

Contrasting growth and water use efficiency after thinning in mixed *Abies pinsapo*–*Pinus pinaster*–*Pinus sylvestris* forests

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ABSTRACT: Foresters frequently lack sufficient information about thinning intensity effects to optimize semi-natural forest management and their effects and interaction with climate are still poorly understood. In an *Abies pinsapo*–*Pinus pinaster*–*Pinus sylvestris* forest with three thinning intensities, a dendrochronological approach was used to evaluate the short-term responses of basal area increment (BAI), carbon isotope ($\delta^{13}\text{C}$) and water use efficiency (iWUE) to thinning intensity and climate. Thinning generally increased BAI in all species, except for the heavy thinning in *P. sylvestris*. Across all the plots, thinning increased ^{13}C -derived water-use efficiency on average by 14.49% for *A. pinsapo*, 9.78% for *P. sylvestris* and 6.68% for *P. pinaster*, but through different ecophysiological mechanisms. Our findings provide a robust mean of predicting water use efficiency responses from three coniferous species exposed to different thinning strategies which have been modulated by climatic conditions over time.

Keywords: basal area increment; carbon isotopes; dendroecology; naturalization

According to climate predictions, temperature and related aridification are expected to rise in the Mediterranean area within the current century. Climate warming and increasing carbon availability threaten Mediterranean forests (LINDNER et al. 2010). Mountain conifer forests in southern Europe might be some of the most vulnerable ecosystems, such as the case of *Abies pinsapo* Boiss. (Spanish fir)

forests; a relict drought-sensitive fir species present only in the southwestern Mediterranean (AUSSENAC 2002).

Climatic stressors interact in *A. pinsapo* forests with historical land-use changes that have shaped the current structure of fir forests (LINARES et al. 2011). Traditional land uses (overexploitation through logging, grazing, and charcoal burning) by

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local populations led to the historical deforestation of *A. pinsapo* forests, thus prompting the Spanish government to protect these relict forests in the 1970s (ESTEBAN et al. 2010). Massive reforestation using pine species was subsequently carried out to promote fir regeneration in many degraded *A. pinsapo* forests (ESTEBAN et al. 2010). These reforestation produced promising mixed fir-pine stands, but often resulting in high-density stands that are highly vulnerable to drought stress (MARTÍN-BENITO et al. 2010, 2011).

In these dense stands, crown competition affects the space available for growth as well as the amount of light, whereas root competition reduces the amount of water and soil nutrients available to each tree. Water is the most limited resource for mixed fir-pine stands in southern Spain, and is predicted to become even scarcer in coming years. To overcome this problem, forest managers use thinning (of different intensity) to reduce tree competition for water within stands (MARTÍN-BENITO et al. 2011; DEL CAMPO et al. 2014). When properly used, thinning reduces long-term stress by competition, but it also reduces the vulnerability of trees to extreme drought events (LINARES et al. 2011; SÁNCHEZ-SALGUERO et al. 2012). Adaptive management could enhance growing conditions, and thus alleviate water stress that constrains photosynthetic activity and growth (McDOWELL et al. 2003). Besides improving the tree productivity, thinning has important effects on understory, fauna and soil nutrient cycle and ecology (MARTÍN-BENITO et al. 2011). However, the advantages of thinning for growth may not be the same for trees of different species (MORENO-FERNÁNDEZ et al. 2014), mainly due to different duration of the growing period (CAMARERO et al. 2011).

Given that precipitation is predicted to decrease or temperatures to increase in the Mediterranean, proactive adaptive management has become a basic strategy to either maintain or to gradually adapt current forest ecosystems (LINDNER et al. 2010). Such management strategies need to be grounded in the long-term and realistic ecological knowledge provided by long-term management experiments (MARTÍN-BENITO et al. 2010). Although these experiments offer extremely valuable information, they are time-consuming to measure and expensive to set up and maintain. Furthermore, very few of these trials provide long-term physiological data, and rarely use time spans longer than a decade (BROOKS, MITCHELL 2011). Implementing the tree-ring growth analysis combined with stable isotopes in cellulose allows exploring long-term

physiological responses to forest management and recent climate changes (McCARROLL, LOADER 2004). As stable isotopes record physiological and environmental processes at the time the ring was formed (McCARROLL, LOADER 2004), remnant trees are expected to increase their annual growth, reduce the $^{13}\text{C}/^{12}\text{C}$ isotope ratio in fixed wood cellulose ($\delta^{13}\text{C}$) although there is not a clear evidence on the long-term effects of thinning on iWUE (McDOWELL et al. 2003; FERNANDES et al. 2015). Due to the variability of interspecific growth responses to climate change in Mediterranean forests (GRANDA et al. 2014), further studies are needed to provide additional information about the utility of adaptive management strategies to regulate the interspecific ecophysiological responses involved in iWUE and growth in mixed forests. How thinning may help to improve the resilience and recovery of pure native *A. pinsapo* forests under climatically contrasting years in drought-prone Mediterranean areas remains unexplored (LINARES et al. 2011).

We propose comparing species-specific responses to thinning intensity and climate in mixed *A. pinsapo*–*P. pinaster*–*P. sylvestris* forests, and their consequences for forest management using a combination of dendrochronological and stable isotope methods. Specifically, we attempt to address the following questions: (i) Do trees of different species respond differentially to thinning intensity and climate? (ii) Does water use efficiency increase equally in the three species after thinning? and (iii) Does adaptive management facilitate the recovery of native *A. pinsapo* forests? We hypothesize that heavy thinning would expose *A. pinsapo* saplings to dry summer conditions at too an early stage, which would show high water stress levels in dry years. Moreover, competition with the other two pine species in unthinned plots could reduce the availability of resources for the *A. pinsapo*. Therefore, we expect light thinning intensity to be a good compromise between stress protection and reduced competition that would allow *A. pinsapo* to achieve better performance.

MATERIAL AND METHODS

Study area. The study area is located in the Sierra de las Nieves Natural Park of Malaga, Spain (36°43'N, 4°58'W) (see supplementary material – Fig. S1); a mountainous area situated on the north-facing slopes ranging in elevation from 1,126 to 1,562 m a.s.l. It is dominated by *A. pinsapo* forests and mixed forests containing other species such

as oak species (*Quercus ilex*, *Q. faginea*, *Q. canariensis*) and planted pine species (*Pinus halepensis*, *P. pinaster*, *P. sylvestris*). The area is subjected to a Mediterranean climate characterized by a shortage of water and warm temperatures in the summer months, with mean annual precipitation and temperature of 1,089 mm and 11.6°C, respectively. Soils are predominantly calcareous brown forest soils.

