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# Long-term thinning effects on tree growth, drought response and water use efficiency at two Aleppo pine plantations in Spain



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# HIGHLIGHTS

# GRAPHICAL ABSTRACT

- Thinning enhanced tree growth on Mediterranean Aleppo pine forests.
- Thinning reduced growth dependence on water stress (SPEI).
- Thinning improved drought resilience, making pines better adapted to unfavourable conditions.
- Effects of thinning remained at least for 20 years, especially in the heavy thinning treatment.



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# ABSTRACT

In Mediterranean areas where drought-induced forest dieback and tree mortality have been widely reported, it is still under debate how the likely risks of climate change will affect tree growth and consequently forest productivity. Increasing tree mortality has been associated not only to increased drought, but also to a lack of management in many dense pine forests and plantations, where warming may intensify tree-to-tree competition for soil water. This emphasizes the need of using silviculture to adapt dense stands of Mediterranean pine reforestations to warmer and drier conditions. Here we combined dendrochronology and C and O isotope analyses of wood in two Aleppo pine (*Pinus halepensis*) plantations, growing under semiarid conditions and experimentally thinned at high and moderate intensities along with control. The main aim was to understand the responses of radial growth and water use efficiency (WUEi) to different thinning intensities, and to analyze the effectiveness of thin ning to enhance post-drought growth resilience. Thining had a positive effect on growth, produced an increase of  $\delta^{18}$ O, reduced growth sensitivity to drought and decreased WUEi, suggesting a reduction of drought stress. These results were consistent across sites, and were significant even 20 years after the intervention took place. Considering the climate effects on growth through the SPEI drought index to calculate resistance and recovery indices, an increase of resistance after thinning was observed. We conclude that high thinning intensity (50%

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of basal area removed) is a useful silviculture intervention on Mediterranean Aleppo pine plantations that enhances their growth, and makes them less dependent on harsh climatic conditions, improving their resilience against drought and consequently making them better adapted to more unfavourable conditions.

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# 1. Introduction

Aleppo pine (Pinus halepensis Mill.) has been used extensively in afforestation programs across the Mediterranean Basin during the last 100 years (Maestre and Cortina, 2004). Covering over 25,000 km<sup>2</sup> in the Mediterranean Basin (Quézel, 2000). It is one of the most used tree species for reforestation under semiarid conditions (Pemán et al., 2009), being for instance representative in a 70% of the reforested area in eastern Spain (Vallejo and Alloza, 1998). Aleppo pine forests provide hydrological cycle stabilization, soil protection (Fady et al., 2003) and wood products. In some cases, afforestation with P. halepensis has promoted the establishment of high-density plantations characterized by slow-growing rates which are prone to fire and pest attacks (Maestre and Cortina, 2004), and more predisposed to forest dieback following extreme drought events (Carnicer et al., 2011; García de la Serrana et al., 2015). Severe canopy dieback and increasing tree mortality in pine plantations has been associated not only to increased drought but also to a lack of forest management, which may intensify intertree competition (Sánchez-Salguero et al., 2012, 2015).

Understanding how forest structure modulates the physiological response of trees to drought could help managers to adopt strategies for improving the resistance and resilience against drought (Moreno-Gutiérrez et al., 2011). This is especially critical in Mediterranean areas within the context of climate change, where is still debated how climate change impacts will affect tree growth and forest productivity in addition to more recurrent events of tree mortality and forest dieback (Martinez-Vilalta and Pinol, 2002; Allen et al., 2010; McDowell and Allen, 2015).

Forest density reduction treatments (i.e., thinning) offer a strategy for potentially mitigating these effects not only by improving forest health but also the hydrological balance through increased soil infiltration and streamflow (del Campo et al., 2019; Tague et al., 2019). By means of reducing the competition for water, light and nutrients, thinning has been observed to increase both the resistance (i.e., the capacity to maintain growth levels during drought) and the resilience (i.e., the ability to recover to the pre-drought growth levels) of trees in case of drought (Aussenac, 2000; Giuggiola et al., 2013; Sohn et al., 2013; Olivar et al., 2014). However, the response of trees to drought varied with thinning intensity (Molina and del Campo, 2012), stand density, age, the site climate conditions (Sohn et al., 2016), and the time since thinning intervention (del Campo et al., 2014). Other policy-relevant questions remain open, such as what the optimum thinning intensity is and how long the effects last. In order to answer these questions, long-term thinning experiments with different intervention intensities are required. For example, Moreno-Gutiérrez et al. (2011) assessed the impacts of thinning on growth in *P. halepensis* in a very short-term scale (12 months after thinning), and del Campo et al. (2018, 2019) for a period of 3 years after thinning. Long-term thinning studies are scarce and limited to other pine species, generally growing in highproductivity regions (Mäkinen and Isomäki, 2004; Prescott et al., 2019). Also, these studies focused on stem production instead of on understanding physiological mechanisms related to growth. To the authors' knowledge, no long-term results have been reported specifically for Aleppo pine.

Analysis of radial-growth rates using tree rings offers a convenient way to characterize tree responses to environmental stressors and it can be used as a vitality indicator, as growth decline can be considered an early-warning signal of forest dieback (Dobbertin, 2005; Camarero et al., 2015). In addition, the analysis of stable isotopes (C, O) in treering wood may allow to obtain complementary information about the water and carbon economy of trees (Scheidegger et al., 2000; McCarroll and Loader, 2004; Gessler et al., 2018). The composition of carbon isotopes ( $\delta^{13}$ C) is a proxy of intrinsic water-use efficiency (WUEi) in C3 plants (Farquhar et al., 1989; Klein et al., 2005). The WUEi is the ratio between the net photosynthetic rate (*A*) and the stomatal conductance ( $g_s$ ) (Farquhar et al., 1989). Reduction of WUEi after a thinning treatment can be expected as a result of decreasing competition for soil water, and such an effect has been observed in several empirical studies regarding pine species (McDowell et al., 2006; Giuggiola et al., 2016; Navarro-Cerrillo et al., 2019). However, other authors reported no variation in WUEi after thinning (Martin-Benito et al., 2010; Moreno-Gutiérrez et al., 2011), so the question remains open.

Several physiological adaptations determine WUEi adjustments after thinning: (i) variations in  $g_s$  at constant A; (ii) variations in A at constant  $g_s$ ; and (iii) variations of both, with that of  $g_s$  or A being stronger. The dual-isotope approach based on the simultaneous analysis of  $\delta^{13}$ C and oxygen stable isotopes ( $\delta^{18}$ O) has been proposed as a means to disentangle the influences of A and  $g_s$  on WUEi, since  $\delta^{18}$ O is related to  $g_s$  (Scheidegger et al., 2000; Barbour, 2007), especially in droughtsensitive tree species (Battipaglia et al., 2014). However, while  $\delta^{13}$ C responses to thinning have been described in previous studies considering different pine species and a variety of precipitation regimes, only a few of them combined  $\delta^{13}$ C and  $\delta^{18}$ O (McDowell et al., 2006; Martin-Benito et al., 2010; Moreno-Gutiérrez et al., 2011; Giuggiola et al., 2016; Fernandes et al., 2016), with varying responses and interpretation of the results.

