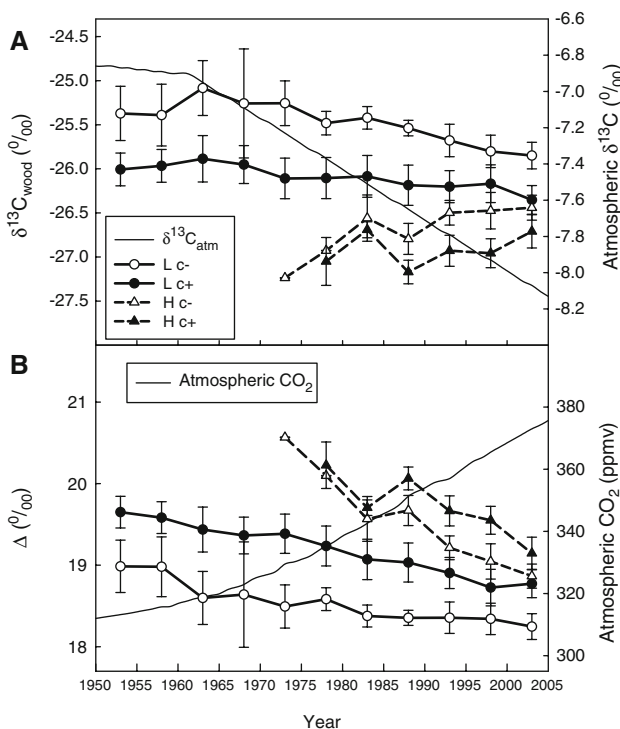
Fig. 4 Variations in the $\delta^{13}C_{wood}$ raw data (**a**) and Δ (**b**) for trees subjected to low (c^-) and high (c^+) competition, located either in the H or in the L sites. Atmospheric $\delta^{13}C$ and CO_2 concentrations from McCarroll and Loader (2004) are also shown. *Error bars* represent the SE. Note that C isotope series are shorter than those of tree-ring width because the first 15–20 tree-rings of each tree were discarded in order to avoid the juvenile effect. For other abbreviations, see Fig. 2

importance of the stand structure as a modulating factor of tree function (Canham et al. 2004). These findings should be taken into account when performing studies on Mediterranean conifers that relate $\delta^{13}C_{wood}$ values with climatic information (Andreu et al. 2008).

The negative association between competition and the $\delta^{13}C_{wood}$ could be explained by a canopy closure effect on intercepted radiation. The dbh range for trees classified as c^+ fitted entirely within the one for c^- trees (Table 2), and just adult, dominant or co-dominant trees were sampled.

However, even trees within these stand classes may experience a variety of light environments because of relative differences in the distances to, and the number and sizes of, other trees in their vicinity. A relatively higher mean radiation reaching trees subjected to low competition (c^-) would allow for a higher rate of photosynthesis and lower C_i/C_a ratios, which agree with their higher WUEi values (Fig. 5). Because the net assimilation rate (A) is constrained both by the atmospheric CO_2 concentration and the amount of photosynthetically active radiation (Wullschleger 1983), trees subjected to higher shading would have a limited capacity to increase their C assimilation rate in response to rising levels of CO_2.

This hypothesis is supported by the small spatial scale (2–5 m) at which the maximum correlation between the $\delta^{13}C_{wood}$ and competition values was found, which suggests a local tree response to shading by their nearest neighbors (Fig. 3). An additional factor to be considered is that the CO_2 fixed by trees may not have the same $\delta^{13}C$ value as the $\delta^{13}C_{atm}$ due to the mixing of respired CO_2 from the soil and plants into the local atmosphere. This effect is likely to be much more important for young trees growing below the canopy (in our study, this source of confusion was minimized by the exclusion of the first 15–20 years of tree-rings), but it may also be significant for crowded trees living within closed canopies. Furthermore, the mean differences in the $\delta^{13}C_{wood}$ between trees subject to high (c^+) and low (c^-) competition intensities were constant over most of the study period (Fig. 4a), which allows us to assume that a juvenile effect on C isotope discrimination was negligible.