We used local monthly climatic data (mean temperature and total precipitation) to characterize the climatic conditions in the study area. For that purpose, we obtained climate data of precipitation (period 1964–2010) and temperature (period 1983–2010) from four meteorological stations located within a radius of 10 km in the study area (Agencia Estatal de Meteorología, see <http://www.aemet.es/es/>) (see supplementary material – Fig. S1).

Experimental design. The site was planted with 2-years-old *P. pinaster* and *P. sylvestris* in 1970 at an initial stand density of 2,200 trees·ha⁻¹ mixed with the natural *A. pinsapo* regeneration present there. Light thinning (removal of 20–28% of the trees with smaller diameters and tree forks) was applied in 1979. Ten years later, in 1989, a thinning experiment was performed. The thinning treatments were carried out on three experimental plots: control plot (T0); plot with light thinning where 30% of the pine basal area was removed (T30), and plot with heavy thinning where 70% of the pine basal area was removed (T70) (Table 1). Each treatment was applied in one 900 m² plot with a 10 m treated buffer around each plot. The experimental design was justified because a forest stand comprising three species was used. Thinning activities were carried out manually, and selected trees were small, dying or suppressed (Table 1).

No significant differences in tree density existed between treatments within each plot before the 1989 thinning (one-way ANOVA, $F = 25.34$, $P = 0.374$). Suppressed trees and trees with physical damage due to storms or windy conditions were excluded from the analyses.

Dendrochronological analysis and climate-growth relationships. In May 2011, the three thinning plots were inventoried. All trees located within each plot were measured (DBH, tree height) (Table 1). In each plot, at least 5 dominant trees for each species with a diameter larger than 15 cm at 1.3 m (DBH) were cored at 1.3 m with a Pressler increment borer and two cores were sampled per tree in the direction perpendicular to the maximum slope (FRITTS 2001). Cores were air-dried, sanded with papers of progressively finer grain until tree rings were clearly visible and visually cross-dated (YAMAGUCHI 1991). Individual tree-ring width series were measured to the nearest 0.01 mm with a LINTAB measuring device (Rinntech, Heidelberg, Germany). Cross-dating quality was done using COFECHA software (HOLMES 1983) by checking the consistency of the different ring-width series among trees coexisting within the same plots. To assess the quality of the tree-ring width series, several dendrochronological statistics (FRITTS 2001) were calculated considering the common interval after thinning 1990–2010 (Table 1): first-order autocorrelation of raw width data (AC); mean sensitivity (MS) of indexed growth values; mean correlation between trees (*r_{bt}*); age at breast height (Age); and the expressed population signal (EPS), which measures the statistical quality of the mean site chronology as compared with a perfect infinitely replicated chronology (WIGLEY et al. 1987). The segments of the site chronologies that reached EPS values equal to or greater than 0.85

Table 1. Measured parameters in treatments in years

	AP/PS/PP		
	control (C)	light thinning (T30)	heavy thinning (T70)
2011			
DBH (cm)	15.3/16.3/23.5	13.6/20.3/30.2	14.7/15.7/30.4
BAI (cm ² ·yr ⁻¹)	5.8/7.4/13.8	5.6/10.4/23.4	6.2/6.8/34.4
Age at 1.3 m (yr)	34/33/37	37/36/38	37/34/36
1980–2010			
<i>R_{bt}</i>	0.667/0.414/0.490	0.688/0.475/0.711	0.663/0.657/0.773
MS	0.209/0.222/0.213	0.217/0.216/0.215	0.225/0.212/0.217
AC	0.628/0.802/0.931	0.608/0.874/0.812	0.555/0.833/0.807
EPS	0.947/0.860/0.906	0.974/0.853/0.961	0.981/0.905/0.964

AP – *Abies pinsapo*, PS – *Pinus sylvestris*, PP – *Pinus pinaster*, DBH – diameter at breast height, BAI – basal area increment, *r_{bt}* – mean between-trees correlation of ring-width indices, MS – mean sensitivity, AC – first-order autocorrelation, EPS – expressed population signal

were regarded as reliable for their use in the subsequent climate-growth analyses.

The trend due to the geometrical constraint of adding a volume of wood to a stem of increasing radius was corrected by converting tree-ring widths into BAI using Eq. (1):

$$\text{BAI} = \pi (R_t^2 - R_{t-1}^2) \quad (1)$$

where:

R – radius of the tree,

t – year of tree-ring formation.

Individual species growth responses to thinning effects were evaluated with annual BAI series.

To evaluate the short-term responses of BAI to thinning, we performed repeated measures analysis of variance (one-way ANOVA). To do so, we selected periods of equal length, defined as pre-treatment (1974–1989) and post-treatment (1990–2005), to calculate mean BAIs, which were used as within-subject factors (Table 2).

Tree-ring isotope analysis and intrinsic water use efficiency. To assess the response of different species to thinning intensity and climate, an isotopic analysis was performed on each cored tree to study the long-term effects of thinning on iWUE (MARTÍN-BENITO et al. 2010; MORENO-GUTIÉRREZ et al. 2012). For that purpose, we analysed $\delta^{13}\text{C}$ in individual rings 3 years before thinning (1987–1989) and 9 years after thinning (1990, 1995, 1996, 1999–2001, 2003, 2005, 2007) to explore the effects of management on iWUE. To evaluate climate effects in this period, we selected three climatically contrasting years following GRANDA et al. (2014) (using mean temperature and accumulated precipitation during the spring and summer growing period; [see supplementary material – Fig. S1](#)): Unfavourable years (i.e. years with a combination of low precipitation and high temperatures: 1987, 1995, 1999, 2005); Average years (i.e.

years with average precipitation and temperature: 1988, 1990, 2003, 2007) and Favourable years (i.e. years with a combination of high precipitation and low temperatures: 1989, 1996, 2000, 2001). Complete dated rings (including both early and late wood) were cut and detached with a sharp blade from cores under a stereomicroscope to ensure no contamination from adjacent rings.