We used dendrochronology and stable isotopes ( $\delta^{13}C, \delta^{18}O$ ) analyses to evaluate the growth and drought-climate relationships and to assess the physiological responses under different thinning intensities (medium and high intensity thinning treatments) and different times after thinning intervention (ten and twenty years after the thinning treatments) in two planted stands of Aleppo pine in Spain. We selected two sites with similar Mediterranean climatic conditions and performed the same analyses in order to determine the robustness of the results (that is, we treated the sites as replicates). The main objectives of this research were: (i) to assess the response of radial growth and WUEi to different thinning intensities and different time since thinning intervention and their relationship with climate; (ii) to disentangle the effects of thinning on WUEi through complementary measurements of oxygen stable isotopes; and (iii) to analyze the effectiveness of thinning on improving growth indices. We hypothesize that thinning will improve radial growth and resilience of trees against drought, attributable to a relaxation of drought stress. In thinned plots, we expect a decrease of WUEi, caused by a reduction of tree competition for soil water and nutrients. Regarding the physiological mechanisms, we hypothesize that combined analysis of  $\delta^{13}C$  and  $\delta^{18}O$  would allow us to determine the relative influence of changes in *A* and *g*<sub>s</sub> on WUEi and growth.

## 2. Material and methods

## 2.1. Study sites and thinning experiments

We selected two areas afforested with *P. halepensis* located in eastern Spain: Tarazona (41° 52′ N, 1° 50′ W, 798 m a.s.l.) and La Hunde (39° 05′ N, 1° 12′ W, 950 m a.s.l.; Fig. 1). The two sites are characterized by similar Mediterranean climatic conditions. To describe the climatic



Fig. 1. Location of sites at eastern Spain and view of the thinning treatments studied in Tarazona and La Hunde. Note that pictures were taken in 2018 for Tarazona while in 2008 for La Hunde (with time periods of 20 years and 1 month from thinning intervention, respectively).

characteristics of both sites we used the 1.1-km<sup>2</sup> gridded climate data for the 1981 to 2010 period of temperature, precipitation (Vicente-Serrano et al., 2017) and reference evapotranspiration (Tomas-Burguera et al., 2019). Mean annual temperature ranges from 12.4 °C (s.d. of 0.5 °C) at Tarazona to 13.2 °C (s.d. of 0.6 °C) at La Hunde. In Tarazona and La Hunde, the mean annual Penman-Monteith reference evapotranspiration is 1121 mm and 1217 mm, respectively, whereas the mean annual precipitation varies from 415 mm (s.d. of 95 mm) to 484 mm (s.d. of 143 mm). The hydrologic balance (annual precipitation minus potential evapotranspiration) is -706 mm (s.d. 117 mm) in Tarazona, and -733 mm (s.d. 167 mm) in La Hunde. Both locations have similar seasonal temperature and precipitation distribution (Fig. S2), characterized by a strong intra-annual variation with a dry period in summer and two maxima in spring and fall. The maximum rainfall in La Hunde occurs in fall (October, 62 mm) due to its closer location to the Mediterranean Sea, while Tarazona shows its maximum in spring (May, 58 mm), which is a typical feature of a more continental Mediterranean climate subjected to Atlantic influences. In fact, following the Köppen classification, only La Hunde adjusts to a purely Mediterranean climate ('Csa'), while Tarazona is classified as Temperate Oceanic ('Cfb'). Despite this difference in the Köppen classification, both sites were considered as Mediterranean as both showed a marked arid summer period.

The studied *P. halepensis* plantations were established at both sites between the late 1940s and the early 1960s with the main objectives of improving the ecological status and reducing soil erosion. No forest management has been carried out until the thinning experiments took place. In the Tarazona site the plots contained scattered sclerophyllous shrubland vegetation (*Quercus coccifera* L., *Juniperus thurifera* L., *Rhamnus alaternus* L., and *Rhamnus lycioides* L.), whereas in La Hunde the plots presented scant presence of understory species (e.g., *Quercus ilex* sbsp. *Ballota* (Desf.) Samp., *Juniperus oxycedrus* L. and *Juniperus phoenicea* L.). At both sites understory was not removed during the thinning intervention nor afterwards. The soils, according to the World Reference Base for Soil Resources (WRB, 2014), correspond to Cambisols and Leptosols for Tarazona and La Hunde, respectively. The soil depth is around 60 cm in both sites. In both study sites the slope gradient is <5%.

Experimental thinning treatments took place in different years and included several intensities at both sites. In Tarazona the experimental thinning was carried out in 1998 while in La Hunde was performed in 2008. In Tarazona the initial canopy cover of 81% (control plot, C) was

diminished to 62% (moderate intensity plot, M) and 40% (high intensity plot, H). In La Hunde the initial canopy cover was reduced from approximately 84% (control plot, C) to 68% (moderate thinning intensity plot, M) and 50% (high thinning intensity plot, H). For additional details of thinning intervention in La Hunde see previous works by Molina and del Campo (2012), and del Campo et al. (2014) and for Tarazona see Hernández and Arrechea (2017). In both sites the plots show similar forest structure and tree characteristics (Table 1).

# 2.2. Dendrochronology

In 2018 we selected 15 *P. halepensis* trees per site and per treatment and extracted 2 cores per tree at breast height (1.3 m) separated by 180° using Pressler borers. A total of 180 samples from 90 trees (two samples  $\times$  15 trees  $\times$  three treatments  $\times$  two sites) were thus obtained. Samples were taken from dominant and mature trees showing no visible signs of damage. The diameter at 1.3 m of all sample trees and the total height of 5 selected trees per plot were measured using tapes and clinometers, respectively.

The wood samples were visually cross-dated, and total tree-ring width (RW) was measured under a stereo microscope with an accuracy of 0.01 mm using the TSAP-Win program LINTAB measuring device (Rinntech, Heidelberg, Germany). The dating was checked using the COFECHA program (Holmes, 1983). The RW was transformed into

#### Table 1

Characteristics of the *Pinus halepensis* stands and trees immediately after thinning interventions in the study sites (values are means). Note that relative changes when comparing forest structure metrics among control and thinned treatments are consistent between the sites.

Site/thinning treatment	Diameter at 1.3 m (cm)	Tree height (m)	Canopy cover (%)	Density (trees ha <sup>-1</sup> )	Basal area (m² ha <sup>-1</sup> )
Tarazona Control Moderate High	13.6 14.5 14.6	9.2 9.3 8.9	81 62 40	2000 1050 975	31.0 21.3 15.5
La Hunde Control Moderate High	16.9 17.7 17.5	11.5 12.2 11.3	84 68 50	1489 744 478	40.1 27.2 18.2

basal area increment (BAI) which is biologically more meaningful to quantify growth variations between years and treatments. BAI values were calculated annually from each RW measurements assuming a circular shape of stems as the difference between consecutive annual cross-sectional basal areas. We are aware that such assumption may introduce additional uncertainty since stems are not perfectly circular. However, BAI is still a better biological descriptor of growth that treering width. The mean BAI of the two cores for each tree was computed, and the resulting mean BAI series were further used in all statistical analyses. The years prior to 1965 were not considered due to the juvenile age of the trees.

