

Stand structure modulates the long-term vulnerability of *Pinus halepensis* to climatic drought in a semiarid Mediterranean ecosystem

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ABSTRACT

We investigated whether stand structure modulates the long-term physiological performance and growth of *Pinus halepensis* Mill. in a semiarid Mediterranean ecosystem. Tree radial growth and carbon and oxygen stable isotope composition of latewood ($\delta^{13}\text{C}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$, respectively) from 1967 to 2007 were measured in *P. halepensis* trees from two sharply contrasting stand types: open woodlands with widely scattered trees versus dense afforested stands.

In both stand types, tree radial growth, $\delta^{13}\text{C}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$ were strongly correlated with annual rainfall, thus indicating that tree performance in this semiarid environment is largely determined by inter-annual changes in water availability.

However, trees in dense afforested stands showed consistently higher $\delta^{18}\text{O}_{\text{LW}}$ and similar $\delta^{13}\text{C}_{\text{LW}}$ values compared with those in neighbouring open woodlands, indicating lower stomatal conductance and photosynthesis rates in the former, but little difference in water use efficiency between stand types. Trees in dense afforested stands were more water stressed and showed lower radial growth, overall suggesting greater vulnerability to drought and climate aridification compared with trees in open woodlands.

In this semiarid ecosystem, the negative impacts of intense inter-tree competition for water on *P. halepensis* performance clearly outweigh potential benefits derived from enhanced infiltration and reduced run-off losses in dense afforested stands.

Key-words: $\delta^{13}\text{C}$; $\delta^{18}\text{O}$; climate change; competition; plant–climate interactions; stomatal conductance; tree-ring; water use efficiency.

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INTRODUCTION

Climate change scenarios predict large increases in temperature and decreases in precipitation by the end of the 21st century in the Mediterranean region (Giorgi & Lionello 2008). Greater drought and heat stress associated with climate change have been already related to unprecedented episodes of forest decline (Peñuelas, Lloret & Montoya 2001; Martínez-Vilalta & Piñol 2002; Linares, Camarero & Carreira 2009; Allen *et al.* 2010). In the Mediterranean region, extensive reforestation with pines (3.5 million ha reforested with conifers since 1940 in Spain) together with land abandonment has led to the establishment of dense, uniform, early successional forest vegetation over large areas that may be particularly vulnerable to climate change (Cortina *et al.* 2011). Understanding how plant community structure modulates the physiological response of trees to drought can help forest managers to adopt strategies for improving the resistance and resilience of these forests to the predicted increase in climatic stress. This is especially important for *Pinus halepensis* Mill. plantations in semiarid areas of the Mediterranean region, where this species has been extensively used in afforestation programmes due to its remarkable ability to withstand drought stress (Maestre & Cortina 2004; Cortina *et al.* 2011).

In dense afforested plantations, intense inter-tree competition for soil moisture and significant canopy interception of precipitation (Chirino *et al.* 2006) may exacerbate the severity of water stress experienced by trees. Linares, Camarero & Carreira (2010) found that Mediterranean fir (*Abies pinsapo*) trees already suffering from intense inter-tree competition (a long-term stress) were predisposed to decline during severe drought episodes. Thinning experiments have highlighted the importance of inter-tree competition for soil water in semiarid environments (McDowell *et al.* 2006; Moreno-Gutiérrez *et al.* 2011). Stand density reductions with thinning have been extensively used to enhance tree water status and growth by means of increasing soil water availability, which is achieved as a

consequence of reduced stand transpiration and canopy interception of precipitation (Aussenac & Granier 1988; Brèda, Granier & Aussenac 1995; Misson, Vincke & Devillez 2003). However, improved water status is not always evident in the remaining trees, and some studies have reported no effect of thinning on leaf water potential (Cregg, Hennessey & Dougherty 1990; Schmid *et al.* 1991). Moreover, several studies conducted in water-limited conifer forests found no evidence for greater drought-induced tree mortality in high-density stands under prolonged drought conditions (van Mantgem & Stephenson 2007; Clifford *et al.* 2008; Floyd *et al.* 2009; van Mantgem *et al.* 2009; Ganey & Vojta 2011). These findings question the widely held assumption that increased stand densities in semiarid conifer woodlands predispose trees to experience more intense drought stress during periods of water shortage.

In fact, dense afforested stands are characterized by enhanced run-off infiltration and retention capacities, decreased run-off losses and more mesic microclimate compared with adjacent areas (Aussenac 2000; Van Dijk & Keenan 2007). Recently, Gea-Izquierdo *et al.* (2009) reported that radial growth in *Quercus ilex* L. was becoming more sensitive to summer drought in the last decades, especially in low-density stands, and suggested that denser stands could buffer the influence of extreme climatic events on tree performance. In semiarid ecosystems, the benefits of canopy closure might buffer or offset the negative effects of intense inter-tree competition for water on tree performance in dense afforested stands, depending on annual climatic conditions.

Dendroecological methodologies allow the long-term study of plants' performance and their interaction with changing climate. The stable isotope composition of wood provides insight into the ecophysiological processes involved in the response of trees to past environmental conditions. Plant carbon stable isotope composition ($\delta^{13}\text{C}$) provides a time-integrated proxy of plant intrinsic water use efficiency (WUE_i) during the growing season (Farquhar, Ehleringer & Hubick 1989; Dawson *et al.* 2002; Klein *et al.* 2005). Plant WUE_i is determined by the ratio between photosynthetic rate (A) and stomatal conductance (g_s). In dry environments, this ratio is dominated by water availability (which will determine changes in g_s and subsequently in A), and tree-ring $\delta^{13}\text{C}$ has been found to be strongly and negatively correlated with atmospheric relative humidity and precipitation amount (Saurer, Siegenthaler & Schweingruber 1995; McCarroll & Loader 2004).

The oxygen stable isotope composition ($\delta^{18}\text{O}$) of tree rings is influenced by the $\delta^{18}\text{O}$ of source water, the evaporative enrichment at the leaf level, the biochemical fractionation during sucrose formation in the leaves and the re-exchange of oxygen atoms with xylem water during heterotrophic cellulose synthesis (Roden, Lin & Ehleringer 2000; McCarroll & Loader 2004). The evaporative enrichment of leaf water is strongly affected by changes in g_s (Barbour 2007; Farquhar, Cernusak & Barnes 2007) and produces a strong signal that is reflected in the $\delta^{18}\text{O}$ values

of tree rings. Many studies have found that $\delta^{18}\text{O}$ values in tree rings are related to water availability during the growing season (Saurer, Borella & Leuenberger 1997) and are significantly correlated with precipitation amount, relative humidity and vapour pressure deficit (VPD) (McCarroll & Loader 2004; Ferrio & Voltas 2005; Reynolds-Henne, Saurer & Siegwolf 2009; Hilasvuori & Berninger 2010). Furthermore, the combined analysis of plant $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ can provide valuable complementary information, as $\delta^{18}\text{O}$ is related to g_s but is unaffected by A , and can thus help separate the independent effects of A and g_s on $\delta^{13}\text{C}$ (Scheidegger *et al.* 2000; Dawson *et al.* 2002; Querejeta *et al.* 2006, 2007, 2008; Barbour 2007; Battipaglia *et al.* 2007, 2009; Grams *et al.* 2007).