Samples were ground to a fine powder using a ball mill (Spex 5300, Metuchen, USA) and 400–600 μg of cellulose were weighed in tin cups for carbon isotopes ($\delta^{13}\text{C}$) (LEAVITT, DANCER 1993). Isotope subsamples were combusted in an elemental analyser (Thermo Fisher, Basingstoke, UK) for $\delta^{13}\text{C}$ and the resulting gases were analysed on an isotope ratio mass spectrometer (IRMS; ThermoFisher Delta V Advanced, Basingstoke, UK) located at the LI-SEEM laboratory (IFAPA, Cordoba, Spain).

The results of the carbon (^{13}C) isotope ratio analyses were reported as per thousand (‰) enrichment relative to the Vienna Pee Dee Belemnite (V-PDB) international standard for carbon isotope ratio according to the following Eq. (2):

$$\delta^{13}\text{C}_{\text{sample}} = \left[\left(\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{V-PDB}}} - 1 \right) \times 1,000 \right] (\text{‰}) \quad (2)$$

where:

$(^{13}\text{C}/^{12}\text{C})_{\text{sample}}$ – measured in comparison with the standard $(^{13}\text{C}/^{12}\text{C})$.

The samples of tree rings were analysed in triplicate with a SD of less than 0.20% for ^{13}C measurements. Δ was then calculated as Eq. (3):

$$\Delta = (\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{plant}}) / 1 + \delta^{13}\text{C}_{\text{plant}} / 1,000 \quad (3)$$

where:

$\delta^{13}\text{C}_{\text{atm}}$ – relative concentration of ^{13}C in the atmosphere (in this case, La Jolla-California) (FARQUHAR, RICHARDS 1984).

Table 2. Mean values (\pm SE) of basal area increment (BAI) and intrinsic water-use efficiency (iWUE) for each species and thinning treatment

Treatment	BAI ($\text{cm}^2\cdot\text{yr}^{-1}$)			iWUE ($\mu\text{mol}\cdot\text{mol}^{-1}$)		
	AP	PS	PP	AP	PS	PP
Pre-thinning (period 1974–1989)						
Control (C)	2.85 ± 0.32^a	5.24 ± 0.78^b	15.45 ± 3.55^b	69.08 ± 2.06^b	87.16 ± 1.41^b	86.08 ± 0.98^a
Light thinning (T30)	2.24 ± 0.27^a	9.24 ± 0.50^a	17.70 ± 1.55^b	67.66 ± 4.34^b	82.51 ± 1.30^c	85.98 ± 2.01^a
Heavy thinning (T70)	2.44 ± 0.48^a	5.49 ± 1.04^b	22.92 ± 1.46^a	76.32 ± 3.59^a	93.40 ± 2.45^a	88.45 ± 2.53^a
Post-thinning (period 1990–2005)						
Control (C)	7.06 ± 0.32^a	9.33 ± 2.07^b	12.52 ± 2.36^c	74.99 ± 1.14^c	95.69 ± 3.34^a	91.96 ± 1.70^a
Light thinning (T30)	5.64 ± 0.84^b	10.82 ± 1.69^a	24.40 ± 3.04^b	77.47 ± 1.63^b	83.15 ± 2.73^b	91.73 ± 1.94^a
Heavy thinning (T70)	5.83 ± 0.97^b	7.63 ± 1.91^c	32.20 ± 2.20^a	80.40 ± 1.99^a	98.02 ± 2.75^a	93.31 ± 1.70^a

AP – *Abies pinsapo*, PS – *Pinus sylvestris*, PP – *Pinus pinaster*, different letters indicate significant differences ($P < 0.05$) between treatments in repeated measures ANOVA Tukey's test for the same period

Finally, intrinsic water use efficiency ($\mu\text{mol}\cdot\text{mol}^{-1}$) was calculated following FARQUHAR and RICHARDS (1984) as Eq. (4):

$$i\text{WUE} = C_a - (b - \Delta)/1.6 (b - a) \quad (4)$$

where:

- C_a – internal and atmospheric concentrations of CO_2 ,
- b – fractionation associated with reactions by Rubisco and phosphoenolpyruvate carboxylase (27%),
- a – fractionation during CO_2 diffusion through the stomata (4.4‰).

Statistical analysis. To understand how co-occurring species respond to thinning and climate, all the analyses were performed for each separate species. For the statistical analysis, the BAI and iWUE measurements were normalized. Pearson correlation between $\delta^{13}\text{C}$ and seasonal climatic variables (precipitation and mean temperature) was calculated to estimate which differences in specific environmental responses were statistically significant after thinning. Moreover, Pearson correlation coefficients were calculated for the period 1980–2010 to quantify thinning effects in climate-growth relationships between the BAI series and monthly climate data (mean temperature and precipitation). ANOVAs and *post hoc* comparisons (Tukey-Kramer HSD test) by STATISTICA (SPSS, Tulsa, USA) were used to evaluate if there were differences between species by contrasting climate conditions and thinning treatments ($P < 0.05$). The trends of iWUE were explored by linear regressions. For BAI trends, the analyses were performed on log-transformed values to normalize the variable.

Generalized linear mixed models (GLMMs) for BAI and iWUE (period 1990–2010) were calculated to assess the thinning treatments and climate effects, as well as their significant interactions for each species. The factors treatment, year and tree were introduced as random effects to account for the spatial and temporal autocorrelation in the model. The best fitted models were considered those showing the lowest Akaike Information Criterion (AIC) (BURNHAM, ANDERSON 2002). GLMMs were fitted using the lme4 package (BATES et al. 2013) for R platform v. 3.1 (R Core Development Team 2014).

RESULTS

Radial growth and iWUE responses to thinning

The quality of the BAI series was better in *A. pinsapo* than in *P. pinaster* and *P. sylvestris*, respectively, and showed a higher inter-tree synchrony of growth (*r_{bt}*, EPS), which increased with intensity of thinning for

the three species (Table 1). *P. pinaster* trees were larger than those of the other species, which may explain why they formed wider tree rings (BAI) (Table 1). A year-to-year persistence (AC) of growth was higher in *P. pinaster* and *P. sylvestris* than in *A. pinsapo* trees. Similar inter-annual variability (MS) of growth was also observed for the three species, thus suggesting the increasing importance of climatic constraints on growth in the study site (Table 1). The remaining dendrochronological statistics confirm a common and coherent growth response of trees to climatic variability along the thinning gradient.