# 2.3. Climatic data and identification of drought years

The Standardized Precipitation Evapotranspiration Index (SPEI, unitless), was used to characterize drought conditions at several time scales (1 to 48 months). The SPEI is a multi-scalar drought index based on anomalies of the climatic water balance with respect to the normal conditions at a given site, and it allows determining the onset and magnitude of droughts (Vicente-Serrano et al., 2010). The SPEI is computed as the difference between precipitation and potential evapotranspiration (the climatic water balance) accumulated over a given time period (the so called 'time scale'). The values are standardized after fitting a log-logistic distribution to the data, so the SPEI is finally expressed as standard deviations following a standard Gaussian distribution (Beguería et al., 2014). SPEI weekly time series were downloaded from http://monitordesequia.csic.es (Vicente-Serrano et al., 2017) using the sites' coordinates.

Since the SPEI data consisted on weekly values, and there are different variants of the index depending on the time scale (i.e., the period over which the climatic water balance is calculated), we undertook a preliminary analysis in order to determine the time scale and week of the year that had the best correlation with the BAI. Pearson correlation coefficients between alternative SPEI and BAI time series (from October of the previous year to December of the growing year, that is a period of 15 months) were thus computed, allowing to determine the best combination at each site. Based on the SPEI data, the 1986–1987, 1994–1995, 2005–2006 and 2012 droughts were considered as the most severe in the two study sites. In addition, for La Hunde 1999–2000 and 2014 were also included as droughts.

### 2.4. Resistance, recovery and resilience indices

The set of selected droughts included events before and after the thinning treatments. In order to quantify the responses of BAI to the selected droughts and to explore the effects of management on growth, we followed Lloret et al. (2011) who defined resistance, recovery and resilience indices. The resistance index (Rt) quantifies the capacity to maintain growth levels during a drought; the recovery index (Rc) measures the growth increase after a drought; and the resilience index (Rs) reports the ability to recover pre-drought growth levels after a drought. The indices are computed as follows:

$$Resistance (Rt) = BAI_D / BAI_{Pre-D}$$
(1)

$$Recovery (Rc) = BAI_{Post-D}/BAI_D$$
(2)

$$Resilience (Rs) = BAI_{Post-D}/BAI_{Pre-D}$$
(3)

where BAI<sub>D</sub> corresponds to BAI during the drought year, and the BAI<sub>Pre-D</sub> and BAI<sub>Post-D</sub> correspond to the average BAI of the 2 years previous to the drought event and after the drought, respectively. Sohn et al. (2016) suggested to use 3 years before and after the drought event, however we selected 2-years long for the pre- and post-drought periods in order to avoid drought events overlap and following Camarero et al. (2018). This is also in agreement with 2-year period established as a

global reference for post-drought recovery by Anderegg et al. (2015). The resilience indices were calculated for the selected drought episodes.

#### 2.5. Stable isotope analysis and intrinsic water use efficiency

Wood tissue samples were taken for C and O isotope analyses, as studies comparing whole wood and cellulose show similar trends (Saurer et al., 2004). For each treatment (Control and Heavy thinning), we selected the five trees whose cross-dated tree-ring width series presented the highest correlation with the mean series. For the five trees selected per treatment, rings were separated in years using scalpels, starting in 1992 and sampling every year onwards. Then, year pools were obtained by making composite samples of the five selected trees. The resulting wood samples were homogenized to a fine powder using a ball mixer mill (Retsch MM301, Haan, Germany). Wood aliquots (0.001 g) were weighted on a microbalance (AX205 Mettler Toledo, OH, USA). In the case of  $\delta^{13}$ C, sampled were stored in tin foil capsules and combusted to CO<sub>2</sub> using a Flash EA-1112 elemental analyser interfaced with a Finnigan MAT Delta C isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). In the case of  $\delta^{18}$ O, samples were stored into silver foil capsules and combusted in an elementary PyroCube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Isotope analyses were performed at the Stable Isotope Facility of the University of California at Davis (USA). Stable isotope ratios were expressed as per mil ( $\infty$ ) deviations using the  $\delta$  notation relative to VPDB (for carbon) and VSMOW (for oxygen) standards. The standard deviation for repeated analyses was  $\pm 0.06\%$  and  $\pm 0.25\%$  for  $\delta^{13}$ C and  $\delta^{18}$ O, respectively.

We calculated WUEi values from tree-ring wood C isotopes using the following framework. We calculated C isotope discrimination in wood ( $\Delta^{13}$ C) based on changes in  $\delta^{13}$ C of atmospheric CO<sub>2</sub> ( $\delta^{13}c_a$ ) and wood  $\delta^{13}$ C ( $\delta^{13}c_w$ ) following Farquhar and Richards (1984):

$$\Delta^{13} C = \left( \delta^{13} c_a - \delta^{13} c_w \right) / \left( 1 + \delta^{13} c_w / 1000 \right)$$
(4)

 $\delta^{13}c_a$  was obtained from McCarroll and Loader (2004). Then, we calculated WUEi (in µmol mol<sup>-1</sup>) using the equation proposed by Farquhar et al. (1982):

WUEi = 
$$c_a \left[1 - (c_i/c_a)\right] 0.625 = (c_a - c_i)/1.6$$
 (5)

where  $c_a$  is the atmospheric CO<sub>2</sub> concentration,  $c_i$  is the CO<sub>2</sub> concentration in the sub-stomatal cavity of leaves, and 0.625 is the relation among the conductance of H<sub>2</sub>O compared to the conductance of CO<sub>2</sub>. To determine  $c_i$  we followed Francey and Farquhar (1982):

$$c_i = \left[ \left( \Delta^{13} \mathbf{C} - \mathbf{a} \right) \times c_a \right] / (\mathbf{b} - \mathbf{a})$$
(6)

where *a* is the diffusion fractionation across the boundary layer and the stomata (4.4%) and *b* is the Rubisco fractionation (27.0%).

The relationships between  $\delta^{13}$ C and  $\delta^{18}$ O were analysed by means of the differences between thinned and control plots ( $\Delta \delta^{13}$ C and  $\Delta \delta^{18}$ O, respectively), during the pre- and post-thinning periods.

## 2.6. Statistical analyses

Univariate ANOVA was used for testing for differences in BAI,  $\delta^{18}$ O and WUEi comparing between pre- and post-treatment periods and between treatment levels. In Tarazona, differences were tested for two post-treatment periods of ten years each. ANOVA was also used to test for differences between treatments in resistance, recovery and resilience indices for each drought year, using the Tukey's significant difference post hoc test. For multi-year droughts the last year was used, and prior to the analysis we examined all the indices for normality and homoscedasticity.

One problem with ANOVA was that it did not allow for controlling for the influence of other relevant factors besides the period or the treatment levels, such as the climate. Climate has a strong influence on both the growth and water use efficiency of trees, and differences in climate between pre and post thinning periods could alter the results when comparing based on ANOVA. Therefore, we used a set of multivariate linear models in order to test for treatment differences in BAI, WUEi and  $\delta^{18}$ O while controlling for other relevant factors such as differences in climate (SPEI) or random differences among plots or trees. This also allowed us to test for differences in the sensitivity to the SPEI between periods or among treatments.