The aim of this study is to evaluate whether stand structure modulates the long-term physiological performance and growth of *P. halepensis* trees in a semiarid Mediterranean ecosystem. We measured tree-ring widths (TRWs) and the oxygen and carbon isotopic composition of latewood ($\delta^{18}\text{O}_{\text{LW}}$ and $\delta^{13}\text{C}_{\text{LW}}$, respectively) from 1967 to 2007 in two types of stands with sharply contrasting structure and density: a dense afforested plantation, and neighbouring open woodlands with widely scattered pine trees and a well-developed shrub understorey. We hypothesized that inter-annual rainfall variability would be the major control on tree physiological status and radial growth in both stand types in this semiarid ecosystem. Secondly, we hypothesized that pines in dense afforested stands would be consistently more water stressed than those in neighbouring open woodlands due to more intense inter-tree competition for soil water in the former, regardless of large inter-annual climate variability during the 40 year period evaluated. Because *P. halepensis* is a drought-avoiding, isohydric species with tight stomatal control of transpiration and photosynthesis (Borghetti *et al.* 1998; Ferrio *et al.* 2003), we predicted that trees in afforested stands would show chronically lower g_s , A and radial growth and higher $\delta^{18}\text{O}_{\text{LW}}$ than those in open woodlands, with little difference in $\delta^{13}\text{C}_{\text{LW}}$ between stand types.

MATERIALS AND METHODS

Study sites

The study was conducted near the city of Murcia (SE Spain) in a dense 60-year-old *P. halepensis* Mill. plantation and in a nearby open woodland with scattered *P. halepensis* trees. The open woodland has an understorey dominated by *Stipa tenacissima* L., *Rosmarinus officinalis* L. and *Anthyllis cytoides* L. The plantation had an initial density of approximately 1150 trees ha^{-1} but was thinned in 2004 to a final density of 770 trees ha^{-1} . In sharp contrast to afforested stands, tree density in neighbouring open woodland stands is less than 20 trees ha^{-1} . The terrain in the experimental area is hilly, with low hills (140–170 m asl, <20% slopes) and dry ravines between them. The climate is semiarid Mediterranean, with mean annual precipitation of 288 mm and an average annual temperature of 19 °C (Fig. 1; data from the

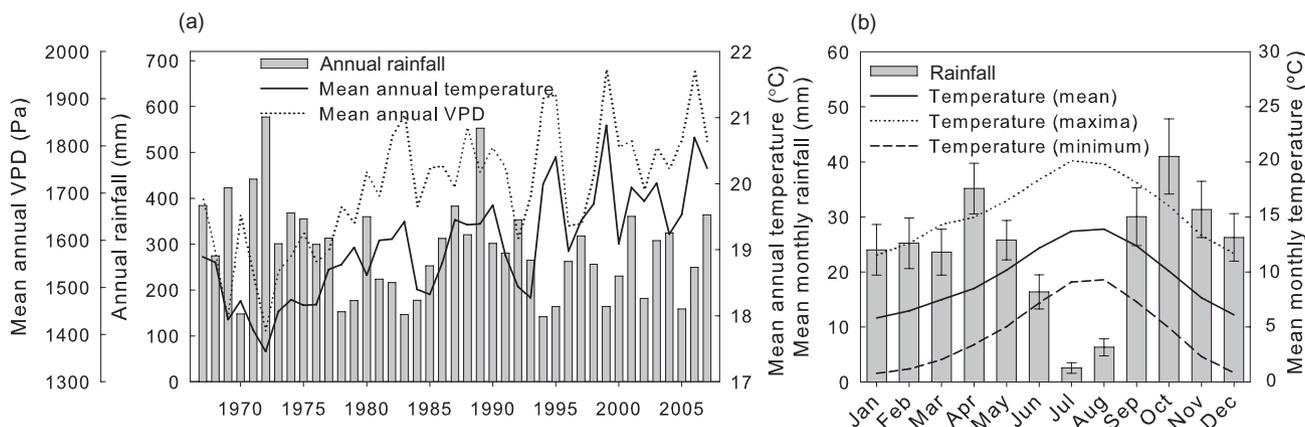


Figure 1. (a) Annual rainfall, mean annual temperature and mean annual vapour pressure deficit (VPD) from 1967 to 2007. (b) Mean monthly rainfall and mean monthly temperatures (maxima, mean and minimum temperatures) for the period from 1967 to 2007, error bars represent ± 1 SE. Data from 'Embalse de Santomera' weather station in Murcia, provided by the Spanish National Meteorological Agency (AEMET).

Spanish National Meteorological Agency, AEMET). Soils in the area are mostly haplic calcisols, with some lithic leptosols (according to the classification of the Food and Agriculture Organization of the United Nations).

Sampling

In May 2008, 10 trees were sampled in each stand type (20 trees in total; mean tree ages are shown in Table 1). EPS values (*expressed population signal*, see following section) were used to assess whether the samples were representative for building a population chronology. Dominant trees located in the valley floors of independent micro-catchments were chosen for sampling. Three cores per tree were sampled with an increment borer (Haglöf, Långsele, Sweden) of 0.5 cm in diameter. The cores were collected at 20 cm height, in order to sample the maximum possible number of tree rings, and oriented at 90° to each other, trying to avoid compression wood (Schweingruber 1988). Samples were air dried and sanded for later analyses of tree rings.

In April 2010, leaf gas exchange and stem water potential measurements were performed at midday in 8 and 10 trees of open woodland and the afforested stands, respectively. From the same trees, we also collected lignified twig sections (2 per tree, approximately 10 mm in diameter and 20 mm long) for stem water extractions. After collection,

samples were immediately placed in capped vials, wrapped with Parafilm and stored in the freezer until water extraction.

Dendroecological analysis

As with other Mediterranean species (Cherubini *et al.* 2003), tree-ring dating in *P. halepensis* was difficult but possible. Tree rings from all cores (3 cores per tree) were visually cross-dated (Raventós *et al.* 2004) 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; Rinn 1996). Cross-dating was statistically verified using the programs TSAP (by the Gleichläufigkeit, GLK: percentage of slope intervals with equal sign in two time series) and COFECHA (Holmes 1983). Single-core ring-width series were cross-dated with the mean of all individual tree growth series from the same stand type. GLK values were always significant ($P < 0.01$) and higher than 60%. In each stand type, individual tree growth series were standardized with ARSTAN (Cook & Holmes 1984; Holmes 2001) using a two-step detrending after stabilizing the variance ('Briffa/Osborn' variance adjusted version, computed in ARSTAN; Osborn, Briffa & Jones 1997): firstly, a negative exponential function was applied, and secondly, a cubic smoothing spline with a 50%

Characteristics	Open woodland	Afforested plantation
Mean tree age and SE in 2007	76.6 (8.5)	59.5 (0.4)
Mean DBH and SE in 2007	38.1 (3.3)	26.3 (1.2)
Mean EPS (residual)	0.890	0.980
Mean Rbar (residual)	0.427	0.675
Mean sensitivity (residual)	0.375	0.510
Standard deviation (residual)	0.355	0.497
First-order partial autocorrelation (residual)	0.142	0.126