A dependence of the C isotope ratio on elevation was also observed, as expected, considering that precipitation increases with elevation. The mean annual precipitation was ca. 500 mm higher in the H than in the L site, which was concurrent with a decrease of ca. 0.96‰ in the $\delta^{13}C_{wood}$ at the H site. Roden et al. (2005) obtained a similar slope of −0.90‰ for each increase of 500 mm of mean annual precipitation for conifer trees along a rainfall gradient in Oregon. Panek and Waring (1997) also obtained

Table 3 Trends in intrinsic water use efficiency (WUEi) for trees at the H and L sites. Linear regressions[a] between WUEi and time were fitted separately for trees subjected to low (c^-) and high (c^+) competition

Site	Tree competition class	a	b	Residual SD	R^2(adj)
H	c^-	−1,364.81 (135.20)	0.72 (0.07)	3.13	0.79a
H	c^+	−1,129.60 (161.29)	0.60 (0.08)	3.42	0.68a
L	c^-	−624.63 (64.03)	0.36 (0.03)	3.94	0.69b
L	c^+	−683.41 (91.00)	0.38 (0.05)	4.72	0.54b

[a] The four regressions were highly significant ($P < 0.001$)

The SDs of the fitted parameters are shown *in parentheses*. *Different letters* denote significant differences between slopes (analysis of covariance, $P < 0.05$). For other abbreviations, see Table 1

Table 4 Model[a] selection criteria for Δ of *Abies pinsapo* trees in H ($n = 11$ groups and 58 samples) and L ($n = 13$ groups and 116 samples) sites

	Model	AICc	ΔAICc	$L(g_i/x)$	Wi (%)
Δ at the H site	C_i constant (linear model)	**83.6**[b]	**0.00**	**1.00**	**53.6**
	C_i constant (classic model)	**83.9**	**0.29**	**0.87**	**46.4**
	$C_a - C_i$ constant (classic model)	107.8	24.2	0.00	0.00
	$C_a - C_i$ constant (classic model)	110.0	26.4	0.00	0.00
	Null model	110.3	26.7	0.00	0.00
	C_i/C_a constant (linear model)	112.7	29.1	0.00	0.00
	C_i/C_a constant (classic model)	113.1	29.5	0.00	0.00
Sum				1.87	
Δ at the L site	C_i constant (classic model)	**96.5**	**0.00**	**1.00**	**49.7**
	C_i constant (linear model)	**97.2**	**0.64**	**0.73**	**36.0**
	C_i/C_a constant (linear model)	100.1	3.56	0.17	0.84
	C_i/C_a constant (classic model)	100.9	4.39	0.11	0.55
	$C_a - C_i$ constant (classic model)	107.9	11.4	0.00	0.02
	Null model	108.0	11.5	0.00	0.00
	$C_a - C_i$ constant (linear model)	108.5	12.0	0.00	0.00
Sum				2.01	

[a] We tested the three models proposed by Saurer et al. (2004), using Eqs. 2 (linear model, Δ_{lin}) and 5 (classic model, Δ_{class}). The scenarios for the theoretical regulation of plant gas exchange at increased atmospheric CO_2 mole fraction (C_a) were fitted to achieve the following: (1) constant intercellular CO_2 mole fraction (C_i); (2) constant C_i/C_a; and (3) constant $C_a - C_i$. These theoretical scenarios were compared with Δ values obtained from using the values of $\delta^{13}C_{wood}$. A null model (Δ = constant) was also included

[b] Values *in bold* correspond to models with substantial support

AICc Akaike information criterion corrected for small sample size and K parameters, including each theoretical model, intercept and error term, *ΔAICc* difference in AICc with respect to the best model; *$L(g_i/x)$* likelihood of a model g_i given the data x; *Wi* relative probability, expressed as a percentage, that the model i is the best model given the observed data; for other abbreviations, see Table 1

differences of about 4‰ for *Pseudotsuga menziesii* related to precipitation gradients of ca. 2,000 mm. Furthermore, the C isotope ratio has been shown to increase in response to the effect of a decrease in either air humidity or soil moisture (Warren et al. 2001), primarily as a result of reduced stomatal conductance (Wullschleger et al. 2002). A reduction in stomatal conductance may also contribute to higher $\delta^{13}C_{wood}$ values (lower Δ) in the low-elevation and more xeric *A. pinsapo* site (Fig. 4).