In the case of BAI we fitted a series of generalized linear mixedeffects models (GLMM) including the treatment intensity (control, C; medium, M; and high, H) and the time period (previous to thinning, *preT*; and posterior to thinning, *postT*) and the SPEI as fixed factors, and the tree labels as random factors. A Gamma GLMM with a logarithmic link was chosen as the model better fitting the skewed shape of the data. Separated models were fitted for each site, leading to two different sets of parameters. Model configurations of increasing complexity were fitted:

$$M0: \quad \mu(t,p,n) = \beta_0(t,p) + \beta_1 SPEI_i + \upsilon(n) + \varepsilon$$

$$M1: \quad \mu(t,p,n) = \beta_0(t,p) + \beta_1(p) SPEI_i + \upsilon(n) + \varepsilon$$

$$M2: \quad \mu(t,p,n) = \beta_0(t,p) + \beta_1(t) SPEI_i + \upsilon(n) + \varepsilon$$

$$M3: \quad \mu(t,p,n) = \beta_0(p) + \beta_1(t,p) SPEI_i + \upsilon(n) + \varepsilon$$

$$(7)$$

 $\text{M4}: \hspace{0.3cm} \mu(t,p,n) \hspace{0.3cm} = \hspace{0.3cm} \beta_0(t,p) + \beta_1(t,p) \hspace{0.3cm} \textit{SPEI}_i + \upsilon(n) + \varepsilon$ 

where  $\mu(t, p, n) = \log(BAI(t, p, n))$  is the dependent variable for a given site, treatment (*t*), time period (*p*) and tree (*n*);  $\beta_0$  is an intercept;  $\beta_1$ is the climate effect (SPEI); v is the individual-level random effect following a Normal distribution with mean 0; and  $\varepsilon$  is a Gammadistributed random error. The subscripts (t, p) refer to interactions between the model coefficients ( $\beta$ ) and the treatment and time period factors, so for instance  $\beta_1$  SPEI indicates that the coefficient does not vary between periods and treatments, while  $\beta_1(t,p)$  SPEI indicates a fullinteraction configuration in which the coefficient varies between both treatments and periods. The different models include, thus, configurations with equal slopes and different intercepts; equal intercepts and different slopes; and different slopes and intercepts. The models were fitted using the lme4 R package (Bates et al., 2015), and the Akaike's Information Criterion (AIC) was used to compare between them. The AIC was used because it provides a balance between goodness-of-fit and model complexity. It must be noted that mixed models do not allow for computing *p*-values of the fixed-effects model coefficients due to the existence of random effects, so we used the overlapping of the 95% confidence bands around the model coefficients to determine the significance of differences between treatment levels and between periods.

For  $\delta^{18}$ O and WUEi we fitted linear models, since there were no data for individual trees (as stated, a pooled sample was used for the isotopic analysis), so using a mixed-effects model was not possible. Similar model configurations were compared:

M0 :	$\mu(t,p)$	=	$\beta_0(t,p) + \beta_1 SPEI_i + \varepsilon$	
M1 :	$\mu(t,p)$	=	$eta_0(t,p) + eta_1(p) \ SPEI_i + arepsilon$	
M2 :	$\mu(t,p)$	=	$eta_0(t,p) + eta_1(t) \ \textit{SPEI}_i + arepsilon$	(8)
M3 :	$\mu(t,p)$	=	$\beta_0(p) + \beta_1(t,p) \operatorname{SPEI}_i + \varepsilon$	
M4 :	$\mu(t,p)$	=	$\beta_0(t, p) + \beta_1(t, p) SPEI_i + \varepsilon$	

where  $\mu(t,p)$  equals  $\partial O^{18}(t,p)$  or WUEi(t,p), respectively, and the error term  $\varepsilon$  follows a Normal distribution.

# 3. Results

#### 3.1. Thinning effects on radial growth

The BAI series showed very similar patterns for all treatment plots before thinning as indicated by the non-significant differences (Fig. 2 and Table 2). As expected, lower values of BAI were recorded during drought years (e.g. 1994–1995, 2005–2006, 2012), while high BAI values were observed during wet years such as 1997 (Fig. S2). The lowest values of BAI occurred in 1994 in Tarazona (0.72 cm<sup>2</sup>) and in 2005 in La Hunde (0.80 cm<sup>2</sup>).

Differences between treatments were apparent after the thinning in both sites. BAI was enhanced in both thinning intensities plots, although the greatest increases occurred on the high intensity thinning plots (H). On the other hand, the moderate intensity treatment (M) in Tarazona showed increasing BAI annual values in the *postT10* period, while in the last period the differences with the control disappeared, decreasing with the time elapsed since thinning (Fig. 2). In La Hunde, differences were significant compared with the *postT* period. On the contrary, in the control plots the differences between the periods were negligible.

# 3.2. Combined effects of drought and thinning on radial growth

In both sites the highest correlation between BAI and SPEI time series in control plots was found during summer (see Fig. S1 in the Supporting Information). The SPEI at the time scale of 9 months and week 31 of the year (mid-August) had the highest correlation in Tarazona, with a positive and significant Pearson correlation coefficient of 0.75, while in La Hunde the highest correlation was found in week 24 (mid-June) at the same time scale of 9 months, with a positive and significant correlation coefficient of 0.55 (Fig. S3). SPEI data for those weeks and time scales were therefore used in the regression analysis as independent variables.

The results of the linear regression analysis confirmed that BAI was significantly affected by the SPEI and the thinning treatments (Table S1, Fig. 3). It is interesting to compare the model coefficients between *preT* and *postT* periods for a given treatment, while the comparison between the two sampling sites confirms the robustness of the results.

Between the different model configurations tested, the lowest AIC was obtained by model M4 with full interaction between the intercept and the slope coefficients and the two factors (period and treatment). The relationship between BAI and SPEI was positive in all cases, so high values of the SPEI, indicative of a wetter-than-normal year, were associated with high values of BAI and vice versa (Fig. 3a). The regression coefficients for SPEI were in general higher in Tarazona than in La Hunde, indicating a stronger influence of climate on BAI. In the *postT* period the coefficients were lower in the thinned plots than in the control, although this difference was only significant in La Hunde between control and heavy thinning. While the coefficients were not significantly different between both periods in the control plots, they were significantly different in the thinned plots, in both sites.

There were differences in the model intercepts, too, indicative of differences in mean BAI for controlled SPEI conditions (since a value of SPEI = 0 represents average water balance). The coefficients were significantly higher for thinned plots in the *posT* period, providing evidence that BAI increased on average after thinning (Fig. 3a). This increase was moderately higher (albeit not significantly so) in the heavy thinned than in the moderately thinned plots. Thinning increased BAI by around 0.50 to 0.75 cm<sup>2</sup> in moderately and heavy thinned plots in Tarazona, and by 0.75 and 1.25 cm<sup>2</sup> in La Hunde. For comparison of the magnitude of the effects, this is a similar difference as between moderately favourable (SPEI = 0.5) and unfavourable (SPEI = -0.5) years in the control plot.

Fig. 3b shows the resulting GLM models of BAI against SPEI, only for the post period. The higher sensitivity of BAI to SPEI in Tarazona is reflected by the steeper lines, while differences in BAI between



**Fig. 2.** Basal area increment (BAI) series of *Pinus halepensis* plantations in Tarazona and La Hunde for control plots (squares), moderately (grey triangles) and heavily thinned (black circles) plots. Bars represent standard errors. Vertical dashed lines indicate the date of the thinning interventions (1998 in Tarazona and 2008 in La Hunde). Significant differences in mean BAI values among treatments before thinning (*preT*) and after thinning (*postT*) are indicated with letters (*p*-value < 0.05) in the right-hand plots.

treatment levels are also apparent. The regression line for the heavy thinned plot in La Hunde is slightly negative, but the coefficient's confidence interval did not exclude zero so the line can be considered as flat, indicating that a negligible influence of SPEI on BAI existed.