Table 1. Mean tree age (years), mean DBH (cm) and dendrochronological characteristics of the residual mean chronology (calculated with ARSTAN, Holmes 2001) of *Pinus halepensis* in open woodland and afforested stands

DBH, diameter at breast height; EPS, expressed population signal.

frequency response over 25 years. Afterwards, an autoregressive model was applied to remove the autocorrelation with the previous year ring width. Individual series within each stand type were averaged with a robust (bi-weight) estimation of the mean (Cook 1985). The following parameters were calculated within each stand type: EPS (indicates the level of coherence of the constructed chronology and how it portrays the hypothetical perfect population chronology), \bar{r} (mean correlation among all possible pairings of individual series within a chronology) and MS (*mean sensitivity*, indicates the degree to which TRW changes from year to year and how it is influenced by high-frequency climatic variations). Standardized residual values (TRW_{res}) were used for assessing correlations with climatic data. In order to perform tree radial growth comparisons between stand types, we calculated basal area increments (BAIs), which remove variations in radial growth attributable to size and age effects (Van Deusen 1992; Piovesan *et al.* 2008; Linares *et al.* 2009). BAI values of individual trees from 1967 to 2007 were calculated using the formula: $BAI = \pi(r_t^2 - r_{t-1}^2)$, where r is the tree radius and t is the year of tree-ring formation.

Tree-ring cellulose extraction and isotopic analysis

The stable isotope composition of tree rings formed between 1967 and 2007 was measured with annual resolution on cellulose extracted from latewood of individual trees (5 trees per stand type). Latewood was carefully split from earlywood under a stereomicroscope. Earlywood was not analysed for stable isotopes due to possible influence of compounds formed during the previous year (Hill *et al.* 1995; Robertson *et al.* 1996). Latewood of the same year from 2 cores per tree was pooled together. There was not enough wood to conduct isotopic analyses for years 1994, 1995 and 2003.

The cellulose was extracted with a double step digestion, with a 5% NaOH solution at 60 °C for 2 h followed by a 7% NaClO₂ + acetic acid solution at 60 °C for a minimum of 36 h (Rinne *et al.* 2005; Boettger *et al.* 2007; Battipaglia *et al.* 2008).

Isotopic analyses were conducted at the Stable Isotope Facility at Paul Scherrer Institut (Switzerland). $\delta^{18}\text{O}$ was measured using a continuous-flow pyrolysis system (Saurer *et al.* 1998). $\delta^{13}\text{C}$ was determined using an elemental analyser linked to an isotopic ratio mass spectrometer (MS, Delta S; Finnigan Mat, Bremen, Germany) via a ConFlo II interface (Finnigan Mat). Isotopic compositions are expressed in delta notation (‰) relative to an accepted reference standard: Vienna PeeDee belemnite for carbon isotope values and Vienna Standard Mean Ocean Water (VSMOW) for oxygen isotope values. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was better than 0.1‰ for carbon and better than 0.3‰ for oxygen.

Data provided by Francey *et al.* (1999) and McCarroll & Loader (2004) were used to remove the decline in the $\delta^{13}\text{C}$

of atmospheric CO₂ due to fossil fuel emissions from the carbon isotope data series. The corrected series were then employed in all the statistical analyses.

Stem water content and isotopic composition

Stem water from lignified twigs collected in April 2010 was extracted using a cryogenic vacuum distillation line (Ehleringer, Roden & Dawson 2000), and stem water content was calculated gravimetrically. 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 $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{stem water}}$) was conducted at the Center for Stable Isotope Biogeochemistry, University of California-Berkeley (USA), by equilibrium of a 0.2 mL sample of stem water with an atmosphere of 0.2% of CO₂ for 48 h at room temperature (21–23 °C), using a continuous-flow isotope ratio mass spectrometer (Finnigan MAT Delta Plus XL; Thermo Instruments Inc., Bremen, Germany). The long-term external precision was $\pm 0.12\text{‰}$. $\delta^{18}\text{O}$ values are expressed in delta notation (‰) relative to the international standard VSMOW.

Gas exchange and water potential measurements

Net photosynthetic rate (A) and stomatal conductance (g_s) were measured in April 2010 with a portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA) equipped with a LI-6400-40 Leaf Chamber Fluorometer and a Li-Cor 6400-01 CO₂ injector. *P. halepensis* trees show maximum physiological activity during spring (Maseyk *et al.* 2008), so leaf gas exchange measurements were taken in April when differences between stand types are expected to be greatest. Gas exchange was measured on 1-year-old, fully sun-exposed needles from intact, attached (non-excised) shoots from the low-middle part of the tree crown (approximately 2 m height). Approximately 20 attached needles were placed in a 2 cm² leaf cuvette for gas exchange measurements. The CO₂ concentration in the cuvette was maintained at 380 $\mu\text{mol mol}^{-1}$ CO₂. Measurements were done at saturating light of 1.500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and at ambient air temperature and relative humidity. The leaf-to-air water vapour pressure difference ranged between 0.75 and 1.2 mmol mol⁻¹ for all measurements, and the air flow was set to 350 $\mu\text{mol s}^{-1}$. All leaf gas exchange measurements were conducted at mid-morning between 0900 and 1100 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 analysed 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 cm²) than the area of the leaf cuvette (2.00 cm²). All gas exchange parameters were expressed on a total needle surface area basis. WUE_i was calculated as A/g_s.

Stem water potential was measured at midday with a pressure chamber device (Scholander *et al.* 1965) in 3 small twigs per tree. The twigs had been previously covered with aluminium foil and enclosed in plastic bags to prevent transpiration.

Enhanced vegetation index at the stand level

We obtained enhanced vegetation index (EVI) values for each stand type from the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite, and used them as a surrogate of stand transpiration. EVI was extracted from the MOD13Q1 land product which represents 16 d composites of EVI values for a pixel size of 250 m. Data for each stand type from October 2000 to September 2007 were downloaded using the 'MODIS Global Subsets: Data Subsetting and Visualization' tool at the ORNL-DAAC (<http://daac.ornl.gov/>). In drylands, EVI has been shown to be well correlated with structural vegetation properties (e.g. leaf area index, fractional projective cover) and physiological processes directly related to photosynthetically active radiation (PAR) absorption by vegetation (e.g. photosynthesis and transpiration; Glenn *et al.* 2008; Contreras *et al.* 2011).

Meteorological data

Meteorological data were provided by the AEMET. Monthly values of mean (T), maximum (T_{max}) and minimum (T_{min}) temperatures and precipitation from 1967 to 2007 were obtained from the 'Embalse de Santomera' meteorological station (38°05' N, 1°05' W, 90 m asl), located near the sampling sites (<5 km). Missing data were obtained by simple linear regression with the nearby meteorological stations of 'Santomera' (38°03' N, 1°02' W, 36 m asl) and 'Murcia-Alfonso X' (37°59' N, 1°07' W, 90 m asl). Atmospheric VPD was calculated using the model of Ferrio & Voltas (2005) for the Mediterranean region. From monthly meteorological data, we calculated seasonal values (three month periods: January–March, April–June, July–September, October–December), annual values (January–December) and values for the hydrological year (from October of the previous year to September of the current year, oct-Sept) of every measured variable (T, T_{min}, T_{max}, P, VPD).

