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Leaf $\delta^{18}O$ of remaining trees is affected by thinning intensity in a semiarid pine forest

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ABSTRACT

Silvicultural thinning usually improves the water status of remaining trees in water-limited forests. We evaluated the usefulness of a dual stable isotope approach ($\delta^{13}C$, $\delta^{18}O$) for comparing the physiological performance of remaining trees between forest stands subjected to two different thinning intensities (moderate versus heavy) in a 60-year-old *Pinus halepensis* Mill. plantation in semiarid south-eastern Spain. We measured bulk leaf $\delta^{13}C$ and $\delta^{18}O$, foliar elemental concentrations, stem water content, stem water $\delta^{18}O$ ($\delta^{18}O_{stem\ water}$), tree ring widths and leaf gas exchange rates to assess the influence of forest stand density on tree performance. Remaining trees in low-density stands (heavily thinned) showed lower leaf $\delta^{18}O$, and higher stomatal conductance (g_s), photosynthetic rate and radial growth than those in moderate-density stands (moderately thinned). By contrast, leaf $\delta^{13}C$, intrinsic water-use efficiency, foliar elemental concentrations and $\delta^{18}O_{stem\ water}$ were unaffected by stand density. Lower foliar $\delta^{18}O$ in heavily thinned stands reflected higher g_s of remaining trees due to decreased inter-tree competition for water, whereas higher photosynthetic rate was largely attributable to reduced stomatal limitation to CO_2 uptake. The dual isotope approach provided insight into the early (12 months) effects of stand density manipulation on the physiological performance of remaining trees.

Key-words: *Pinus halepensis*; competition; drought stress; dual isotope approach; oxygen isotopic composition; stomatal conductance.

INTRODUCTION

Pinus halepensis Mill. is a drought-adapted species that has been extensively used in afforestation programmes in semiarid areas of the Mediterranean basin during the last 100 years (Maestre & Cortina 2004). In semiarid south-eastern (SE) Spain, afforestation with *P. halepensis* has been primarily aimed at preventing catastrophic floods, reducing soil erosion rates and combating desertification. In many instances, afforestation of degraded soils with *P. halepensis* has led to the establishment of high-density plantations with slow-growing, even-aged trees which are prone to fire and pest attacks (Maestre & Cortina 2004). Silvicultural thinning is a suitable and widely used forest management alternative in this context, as it can enhance resource (light, nutrients, water) uptake and growth in remaining trees (Brèda, Granier & Aussenac 1995; Kolb *et al*. 2007). Thinning in drought-prone forests is primarily aimed at increasing water availability for remaining trees through reduced canopy interception of rainfall and decreased inter-tree competition for soil water (Brèda *et al*. 1995; McDowell *et al*. 2006; Martín-Benito *et al*. 2010). In semiarid conifer forests, enhanced soil water availability is to a large extent responsible for increased growth of remaining trees after thinning (Klein *et al*. 2005; McDowell *et al*. 2006). Several studies have shown that thinning stimulates *P. halepensis* growth by reducing inter-tree competition for growth-limiting soil resources (water and nutrients; Ne'eman, Lahavb & Izhaki 1995; González-Ochoa, López-Serrano & De Las Heras 2004; Moya *et al*. 2008). However, diameter growth responses to thinning can take several years to become noticeable in semiarid *P. halepensis* forests due to very slow tree radial growth. It thus becomes difficult to evaluate the effectiveness of stand density manipulation for improving the performance of remaining trees in these low-productivity forests. Stable isotope measurements in bulk leaf material could help overcome this limitation.

Stable isotope techniques are a powerful research tool in plant ecophysiological studies, as isotopic signatures provide time-integrated information on plant resource uptake and plant responses to the changing biotic and abiotic environments (Dawson *et al*. 2002). In C_3 plants, carbon stable isotope composition ($\delta^{13}C$) is inversely and linearly correlated with c_i/c_a, the ratio of intercellular to atmospheric CO_2 concentrations in leaves (Farquhar, Ehleringer & Hubick 1989). This ratio reflects the relative magnitudes of net photosynthetic rate (A) and stomatal conductance (g_s), and thus, $\delta^{13}C$ is a good indicator of plant intrinsic water-use efficiency (WUE_i), which is given by the ratio A/g_s (Farquhar *et al*. 1989; Dawson *et al*. 2002; Klein

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et al. 2005). The oxygen stable isotope composition ($\delta^{18}O$) of plant organic material is strongly influenced by the isotopic signature of source water, which may change with depth of water uptake due to evaporative isotopic enrichment of soil water near the surface (Dawson *et al*. 2002; Barbour 2007). Plant $\delta^{18}O$ is also inversely related to e_a/e_i, the ratio of atmospheric to leaf intercellular water vapour pressure, and thus is strongly affected by changes in g_s (Barbour 2007; Farquhar, Cernusak & Barnes 2007). Since plant $\delta^{18}O$ is related to g_s but is unaffected by A, it can help separate the independent effects of A and g_s on $\delta^{13}C$ (Barbour 2007): simultaneous measurement of $\delta^{18}O$ and $\delta^{13}C$ in plant tissues allows discrimination between biochemical and stomatal limitations to photosynthesis (Scheidegger *et al*. 2000; Dawson *et al*. 2002; Querejeta *et al*. 2006; 2007, 2008; Grams *et al*. 2007).

$\delta^{13}C$ measurements in plant material (leaves and/or tree growth-rings) have been often used to assess the effects of stand density manipulation on the ecophysiological performance of remaining trees, with different results (Warren, McGrath & Adams 2001; McDowell *et al*. 2003; Sala *et al*. 2005). Reduced competition for water and increased soil water availability after thinning may lead to reduced WUE_i due to differential enhancement of g_s over A in remaining trees (Meinzer, Goldstein & Grantz 1993; McDowell *et al*. 2003; Skov, Kolb & Wallin 2004; Ferrio *et al*. 2005). However, forest thinning can also increase foliar nutrient concentrations (López-Serrano *et al*. 2005) and incident light levels in remaining trees, thus enhancing their A and WUE_i (Warren *et al*. 2001). Increased soil water availability, on the one hand, and enhanced nutrient and/or light availability, on the other, can exert effects of opposite sign on the WUE_i of remaining trees after silvicultural thinning. Unsurprisingly, different studies have found widely different responses of WUE_i and $\delta^{13}C$ to forest thinning. McDowell *et al*. (2003) reported depletion of $\delta^{13}C$ values in *Pinus ponderosa* after forest thinning, due to a greater increase in g_s than in A that led to reduced WUE_i in remaining trees. By contrast, Warren *et al*. (2001) and Powers, Pregitzer & Palik (2008) reported foliar $\delta^{13}C$ enrichment in various *Pinus* species after thinning, which they attributed to differential enhancement of A over g_s due to increased foliar nutrient concentrations and greater canopy light interception in remaining trees. Other studies have reported no change in the $\delta^{13}C$ values of remaining pine trees after thinning (Wallin *et al*. 2004; Sala *et al*. 2005; McDowell *et al*. 2006), allegedly due to roughly parallel increases in both g_s and A, without significant change in WUE_i.

Simultaneous measurement of $\delta^{18}O$ and $\delta^{13}C$ in bulk leaf material could help elucidate which physiological processes (A, g_s, WUE_i) are most strongly affected by stand density manipulation in each particular situation. Huang *et al*. (2008) and Brooks & Coulombe (2009) recently used this dual isotope approach to investigate the effects of various forest management practices (weed control, fertilizer addition) on tree physiological status. However, to the best of our knowledge, only Powers *et al*. (2008) have used both foliar $\delta^{18}O$ and $\delta^{13}C$ to evaluate the effects of silvicultural thinning on the performance of remaining trees. Powers *et al*. (2008) found increased $\delta^{13}C$ and no significant change in $\delta^{18}O$ in response to thinning, which they interpreted as evidence of enhanced A (and to a lesser extent, g_s) in remaining pine trees.