Throughout the study period, *P. pinaster* showed the highest average BAI before ($18.69 \text{ cm}^2\cdot\text{yr}^{-1}$) and after thinning ($23.04 \text{ cm}^2\cdot\text{yr}^{-1}$), with significantly lower values in the control plot ($15.45 \text{ cm}^2\cdot\text{yr}^{-1}$) and increasing BAI with intensity of thinning (Table 2, Fig. 1). In contrast, *A. pinsapo* presented the lowest BAI values before ($2.51 \text{ cm}^2\cdot\text{yr}^{-1}$) and after thinning ($6.18 \text{ cm}^2\cdot\text{yr}^{-1}$) in the three plots. *P. sylvestris* showed an intermediate response to thinning, with the highest BAI value in plot T30 with light treatment ($10.82 \text{ cm}^2\cdot\text{yr}^{-1}$) (Table 2, Fig. 1). These increases were statically significant in comparison with the pre-thinning BAI for all species: *A. pinsapo* ($F = 3.14$; $P < 0.01$), *P. pinaster* ($F = 2.19$; $P < 0.01$), and *P. sylvestris* ($F = 1.54$; $P < 0.01$).

As regards iWUE, *P. sylvestris* showed the highest values before ($93.40 \mu\text{mol}\cdot\text{mol}^{-1}$) and after thinning ($98.02 \mu\text{mol}\cdot\text{mol}^{-1}$), with significantly lower values in the lightly thinned T30 plot (Table 2; Fig. 1). In contrast, *A. pinsapo* showed the lowest iWUE values before ($67.66 \mu\text{mol}\cdot\text{mol}^{-1}$) and after thinning ($74.99 \mu\text{mol}\cdot\text{mol}^{-1}$), which increased with intensity of thinning (Table 2, Fig. 1). *P. pinaster* presented similar values in all the plots before thinning, but significantly higher values in plot T70 after thinning ($93.31 \mu\text{mol}\cdot\text{mol}^{-1}$).

Effects of climate on BAI and iWUE

Significant ($P < 0.05$) and opposite trends in temperature (increasing) and precipitation (decreasing) were observed in the study area (see supplementary material – Fig. S1). The drying trend was mainly due to the long-term decrease in spring rainfall over the second half of the 20th century. The mean temperature increased at average rates of $+0.06^\circ\text{C}\cdot\text{yr}^{-1}$.

Correlations between BAI chronologies and climate data showed that growth strongly depended on winter and spring precipitation and more generally on precipitation from August to September of the

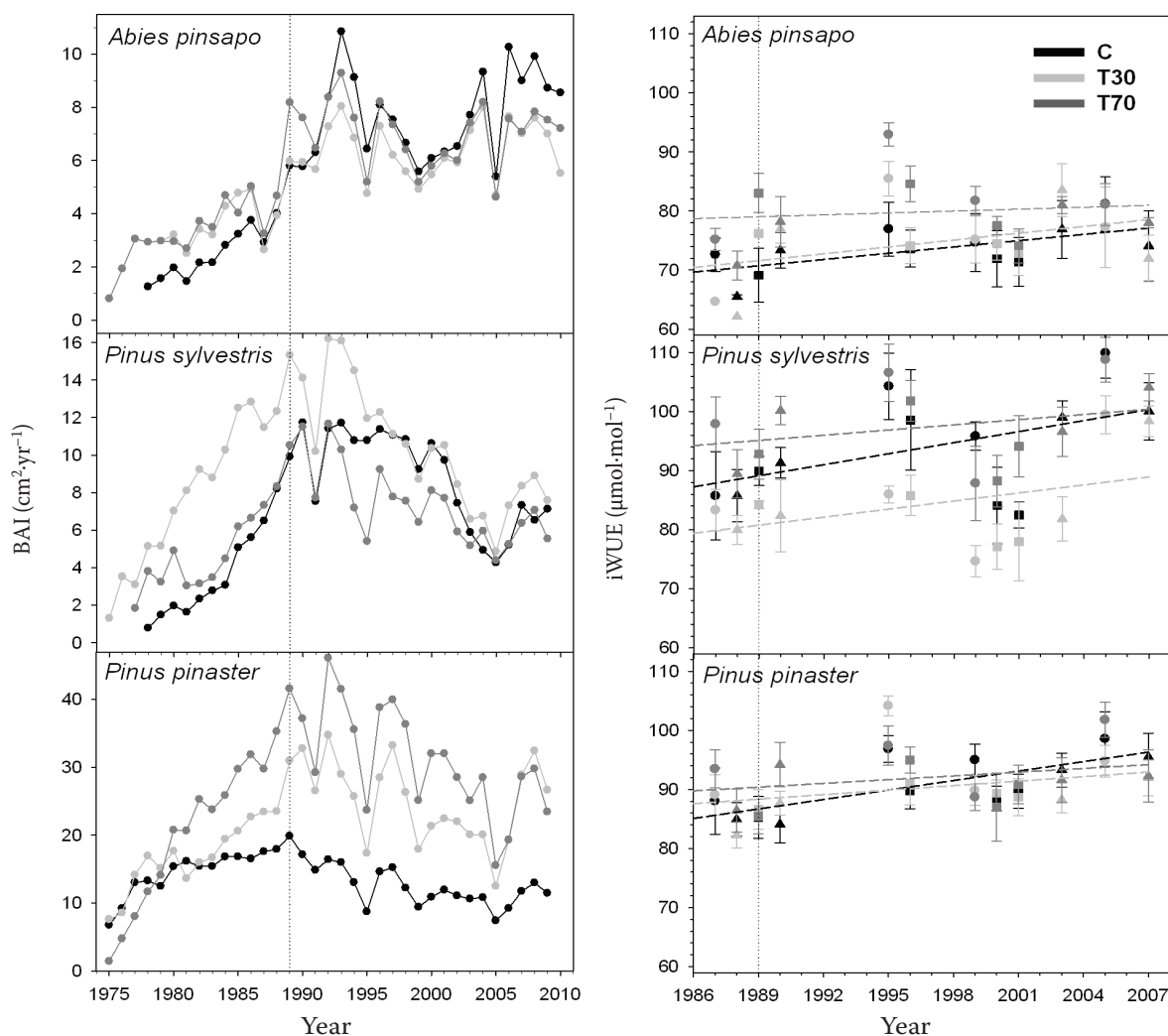


Fig. 1. Mean curves of basal area increment (BAI) chronologies for each species and changes in intrinsic water use efficiency (iWUE) over time for each treatment and random years with favourable (squares), average (triangles) and unfavourable (dots) year climate, vertical dotted lines correspond to the 1989 thinning treatment
C – control, T30 – light thinning, T70 – heavy thinning (left figure)