Statistical analyses

All statistical analyses were performed with SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). The responses of BAI, δ¹³C_{LW} and δ¹⁸O_{LW} from 1967 to 2007 were analysed with a linear mixed-effect model, with stand type as the main effect (fixed factor), years as the variable to identify repeated observations and an ARMA (first-order

autoregressive moving average) covariance structure. The significance of the fixed-effect term was assessed with Wald test and likelihood-ratio test. BAI values were log transformed in order to satisfy assumptions of normality. Individual trees were considered subjects (20 trees for BAI and 10 trees for δ¹⁸O_{LW} and δ¹³C_{LW}). The effect of stand structure on leaf gas exchange parameters and stem water δ¹⁸O (measured in a single year) was tested using Student's *t*-test. A Wilcoxon matched-pairs signed-ranks test was used to compare EVI values between stand types. Simple Pearson correlations were used to examine the relationships between pairs of measured variables (meteorological variables and TRW_{res}, δ¹⁸O_{LW} and δ¹³C_{LW} chronologies) from 1967 to 2007 for each stand type separately. The relationship between A and g_s was assessed across trees from both stand types with simple linear regression.

RESULTS

Tree-ring growth

There was no significant relationship between tree age and mean BAI from 1967 to 2007, either within or across stand types. Mean BAI from 1967 to 2007 was nearly twice larger in the scattered trees from open woodland stands than in trees from dense afforested stands (11.21 ± 0.50 cm² year⁻¹ versus 6.68 ± 0.32 cm² year⁻¹, respectively; *P* = 0.007; Fig. 2c). Radial growth differences between open woodland and afforested stands seem to have decreased over the last few years, but our chronologies are too short to clearly identify long-term trends in this respect. It should be noted that the afforested stands were thinned in the fall of 2004, which thereafter led to enhanced radial growth in the remaining trees due to competition release (see Moreno-Gutiérrez *et al.* 2011). In addition, 1994 and 1995 were extremely dry years in which most sampled trees did not show any detectable radial growth (only 1 tree out of 10 in the afforested stands and 4 trees out of 10 in the open woodland stands showed any detectable growth). This exceptionally severe drought may have damaged the trees in both stand types, thus leading to smaller differences in growth between stand types during subsequent years.

The detrended TRW chronologies (TRW_{res}) of both stand types were strongly correlated with one another (*r* = 0.876; *P* < 0.01) during the period from 1967 to 2007 (see Fig. 2d). High EPS values (Table 1; greater than 0.85) in both types of stands indicate that constructed chronologies from detrended individual TRW series (TRW_{res}) were representative of radial growth variations of the whole population of trees (Wigley, Briffa & Jones 1984). There was also good coherence among individual growth series (high mean *r*-bar; Table 1). The *r*-bar and MS values were higher in the afforested stands than in the open woodland stands (Table 1), thus indicating that in afforested stands there is a stronger common growth signal and greater year-to-year radial growth variability associated to inter-annual changes in climatic conditions. Furthermore, the standard deviation of the residual chronology was larger in afforested than in

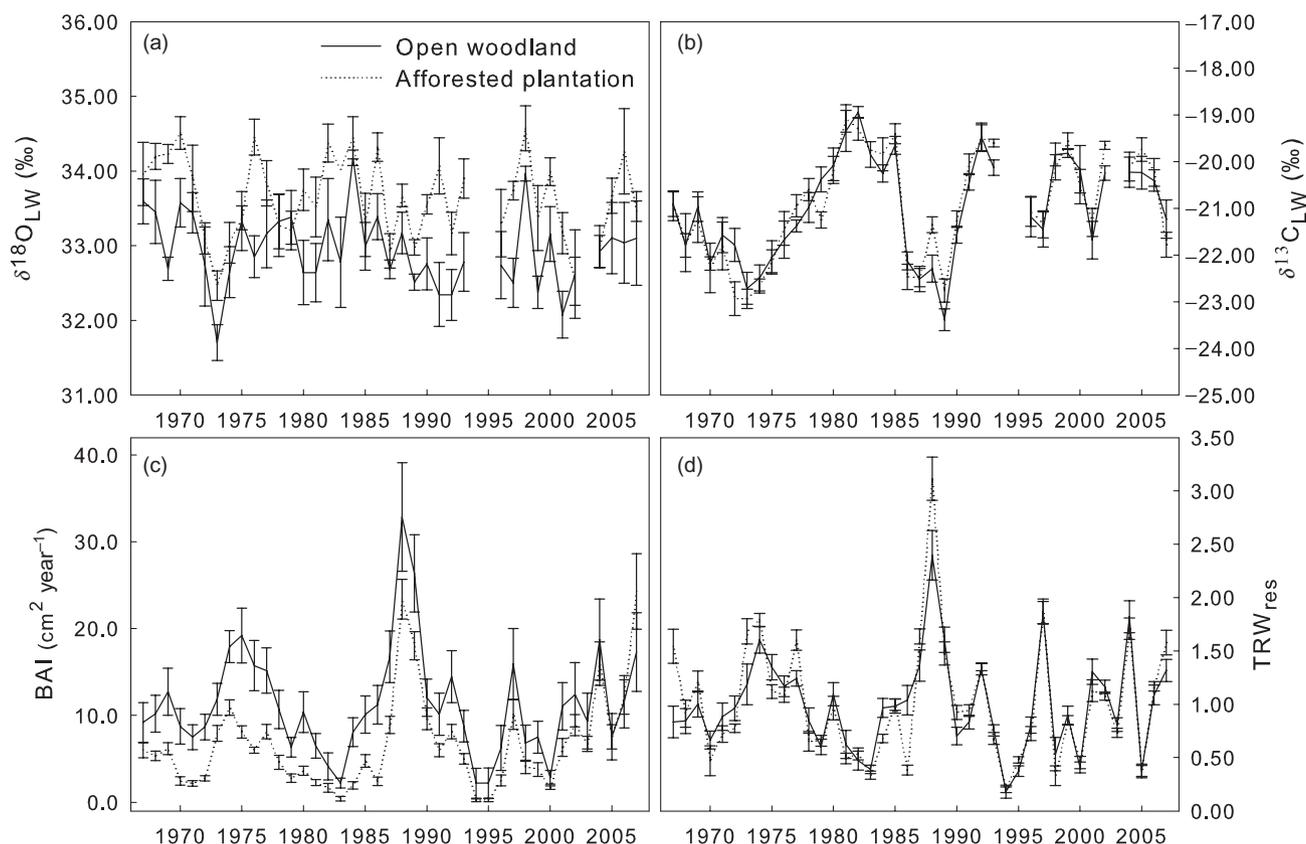


Figure 2. Open woodland and afforested stands mean chronologies of (a) oxygen isotopic composition of latewood ($\delta^{18}\text{O}_{\text{LW}}$, $n = 5$ in each stand type), (b) carbon isotopic composition of latewood ($\delta^{13}\text{C}_{\text{LW}}$, $n = 5$ in each stand type), (c) basal area increments (BAIs, $n = 10$ in each stand type) and (d) residual tree-ring widths (TRW_{res} , $n = 10$ in each stand type) from 1967 to 2007. The afforested stands were thinned in the fall of 2004 (from 1150 trees ha^{-1} to a final density of 770 trees ha^{-1} ; tree density in open woodland stands is less than 20 trees ha^{-1}). $\delta^{13}\text{C}_{\text{LW}}$ values were corrected according to Francey *et al.* (1999) and McCarroll & Loader (2004). Error bars represent ± 1 SE.

open woodland stands (Table 1), suggesting that radial growth responses to extreme climatic events are stronger in the former.