Inspection of the random effects revealed that there were remarkable differences between individuals, with extreme values ranging between -0.2 and  $0.2 \text{ cm}^2$ . In general, thinning did not seem to modify significantly the variability between trees in the *postT* period.

# 3.3. Resistance, recovery and resilience indices

Drought resistance (i.e., the ratio between BAI values of the drought year and the two previous years, Rt) tended to be, as expected, lower

than one, indicating reduced growth during a drought. There were exceptions to this, however, such as in the 1995 episode in Tarazona were values were unexpectedly higher than one. In general, *Rt* was increased by thinning, but only significantly so in La Hunde (Fig. 4a, b). Differences were also found during the *preT* period in both sites, with thinning plots having lower *Rt* in general (but only significantly so in one drought episode on each site).

Drought recovery (i.e. the ratio between BAI values posterior to and during the drought, *Rc*) was higher than one in all cases, indicating a good ability to recover to the pre-drought situation in general. Values between 2.5 and 5 were normal, although in some episodes (2006 in Tarazona, 2000 in La Hunde) *Rc* was almost one implying very limited growth recovery. No significant differences were found between

#### Table 2

Basal Area Increment (BAI), intrinsic water-use efficiency (WUEi) and oxygen isotope ratio ( $\delta^{18}$ O) values calculated for the two study sites and treatments (*preT* and *postT* refer to the preand post-thinning periods, *postT10* and *postT20* in Tarazona). Values are means  $\pm$  SE.

Thinning intensity and $period^*$	Tarazona			La Hunde	
	preT (1965–1997)	postT10 (1998–2007)	postT20 (2008–2017)	preT (1965–2007)	postT (2008–2017)
BAI (cm <sup>2</sup> )					
Control	$3.51 \pm 0.30$	$2.77\pm0.29a$	$3.50 \pm 0.47a$	$3.45 \pm 0.19$	$3.24\pm0.26a$
Moderate	$3.26 \pm 0.26$	$4.97 \pm 0.59b$	$4.91 \pm 0.48a$	$4.00 \pm 0.23$	$7.45 \pm 0.55b$
Heavy	$2.60\pm0.26$	$5.52\pm0.73b$	$6.93\pm0.61b$	$4.16\pm0.26$	$9.87\pm0.10c$
WUEi (µmol mol $^{-1}$ )					
Control	$109.10 \pm 3.04$	$107.84 \pm 1.96$	$107.74 \pm 2.29$	102.36 ± 1.56b	102.83 ± 2.19b
Thinned	$106.80 \pm 2.08$	$103.49 \pm 2.17$	$101.45\pm1.94$	$95.25\pm1.05a$	$95.59\pm1.95a$
δ <sup>18</sup> O (‰)					
Control	$27.47 \pm 0.11b$	$27.45 \pm 0.10$	$26.93 \pm 0.16a$	27.86 ± 0.12b	$27.80 \pm 0.12a$
Thinned	$26.58\pm0.26a$	$27.73 \pm 0.15$	$27.57 \pm 0.13b$	$27.44\pm0.14a$	$28.41\pm0.25b$

\* Letters (a, b, c) indicate significant differences between treatments at the 95% confidence level (p-value < 0.05), according to ANOVA using post hoc Tukey test for multiple comparisons.

treatments after thinning (*postT*), although the values were consistently lower on thinned plots suggesting reduced recovery (Fig. 4c, d). This interpretation is reinforced by the fact that, before thinning (*preT* period), *Rc* was higher in the thinning plots, especially in Tarazona.

Regarding the resilience index (i.e. the ratio between BAI values posterior to and before the drought. *Rs*), values higher than one were the norm although there were exceptions in some droughts and plots. Significant differences between treatments were found after eight and four years in Tarazona and La Hunde, respectively, with lower resilience corresponding to thinned plots (Fig. 4e, f). In Tarazona, again, differences between plots were also found before thinning (*preT* period), although the variability between trees was very high and these values must be considered with care.

# 3.4. Combined effects of thinning and SPEI on WUEi and $\delta^{18}$ O

Analysis of the time series of WUEi showed lower average values on thinned plots even during the *preT* period (Fig. 5 and Table 2), most notably in La Hunde. This means that there were differences between the plots (control and thinned) before the treatment took place, so the most valuable comparison has to be between *preT* and *postT* periods. In Tarazona WUEi decreased from 106.8 to 103.49 µmol mol<sup>-1</sup> in *postT10* and to 101.45 µmol mol<sup>-1</sup> *postT20* for thinned trees, while in La Hunde the differences were lower. At the same time, WUEi on the control plot remained remarkably constant during the two periods. In all cases the WUEi increased during dry years in both sites (e.g. 1994–1995, 2005–2006, 2014), especially in the control plots.

The best linear model configuration according to the AIC statistic corresponded to model M4, with full interaction between the intercept and the slope and the treatment and period factors. The SPEI had a negative effect on WUEi, indicating increased water use efficiency during dry periods (Fig. 6a and Table S3). There were differences in the SPEI model coefficients between treatments both during the *preT* and *postT* periods in both sites, although their confidence ranges highly overlapped. Differences in the SPEI coefficients between *preT* and *postT* periods were only significant in the thinned plots, and especially so in the case of Tarazona, with more negative values in the latter period suggesting that thinning increased the sensitivity of WUEi to the SPEI. The model intercepts were also different between treatments both during the *preT* and *postT* periods, and especially so in La Hunde. Only in Tarazona the intercept was around 4 µmol mol<sup>-1</sup> lower in the *postT* period with respect to the *preT* period in the thinned plot, while in the control plot, as well as in La Hunde for both plots, the intercepts remained fairly constant during the two periods.

The relationship between WUEi and SPEI during the *postT* period is more clearly seen in Fig. 6b. The range of variation of WUEi with SPEI was similar in both sites, ranging roughly between 115 and 90 mmol mol<sup>-1</sup> for SPEI values of -2 (very dry conditions) and 2 (very wet conditions), respectively. WUEi was consistently lower in the thinned plots in both sites, and only in Tarazona the slope of the model was slightly different, so during droughts (negative SPEI) the difference in WUEi was small but increased rapidly as the conditions improved. This suggests a more elastic response of thinned trees to the climatic conditions, at least on this site, although as it was mentioned before this difference was not significant since the confidence bands of the coefficients slightly overlapped.

With respect to  $\delta^{18}$ O, there were also differences between control and thinned plots even before the thinning treatment (*preT* period), with lower values in the thinned plots in both sites (Fig. 7 and Table 2). However, in both sites  $\delta^{18}$ O increased in the thinning plots after the thinning treatment, while in the control plots it remained constant or even decreased (Tarazona). In Tarazona  $\delta^{18}$ O was significantly



Fig. 3. Model coefficients for the fixed effects (mean values and 95% confidence intervals) for basal area increment (BAI) in Tarazona and La Hunde, for three treatment levels during pre-(*preT*) and post-thinning (*postT*) periods (a), and generalized linear models of BAI against SPEI for different treatment levels in the *posT* period (b).