Several recent studies have successfully used $\delta^{18}O$ and $\delta^{13}C$ measurements to investigate the intensity of interspecific or intraspecific competition for soil resources among plants (Grams *et al*. 2007; Huang *et al*. 2008; Ramírez, Querejeta & Bellot 2009). The main goal of the present study was to evaluate the usefulness of the dual stable isotope approach for early (≈1 year) detection of differences in tree physiological status between forest stands subjected to different thinning levels in a semiarid environment. We hypothesized that heavy thinning in a 60-year-old *P. halepensis* plantation would reduce inter-tree competition for water and would improve the water status of remaining trees to a greater extent than moderate thinning. We predicted that remaining trees in low-density stands (subjected to heavy thinning) would show higher g_s and A, and more depleted foliar $\delta^{18}O$, than those in moderate-density stands (subjected to moderate thinning). Furthermore, we expected to find no differences in WUE_i or foliar $\delta^{13}C$ between treatments due to tightly coupled changes in g_s and A. We also expected that trees in heavily thinned stands would show higher radial growth rates than those in moderately thinned stands.

MATERIALS AND METHODS

Study site

The study was conducted in a 60-year-old *P. halepensis* Mill. plantation ('Los Cuadros', 38 °05′ N, 1 °06′ W) located near the city of Murcia in SE Spain. Elevation in the experimental area ranges from 140 to 170 m above sea level. The climate is semiarid Mediterranean, with mean annual precipitation of 280 mm and average annual temperature of 18.2 °C (data from the Spanish Agencia Estatal de Meteorología, Ministerio de Medio Ambiente, y Medio Rural y Marino). Potential annual evapotranspiration, calculated by the Thornthwaite method (e.g. Dunne & Leopold 1978), is 932 mm. Soils in the area are mostly haplic calcisols, with some lithic leptosols (Alías *et al*. 1998). The *P. halepensis* plantation was established on abandoned agricultural lands in the 1940s and 1950s, with an initial planting density of 1400 trees ha^{-1}. Tree growth is very poor, with heights ranging from 4 to 8 m and basal diameters ranging from 7 to 20 cm (2003 data).

The year 2005 was extremely dry in the experimental area, with a total annual precipitation of only 158.8 mm. Years 2004 and 2007 were moderately wet (324.7 and 363.6 mm annual precipitation, respectively) but had very different rain distributions: whereas 2004 had a rainy spring, 2007 had a dry spring and above-average rainfall during late summer and early fall (Fig. 1).

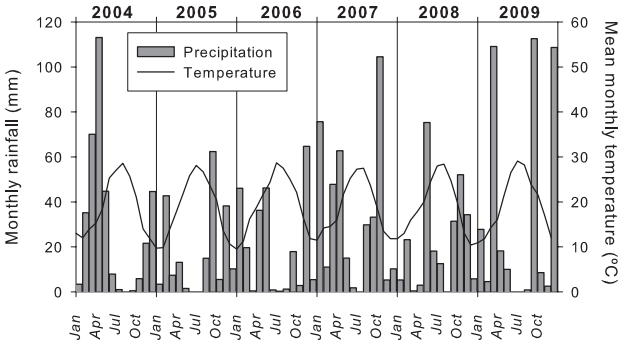

Figure 1. Monthly rainfall and mean monthly temperatures between January 2004 and December 2009 (data from Murcia weather station provided by the Spanish Agencia Estatal de Meteorología).

Experimental and sampling design

The forest thinning experiment is a randomized block design with one factor (thinning intensity) and three replicate blocks. All replicate blocks (60 × 120 m each) are located on moderately steep (less than 20%) slopes. One replicate block is located on a site with north-west (NW) aspect and the other two on sites with north-east (NE) aspect. Each replicate block was divided in two adjacent plots of 60 × 60 m each, and each plot was assigned one of two different thinning treatments: heavy thinning, with approximately 50% reduction of initial basal area, and a final tree basal area of 4.5 m^2 ha^{-1} left standing; or moderate thinning, with approximately 33% reduction of initial basal area, and a final tree basal area of 6.6 m^2 ha^{-1} left standing. Thinning treatments were applied between August and November 2004. After thinning, some of the slash was left on site. Final tree density was approximately 770 trees ha^{-1} in the moderate thinning treatment, versus approximately 550 trees ha^{-1} in the heavy thinning treatment. The effects of thinning treatments on the carbon and water relations of the remaining trees were assessed within a 35 × 35 m core area in each experimental plot, which allowed for a 25 m wide buffer zone around the core area to avoid boundary or edge effects.

Bulk leaf material was collected in the fall of 2005 and 2007 for isotopic and elemental analyses. In November 2005, about 1 year after thinning, pine needle samples were collected from six randomly selected trees in each plot (six trees × two treatments × three replicate blocks = 36). All sampled trees had diameters at breast height ranging from 10 to 20 cm. Current-year needles (2005 leaf cohort) and needles formed during the previous year (2004 leaf cohort) were collected from south-facing branches in the upper portion of the tree crown, using a telescopic pruner. In November 2007, current-year needles (2007 leaf cohort) and lignified stem sections were collected from 10 trees in each plot (10 trees × two treatments × three replicate blocks = 60 trees, including the same 36 trees sampled in 2005). New leaves of *P. halepensis* flush in spring and grow throughout the summer and into early fall (Weinstein 1989; Dougherty, Whitehead & Vose 1994). Foliar samples were taken in November, when all the pine needles were fully expanded. In May 2009, leaf gas exchange measurements (A, g_s, WUE_i) were conducted in order to validate the interpretation of isotopic data. Leaf gas exchange measurements were made on 9–12 remaining trees per treatment in each replicate block (~30 trees per thinning treatment overall). In October 2010, two increment cores were taken at ~1.30 m height from each of the same 36 pines sampled in 2005, for tree ring growth analysis.

Leaf elongation, nutrient concentrations and isotopic composition

The length of 10 randomly selected pine needles per sampled tree was measured with a precision of 1 mm for each leaf cohort (2004, 2005 and 2007). Bulk leaf samples were oven-dried at 60 °C and ground to a fine powder using a ball mill. Finely ground bulk leaf samples were weighed using a precision balance, and encapsulated in tin capsules for stable isotope analyses. Bulk leaf material contains varying proportions of organic compounds with different isotopic signatures; however, several studies measuring $\delta^{18}O$ and $\delta^{13}C$ in both bulk leaf material and leaf cellulose have found that the stable isotope compositions of these materials are strongly positively correlated (e.g. Barbour *et al*. 2000; Sullivan & Welker 2007; Powers *et al*. 2008; although cellulose extraction may be necessary when comparing plants grown under different environmental conditions, as in Grams *et al*. 2007).

The carbon isotope ratio of bulk leaf material ($\delta^{13}C$) was analysed using elemental analyzer/continuous flow isotope ratio mass spectrometry (ANCA/SL elemental analyzer coupled with a Finnigan MAT Delta PlusXL IRMS, Bremen, Germany). The oxygen isotope ratio of the bulk leaf material ($\delta^{18}O$) was determined with a Finnigan MAT Delta Plus XL following the method described in Farquhar, Henry & Styles (1997). This method uses a high-purity alumina pyrolysis tube, a pyrolysis temperature of 1130 °C, chloropentane doping of the carrier gas and a Porapak Q gas chromatography (GC) column before the molecular sieve column. The Porapak Q column separates N_2/CO_2 from any organic compounds, the N_2 and CO_2 is allowed to proceed to and be separated on the molecular sieve column, then both columns are back flushed to remove any contaminants before the next sample is analysed. Isotope analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley (USA). Isotope ratios are expressed in delta notation (‰), where the isotopic composition of a material relative to that of an internationally accepted reference standard is given by $\delta^{xx}E = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the molecular ratio of heavy to light isotope forms (Dawson *et al*. 2002). The standard used for $\delta^{13}C$ is PeeDee Belemnite (V-PDB) and the standard for $\delta^{18}O$ is Vienna-standard mean ocean water (V-SMOW). Long-term (3+ years) external precisions for carbon and oxygen isotope analyses are 0.14 and 0.23‰, respectively.

Foliar nitrogen and carbon concentrations were determined with a Thermo Finnigan Flash 1112 elemental analyzer (Franklin, MA, USA). Foliar phosphorus and potassium concentrations were measured at Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC) by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with HNO_3:H_2O_2 (4:1, v:v).