Tree-ring stable isotope composition

Latewood carbon isotope composition chronologies ($\delta^{13}\text{C}_{\text{LW}}$) were tightly coupled between open woodland and afforested stands (Fig. 2b) and were strongly correlated with each other for the period 1967–2007 ($r = 0.932$, $P < 0.01$). Compared with $\delta^{13}\text{C}_{\text{LW}}$, latewood oxygen isotope composition chronologies ($\delta^{18}\text{O}_{\text{LW}}$) showed a significant but weaker correlation between stand types (Fig. 2a; $r = 0.607$, $P < 0.01$, from 1967 to 2007) due to greater variability among the $\delta^{18}\text{O}_{\text{LW}}$ time series of individual trees.

From 1967 to 2007, pines in open woodland stands showed consistently lower $\delta^{18}\text{O}_{\text{LW}}$ values than those in afforested stands (Fig. 2a; mean values were $32.95 \pm 0.18\%$ versus $33.63 \pm 0.18\%$, respectively; $P = 0.022$). By contrast, there was no difference in $\delta^{13}\text{C}_{\text{LW}}$ between both stand types (Fig. 2b).

Within each stand type, correlations of the $\delta^{13}\text{C}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$ chronologies with TRW_{res} chronologies were

significant or marginally significant and of negative sign (Table 2). $\delta^{13}\text{C}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$ chronologies were not significantly correlated with each other within or across stand types (Table 2).

Relationships with climatic variables

Tree-ring chronologies (TRW_{res} , $\delta^{13}\text{C}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$) from both stand types showed similar correlation patterns with meteorological variables for the 1967–2007 period. TRW_{res} was strongly positively correlated with precipitation in both stand types (Figs 3a & 4a). TRW_{res} was particularly strongly correlated with precipitation of the whole hydrological year (from October of the previous year until September of the current year) in both stand types. TRW_{res} was also strongly affected by water availability during spring months, as shown by tight correlations with total precipitation of spring (April–June; Figs 3a & 4a). Relationships of TRW_{res} with temperature and atmospheric VPD were not significant, but they were mainly of negative sign (Figs 3a & 4a). In both stand types, TRW_{res} was marginally negatively correlated with VPD of July (Figs 3a & 4a), thus indicating that tree-ring growth in *P. halepensis* is also influenced by

Table 2. Pearson's correlation coefficients (r) and P -values (in parentheses) of the relationship between the chronologies of residual tree-ring widths (TRW_{res}) and carbon isotopic composition of latewood ($\delta^{13}C_{LW}$), TRW_{res} and oxygen isotopic composition of latewood ($\delta^{18}O_{LW}$), and $\delta^{13}C_{LW}$ and $\delta^{18}O_{LW}$ from 1967 to 2007. $\delta^{13}C_{LW}$ values were corrected according to Francey *et al.* (1999) and McCarroll & Loader (2004). Chronologies from each stand type as well as the averaged chronology across stand types are considered

	TRW_{res} versus $\delta^{13}C_{LW}$	TRW_{res} versus $\delta^{18}O_{LW}$	$\delta^{13}C_{LW}$ versus $\delta^{18}O_{LW}$
Open woodland	-0.503 (0.001)	-0.295 (0.065)	0.135 (0.405)
Afforested plantation	-0.288 (0.072)	-0.406 (0.009)	0.198 (0.220)
Both stand types	-0.398 (0.011)	-0.411 (0.008)	0.177 (0.276)

Significant values ($P < 0.05$) are highlighted in bold.

weather conditions during the summer months. Minimum temperature of January (the coldest month; Fig. 1) was marginally positively correlated with TRW_{res} ($r = 0.300$, $P = 0.057$, $n = 41$ in open woodland stands; $r = 0.299$, $P = 0.058$, $n = 41$ in afforested stands), thus suggesting that mild winter temperatures enhance radial growth in *P. halepensis*.

$\delta^{13}C_{LW}$ was negatively correlated with precipitation, while it was positively correlated with temperature and VPD in both stand types (Figs 3b & 4b). $\delta^{13}C_{LW}$ was strongly correlated with precipitation of the whole current hydrological year (oct-Sept) in both stand types, and also with precipitation during several particular periods within the growing season (best correlations with precipitation of March, September and autumn; Figs 3b & 4b). $\delta^{13}C_{LW}$ was positively correlated with mean VPD during the whole hydrological year, and during several months of spring and autumn (Figs 3b & 4b). In both stand types, $\delta^{13}C_{LW}$ was positively correlated with mean temperature of April (Figs 3b & 4b) and of the whole hydrological year (oct-Sept, although this correlation is only marginally significant in open woodland stands).

Although inter-tree variability in $\delta^{18}O_{LW}$ values was high, the mean $\delta^{18}O_{LW}$ chronologies from both stand types still contained a clear climatic signal, even if correlations with meteorological variables were weaker than for $\delta^{13}C_{LW}$. The $\delta^{18}O_{LW}$ chronologies from both stand types were strongly negatively correlated with precipitation of the whole hydrological year (Figs 3c & 4c). Negative correlations were also found with precipitation of September and summer (July–September) in both stand types (Figs 3c & 4c). VPD of July strongly influenced $\delta^{18}O_{LW}$ in both stand types (positive correlation; Figs 3c & 4c). A positive correlation was also found between $\delta^{18}O_{LW}$ and maximum temperatures of July in afforested ($r = 0.366$, $P = 0.024$, $n = 38$) and open woodland stands ($r = 0.511$, $P = 0.001$, $n = 38$). Paradoxically, $\delta^{18}O_{LW}$ was negatively correlated with VPD ($r = -0.340$, $P = 0.037$, $n = 38$) and T_{max} ($r = -0.379$, $P = 0.019$, $n = 38$) from April to June in the open woodland stands, but not in the afforested stands.

Stem water potential, content, and $\delta^{18}O$ and leaf gas exchange data

Stem water potential at midday was significantly higher ($P = 0.007$) in trees from open woodlands than in those

from afforested stands (Fig. 5d) during peak growing season (April 2010). Trees in open woodlands also had higher stem water contents than those in afforested stands ($51.20 \pm 0.82\%$ and $43.84 \pm 1.04\%$, respectively; $P < 0.001$). The oxygen stable isotope composition of stem water ($\delta^{18}O_{stem\ water}$) differed between plant communities ($P = 0.029$), with more enriched values in the afforested stands ($-6.46 \pm 0.22\%$) than in open woodlands ($-7.12 \pm 0.13\%$).