previous year and May of the current year for *A. pinsapo* and June for *P. sylvestris* and *P. pinaster* (Fig. 2). Temperature was observed to have a negative effect on growth, particularly during winter and summer (Fig. 2). The influence of winter temperature was notably different between species, with positive signifi-

cant correlations being higher in *A. pinsapo* than in *P. sylvestris* and *P. pinaster* (Fig. 2).

After the thinning treatment in 1989, the remaining *A. pinsapo* trees in the thinned plots showed a stronger dependence on precipitation and winter temperature than in the control plot, whereas the opposite

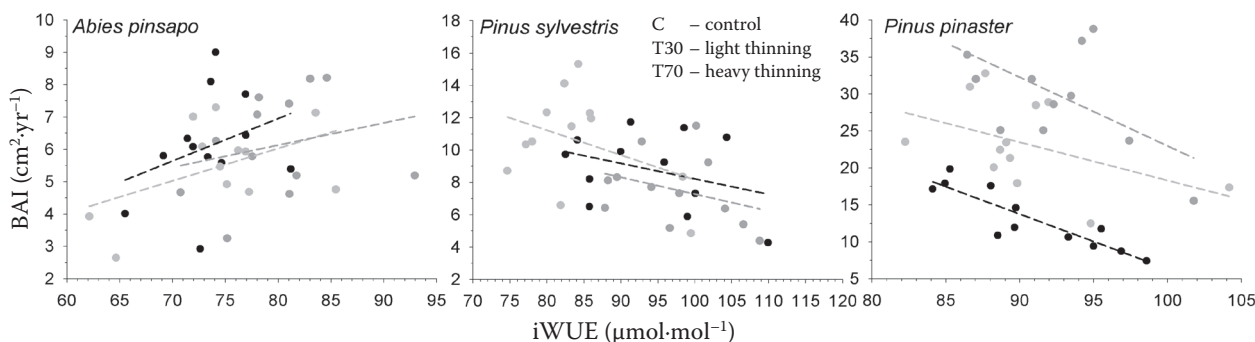


Fig. 2. Relationship between intrinsic water use efficiency (iWUE) and basal area increment (BAI) in response to C, T30 and T70

Table 3. Pearson's correlation (R^2) obtained by relating $\delta^{13}\text{C}$ isotope series and seasonal climatic data (precipitation and mean temperature) for the post-thinning period (1990–2010) (in AP/PS/PP)

Period	Precipitation			Temperature		
	C	T30	T70	C	T30	T70
Winter	-0.06/-0.09/- 0.35	-0.17/0.13/-0.15	0.00/0.21/0.24	-0.23/- 0.57 /- 0.39	0.04/- 0.25 /-0.03	-0.19/-0.05/-0.06
Spring	-0.26 /- 0.41 /- 0.80	0.08/- 0.32 /- 0.40	-0.21/- 0.29 /- 0.29	0.15/-0.20/ 0.41	-0.26 /-0.22/0.02	-0.27 /-0.08/0.13
Summer	0.50 /0.17/ 0.71	0.41 / 0.26 / 0.67	0.38 / 0.46 / 0.56	0.25 /0.03/-0.06	0.50 /-0.35/-0.14	0.56 /- 0.27 /-0.17
Autumn	-0.09/-0.11/-0.24	0.22/-0.01/-0.17	0.18/-0.15/-0.21	-0.16/-0.16/-0.13	-0.06/ 0.31 /0.04	-0.13/0.18/0.04
Annual	0.31 /-0.10/- 0.32	0.23/0.13/0.03	0.35 /0.10/0.00	-0.04/- 0.32 /-0.05	0.06/0.08/0.07	-0.25 /0.02/-0.02

AP – *Abies pinsapo*, PS – *Pinus sylvestris*, PP – *Pinus pinaster*, C – control, T30 – light thinning, T70 – heavy thinning, in bold – significant at $P < 0.05$

trend was observed for the relationships between growth and June temperature (Fig. 2).

The effects of precipitation and temperature on *P. sylvestris* growth were lower in the thinned plots (Fig. 2), with the exception of June precipitation. As regards the growth of *P. pinaster*, precipitation and temperature were found to have higher, positive effects in the thinned plots than in the control plot. Furthermore, we found an overall significant

increment in the correlations with intensity of thinning.

The $\delta^{13}\text{C}$ isotopic ratio showed stronger relationships with spring and summer climate (Table 3) for the three species. The strongest significant correlations were found between summer precipitation and $\delta^{13}\text{C}$ in *A. pinsapo*, *P. pinaster* and *P. sylvestris*, respectively, which decreased with thinning intensity. However, spring and summer