**Fig. 4.** Temporal evolution of tree resistance (a, b), recovery (c, d) and resilience (e, f) indices in response to selected droughts calculated for Tarazona (left panels) and La Hunde (right panels) growth data (BAI) considering control plots (C), moderately (M) and heavily thinned (H) plots. Values are shown before (circles, Pre) and after thinning (squares, Post) treatments. Vertical dashed lines indicate the date of thinning treatments. Bars represent standard deviations. Significant differences among thinning treatments are indicated with letters (*p*-value < 0.05).

higher (27.57  $\pm$  0.13‰, *p*-value < 0.05) for thinned trees in the *postT20* period and was especially responsive during dry periods (2005–2006 and 2012). In La Hunde it was also significantly higher (28.41  $\pm$  0.25‰, p-value < 0.05) for thinned trees, and remarkably higher during the dry year 2012.

Regression analyses revealed a negative relationship between  $\delta^{18}$ O and SPEI, implying that  $\delta^{18}$ O decreased during favourable years (positive SPEI) and increased during droughts (Table S3, Fig. 8). The exception to that was Tarazona during the *preT* period, which had a positive coefficient. There were marked differences between sites in the evolution between preT and postT periods: while in Tarazona the SPEI coefficient experienced a decrease, in La Hunde the opposite was found. No significant differences were found between thinned and control plots in the slope of the regression, and in fact model M1, with no interaction between  $\beta_1$  and treatment, was selected by the AIC statistic as the best configuration. Once the effect of the climatic conditions (SPEI) were accounted for we found significant evidence of increasing  $\delta^{18}$ O values after thinning, as revealed by the model intercepts. Thus, while in the control plots the model intercepts decreased in the period after thinning (only significantly in Tarazona), the thinned trees experienced a strong and significant increase in both sites. The difference between control and thinned trees was about 0.5% in both sites, as shown in Fig. 8b.

We analysed the changes in isotopic concentrations after thinning according to the dual isotope interpretation of Scheidegger et al. (2000). Fig. 9 shows annual and mean differences in  $\delta^{13}$ C and  $\delta^{18}$ O with respect to the control plot ( $\Delta\delta^{13}$ C and  $\Delta\delta^{18}$ O, respectively) after thinning. The predominant signal was a positive movement in the

 $\Delta\delta^{18}$ O axis, while in the  $\Delta\delta^{13}$ C there was larger variability between years and the average difference was almost zero. There was no relationship between dry/wet years and the positive or negative sign of  $\Delta\delta^{13}$ C, and the only effect seen at the annual level was a tendency of the year or years after the thinning to have null or negative values of  $\Delta\delta^{18}$ O, as it was the case of 1998 in Tarazona or 2008 and 2009 in La Hunde.

## 4. Discussion

# 4.1. Thinning effects on radial growth

Thinning enhanced radial growth at both experimental sites, and slightly more so in the heavy thinning plot than in the moderately thinned plot. Reduction of intra-species competition as induced by thinning clearly benefited the remaining trees, a result that can be explained by the fact that resource availability (water, nutrients, light) increases with the growing space (Breda et al., 1995; Aussenac, 2000). The positive effect of thinning on BAI has already been reported for *P. halepensis* in semiarid conditions and similar thinning intensity reductions of basal area by Martin-Benito et al. (2010), del Campo et al. (2014), Fernandes et al. (2016) and Tsamir et al. (2019), where the increase of BAI represented a mean annual increment of 7.01 cm<sup>2</sup> year<sup>-1</sup> for heavy thinning treatments and 2.85 cm<sup>2</sup> year<sup>-1</sup> for moderate thinning in other planted pine species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the second state of the species such as *P. nigra* (Navarro-Cerrillo et al., and the species such as *P. nigra* (Navarro-Cerrillo et al., and the species such as *P. nigra* (Navarro-Cerrillo et al., and the species such as *P. nigra* (Navarro-Cerrillo et al., and the species such as *P. nigra* (Navarro-Cerrillo et al., and the species such as *P. nigra* (Navarro-Cerrillo et al., and the spe



**Fig. 5.** Time series of the intrinsic water-use efficiency (WUEi) at Tarazona and La Hunde according to thinning treatments: control (empty triangles) and heavy thinning (filled dots). In both cases, n = 5 trees per treatment. Vertical dashed lines indicate the date of thinning treatments (1998 in Tarazona and 2008 in La Hunde). Right margin plots show mean and standard errors for both treatments during pre- (*preT*) and post-thinning (*postT*) periods, with significant (p-value < 0.05) differences among treatments during each period indicated with letters.

2019), *P. sylvestris* (Giuggiola et al., 2016; Navarro-Cerrillo et al., 2019) and *Pinus ponderosa* (McDowell et al., 2003; McDowell et al., 2006).

It is crucial to examine the positive effect of thinning in long-term experiments to assess the persistence of the effects of thinning with time elapsed since the intervention (Bottero et al., 2017). We found that the effect of thinning enhanced growth even 20 years after the treatment in the heavy thinning treatment in Tarazona, while in the moderate treatment the effects started to fade (the growth difference with respect to control was not significant, albeit it was still positive). This persistent effect has already been reported for *Pinus contorta* where the enhanced growth was sustained after 18 years (Prescott et al., 2019), and for *P. pinaster* throughout all the study period (15 years) (Navarro-Cerrillo et al., 2016). The maintenance of benefits of thinning are common for short-term trials and have been described



Fig. 6. Model coefficients for the fixed effects (mean values and 95% confidence intervals) for intrinsic water use efficiency (WUEi) in Tarazona and La Hunde, for two treatment levels during pre- (*preT*) and post-thinning (*postT*) periods (a), and generalized linear models of WUEi against SPEI for control and heavy thinning treatment levels in the *posT* period (b).



Year

**Fig. 7.** Time series of  $\delta^{18}$ O values at Tarazona and La Hunde according to thinning treatments: control (empty triangles) and heavy thinning (filled dots). In both cases, n = 5 trees per treatment. Vertical dashed lines indicate the date of thinning treatments, and margin plots show the mean and standard deviation for each treatment during pre- (*preT*) and post-thinning (*postT*) periods, with significant (p-value < 0.05) differences among treatments during each period indicated with letters.

from 5 to 10 years and in several pine species, such as *P. halepensis* (Ruano et al., 2013; Tsamir et al., 2019), *P. sylvestris* and *P. nigra* (Navarro-Cerrillo et al., 2019) and *P. ponderosa* and *P. resinosa* (Bottero et al., 2017).

As a novelty with respect to previous studies, we also considered the effect of climate (SPEI drought index) on growth (BAI) under the different treatments. Growth was reduced during dry years (negative SPEI

values) as expected, but we also found a lower sensitivity of BAI to SPEI after thinning, with regression coefficients closer to zero (and more so in the heavy thinning than in the moderate thinning). This can be interpreted as the climate affecting less in the thinned plots where soil water availability increases. Lower climate sensitivity implies reduced stress during non-favourable periods (droughts), which have important positive consequences for forest vitality and health. We also



**Fig. 8.** Model coefficients for the fixed effects (mean values and 95% confidence intervals) for  $\delta^{18}$ O in Tarazona and La Hunde, for two treatment levels during pre- (*preT*) and post-thinning (*postT*) periods (a), and generalized linear models of  $\delta^{18}$ O against SPEI for control and heavy thinning treatment levels in the *posT* period (b).