The AICc model selection supported the hypothesis that the increase in atmospheric CO_2 concentration is the main driver for the observed long-term increase in WUEi in *A. pinsapo* (Table 4). Although additional parameters can influence WUEi changes through their effects on stomatal aperture (Seibt et al. 2008), the significant increase in the WUEi for all trees and both sites supports our conclusions. The increase in the WUEi over the second half of the 20th century (ca. 34 and 20% in the H and L sites, respectively) is within the average range of those published for other regions, where an improvement in WUEi has been prevalent during the past century (Bert et al. 1997; Duquesnay et al. 1998; Feng 1998; Saurer et al. 2004). Nevertheless,

Table 5 Observed changes in $\delta^{13}C_{wood}$, Δ and WUEi, and the results based on the models found to have substantial support using an information-theoretic approach to multimodel selection according to the AIC (see Table 3)

|			Site	L		H	
			Competition	c^-	c^+	c^-	c^+
Values obtained from $\delta^{13}C_{wood}$ using Eqs. 2–3	Measured mean value in 2001–2005 sample	$\delta^{13}C$		$-25.85 \pm 0.15a$	$-26.35 \pm 0.16b$	$-26.44 \pm 0.14b$	$-26.71 \pm 0.19c$
		Δ		$18.25 \pm 0.16a$	$18.77 \pm 0.17b$	$18.87 \pm 0.15b$	$19.15 \pm 0.20c$
		WUEi		$90.26 \pm 1.63a$	$84.84 \pm 1.76b$	$83.88 \pm 1.52b$	$80.99 \pm 2.04c$
	Changes over 1971–2005	Δ		$-0.31A$	$-0.74A$	$-1.66B$	$-1.29B$
		WUEi (%)		$16.69A$	$22.52A$	$34.94B$	$32.59B$
Constant C_i scenario using Eqs. 2–3 (linear model)	Predicted mean value for 2001–2005 sample	Δ		$16.91 \pm 0.21a$	$17.39 \pm 0.17a$	$18.47 \pm 0.15b$	$18.75 \pm 0.19b$
		WUEi		$103.24 \pm 2.01a$	$99.08 \pm 1.79a$	$87.92 \pm 1.55b$	$85.05 \pm 2.00b$
	Predicted changes over 1971–2005	Δ		$-1.90A$	$-1.99A$	$-2.23B$	$-1.99A$
		WUEi (%)		$35.50A$	$38.48A$	$42.59B$	$39.24A$
Constant C_i scenario using Eqs. 4–5 (classic model)	Predicted mean value for 2001–2005 sample	Δ		$16.84 \pm 0.22a$	$17.35 \pm 0.18a$	$18.48 \pm 0.16b$	$18.77 \pm 0.17b$
		WUEi		$99.95 \pm 1.80a$	$96.28 \pm 1.59a$	$85.28 \pm 1.36b$	$84.84 \pm 1.76b$
	Predicted changes over 1971–2005	Δ		$-1.89A$	$-1.99A$	$-2.23B$	$-1.99A$
		WUEi (%)		$35.50A$	$35.88A$	$38.92B$	$35.94A$

The presented values correspond both to the 2003 segment (tree-rings of the period 2001–2005) and to the trend obtained for the 1971–2005 period. *Different letters* within each row indicate significant differences (*P*-level = 0.05); *lowercase letters* denote mean comparison tests; *capital letters* denote two-slope comparison tests. For abbreviations, see Tables 2, 3 and 4

some studies found a progressively diminishing response to increasing atmospheric CO_2 concentrations (Waterhouse et al. 2004; Peñuelas et al. 2008), as we also report here for *A. pinsapo* in the L site, or even no change in WUEi at all, as was the case in a study on three coniferous species in the western USA (Marshall and Monserud 1996).

For the H site, the changes in WUEi of *A. pinsapo* were well-represented by the theoretical scenario of a constant C_i

(Fig. 5), which implies a particularly strong improvement in WUEi that has not been reported for many other sites (see Saurer et al. 2004 and references therein). It has been argued that at high elevation, because of the lower atmospheric pressure, any increase in the partial pressure of CO_2 would have a larger effect on the rate of photosynthesis than at lower elevations (Hultine and Marshall 2000). Along this line of reasoning, Woodward and Bazzaz (1988) pointed out