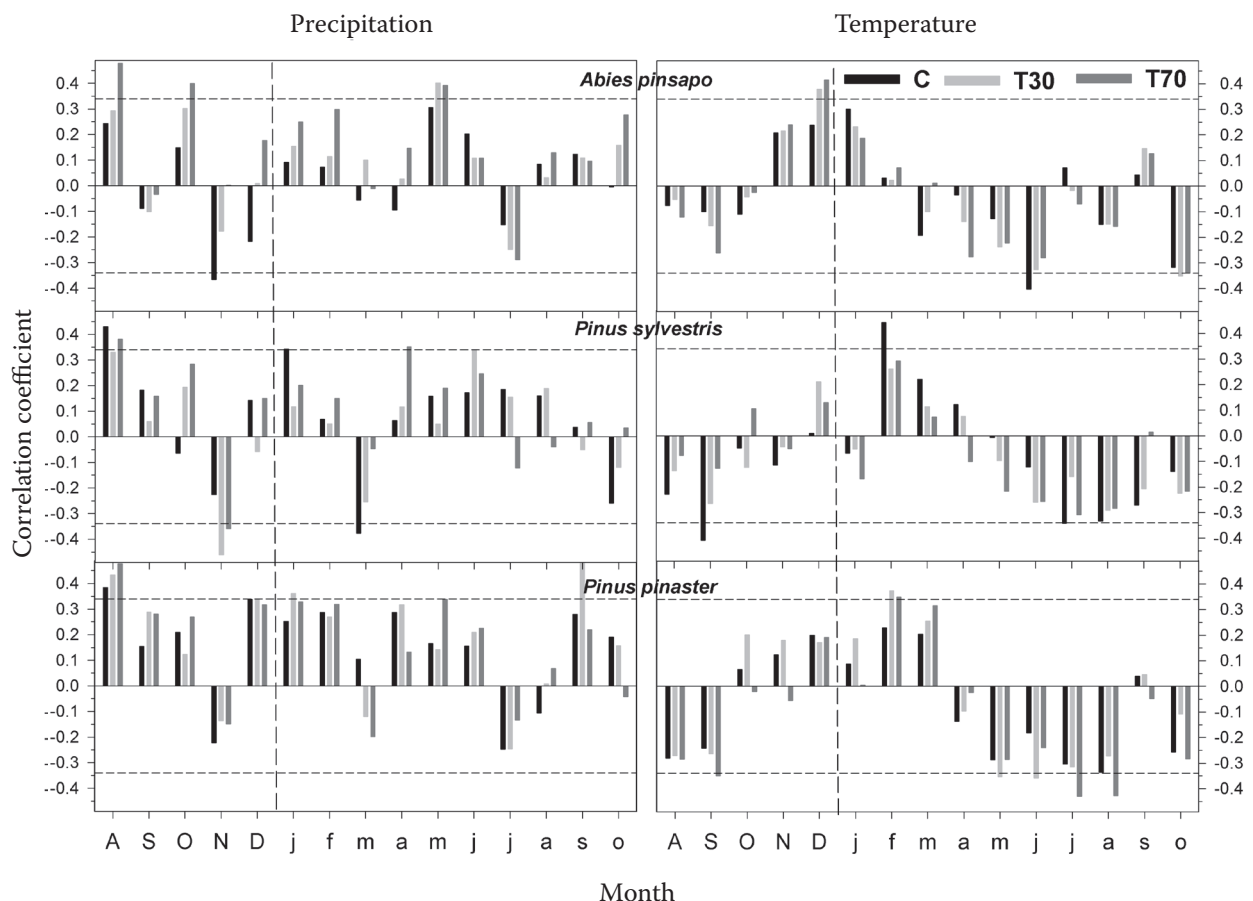


Fig. 3. Correlation coefficients obtained by relating basal area increment (BAI) series and monthly climatic data (precipitation and mean temperature) for each species, BAI is related with monthly climatic data from the previous year (months abbreviated by uppercase letters) up to the year of tree-ring formation (months abbreviated by lowercase letters), bars surpassing dashed lines indicate significant ($P < 0.05$) correlation coefficients

C – control, T30 – light thinning and T70 – heavy thinning for

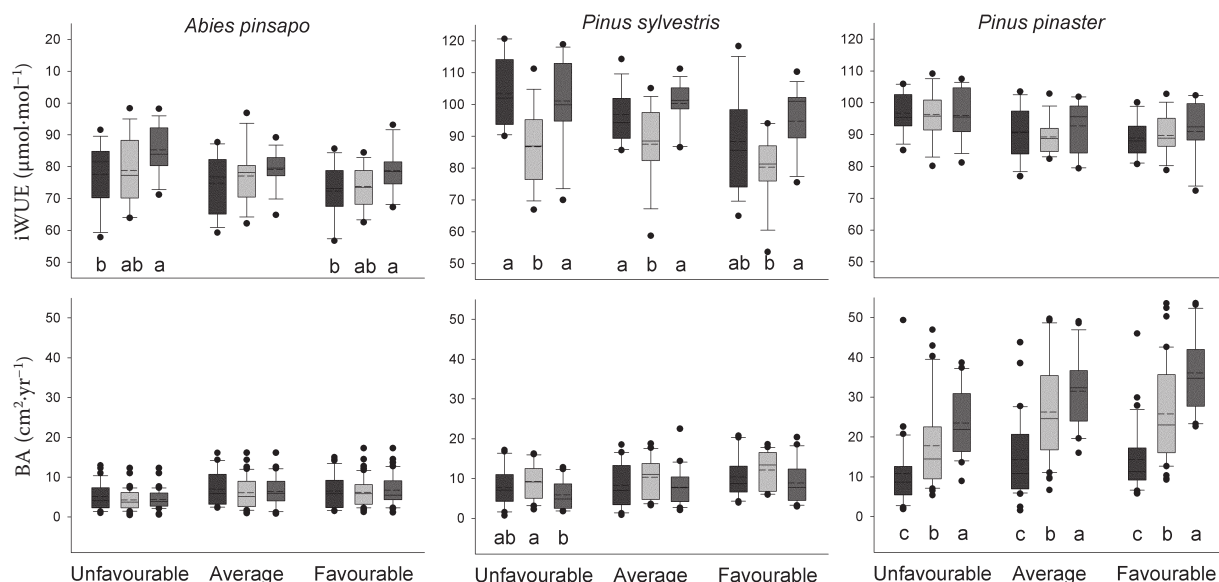


Fig. 4. Responses of intrinsic water use efficiency (iWUE) and basal area increment (BAI) for the studied species after thinning treatment for C – control (black), T30 – light thinning (grey) and T70 – heavy thinning (dark-grey) according to climatically unfavourable, average and favourable years during the post-thinning period (1990–2010). Different letters indicate significant differences ($P < 0.05$) between treatments. Box plots show: the median (horizontal line within the box), mean (dashed horizontal line), the 25th and 75th percentiles (lower and upper box boundaries), the 10th and 90th percentiles (error bars), and the 5th and 95th percentiles (outliers)

temperature showed significant positive correlations with $\delta^{13}\text{C}$ only in *A. pinsapo*, which increased with intensity of thinning (Table 3). $\delta^{13}\text{C}$ was only correlated with winter temperature in *P. sylvestris* and *P. pinaster*.