Stem water content and isotopic composition

In November 2007, lignified stem sections were collected from the same trees sampled for bulk leaf material. Lignified stem sections from remaining trees in both treatments were also collected in May 2008 ($n = 24$) and September 2008 ($n = 14$) in order to obtain stem water content and isotopic composition values representative of the growing season. Two stem sections per tree, approximately 10 mm in diameter and 20 mm long, were collected for stem water extractions, and all leaf and green stem tissue was removed from them to avoid contamination of xylem water with isotopically enriched water (Ehleringer & Dawson 1992). Upon collection, stem samples were immediately placed in capped vials, wrapped with parafilm, and stored in the freezer until water extraction. Stem water was extracted using a cryogenic vacuum distillation line (Ehleringer, Roden & Dawson 2000). Stem water content was calculated from the weight difference before and after oven-drying stem samples at 100 °C. In semiarid ecosystems, interplant differences in stem water content often reflect differences in plant water status (e.g. plant water potential; Querejeta, Egerton-Warburton & Allen 2009).

Analysis of stem water $\delta^{18}O$ ($\delta^{18}O_{stem\ water}$) was conducted at the Stable Isotope Laboratory of the Department of Earth and Planetary Sciences, University of New Mexico (USA), using the CO_2 equilibrium technique. The water samples (1 mL each) were injected into borosilicate vials equipped with rubber septa, which were previously purged with He–CO_2 gas mixture (0.5% CO_2). After 24 h equilibration at 25 °C, the CO_2 was measured by continuous flow isotope ratio mass spectrometry using an automated CombiPal–Gas Bench system coupled to a Finnigan Mat Delta Plus mass spectrometer. The results were corrected using three laboratory standards (calibrated against international water standards). Reproducibility was better than 0.1% based on repeats of laboratory standards. $\delta^{18}O$ values are expressed in delta notation (‰) relative to the international standard V-SMOW.

Since leaf $\delta^{18}O$ is strongly influenced by the isotopic signature of the source water used by the plant (Dawson *et al*. 2002; Barbour 2007), it is standard practice to report the oxygen isotopic composition of leaf tissue as oxygen isotope enrichment above that of source water, in order to account for this effect. Leaf oxygen isotope enrichment above source water ($\Delta^{18}O$; Barbour *et al*. 2000) is calculated as:

$$\Delta^{18}O = \text{bulk leaf } \delta^{18}O\text{-}\delta^{18}O_{stem\ water}$$

We used the $\delta^{18}O_{stem\ water}$ values measured in November 2007 to calculate leaf $\Delta^{18}O$, although it should be noted that these $\delta^{18}O_{stem\ water}$ values may not be exactly representative of mean source water $\delta^{18}O$ integrated over the entire leaf growing season (spring to fall).

Leaf gas exchange measurements

A and g_s were measured in May 2009 with a portable photosynthesis system (LI-6400, Li-Cor, Inc., Lincoln, NE, USA) equipped with a LI-6400–40 Leaf Chamber Fluorometer (LI-6400, Li-Cor) and a Li-Cor 6400–01 CO_2 injector. Gas exchange was measured on 1-year-old sunlit needles from apical shoots in southerly oriented branches in the middle part of the crown. Approximately 20 attached needles were placed in a 2 cm^2 leaf cuvette for gas exchange measurements. The CO_2 concentration in the cuvette was maintained at 380 μmol mol^{-1} CO_2. Measurements were done at saturating light of 1.500 μmol m^{-2} s^{-1}, and at ambient air temperature and relative humidity. The leaf-to-air water vapour pressure difference was between 0.75 and 1.2 mmol mol^{-1} for all measurements and the air flow was set to 350 μmol s^{-1}. All leaf gas exchange measurements were conducted at mid-morning between 9:00 and 11:00 h (local standard time; 7:00–9:00 GMT) on sunny days. Pine needles were collected after leaf gas exchange measurements, and the leaf sections enclosed in the leaf cuvette of the Li-Cor 6400 were digitized by scanning on A3 flatbed scanner (HP Deskscan) fitted with a transparency adaptor at 300 dpi, using an 8-bit greyscale. We analyzed the images with specific software (WinRhizo, Regent Instruments Inc., Québec, Canada) to obtain needle surface area (and needle average diameter; Li, Kräuchi & Dobbertin 2006; Fuentes *et al*. 2007). Total needle surface area values measured by this method were on average 7.5% higher (2.15 cm^2) than the area of the leaf cuvette (2.00 cm^2). All gas exchange parameters were expressed on a total needle surface area basis. WUE_i was calculated as A/g_s.

Tree ring growth analysis

Increment cores taken in October 2010 were mounted, air dried and fine-sanded until tree rings were clearly visible under a binocular microscope. Tree rings were visually cross-dated and measured to the nearest 0.01 mm with a measuring table (LINTAB, Frank Rinn, Heidelberg, Germany) coupled with the TSAP software package (Frank Rinn, Heidelberg, Germany) (Rinn 1996). Obtained tree ring series were compared with each other and with a previously constructed chronology for the same species in a neighbouring site (de Luis *et al*. 2009) to ensure accuracy of cross-dating. Finally, cross-dating was statistically verified using the program COFECHA (Laboratory of Tree-Ring Research, University of Arizona, Tucson, AZ, USA) (Holmes 1983). For each tree, ring widths of years 2004 to 2009 were converted to standardized index values [tree ring width (TRW index)] by dividing them by the average

annual radial growth increment of that tree during the period 1983–2003 (pre-thinning reference period).

Statistical analyses

All statistical analyses were performed with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). Repeated measures analysis of variance (ANOVA) was used to evaluate the effects of thinning intensity, replicate block (between-subject factors), annual leaf cohort/year (within-subject factor) and their interactions on the dependent variables. Individual trees were considered subjects. The dependent variables were pine needle length, foliar nutrient concentrations and foliar stable isotopes ratios in the two annual leaf cohorts (2005, 2007) produced after application of the thinning treatments (2004) and the natural logarithm of the TRW index of years 2005 to 2009. Treatment effects on leaf gas exchange parameters, stem water content and stem water $\delta^{18}O$ (measured in a single year) were tested using two-way ANOVAs (considering thinning intensity as fixed factor and replicate block as random factor). Mean values ± standard error of measured variables in each treatment are presented. For each leaf cohort/year, linear regression analyses were used to examine the relationships between measured variables across all sampled individuals from both thinning treatments.

RESULTS

Foliar $\delta^{18}O$ and $\delta^{13}C$

Repeated measures ANOVA indicates that thinning intensity significantly affected the foliar $\delta^{18}O$ of remaining trees (Table 1; Fig. 2a). Mean bulk leaf $\delta^{18}O$ and $\delta^{13}C$ in the 2004 leaf cohort (fully formed before thinning) were indistinguishable between trees that were to be assigned to different thinning treatments. However, a striking difference between treatments became apparent shortly (12 months) after thinning: foliar $\delta^{18}O$ in the 2005 leaf cohort (newly formed after thinning) was significantly less enriched in the heavily thinned than in the moderately thinned stands (Fig. 2a, $P = 0.010$). This difference in foliar $\delta^{18}O$ between thinning treatments was still apparent in the 2007 leaf cohort (Fig. 2a, $P = 0.035$). Interestingly, the difference in bulk leaf $\delta^{18}O$ between heavily and moderately thinned stands was largest for the 2005 leaf cohort, which was produced during the driest year of the study period (Fig. 2a).

In contrast to $\delta^{18}O$, bulk leaf $\delta^{13}C$ was unaffected by thinning intensity (Table 1; Fig. 2b). Foliar $\delta^{13}C$ in the 2007 leaf cohort (but not in the 2005 cohort) was positively correlated with foliar $\delta^{18}O$ across individuals from both thinning treatments (Fig. 3a).