Stomatal conductance (g_s) was significantly higher ($P = 0.025$) in open woodlands ($0.098 \pm 0.010\ mol\ m^{-2}\ s^{-2}$) than in afforested stands ($0.075 \pm 0.004\ mol\ m^{-2}\ s^{-2}$; Fig. 5a) during peak growing season (April 2010). Photosynthetic activity (A) was also marginally higher in open woodland stands ($7.75 \pm 0.56\ \mu mol\ m^{-2}\ s^{-2}$) than in afforested stands ($6.63 \pm 0.21\ \mu mol\ m^{-2}\ s^{-2}$; $P = 0.094$, $n = 18$; Fig. 5b). A and g_s were strongly positively correlated with one another (Fig. 6) across individuals from both stand types, thus indicating tight stomatal control of A in *P. halepensis*. WUE_i was marginally lower ($P = 0.067$, $n = 18$; Fig. 5c) in the open woodland stands ($80.59 \pm 3.80\ \mu mol\ CO_2/mol\ H_2O$) than in the afforested stands ($89.44 \pm 2.66\ \mu mol\ CO_2/mol\ H_2O$).

Enhanced vegetation index

Mean annual values of EVI were consistently higher ($\approx 30\%$ on average, $P < 0.001$) in the afforested area (0.234 ± 0.005) than in the open woodland (0.178 ± 0.004), with smallest (15.5%) and largest (40.9%) differences between stand types observed during spring and summer, respectively.

DISCUSSION

Major climatic controls on *P. halepensis* ecophysiology

In severely water-limited ecosystems, the physiological performance of *P. halepensis* is strongly dependent on water availability (Ferrio *et al.* 2003; Maseyk *et al.* 2011). Annual TRW_{res} values from both stand types were indeed tightly positively correlated with precipitation of the entire hydrological year (which comprises several months from the previous calendar year; Figs 3a & 4a). The strong influence of precipitation on tree radial growth clearly overwhelms the influence of temperature in this semiarid environment. Furthermore, in both stand types tree-ring

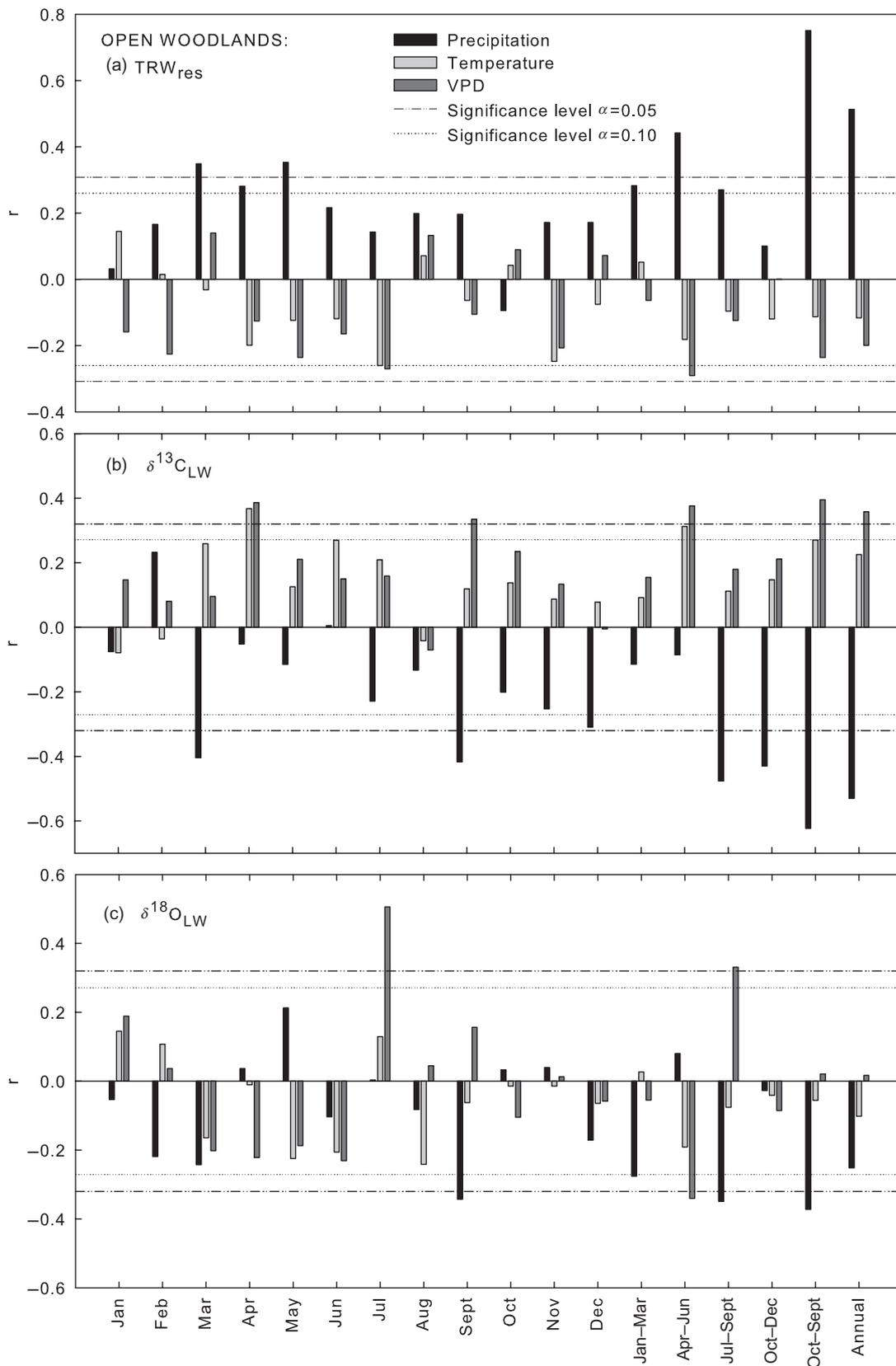


Figure 3. Pearson's correlation coefficients (r) and significance values (dotted lines) for the relationship between monthly/seasonal climatic variables and chronologies of (a) residual tree-ring widths (TRW_{res}), (b) $\delta^{13}C_{LW}$ and (c) $\delta^{18}O_{LW}$ in open woodlands from 1967 to 2007. The $\delta^{13}C_{LW}$ values were corrected according to Francey *et al.* (1999) and McCarroll & Loader (2004). 'oct-Sept' represents the hydrological year, from October of the previous year to September of the current year.