Relationships between iWUE and BAI during climatically unfavourable, average and favourable years highlighted a consistent response between species and thinning treatments (Figs 3 and 4). The three species reached the maximum growth rate in favourable years, although it was related to the intensity of thinning treatments. *A. pinsapo* showed a reduction (increase) in BAI (iWUE) with thinning intensity (Fig. 4). *P. sylvestris* showed higher growth and higher iWUE in the light thinning treatment (T30), while *P. pinaster* growth presented the highest response to the heavy thinning treatment (T70), although very similar responses were found between climatologically contrasting years for iWUE (Fig. 4).

Linear mixed models for BAI and iWUE

The selected linear mixed models (LMMs) for BAI showed negative responses to competition for *A. pinsapo* (AIC = 70.3) (Table 4). However, the divergence in BAI between treatments was evident for *P. pinaster* (AIC = –30.0) and for *P. sylvestris* in T30 (AIC = 149.2). According to these models, BAI

(iWUE) increased (decreased) with spring and summer precipitation in the three species, with these effects being higher with intensity of thinning (Table 4). The interactions included in the models suggest that BAI and iWUE are particularly sensitive to the positive effects of thinning treatments and climatic variability, with *P. pinaster* (AIC = 145.3) being the most sensitive to thinning and *A. pinsapo* (AIC = 141.2) the most sensitive to climate (Table 4).

DISCUSSION

Radial growth and iWUE responses to thinning

The stable isotope analysis was successfully used to clarify the response of *A. pinsapo*–*P. pinaster*–*P. sylvestris* forests to thinning mechanisms related to species-specific responses in terms of tree growth and water use efficiency. The influence of thinning intensity on growth is well understood, but the differential response of mixed forests to thinning has been given less attention (LARSON et al. 2012; PRIMICIA et al. 2013). According to our hypothesis, increasing thinning intensity results in a differential growth rate expressed as BAI related to light species tolerance (*A. pinsapo* < *P. sylvestris* < *P. pinaster*, respectively).

The effect of thinning on basal area increment was highest in *P. pinaster* (i.e. the less tolerant spe-

Table 4. Summary of the general linear mixed-effects models fitted to explain changes in basal area increment – log-transformed values (BAI) and intrinsic water use efficiency (iWUE) of the studied species (*A. pinsapo*, *P. sylvestris*, *P. pinaster*)

<i>Abies pinsapo</i>			<i>Pinus sylvestris</i>			<i>Pinus pinaster</i>		
treatment	estimate	P	treatment	estimate	P	treatment	estimate	P
BAI (cm²·yr⁻¹)								
Intercept	0.513	< 0.001	intercept	1.926	0.045	intercept	2.620	0.004
C	-0.206	< 0.001	C	-0.104	0.058	C	-0.023	0.006
T30	0.107	0.046	T30	0.145	0.005	T30	0.151	< 0.001
T70	-0.086	0.380	T70	0.026	0.437	T70	0.467	< 0.001
P6	0.014	0.011	–	–	–	–	–	–
T7	-0.039	0.005	T7	-0.013	0.057	T7	-0.022	0.048
C × P5	0.002	0.041	T8	-0.037	0.046	T8	-0.031	0.039
T30 × P5	0.012	0.017	C × P6	0.001	0.307	P5	0.002	0.038
T70 × P5	0.014	0.107	T30 × P6	0.002	0.030	C × P4	0.005	0.257
C × T6	-0.037	0.053	T70 × P6	0.001	0.067	T30 × P4	0.070	0.007
T30 × T6	-0.035	0.022	–	–	–	T70 × P4	0.098	0.028
T70 × T6	-0.045	0.035	–	–	–	–	–	–
iWUE (μmol·mol⁻¹)								
Intercept	11.609	< 0.001	intercept	6.551	0.034	intercept	12.970	0.017
C	-10.206	< 0.001	C	-2.104	0.047	C	-0.123	0.006
T30	3.025	0.022	T30	12.034	< 0.001	T30	1.265	< 0.001
T70	-4.755	0.780	T70	3.187	0.239	T70	3.210	0.046
P6	0.250	< 0.001	–	–	–	–	–	–
T7	-2.715	0.008	T7	-7.323	0.049	T7	-1.977	0.048
C × P5	0.031	0.058	T8	-5.065	0.048	T8	-0.341	0.030
T30 × P5	0.054	0.012	C × P6	-0.018	0.397	P5	0.052	0.035
T70 × P5	0.045	0.257	T30 × P6	0.106	0.037	C × P4	-0.016	0.025
C × T6	-1.156	0.043	T70 × P6	0.091	0.045	T30 × P4	0.021	0.024
T30 × T6	-0.405	0.022	–	–	–	T70 × P4	0.031	0.007
T70 × T6	-0.086	0.015	–	–	–	–	–	–

fixed effects (treatments): C – control, T30 – light thinning, T70 – heavy thinning; precipitation: P4 – April, P5 – May, P6 – June; temperature: T6 – June, T7 – July, T8 – August, and interactions; only those factors of the best model obtained by minimizing the Akaike information criterion

cies), which reacted to the increasing availability of resources with a strong growth rate. The BAI obtained in year 9 after thinning was highest in the heavily thinned plots, where many dominated and small-sized trees were removed. This is in line with other results obtained for Mediterranean pine stands, which have shown the positive effect of thinning on growth (MONTERO et al. 2008; DEL CAMPO et al. 2014). In contrast, Scots pine showed that light thinning intensity resulted in a statistically significant higher BAI. This result may contradict those obtained in many studies carried out in central and northern Europe which establish that thinning intensity must be high (e.g. MÄKINEN, ISOMÄKI 2004). However, due to the Mediterranean climatic conditions of the study area, the growth response of Scots pine was more modulated by intermediate light availability as a result of light

thinning (DEL RIO et al. 2008). The BAI in *Abies pinsapo* was also clearly related to thinning intensity and reached the highest value on the unthinned control plots. This is in accordance with the results of LINARES et al. (2011), who reported that plots with no thinning had the highest BAI value.