Leaf growth and elemental concentrations

Repeated measures ANOVA showed no significant difference in pine needle length between thinning treatments (Table 1). However, remaining pines in the heavily thinned stands tended to have somewhat longer needles than those in the moderately thinned stands, and this difference became marginally significant for the 2007 leaf cohort ($P = 0.080$), when average needle length was 7.1 ± 0.1 cm in heavily thinned stands versus 6.8 ± 0.1 cm in moderately thinned stands (Fig. 2c). In both thinning treatments, pine needle length was much shorter in the 2005 cohort than in the other cohorts due to severe drought. In the 2005 leaf cohort, pine needle length was weakly negatively correlated with foliar $\delta^{18}O$ across individuals from both thinning treatments ($r^2 = 0.127$; $P = 0.038$).

Foliar N concentration was highest in 2005 for both thinning treatments, possibly due to a pulse of nitrogen availability derived from decomposition of dead fine roots following tree thinning. However, poor leaf growth due to severe drought may have also contributed to altered leaf stoichiometry and increased foliar N concentration in the 2005 leaf cohort. Foliar elemental concentrations in remaining trees were not significantly different between thinning treatments according to repeated measures ANOVA (Table 1; Fig. 2d–h). However, foliar N concentration became transiently higher in the heavily thinned than in the moderately thinned stands for the 2005 leaf cohort (produced shortly after thinning), whereas the reverse pattern was found in the 2004 (pre-thinning) and 2007 leaf cohorts.

Stem water content and isotopic composition

The $\delta^{18}O$ of stem water in the remaining trees did not differ between moderately and heavily thinned stands in

Table 1. Levels of significance of main effects (*thinning intensity*, *replicate block, leaf cohort* and the interaction between *thinning intensity × leaf cohort*) on the leaf stable isotope composition, needle length and leaf nutrient concentrations of two leaf cohorts produced after thinning (2005 and 2007) in *Pinus halepensis*, as determined by repeated measures analysis of variance

		df	$\delta^{13}C$	$\delta^{18}O$	Needle length	C	N	C/N	P	K
Between subjects effects	Thinning intensity	1	0.108	**0.004**	0.238	0.496	0.196	0.310	0.358	0.556
	Replicate block	2	0.311	**0.003**	0.322	0.663	**0.014**	**0.008**	0.051	**<0.001**
Within subjects effects	Leaf cohort	1	**0.025**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	0.269	0.171
	Leaf cohort × Thinning intensity	1	0.697	0.340	0.866	0.187	**0.034**	0.086	0.427	0.308

Values of $P < 0.05$ appear in bold.

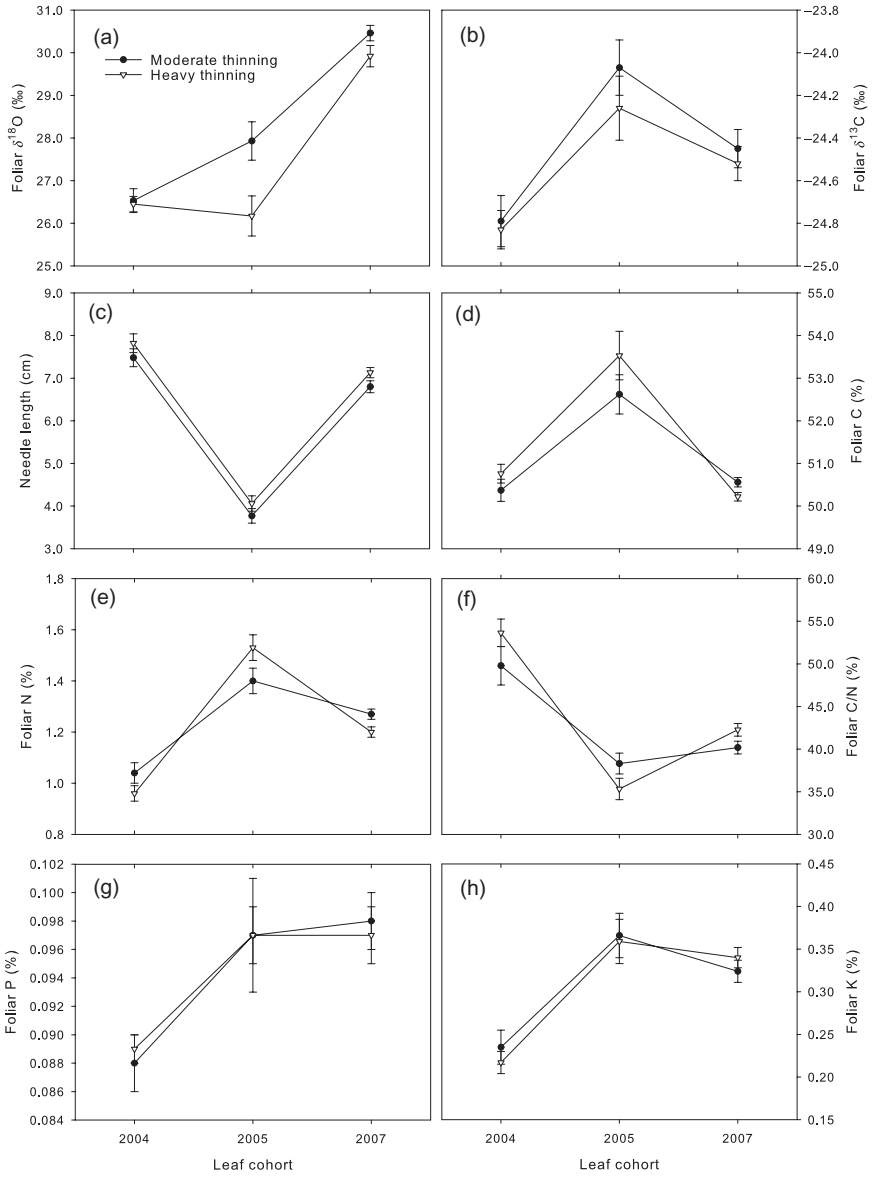

Figure 2. Mean values of (a) bulk leaf δ^{18}O, (b) bulk leaf δ^{13}C, (c) needle length, (d) leaf carbon concentration (C), (e) leaf nitrogen concentration (N), (f) leaf carbon to nitrogen ratio (C/N), (g) leaf phosphorus concentration (P) and (h) leaf potassium concentration (K) in remaining *Pinus halepensis* trees of the heavy and moderate thinning treatments for the 2004, 2005 and 2007 leaf cohorts (error bars indicate ± one SE). Thinning treatments were applied in the fall of 2004.

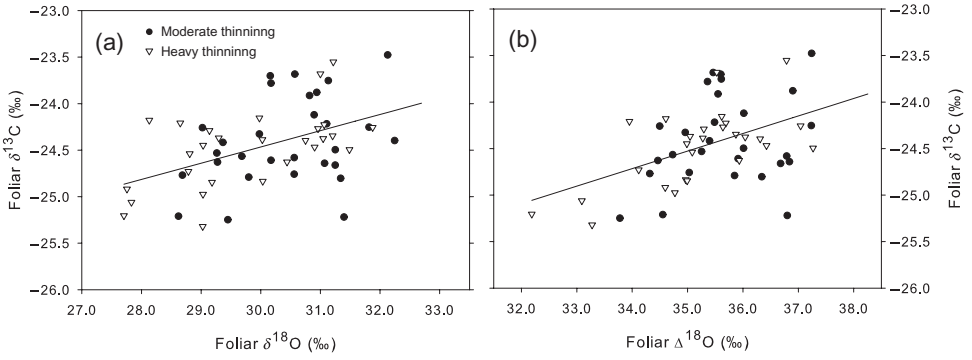

Figure 3. Relationships between (a) bulk leaf δ^{13}C and δ^{18}O ($\delta^{13}C = -29.70 + 0.17\delta^{18}O$; $r = 0.46$, $P < 0.01$, $r^2 = 0.21$, $n = 56$), and (b) bulk leaf δ^{13}C and foliar oxygen isotope enrichment above source water (Δ^{18}O) ($\delta^{13}C = -31.12 + 0.19\Delta^{18}O$; $r = 0.46$, $P < 0.01$, $r^2 = 0.21$, $n = 55$) in the 2007 leaf cohort of remaining *Pinus halepensis* trees, across individuals from both thinning treatments.