Fig. 5 Intrinsic water use efficiency (*WUEi*) for the two stands differing in altitude (H, L). We calculated WUEi using Eqs. 3 (linear model, *left-hand scatter plots*) and 5 (classic model, *right-hand scatter plots*) assuming three scenarios for the theoretical regulation of plant gas exchange at increasing atmospheric CO_2 mole fraction (C_a) to achieve the following: (1) a constant intercellular CO_2 mole fraction (C_i), (2) a constant C_i/C_a, and (3) a constant $C_a - C_i$. These theoretical scenarios were compared with WUEi values obtained from the $\delta^{13}C_{wood}$ in trees subjected to c^- or to c^+ competition, located at H (*top scatter plots*) or L (*bottom scatter plots*). *Error bars* represent the SEs. For other abbreviations, see Fig. 2
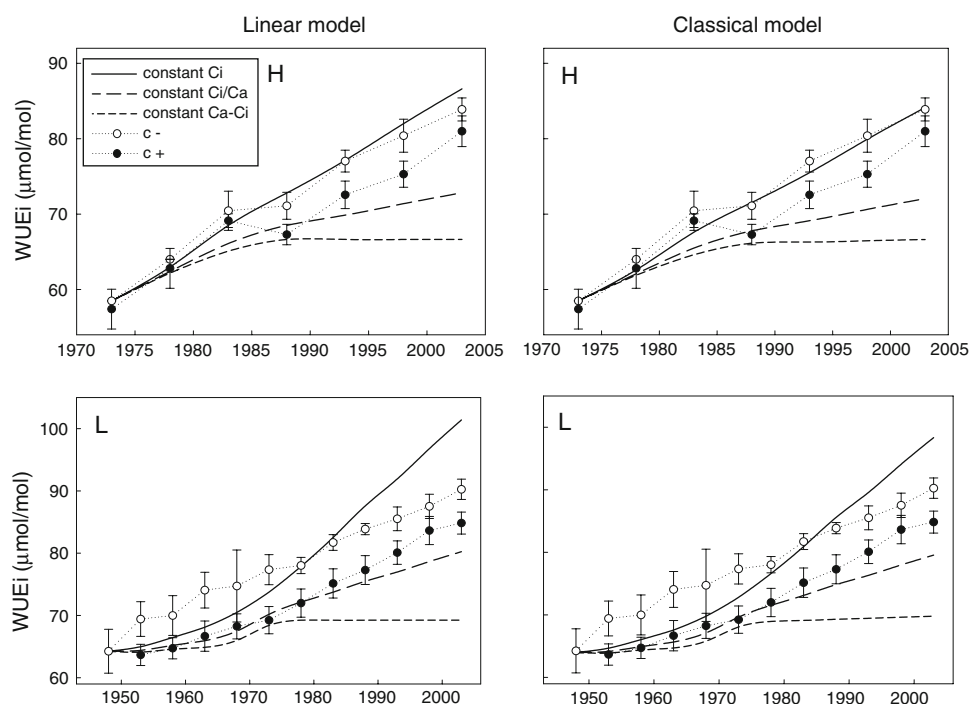
that the dependence of stomatal density on the partial pressure of CO_2 is more sensitive than its dependence on the concentration of CO_2. If the responses of stomatal conductance occur with different sensitivities at elevations with contrasting partial pressures, this would explain the observed variations in WUEi as a function of elevation, in addition to the altitudinal changes in climatic conditions.

On the other hand, in the L site the responses of trees were within the range of changes represented both by the constant C_i and the constant C_i/C_a scenarios (Fig. 5). Saurer et al. (2004) reported that the isotopic responses of conifers in northern Eurasia over the past century were best represented by constant C_i/C_a values, corresponding to a proportional regulation of A and g. If this is the case for *A. pinsapo*, it would mean that there was a slightly lower increase in WUEi than that calculated from isotopic data for the H site, but one similar to that calculated for the L site. The trends obtained were similar when we included the mesophyll conductance and photorespiration, likely due to the fact that the analysis is based on the assumption that only C_a has changed. This is no longer valid if there are concurrent trends in mean temperature, air humidity or light availability. In summary, the use of classic equations rather than linear ones may not be justified in long-term tree-ring research in which environmental variables (such as relative humidity, and thus vapor pressure deficit) or soil water availability are lacking, because WUEi will diverge strongly among the two approaches depending on the physiological parameters that must be assumed or modeled.

In short, since the 1980s, the rate of WUEi improvement for the L stand decreased and stopped following the rising atmospheric CO_2 trend. Although the reason for this decoupling is not well understood, our data show a good agreement between its timing (starting point ca. 1983, for instance; Fig 5), and that of the regional rise of air temperature (Fig. 1b), the succession of severe drought events in the mid 1980s and 1990s (Fig. 1c), and the stabilization and decline of radial growth (Fig. 2). The combined evidence suggests that a threshold in low water availability may exist that limits the WUEi improvement, as some have previously reported (see for instance Peñuelas et al. 2008).