Thinning studies have shown that the availability of resources is relatively higher in thinned stands than in unthinned plots (DEL CAMPO et al. 2014; GEBHARDT et al. 2014). In addition to decreased competition for light, water and nutrients, the presence of logging residues may increase the amount of nutrients available for strong growth increase and change the leaf area index (LAI) as a result of silvicultural treatments (DE LAS HERAS et al. 2012). In the heavy thinning of *P. pinaster*, the total yield increased with increasing thinning intensity as a result of stand opening, and growth resources

were released by the removed trees. In addition, water availability explains, at least partly, the differences as *P. pinaster* is the most drought tolerant species of those studied. On the other hand, the light thinning of *P. sylvestris* resulted in the highest BAI value, showing an intermediate light tolerance response. PRIMICIA et al. (2013) observed a reduction in Scots pine growth in mixed patches as compared with pure ones resulting from competition interactions. *A. pinsapo* is the most shade-tolerant species and usually lives in dense and multistrata forests. This highly shade-tolerant species could not utilize the free growing space as efficiently as *P. pinaster*–*P. sylvestris*.

Thinning and iWUE

The changes in BAI led to different response in iWUE among the species. Stable C isotopes indicated that the iWUE of the Pinus species decreased when BAI increased but the opposite response was observed in *A. pinsapo*. Previous studies have shown a contradictory response of iWUE to thinning treatments in the Pinus species (PEREZ DE LIS et al. 2011; MORENO-GUTIÉRREZ et al. 2012; FERNANDES et al. 2016). These changes could be related to a decrease in stomatal conductance (which more directly influences the hydrological cycle and climate system), as the dominant driver of changing iWUE in Mediterranean forests. Through its effect on stomatal conductance, an increase in thinning intensity may have the effect of reducing iWUE (assuming the assimilation rate remains constant). The higher iWUE values for *P. pinaster* could therefore be attributed to a higher overall assimilation capacity of this species, to a better stomatal control of water losses than in the *P. sylvestris*, or to a combination of both factors (SEIBT et al. 2008; MORENO-GUTIÉRREZ et al. 2012). On the other hand, shade-tolerant species such as *A. pinsapo* generally have stomata that are more responsive to elevated CO₂ than intolerant coniferous species (MAIER et al. 2004). It is this ‘active response’ to thinning that produces the ‘physiological response’ via a reduction in stomatal conductance, and an increase in the carbon assimilation rate (MORENO-GUTIÉRREZ et al. 2012). This physiological response has been found in other Mediterranean pine species exhibiting a more ‘conservative’ water use strategy (low gs?, high iWUE during the peak growing season), which allows them to extract deeper soil water sources (MORENO-GUTIÉRREZ et al. 2012). How-

ever, other factors may be related to physiological response to thinning, such as nutrient limitation, microclimate acclimation, and reallocation of carbohydrates to other tissues with higher priority as C sinks in the xylem (PEÑUELAS et al. 2011). Additionally, LAI directly controls the photosynthesis rate and the amount of carbon sequestered. Several studies have reported an increase in LAI in temperate forests as a result of silvicultural treatments and have related this to increased rates of growth (DE LAS HERAS et al. 2012).

Effect of climate in radial growth and iWUE

δ¹³C were negatively correlated with spring precipitation and temperature in *A. pinsapo* and *P. pinaster*. In particular, *P. pinaster* showed a great sensitivity to spring precipitation in all the thinning treatments. In contrast, summer precipitation was positively correlated with δ¹³C for the three species, except *P. sylvestris* in the control, while *A. pinsapo* was the only species sensitive to summer temperature in the three thinning treatments. These water use responses suggest that the physiological mechanisms involved in the thinning response in relation to climatic conditions are indeed different between species.

These results were corroborated by the LMM analysis. The divergence in BAI between species and treatments was evident for *P. pinaster* and *P. sylvestris*. According to these models, treatment and summer climatic conditions had significant effects on BAI for *P. pinaster*. However, *P. sylvestris* growth was less sensitive to the thinning treatments and climatic conditions. *A. pinsapo* growth was significantly driven by the thinning treatments and June precipitation, with their effects being positive in both cases. These findings suggest that tree growth and the iWUE of *P. pinaster* and *A. pinsapo* were highly sensitive to climatic variables although with a contrary effect. These species showed the highest growth changes at higher (*P. pinaster*) and lower (*A. pinsapo*) thinning intensity; a response which seems to be dependent on the climatic variables related with the specific physiological response (e.g. stomatal closure). The high correlations between the variation in growth and iWUE for these species (negative for *P. pinaster* and positive for *A. pinsapo*) indicate the influence of climatic constraints on inter-annual variation in iWUE under silvicultural practices. In addition to the potential effects of thinning intensity on tree growth and physiological performance, Mediterranean species showed enhanced BAI during unfavourable years as iWUE increased (ANDREU-HAYLES et al. 2011).

The negative relationship between iWUE and BAI for *Pinus* species and thinning intensities and the opposite response in *A. pinsapo* suggest a different specific response to thinning in terms of tree water losses as well as increasing assimilation and productivity (HERRICK et al. 2004). Shade-tolerant species such as *A. pinsapo* generally have stomata that are more responsive to elevated CO₂ than intolerant coniferous species (MAIER et al. 2004). Therefore, the silviculture of a mixed forest must put under consideration different active response to thinning in the context of global change (D'AMATO et al. 2011).

CONCLUSIONS

Thinning intensity provides an opportunity to study iWUE and growth response to the climate of the *A. pinsapo*–*P. pinaster*–*P. sylvestris* forest in Mediterranean mountain ecosystems. The long duration of the experiments and the absence of plot replicas may diminish potential differences between the thinning treatments. It can be concluded that the prevailing thinning methods in *Abies*–*Pine* forest (light to heavy intensities) most probably result in differential growth and physiological response among species. Furthermore, the recommendations regarding thinning treatments require ecological and species-oriented silviculture. The active response to increasing growth has important implications for silviculture in the context of global change (D'AMATO et al. 2011). As described in our work, iWUE obtained from tree-ring data confirms that there is a positive response to thinning intensity, but that the limits to plasticity are likely to be species- and site-specific. This study therefore provides a set of preliminary results for developing silvicultural frameworks adapted to Mediterranean mountain forests in order to ensure more accurate prediction, detection and attribution of future changes in water-plant-carbon interactions.

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