November 2007 (–5.38 ± 0.11‰ and –5.28 ± 0.10‰, respectively; $P = 0.459$), May 2008 (–3.57 ± 0.08‰ and –3.39 ± 0.15‰, respectively; $P = 0.366$) or September 2008 (–2.61 ± 0.32‰ and –2.20 ± 0.36‰, respectively; $P = 0.503$). This indicates that the sources of water used by the remaining pines were indistinguishable between the two thinning treatments. There were no significant differences in stem water content between moderately and heavily thinned stands in November 2007 (51.36 ± 0.49% and 52.00 ± 0.39%, respectively; $P = 0.289$) or May 2008 (49.30 ± 0.43% and 49.97 ± 0.58, respectively; $P = 0.354$). However, mean stem water content was significantly higher in heavily than in moderately thinned stands (49.68 ± 0.68% versus 46.45 ± 1.03%, respectively; $P < 0.01$) in late summer (September 2008).

Oxygen isotope enrichment of bulk leaf material above source water ($\Delta^{18}O$) was not significantly different between thinning treatments in November 2007 (35.32 ± 0.24‰ in heavily thinned stands versus 35.69 ± 0.17‰ in moderately thinned stands; $P = 0.168$). Across individuals from both thinning treatments, foliar $\Delta^{18}O$ was positively correlated with foliar $\delta^{13}C$ (Fig. 3b), whereas stem water content was weakly negatively correlated with bulk leaf $\delta^{18}O$ ($r^2 = 0.204$; $P < 0.001$) and $\Delta^{18}O$ ($r^2 = 0.142$; $P = 0.004$). Stem water content was not correlated with stem water $\delta^{18}O$.

Leaf gas exchange measurements

A and g_s in the remaining trees were both significantly higher in heavily thinned stands than in moderately thinned stands ($P = 0.008$ and $P = 0.001$, respectively; Fig. 4a,b). However, WUE_i was not significantly different between thinning treatments (Fig. 4c), thus suggesting roughly parallel increments of similar magnitude in both A and g_s after heavy thinning. There was a strong positive correlation between A and g_s across individuals from the two thinning treatments (Fig. 5), thus indicating tight stomatal control of both transpiration and carbon assimilation rate in *P. halepensis*.

Tree ring growth

Repeated measures ANOVA indicates that the remaining trees in heavily thinned stands had significantly greater radial growth than those in moderately thinned stands during the period 2005 to 2009 (Fig. 6, $P < 0.001$). Mean annual TRW from 2005 to 2009 were 1.64 ± 0.12 mm in heavily thinned stands versus 1.14 ± 0.11 mm in moderately thinned stands. For the 1983–2003 period (before thinning), there were no significant differences in TRW between stands assigned to different thinning intensities (mean annual TRW for this period is 0.66 ± 0.04 mm in plots assigned to heavy thinning versus 0.71 ± 0.06 mm in plots assigned to moderate thinning; repeated measures ANOVA, $P = 0.520$). Although the thinning treatments were applied in the fall of 2004, significant differences in TRW index between thinning intensities did not appear until 2006 (Fig. 6).

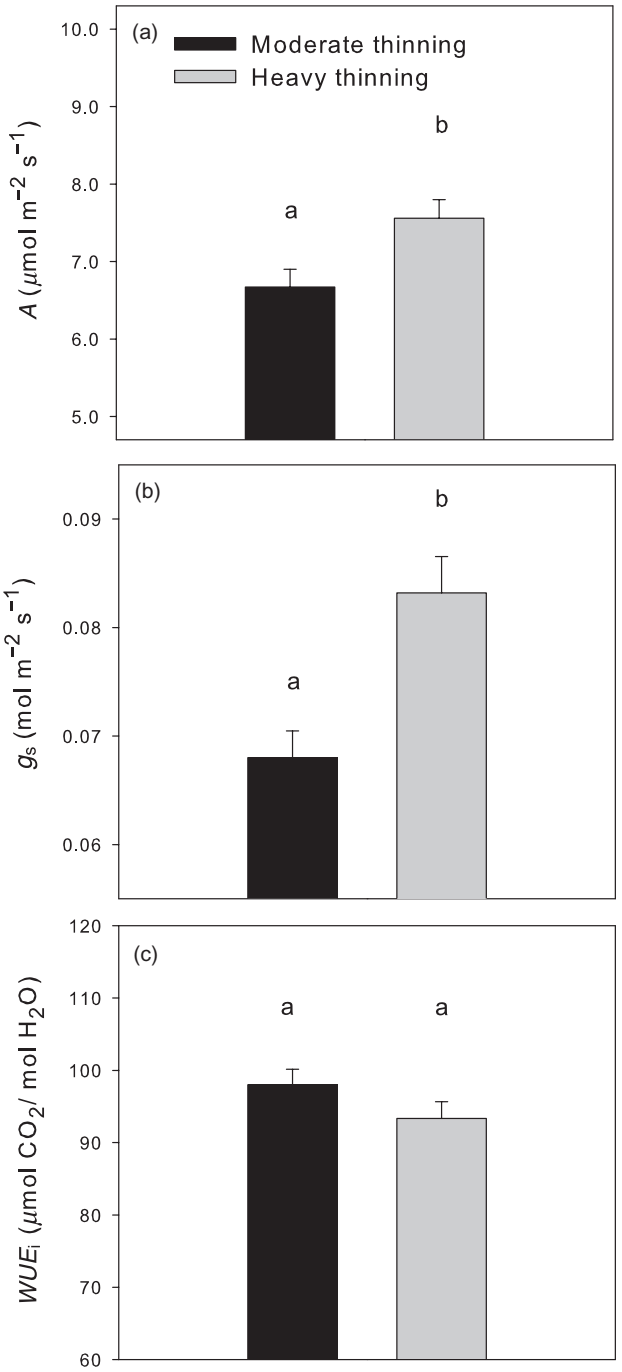

Figure 4. Effects of thinning intensity on the net photosynthetic rate (A), stomatal conductance (g_s) and intrinsic water-use efficiency (WUE_i) of remaining *Pinus halepensis* trees. Different letters above columns indicate significant differences between thinning treatments ($P < 0.05$); error bars represent one SE.

In 2007, TRW index was negatively correlated with foliar $\Delta^{18}O$ (Fig. 7a) and foliar $\delta^{18}O$ (Fig. 7b) across individuals from both thinning treatments. TRW index was also significantly correlated with foliar $\delta^{13}C$ ($r = –0.44$, $P = 0.01$, $r^2 = 0.20$) and stem water content ($r = 0.45$, $P < 0.01$, $r^2 = 0.20$), but not with stem water $\delta^{18}O$.

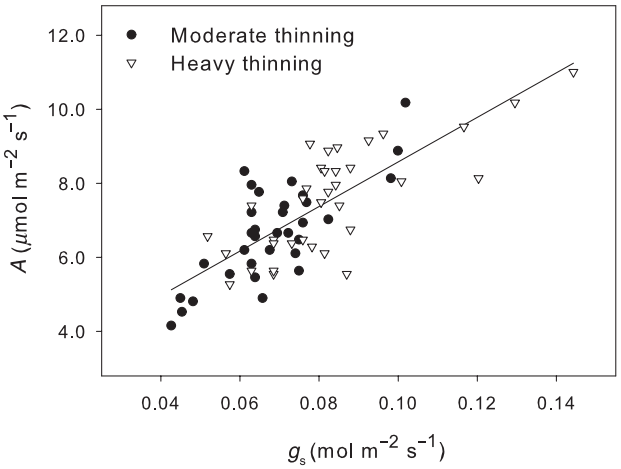

Figure 5. Relationship between net photosynthetic rate (A) and stomatal conductance (g_s) in remaining *Pinus halepensis* trees across individuals from both thinning treatments ($A = 2.56 + 60.18\ g_s$; $r = 0.78$, $P < 0.01$, $r^2 = 0.61$, $n = 68$). The A/g_s regression equation for the heavy thinning treatment was $A = 2.97 + 55.07\ g_s$ ($r^2 = 0.57$; $P < 0.01$, $n = 35$) versus $A = 1.77 + 72.10\ g_s$ ($r^2 = 0.59$; $P < 0.01$, $n = 33$) for the moderate thinning treatment.