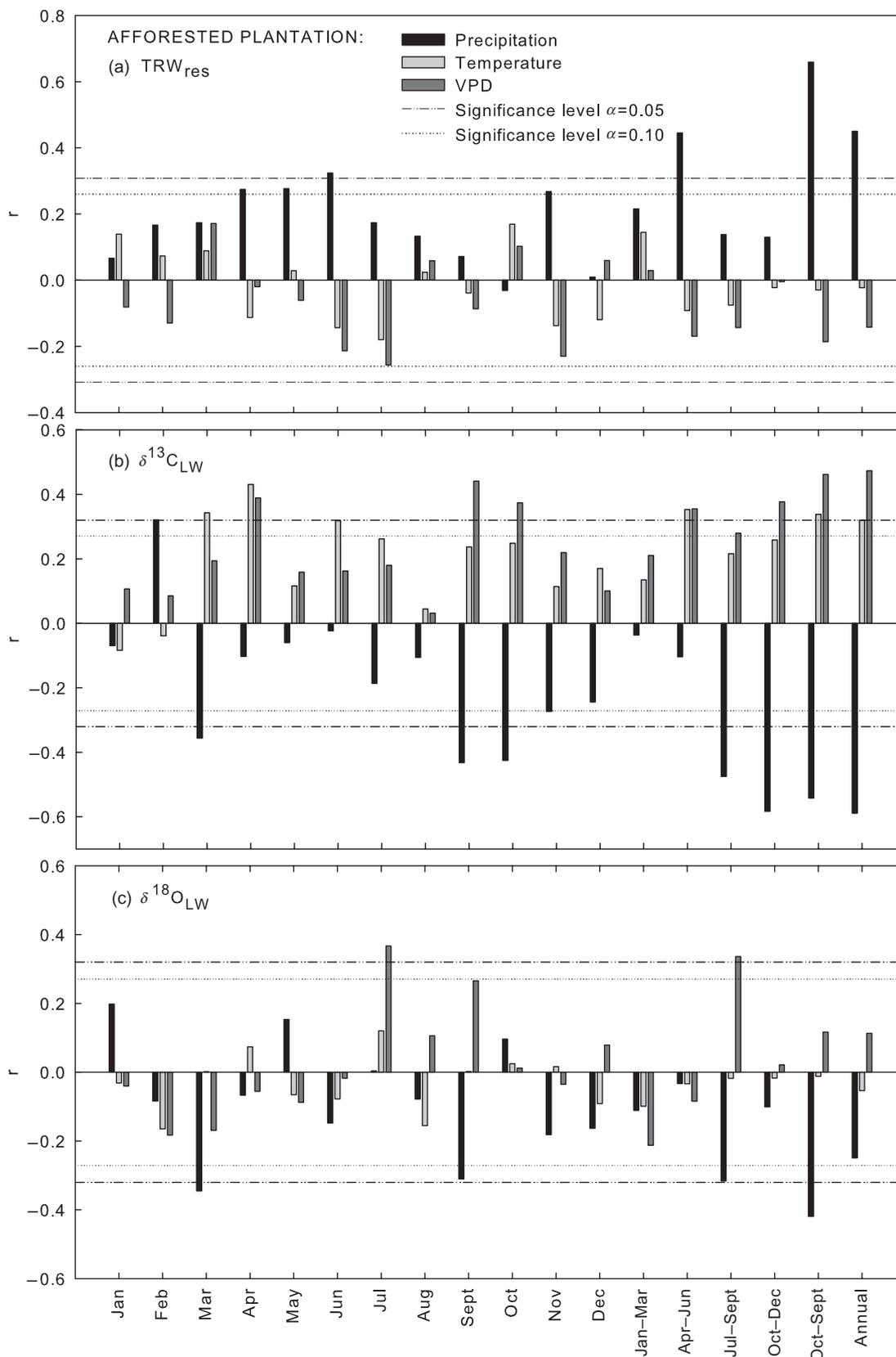


Figure 4. Pearson's correlation coefficients (r) and significance values (dotted lines) for the relationship between monthly/seasonal climatic variables and chronologies of (a) residual tree-ring widths (TRW_{res}), (b) $\delta^{13}C_{LW}$ and (c) $\delta^{18}O_{LW}$ in afforested stands from 1967 to 2007. The $\delta^{13}C_{LW}$ values were corrected according to Francey *et al.* (1999) and McCarroll & Loader (2004). 'oct-Sept' represents the hydrological year, from October of the previous year to September of the current year.

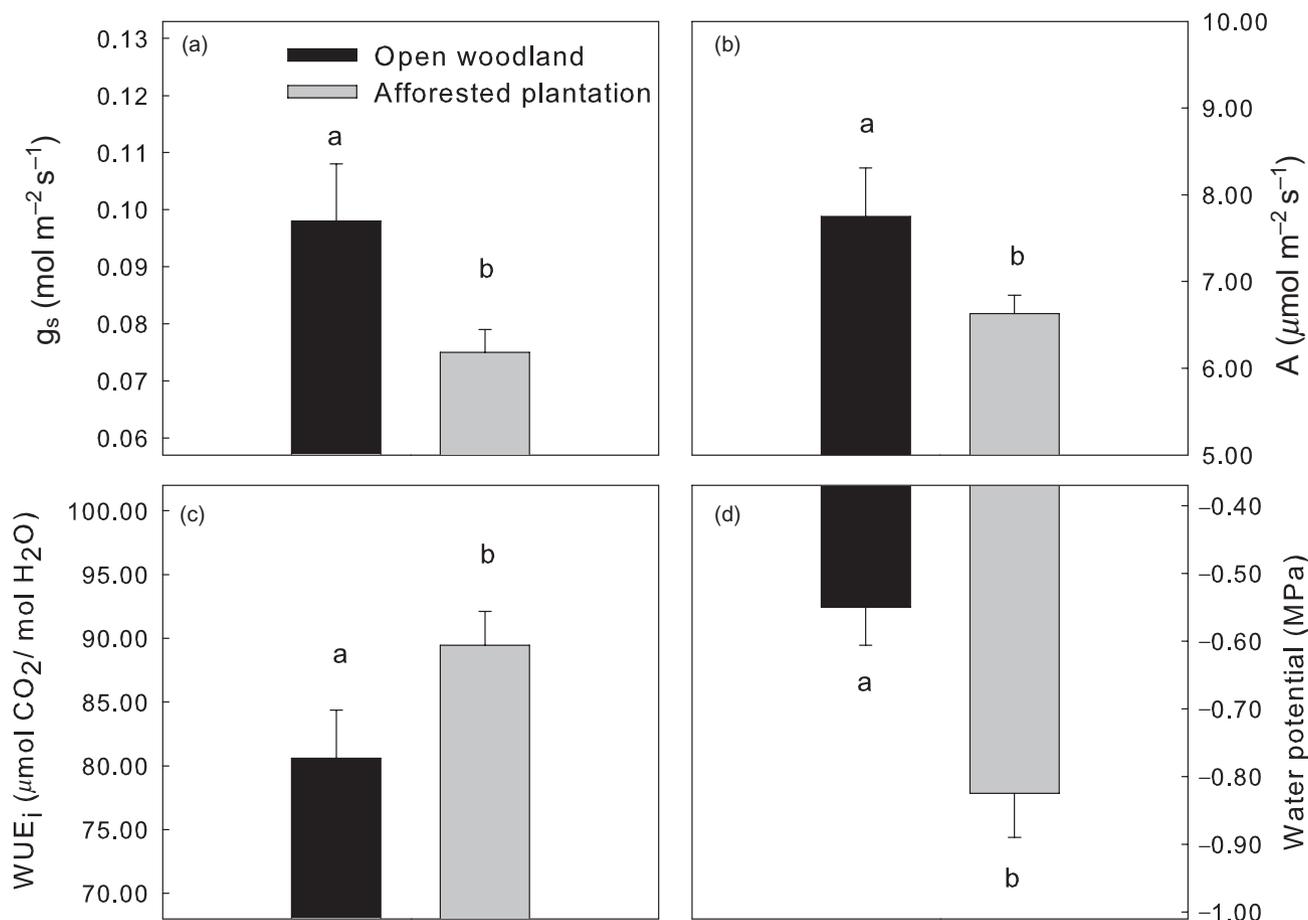


Figure 5. Mean values of (a) stomatal conductance (g_s), (b) net photosynthetic rate (A), (c) intrinsic water use efficiency (WUE_i) and (d) midday stem water potential of *Pinus halepensis* trees from contrasting stand types ($n = 8$ in open woodland stands and $n = 10$ in afforested stands). Differences in g_s and stem water potential between stand types were significant at $P < 0.05$; differences in A and WUE_i between stand types were marginally significant at $P < 0.1$. Error bars represent 1 SE.