**Fig. 9.**  $\delta^{13}$ C and  $\delta^{18}$ O differences from the control (thinned - control). White squares represent the mean values of  $\delta^{13}$ C and  $\delta^{18}$ O during the *preT* period. Grey arrows represent the deviations with respect to these means for all the years in the *postT* period, and the black arrow represents the mean value of the *postT* period. Thinning years (italics).

found differences between sites, as the SPEI had a stronger effect in Tarazona than in La Hunde, suggesting that drought represents a harder constraint in the former site. Despite the two sites having similar Mediterranean climatic conditions (Fig. S2), there are other environmental differences that may help to explain why the trees were more climate-sensitive in one place than in the other. The growing season is on average shorter in Tarazona due to lower winter and spring temperatures. Additionally, growth rates may also be affected by other factors such as different crown classes (Martín-Benito et al., 2008), nutrient limitation, plant acclimation to microclimate conditions or reallocation of carbohydrates after thinning (Peñuelas et al., 2011), which could differ between sites.

Several studies have described climate-growth relationships on Mediterranean pine species (De Luis et al., 2011; Camarero et al., 2013). A strong relationship between BAI and spring-summer precipitation has been reported frequently in water-limited Mediterranean ecosystems (Martin-Benito et al., 2010; Fernandes et al., 2016; Navarro-Cerrillo et al., 2019). There are fewer studies, however, that included the effects of climate and management together. Martin-Benito et al. (2010) found in *P. nigra* that, after thinning, tree growth had a lower correlation with precipitation than before thinning, i.e. lower sensitivity, while trees in their control plot did not show any difference, with the correlation even increasing after thinning. Sohn et al. (2016) and Giuggiola et al. (2016) suggested a weaker growth-climate relationship as thinning intensity increases, at least for *P. sylvestris*. In the case of P. halepensis, Fernandes et al. (2016) found in La Hunde experimental site that thinning made trees less sensitive to soil water shortage, showing that trees in the unthinned plots needed to rely more heavily on current year precipitation than those in thinned plots. Here we used the SPEI instead of only precipitation data, since it provides a more reliable measure of drought severity by considering also the temperaturedriven evaporative demand by the atmosphere (Vicente-Serrano et al., 2010; Beguería et al., 2014), and we also found evidence of a reduced sensitivity to climate induced by thinning.

# 4.2. Thinning effects on water-use efficiency (WUEi)

We found differences in WUEi between control and thinned plots before the thinning took place. Despite this, WUEi was significantly reduced after thinning, while it remained fairly constant in the control plots when comparing the pre- and post-thinning periods. This result was consistent across sites. The reduction of WUEi on thinned stands coincides with the results of Navarro-Cerrillo et al. (2019) for *P. nigra* and *P. sylvestris* and Fernandes et al. (2016) for *P. halepensis*, depicted the last work a reduction from 94.4 µmol mol<sup>-1</sup> to 88.7 4 µmol mol<sup>-1</sup> over 10 years after thinning. McDowell et al. (2003) also found a reduction from 112 µmol mol<sup>-1</sup> to 97 µmol mol<sup>-1</sup> over 6 years after thinning in *P. ponderosa*. Here we found a reduction between 5 and 10 µmol mol<sup>-1</sup> for average climatic conditions (SPEI = 0) in Tarazona and La Hunde, respectively, which is slightly lower. Interestingly, we found that the effect of thinning persisted in Tarazona even 20 years after the treatment.

The decrease in WUEi and the increase of BAI in thinned stands indicate that water deficit due to competition limited tree growth on these plantations (Moreno-Gutiérrez et al., 2012). This interpretation is also supported when analyzing the effect of climate, since WUEi increased during droughts as the trees are affected by water shortage (Moreno-Gutiérrez et al., 2012) and it decreased during wet years. In terms of the regression model, WUEi had a negative relationship with the SPEI implying that lower water use efficiency is related to a reduction in water stress (Klein et al., 2005). In one site (Tarazona), we found that the sensitivity of WUEi to SPEI tended to become higher (i.e., more negative regression coefficient) in the thinned plot, indicating a more elastic response of trees to the climatic conditions. However, this difference was only marginally significant, and this effect was not found in La Hunde, where the regression coefficients were not significantly different between control and thinned plots.

Thinning effects on isotopic composition and physiological interpretation.

Our finding of increasing  $\delta^{18}$ O and constant  $\delta^{13}$ C after thinning in both sites coincides with the results reported by Fernandes et al. (2016) for *P. halepensis*, Martin-Benito et al. (2010) for *P. nigra*, and Brooks and Mitchell (2011) for *Pseudotsuga menziesii*. Giuggiola et al. (2016) found increasing  $\delta^{18}$ O but decreasing  $\delta^{13}$ C after thinning for *P. sylvestris*, and other combinations including constant (Powers et al., 2010) or even decreasing  $\delta^{18}$ O have also been reported by McDowell et al. (2006) for *P. ponderosa* and Moreno-Gutiérrez et al. (2011) for *P. halepensis*.

According to the dual-isotope theoretical model of Scheidegger et al. (2000) (Fig. 9), increasing  $\delta^{18}$ O and constant  $\delta^{13}$ C after thinning should be interpreted as the result of a simultaneous reduction of stomatal conductance  $(g_s)$  and photosynthetic capacity (A). This interpretation, however, would contradict our findings of enhanced BAI and reduced WUEi after thinning. As it has been pointed out by Roden and Siegwolf (2012), this model has some limitations and should only be applied under certain conditions (Barnard et al., 2012). Variability of  $\delta^{18}$ O in plants includes the combined effects of the isotopic composition of the soil water; leaf water enrichment due to transpiration; and biochemical fractionation during the synthesis of organic matter (Scheidegger et al., 2000; Gessler et al., 2014). As their authors point out, the model is based on the assumptions of constant isotopic composition of soil water and atmospheric vapour. We shall examine the validity of these assumptions in our specific case, and in general in the case of thinning treatments.

Especially in dry environments, it is very likely that thinning induces a hotter micro-climate with increased turbulence and air interchange at the stand level, leading to reduced air humidity (Martin-Benito et al., 2010). Therefore,  $\delta^{18}$ O enrichment could be observed that would not be related to changes in stomatal conductance. This mechanism has also been suggested by other authors (Martin-Benito et al., 2010; Fernandes et al., 2016).

Similarly, it could be argued that thinning can also induce changes in soil water uptake depths, again especially in dry environments. Evaporation from the soil causes oxygen and deuterium enrichment near the soil surface, which generally decreases exponentially with soil depth (Barnes and Allison, 1988; Ehleringer and Dawson, 1992). Several authors reported a reduction in shallow water uptake with increasing water deficit, and shallower water uptake during relative wet conditions (Sarris et al., 2013; Grossiord et al., 2017; Barbeta and Penuelas, 2017). We hypothesize that trees on thinned stands present higher rates of shallow water uptake than in unthinned stands, at least under normal conditions. On the contrary in unthinned stands, due to enhanced competition, water is extracted more often from deeper soil layers. This would agree with our observation that thinned trees present weaker growth-climate relationship, while control trees are more restrained by the climatic conditions. This mechanism has also been suggested by Sarris et al. (2013) for thinned P. halepensis subsp. brutia.