DISCUSSION

Differences in the foliar $\delta^{18}O$ of the remaining trees between heavily and moderately thinned stands cannot be plausibly explained in terms of differences in microclimatic conditions. Reducing tree density opens the canopy and changes microclimatic conditions by allowing greater penetration of solar radiation and wind into forest stands (Brèda *et al.* 1995; Aussenac 2000). Thinning leads to significant changes in light availability, temperature and humidity in the forest environment, and these changes in microclimatic conditions could affect the foliar $\delta^{18}O$ of remaining trees (Yakir 1992; Barbour 2007). Forest sites with more open canopies generally show greater light intensity, wider temperature fluctuations and lower air relative humidity than neighbouring sites with more closed canopies (Aussenac 2000). In water-limited conifer forests, low-density stands with more open canopies are usually sunnier, hotter and drier than neighbouring stands with denser canopies (Meyer, Sisk & Covington 2001; Rambo & North 2009; Ma *et al.* 2010; Martín-Benito *et al.* 2010). Higher temperature and lower air humidity [higher vapour pressure deficit (VPD)] would be expected to lead to more enriched foliar $\delta^{18}O$ in semiarid forest with more open canopies (Burk & Stuiver 1981; Yakir 1992; Roden & Ehleringer 1999; Barbour 2007). However, we found that the remaining trees in low-density stands (heavily thinned) with more open canopies showed consistently lower foliar $\delta^{18}O$ values than those in moderate-density stands (moderately thinned) with denser canopies.

Lower foliar $\delta^{18}O$ in heavily thinned stands supports our prediction that a greater reduction in inter-tree competition

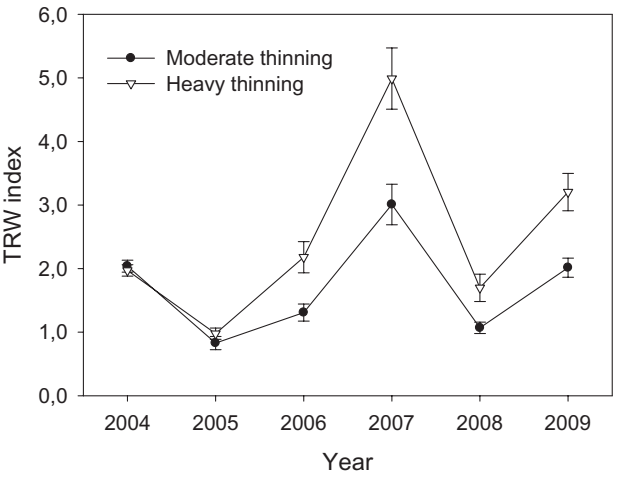

Figure 6. Mean tree ring width index (TRW index) of *Pinus halepensis* trees in the heavily and moderately thinned stands from 2004 to 2009 ($n = 18$; error bars indicate ± one SE). Thinning treatments were applied in the fall of 2004. TRW index is calculated by dividing each annual tree ring width by the average annual radial growth increment of that tree during the period 1983–2003 (pre-thinning reference period).

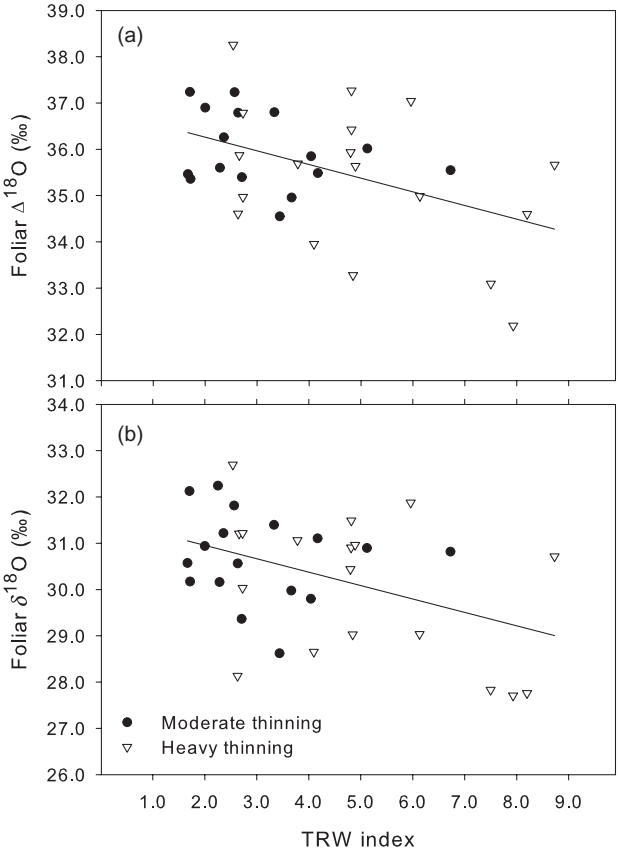

Figure 7. Relationship of tree ring width index (TRW index) in 2007 with (a) foliar oxygen isotope enrichment above source water of the 2007 leaf cohort ($\Delta^{18}O$; $r = -0.45$, $P < 0.01$, $r^2 = 0.20$, $n = 34$; $\Delta^{18}O = 36.85–0.29$TRW index) and (b) bulk leaf $\delta^{18}O$ in the 2007 leaf cohort ($r = -0.43$, $P = 0.01$, $r^2 = 0.18$, $n = 35$, $\delta^{18}O = 31.54–0.29$TRW index) across individuals from both thinning treatments.

for soil resources would be more effective at enhancing the water status of remaining trees (Fig. 2a). *P. halepensis* is a drought-avoiding species with tight stomatal control of transpiration and photosynthesis under water-limiting conditions (Ferrio *et al*. 2003; Klein *et al*. 2005; Maseyk *et al*. 2008; Voltas *et al*. 2008). Lower foliar $\delta^{18}O$ suggests higher leaf-level g_s in stands subjected to heavy thinning than in stands subjected to moderate thinning (Barbour *et al*. 2000; Barbour 2007; Farquhar *et al*. 2007), and this interpretation of isotopic data is well supported by leaf gas exchange data (Fig. 4b). As expected, a greater decrease in canopy interception of rainfall and inter-tree competition for soil water led to higher g_s in the remaining trees of heavily thinned stands (Brèda *et al*. 1995). Whereas foliar $\delta^{18}O$ in the 2004 leaf cohort (produced before thinning) was indistinguishable between plots assigned to different thinning treatments, significant differences were apparent in the 2005 leaf cohort (produced after thinning, Fig. 2a), thus indicating a short response time (≈12 months) of bulk leaf $\delta^{18}O$ to differences in competition intensity in *P. halepensis*. Higher g_s in the remaining trees of heavily thinned stands apparently overwhelmed any potential effects of drier and warmer microclimatic conditions on foliar $\delta^{18}O$.

Differences in bulk leaf $\delta^{18}O$ between treatments were largest in a severe drought year (2005, with annual rainfall of only 159 mm; Fig. 2a). Intense drought likely aggravates the negative effects of inter-tree competition on tree water status in semiarid forests, so heavy thinning may have been particularly beneficial to the water relations of remaining trees during a very dry year. The difference in foliar $\delta^{18}O$ between heavily and moderately thinned stands was less pronounced (but still significant) in a relatively wet year (2007, 364 mm annual rainfall), when inter-tree competition for soil water may have been less intense.

Stem water $\delta^{18}O$ was unaffected by forest stand density, thus indicating that the source water used by the remaining *P. halepensis* trees was indistinguishable between thinning treatments. Stem water $\delta^{18}O$ reflects the isotopic signature of the soil water sources used by the plant, as no fractionation occurs during water uptake by plant roots (Dawson & Ehleringer 1991; Barbour 2007; Ellsworth & Williams 2007). Since pines used soil water of nearly identical $\delta^{18}O$ in heavily and moderately thinned stands, the possibility that differences in the isotopic signature of source water might have been responsible for differences in bulk leaf $\delta^{18}O$ between thinning treatments (Barbour 2007) can probably be ruled out.