The increase in WUEi in response to rising CO_2 and drought may be achieved by a reduction in stomatal conductance; however, if drought-induced stomatal closure is too severe, it also constrains the rate of photosynthesis and tree growth (Wullschleger et al. 2002; Drake et al. 1997). The recent reduction at the L site in the sensitivity of WUEi to increasing C_a suggests a drought-avoidance strategy in the response of *Abies pinsapo* to water stress. If trees react with a severe stomatal closure to the earlier onset of the dry period, the reductions in transpiration and C gain would be proportional despite C_a increases. Low availability of reduced C for growth is reflected in the *A.*

pinsapo, L site by the low radial-growth (BAI) values detected during recent decades, especially during the years with severe droughts (e.g., 1995) (Fig. 2). Furthermore, trees subjected to higher competition within the stand always had lower WUEi values than those of dominant trees, and thus lower values than those predicted under a constant C_i scenario (Fig. 5). However, the slopes of the temporal trends in WUEi were similar within each stand, independent of the degree of competition experienced by focal trees (Table 3). This calls into question our initial hypotheses that differing tree-to-tree competition might alter a tree's WUEi sensitivity to increases in CO_2. However, it still raises the issue that a high and poorly asymmetric competition at the stand level (Tables 1, 2) may act as an important constraint on the adaptive capacity (sensu Linares et al. 2009) of drought-sensitive forests to climatic warming. A loss of adaptive capacity can arise from the effects of competition on precluding the trees to fully express the potential that increased CO_2 concentrations represent in terms of allowing for a reduction in water losses without experiencing a substantial reduction in C gain.

Conclusion

These results strongly suggest that a drought-induced limitation of secondary growth and a decreasing improvement in WUEi are occurring at the lower elevation limit of *A. pinsapo* forests. Moreover, the rate of the increase in WUEi at lower elevations appears to be insufficient to counteract the growth decline induced by warming and more xeric conditions. In contrast, trees in the mesic H sites yielded an improvement in WUEi of about 34% during the past 30 years that, in combination with increasing radial growth, indicates that these trees are able to maintain high radial-growth rates at lower water costs than trees in the L sites. These findings indicate that *A. pinsapo* may not be able to mitigate the negative effects of low water availability by lowering stomatal conductance without a loss in biomass production. Therefore, further growth declines are expected in the low-elevation xeric *A. pinsapo* forests if warming continues. Trees under high competition by their neighbors always had a lower WUEi than trees growing on a less competitive microsite, which suggests that tree-to-tree competition modulates the balance between light and water limitations.

Appendix

See Table 6

Table 6 List of abbreviations used in the paper

Abbreviation	Variable/parameter	Measurement method or reference and units
H	*Abies pinsapo* population at higher elevation	1,749 m a.s.l.
L	*Abies pinsapo* population at lower elevation	1,226 m a.s.l.
dbh	Tree diameter at 1.3 m	Field measurements (cm)
BAI	Basal area increment	From tree-ring width (cm^2)
CI	Competition index	From tree x-y coordinates and dbh, Hegyi (1974)
c^+	Low-competition neighborhood	From CI, recursive partitioning and regression-tree analyses
$c-$	High-competition neighborhood	From CI, recursive partitioning and regression-tree analyses
C_a	CO_2 mole fraction in ambient air	McCarroll and Loader (2004), µmol mol^{-1}
C_i	CO_2 mole fraction in intercellular spaces	From Eq. 1, Farquhar et al. (1982), µmol mol^{-1}
Δ	Net ^{13}C discrimination during photosynthesis	Eq. 1, Farquhar and Richards (1984), ‰
Δ_c	Net discrimination, classic version	Equation 4, Farquhar et al. (1982), ‰
Δ_{lin}	Net discrimination, linear version	Equation 2, Farquhar et al. (1982), ‰
$\delta^{13}C_{atm}$	C isotope composition in atmosphere	McCarroll and Loader (2004), ‰
$\delta^{13}C_{wood}$	C isotope composition of tree-ring wood	Mass spectrometric analysis, ‰
WUEi	Water use efficiency	From Eq. 1, Farquhar et al. (1982), µmol m^{-2} s^{-1}
WUEi$_c$	Water use efficiency, classic version	Equation 5, Farquhar et al. (1982), µmol m^{-2} s^{-1}
WUEi$_{lin}$	Water use efficiency, linear version	Equation 3, Farquhar et al. (1982), µmol m^{-2} s^{-1}
AICc	Akaike information criterion corrected for small sample size	Burnham and Anderson (2002)
ΔAICc	Difference of AICc between models	Burnham and Anderson (2002)

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