It is clear that tree-ring  $\delta^{18}$ O may not always be a good proxy of foliar conditions, and as a consequence should not be used alone to study stomatal behaviour, at least under uncontrolled conditions regarding the source of water or the microclimatic conditions at the stand level. Our findings suggest that  $\delta^{18}$ O was neither constant in the soil water nor in the atmosphere after and before thinning, so that the observed increase in tree-ring  $\delta^{18}$ O does not necessarily imply a reduction of stomatal conductance and photosynthetic capacity as implied by the Scheidegger et al. (2000) model. Araguas-Araguas and Diaz Teijeiro (2005) reported spatial and temporal variability both in rainfall and in soil water in the Iberian Peninsula. Previous results from La Hunde by Molina and del Campo (2012) and del Campo et al. (2014) show that thinning increased the amount of water reaching the soil surface by reducing rainfall interception, but also increased evaporation from the soil.

It is difficult to offer a physiological interpretation of the observed changes in thinned stands in our case study, at least based on isotopic data alone. We observed increasing BAI in thinned trees, which could be explained by increased carbon assimilation. We also observed a reduction in WUEi, which could be a consequence of: i) decreasing *A* at constant  $g_s$ ; ii) increasing  $g_s$  at constant *A*; or iii) increasing  $g_s$  and *A*, with that in  $g_s$  proportionally larger than that in *A*. Therefore, our proposed mechanism for explaining increasing BAI and reduced WUEi is an increase in both  $g_s$  and *A*. The increase in  $g_s$  was proportionally larger than the increase in *A* which could explain the reduction of WUEi. This interpretation agrees with sap flow measurements in La Hunde, which revealed increasing transpiration on thinned trees (del Campo et al., 2014, and Fernandes et al., 2016). The increase in *A* could be related to elevated soil water and nitrogen availability (Feeney et al., 1998; Kaye and Hart, 1998; McDowell et al., 2003; McDowell et al., 2006) and improved nutrient foliar status such as increased N content after thinning (López-Serrano et al., 2005), that generally results in a greater net  $CO_2$  assimilation rate during photosynthesis and increased growth (Warren et al., 2001; Moreno-Gutiérrez et al., 2011). In addition, an increase in *A* may also occur as a result of a water-availability related increase in  $g_s$ , thus allowing for a higher  $CO_2$  uptake (Giuggiola et al., 2016; Moreno-Gutiérrez et al., 2011).

#### 4.3. Reliability of resistance, recovery and resilience indices

Analysis of basal growth inferred from tree-ring series revealed the benefits of thinning, and the analysis of WUEi and stable isotopes allowed interpreting the mechanisms that explain these benefits. It could be expected, therefore, that thinned trees would exhibit enhanced resistance and resilience to drought. Thinning seemed to enhance drought resistance, implying a reduced effect of drought on tree growth, what coincides with the results of regression analysis that indicated reduced climate sensitivity of thinned trees (lower regression coefficients for SPEI).

However, thinning seemed to reduce drought recovery and resilience indices, which seems counter-intuitive. In the case of drought recovery index, we are comparing tree growth after the drought and during the drought. Therefore, if drought affects thinned trees less, then it is to be expected that the recovery index would also be lower. A similar reasoning can be made with respect to drought resilience, where the years after and before the drought are compared.

One problem with drought resistance indices is that the climate conditions of the years before and after a drought are not considered, nor the magnitude of the drought. Climate is an uncontrolled factor in observational studies, and therefore it is difficult to determine how the particular conditions of the years used may affect the indices. In short, it is very different if the year before or after the drought were average years or very good in terms of moisture availability. One benefit of the regression analysis performed is that it allows considering the effect of climate on BAI, through the SPEI drought index. This allows us to redefine at least resistance and recovery indices based on the regression models, using the following expressions:

Resistance $(Rt) = BAI_{SPEI=-2}$	$/BAI_{SPEI=0}$	(9)	)
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$$Recovery (Rc) = BAI_{SPEI=0} / BAI_{SPEI=-2}$$
(10)

where  $BAI_{SPEI=-2}$  is the tree growth expected for a value of the SPEI of -2 (that is, a severe drought), and  $BAI_{SPEI=0}$  is the tree growth expected during normal conditions. Following this approach, we obtain values of Rt of 0.62 and 0.91 for moderately thinned trees in Tarazona and La Hunde, respectively, against 0.50 and 0.75 in the control plot. That is, thinning enhanced drought resistance in both sites following this approach. Regarding Rc, we obtain values of 1.62 and 1.10 in the thinned plot against 2.0 and 1.32 in the control plot, confirming that thinning reduced drought recovery according to the formulation of this index. The resilience index cannot be defined in a similar way, since it would involve normal conditions in the nominator and denominator.

## 5. Conclusions

The findings reported here contribute to the existing knowledge about the effects of thinning on tree growth and functioning, with the general conclusion that thinning had a positive effect on the two Aleppo pine stands analysed by enhancing radial growth. The most interesting result in terms of adaptive silviculture was that thinning helped to reduce growth dependence on drought (SPEI) on the remaining trees. In addition, we found that thinning decreased WUEi, implying a reduction of water stress. Considering the climate and drought (SPEI) effects on BAI to calculate drought resistance index, we observed an increase after thinning that coincided with the observations on selected dry years. Results regarding recovery and resilience indices were compromised by the fact that growth was less affected by drought on thinned trees, so both indices had reduced values when compared to trees in the control plot. We cannot deduce, however, that recovery and resilience were lower on thinned trees, since the effect of drought (and therefore vulnerability) was clearly lower. Due to this effect, we do not recommend the use of these two indices for comparing between thinned and not thinned stands, or in general when different drought vulnerability between groups is suspected. Here we selected two sites of similar climatic conditions to increase robustness of the study, these sites are representative of semiarid conditions, although *P. halepensis* covers a large range of climatic conditions.

Further research should be undertaken to determine to what extent stable isotopes are a reliable tool to describe physiological changes after thinning under uncontrolled environmental conditions. We found that the dual-isotope theoretical model cannot be applied to our case due to non-controlled factors that affect wood  $\delta^{18}$ O composition besides stomatal conductance, such as changes in water uptake depth (and therefore isotopic composition) or micro-climatic effects derived from the thinning. Based on additional physiologic measurements undertaken in one of the two sites, we hypothesize that thinning promoted an increase of both stomatal conductance (transpiration) and photosynthetic rates, with the former being proportionally larger so their ratio (WUEi =  $A/g_s$ ) was reduced.

As a policy recommendation, our findings suggest that thinning (25 to 50% of the initial basal area removed) offers a clear advantage and can be implemented to adapt Mediterranean pine forests to climate variability, improving resistance against drought and promoting a lower dependence of growth on climate conditions. The positive effects of thinning remained at least for 20 years, especially in the heavy thinning treatment, and this value could be used a starting point in order to plan further interventions. This could be critically important to develop adaptation management plans to respond proactively to climate-related global challenges.

## **CRediT** authorship contribution statement

Àngela Manrique-Alba: Conceptualization, Investigation, Writing original draft. Santiago Beguería: Methodology, Formal analysis, Writing - review & editing, Supervision, Funding acquisition. Antonio J. Molina: Validation, Writing - review & editing. María González-Sanchis: Writing - review & editing. Miquel Tomàs-Burguera: Data curation, Writing - review & editing. Antonio D. del Campo: Writing - review & editing. Michele Colangelo: Resources. J. Julio Camarero: Conceptualization, Methodology, Writing - review & editing.

## **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2020.138536.

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