Foliar $\delta^{13}C$ did not differ between thinning treatments, thus suggesting that time-integrated WUE_i in the remaining trees was unaffected by forest stand density. This interpretation is well supported by leaf gas exchange data showing no difference in WUE_i ($WUE_i = A/g_s$) between heavily and moderately thinned stands despite significantly higher A and g_s in the former (Fig. 4). Lack of response of foliar $\delta^{13}C$ to forest thinning in spite of enhanced A and g_s in remaining trees was reported by Sala *et al*. (2005) and McDowell *et al*. (2006), who attributed this result to parallel increases of similar magnitude in both parameters. According to the semi-quantitative dual isotope models developed by Scheidegger *et al*. (2000) and Grams *et al*. (2007), similar foliar $\delta^{13}C$ combined with lower foliar $\delta^{18}O$ in heavily than in moderately thinned stands must be interpreted as indicating higher g_s and A in the former. Once again, this interpretation of isotopic data is in good agreement with leaf gas exchange data (Fig. 4). Furthermore, higher carbon assimilation rate translated into greater radial growth of the remaining trees in heavily thinned stands than in moderately thinned stands (Fig. 6). Interestingly, bulk leaf $\delta^{18}O$ responded to heavy thinning much earlier (Fig. 2a) than tree radial growth.

Foliar $\delta^{13}C$ was positively correlated with both leaf $\delta^{18}O$ and $\Delta^{18}O$ in the 2007 leaf cohort, thus suggesting that $\delta^{13}C$ is (at least partly) under stomatal control in *P. halepensis* (Scheidegger *et al*. 2000; Barbour, Walcroft & Farquhar 2002; Keitel *et al*. 2003; Voltas *et al*. 2008). The strong positive correlation between photosynthetic rate and g_s (Fig. 5) and the negative relationships of TRW index with $\Delta^{18}O$ and $\delta^{18}O$ in 2007 (Fig. 7) across individuals from both thinning treatments further indicate that carbon assimilation rate in *P. halepensis* is largely regulated by changes in stomatal aperture in response to soil water availability. Thus, our data suggest that higher photosynthetic rates in the remaining pines of heavily thinned stands resulted primarily from higher g_s (and therefore lower stomatal limitation to carbon fixation), as compared to moderately thinned stands. However, possible differences in light availability and/or in the biochemical photosynthetic capacity of leaves between thinning treatments may have also played a role (Martín-Benito *et al*. 2010). The photosynthetic capacity of leaves is positively related to plant nutrient status and foliar N concentration because it depends on the concentration of N-containing enzymes, pigments and electron transporters in leaf tissue (Reich *et al*. 1995; Dawson *et al*. 2002). In 2005, the foliar N concentration reached the highest values in both treatments (Fig. 2e), probably as a result of impaired needle growth during this drought year. However, foliar N was transiently higher in heavily than in moderately thinned stands in 2005 (Fig. 2e); since there was no significant difference in mean needle length between treatments, this may have been the result of a larger 'pulse' of nitrogen availability in soil shortly after heavy thinning. Higher carbon assimilation rate in heavily thinned stands may thus have been partly attributable to enhanced biochemical photosynthetic capacity of pine needles due to improved plant N status.

In conclusion, heavy thinning reduced inter-tree competition for water more effectively than moderate thinning, thus resulting in higher g_s, photosynthetic activity and radial growth in the remaining trees. Simultaneous measurement of $\delta^{18}O$ and $\delta^{13}C$ in bulk leaf material helped characterize the leaf-level physiological response of the remaining trees to different thinning intensities at an early stage (≈12 months after stand density manipulation). To the best of our knowledge, this is the first study reporting a significant response of foliar $\delta^{18}O$ to silvicultural thinning intensity. Our results show that the dual isotope approach is

particularly useful in situations where foliar $\delta^{13}C$ is unaffected by thinning treatments due to parallel increases of similar magnitude in both photosynthetic activity and g_s. Bulk leaf $\delta^{18}O$ has a short response time to thinning, which may be an important consideration for managers interested in assessing the effectiveness of silvicultural practices in low productivity forests where tree radial growth responses may take longer to become detectable.

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REFERENCES

Alías L.J., Ortiz R., Sánchez A., Linares P., Martínez M.J. & Marín P. (1998) *Memoria Y Mapa De Suelos Escala 1:100.000 Hoja Número 913 (Orihuela). Proyecto LUCDEME*. Universidad de Murcia y Dirección General de Conservación de la Naturaleza, Ministerio de Medio Ambiente, Murcia, Spain.

Aussenac G. (2000) Interactions between forest stands and microclimate: ecophysiological aspects and consequences for silvicultura. *Annals of Forest Science* **57,** 287–301.

Barbour M.M. (2007) Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology* **34,** 83–94.

Barbour M.M., Fischer R.A., Sayre K.D. & Farquhar G.D. (2000) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Australian Journal of Plant Physiology* **27,** 625–637.

Barbour M.M., Walcroft A.S. & Farquhar G.D. (2002) Seasonal variation in $\delta^{13}C$ and $\delta^{18}O$ of cellulose from growth rings of *Pinus radiata*. *Plant, Cell & Environment* **25,** 1483–1499.

Brèda N., Granier A. & Aussenac G. (1995) Effect of thinning on soil and tree water relations, transpiration and growth in an oak forest (*Quercus petraea* (Matt.) Liebl.). *Tree Physiology* **15,** 295–306.

Brooks J.R. & Coulombe R. (2009) Physiological responses to fertilization recorded in tree rings: isotopic lessons from a long-term fertilization trial. *Ecological Applications* **19,** 1044–1060.

Burk R.L. & Stuiver M. (1981) Oxygen isotope ratios in trees reflect mean annual temperature and humidity. *Science* **211,** 1417–1419.

Dawson T.E. & Ehleringer J.R. (1991) Streamside trees that do not use stream water. *Nature* **350,** 335–337.

Dawson T.E., Mambelli S., Plamboeck A.H., Templer P.H. & Tu K.P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* **33,** 507–559.

Dougherty P.M., Whitehead D. & Vose J.M. (1994) Environmental influences on phenology of pine. *Ecological Bulletins* **43,** 64–75.

Dunne T. & Leopold L. (1978) *Water in Environmental Planning*. WH Freeman and Company, San Francisco, CA, USA.

Ehleringer J.R. & Dawson T.E. (1992) Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell & Environment* **15,** 1073–1082.

Ehleringer J.R., Roden J. & Dawson T.E. (2000) Assessing ecosystem level water relations through stable isotope analyses. In *Methods in Ecosystem Science* (eds O.E. Sala, R.B. Jackson, H.A. Mooney & R.W. Howarth) pp. 181–198. Springer Verlag, New York, NY, USA.

Ellsworth P.Z. & Williams D.G. (2007) Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant and Soil* **291,** 93–107.

Farquhar G.D., Ehleringer J.R. & Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 503–537.

Farquhar G.D., Henry B.K. & Styles J.M. (1997) A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. *Rapid Communications in Mass Spectrometry* **11,** 1554–1560.

Farquhar G.D., Cernusak L.A. & Barnes B. (2007) Heavy water fractionation during transpiration. *Plant Physiology* **143,** 11–18.

Ferrio J.P., Florit A., Vega A., Serrano L. & Voltas J. (2003) $\Delta^{13}C$ and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*. *Oecologia* **137,** 512–518.

Ferrio J.P., Resco V., Williams D.G., Serrano L. & Voltas J. (2005) Stable isotopes in arid and semi-arid forest systems. *Investigación Agraria: Sistemas y Recursos Forestales* **14,** 371–382.

Fuentes D., Disante K.B., Valdecantos A., Cortina J. & Vallejo V.R. (2007) Response of *Pinus halepensis* Mill. seedlings to biosolids enriched with Cu, Ni and Zn in three Mediterranean forest soils. *Environmental Pollution* **145,** 316–323.

González-Ochoa A.I., López-Serrano F.R. & De Las Heras J. (2004) Does post-fire forest management increase tree growth and cone production in *Pinus halepensis*? *Forest Ecology and Management* **188,** 235–247.

Grams T.E.E., Kozovits A.R., Häberle K.H., Matyssek R. & Dawson T.E. (2007) Combining $\delta^{13}C$ and $\delta^{18}O$ analyses to unravel competition, CO_2 and O_3 effects on the physiological performance of different-aged trees. *Plant, Cell & Environment* **30,** 1023–1034.

Holmes R.L. (1983) Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin* **43,** 69–78.

Huang Z., Xu Z., Blumfield T.J. & Bubb K. (2008) Foliar $\delta^{13}C$ and $\delta^{18}O$ reveal differential physiological responses of canopy foliage to pre-planting weed control in a young spotted gum (*Corymbia citriodora* subsp. *Variegata*) plantation. *Tree Physiology* **28,** 1535–1543.

Keitel C., Adams M.A., Holst T., Matzarakis A., Mayer H., Rennenberg H. & Gessler A. (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). *Plant, Cell & Environment* **26,** 1157–1168.

Klein T., Hemming D., Lin T., Grunzweig J.M., Maseyk K., Rotenberg E. & Yakir D. (2005) Association between tree ring and needle $\delta^{13}C$ and leaf gas exchange in *Pinus halepensis* under semiarid conditions. *Oecologia* **144,** 45–54.

Kolb T.E., Agee J.K., Fulé P.Z., McDowell N.G., Pearson K., Sala A. & Waring R.H. (2007) Perpetuating old ponderosa pine. *Forest Ecology and Management* **249,** 141–157.

Li M.H., Kräuchi N. & Dobbertin M. (2006) Biomass distribution of different-aged needles in young and old *Pinus cembra* trees at highland and lowland sites. *Trees* **20,** 611–618.

López-Serrano F.R., De Las Heras J., González-Ochoa A.I. & García-Morote F.A. (2005) Effects of silvicultural treatments and seasonal patterns on foliar nutrients in young post-fire *Pinus halepensis* forest stands. *Forest Ecology and Management* **210,** 321–336.

de Luis M., Novak K., Čufar K. & Raventós J. (2009) Size mediated climate-growth relationships in *Pinus halepensis* and *Pinus pinea*. *Trees-Structure and Function* **23,** 1065–1073.

Ma S., Concilio A., Oakley B., North M. & Chen J. (2010) Spatial variability in microclimate in a mixed-conifer forest before and after thinning and burning treatments. *Forest Ecology and Management* **259,** 904–915.

McDowell N., Brooks J.R., Fitzgerald S.A. & Bond B.J. (2003) Carbon isotope discrimination and growth response of old *Pinus ponderosa* trees to stand density reductions. *Plant, Cell & Environment* **26,** 631–644.

McDowell N.G., Adams H.D., Bailey J.D., Hess M. & Kolb T.E. (2006) Homeostatic maintenance of ponderosa pine gas exchange in response to stand density changes. *Ecological Applications* **16,** 1164–1182.

Maestre F.T. & Cortina J. (2004) Are *Pinus halepensis* plantations useful as a restoration tool in semiarid Mediterranean areas? *Forest Ecology and Management* **198,** 303–317.

Martín-Benito D., Del Río M., Heinrich I., Helle G. & Cañellas I. (2010) Response of climate-growth relationships and water use efficiency to thinning in a *Pinus nigra* afforestation. *Forest Ecology and Management* **259,** 967–975.

Maseyk K.S., Lin T., Rotenberg E., Grünzweig J.M., Schwartz A. & Yakir D. (2008) Physiology–phenology interactions in a productive semi-arid pine forest. *New Phytologist* **178,** 603–616.

Meinzer F.C., Goldstein G. & Grantz D.A. (1993) Carbon isotope discrimination and gas exchange in coffee during adjustment to different soil moisture regimes. In *Stable Isotopes and Plant Carbon–Water Relations* (eds J.R. Ehleringer, A.E. Hall & G.D. Farquhar) pp. 327–348. Academic Press, San Diego, CA, USA.

Meyer C.L., Sisk T.D. & Covington W.W. (2001) Microclimatic changes induced by ecological restoration of Ponderosa pine forests in Northern Arizona. *Restoration Ecology* **9,** 443–452.

Moya D., De las Heras J., López-Serrano F.R. & Leone V. (2008) Optimal intensity and age of management in young Aleppo pine stands for post-fire resilience. *Forest Ecology and Management* **255,** 3270–3280.

Ne'eman G., Lahavb H. & Izhaki I. (1995) Recovery of vegetation in a natural east Mediterranean pine forest on Mount Carmel, Israel as affected by management strategies. *Forest Ecology and Management* **75,** 17–26.

Powers M.D., Pregitzer K.S. & Palik B.J. (2008) $\delta^{13}C$ and $\delta^{18}O$ trends across overstory environments in whole foliage and cellulose of three *Pinus* species. *Journal of the American Society for Mass Spectrometry* **19,** 1330–1335.

Querejeta J.I., Allen M.F., Caravaca F. & Roldán A. (2006) Differential modulation of host plant $\delta^{13}C$ and $\delta^{18}O$ by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment. *New Phytologist* **169,** 379–387.

Querejeta J.I., Allen M.F., Alguacil M.M. & Roldán A. (2007) Plant isotopic composition provides insight into mechanisms underlying growth stimulation by AM fungi in a semiarid environment. *Functional Plant Biology* **34,** 683–691.

Querejeta J.I., Barberá G.G., Granados A. & Castillo V.M. (2008) Afforestation method affects the isotopic composition of planted *Pinus halepensis* in a semiarid region of Spain. *Forest Ecology and Management* **254,** 56–64.

Querejeta J.I., Egerton-Warburton L.M. & Allen M.F. (2009) Topographic position modulates the mycorrhizal response of oak trees to interannual rainfall variability. *Ecology* **90,** 649–662.

Rambo T.R. & North M.P. (2009) Canopy microclimate response to pattern and density of thinning in a Sierra Nevada forest. *Forest Ecology and Management* **257,** 435–442.

Ramírez D., Querejeta J.I. & Bellot J. (2009) Bulk leaf $\delta^{18}O$ and $\delta^{13}C$ reflect the intensity of intraspecific competition for water in a semi-arid tussock grassland. *Plant, Cell & Environment* **32,** 1346–1356.

Reich P.B., Kloeppel B.D., Ellsworth D.S. & Walters M.B. (1995) Different photosynthesis–nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia* **104,** 24–30.

Rinn F. (1996) *TSAP: Reference Manual (Version 3.0)*. Frank Rinn, Heidelberg, Germany.

Roden J.S. & Ehleringer J.R. (1999) Hydrogen and oxygen isotope ratios of tree-ring cellulose for riparian trees grown long-term under hydroponically controlled environments. *Oecologia* **121,** 467–477.

Sala A., Peters G.D., McIntyre L.R. & Harrington M.G. (2005) Physiological responses of ponderosa pine in western Montana to thinning, prescribed fire and burning season. *Tree Physiology* **25,** 339–348.

Scheidegger Y., Saurer M., Bahn M. & Siegwolf R. (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* **125,** 350–357.

Skov K.R., Kolb T.E. & Wallin K.F. (2004) Tree size and drought affect ponderosa pine physiological response to thinning and burning treatments. *Forest Science* **50,** 81–91.

Sullivan P.F. & Welker J.M. (2007) Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of a dual isotope ($\Delta^{13}C$ and $\Delta^{18}O$) conceptual model. *Oecologia* **151,** 372–386.

Voltas J., Chambel M.R., Prada M.A. & Ferrio J.P. (2008) Climate-related variability in carbon and oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests. *Trees – Structure and Function* **22,** 759–769.

Wallin K.F., Kolb T.E., Skov K.R. & Wagner M.R. (2004) Seven-year results of thinning and burning restoration treatments on old ponderosa pines at the Gus Pearson Natural Area. *Restoration Ecology* **12,** 239–247.

Warren C.R., McGrath J.F. & Adams M.A. (2001) Water availability and carbon isotope discrimination in conifers. *Oecologia* **127,** 476–486.

Weinstein A. (1989) Geographic variation and phenology of *Pinus halepensis, P. brutia* and *P. eldarica* in Israel. *Forest Ecology and Management* **27,** 99–108.

Yakir D. (1992) Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. *Plant, Cell & Environment* **15,** 1005–1020.

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