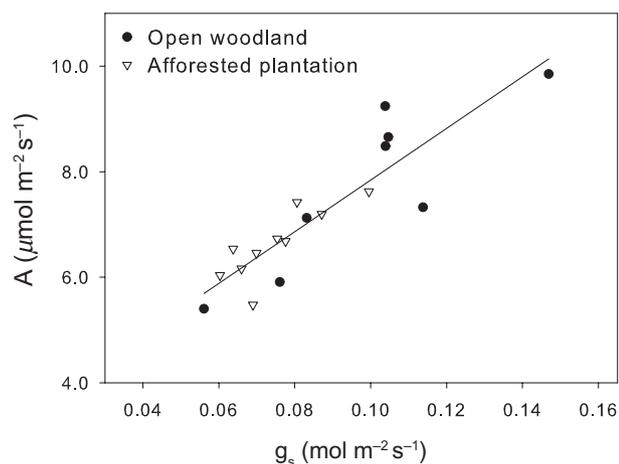


Figure 6. Relationship between net photosynthetic rate (A) and stomatal conductance (g_s) in *Pinus halepensis* trees across stand types ($r = 0.892$, $P < 0.01$, $r^2 = 0.796$; $n = 18$; $A = 2.95 + 48.88g_s$).

$\delta^{13}C_{LW}$ and $\delta^{18}O_{LW}$ were strongly influenced by inter-annual rainfall variability, and both $\delta^{13}C_{LW}$ and $\delta^{18}O_{LW}$ were in turn negatively correlated with TRW_{res} (Table 2). Overall, these relationships indicate that radial growth of *P. halepensis* trees in this semiarid environment shows a strong positive response to increased rainfall due to enhanced stomatal conductance (g_s) and photosynthesis (A) during wet periods.

In both stand types, TRW_{res} was positively correlated with precipitation from April to June (Figs 3a & 4a), thus suggesting that radial growth in *P. halepensis* is largely determined by earlywood formation during the wet spring months. $\delta^{13}C_{LW}$ and $\delta^{18}O_{LW}$ were strongly correlated with climatic conditions during March and September, as well as with conditions during the entire autumn (September–December; Figs 3b,c & 4b,c), which is the time when latewood (the wood fraction that was analysed for stable isotopes) is laid down in *P. halepensis* (De Luis *et al.* 2007). In a nearby location, De Luis *et al.* (2007) found that *P. halepensis* trees had two main growing periods starting in March and September, respectively. Similar seasonal growth patterns have been reported for *P. halepensis* by

Klein *et al.* (2005), De Luis *et al.* (2009), Sánchez-Salguero *et al.* (2010) and Camarero, Olano & Parras (2010).

In semiarid environments, evaporative enrichment of leaf water is expected to dampen the $\delta^{18}\text{O}$ signal of precipitation in tree rings of *P. halepensis* (Ferrio & Voltas 2005). $\delta^{18}\text{O}_{\text{LW}}$ was indeed negatively correlated with rainfall amount in both stand types (Figs 3c & 4c), thus indicating that $\delta^{18}\text{O}_{\text{LW}}$ values reflect changes in stomatal conductance in response to changes in water availability. Tree-ring $\delta^{18}\text{O}_{\text{LW}}$ was positively correlated with VPD of July, thus showing that factors other than low soil water availability (like high VPD) can also decrease A and g_s in *P. halepensis* during dry periods (Klein *et al.* 2005). In both stand types, VPD and temperature were positively correlated to $\delta^{13}\text{C}_{\text{LW}}$ as well, suggesting increased WUE_i due to greater plant physiological stress and reduced stomatal conductance during dry and hot periods.

Despite similar correlation patterns with climatic variables in both stand types, some noteworthy differences suggest that pines in afforested stands are more prone to water shortage than pines in open woodlands. Firstly, the year-to-year variability of TRWs was larger in the afforested plantation (higher MS; Table 1), thus indicating that these pines may rely more heavily on current year rainfall than those in open woodland stands. Secondly, TRW was more strongly correlated with $\delta^{18}\text{O}_{\text{LW}}$ in afforested than in open woodland stands (Table 2), which suggests greater growth dependence on stomatal responses to fluctuations in water availability in the former. Thirdly, during the exceptionally severe drought of 1994–1995, fewer trees showed detectable radial growth in afforested stands than in open woodlands (1 out of 10 versus 4 out of 10, respectively). Fourthly, the paradoxical negative correlation between $\delta^{18}\text{O}_{\text{LW}}$ and VPD from April to June in the open woodlands (but not in afforested stands) indicates greater increases in stomatal conductance (that would cause lower isotopic enrichment of leaf water through an enhanced ‘Péclet effect’; Barbour 2007), and/or greater access to deeper, isotopically depleted water sources (Barbour 2007) during periods of high transpirational demand in spring.

Differences in tree physiological status between stand types

Widely scattered trees in open woodland stands showed consistently lower $\delta^{18}\text{O}_{\text{LW}}$ values than trees in dense afforested stands during the entire period from 1967 to 2007 (Fig. 2a), regardless of large inter-annual climate variability during this 40 year period. Lower $\delta^{18}\text{O}_{\text{LW}}$ values suggest higher stomatal conductance (g_s) in the open woodland stands (Barbour 2007), which is well supported by leaf gas exchange data showing higher g_s values in trees of open woodland stands than in those of afforested stands during peak growing season (Fig. 5a). Higher stem water potential and content in open woodlands than in afforested stands further support that *P. halepensis* trees are considerably less water stressed in the former stand type. Differences in stomatal conductance and in stem water potential and content

between contrasting stand types were quite large in April 2010 despite the thinning conducted in 2004 in the afforested stands, which led to substantial competition release for the remaining trees (Moreno-Gutiérrez *et al.* 2011).

In sharp contrast to $\delta^{18}\text{O}_{\text{LW}}$, there was no significant difference in tree-ring $\delta^{13}\text{C}_{\text{LW}}$ between stand types during the period between 1967 and 2007 (Fig. 2b), thus suggesting that pines in both systems had roughly similar WUE_i (Farquhar *et al.* 1989). Based on the dual isotope model developed by Scheidegger *et al.* (2000) and Grams *et al.* (2007), no difference in $\delta^{13}\text{C}_{\text{LW}}$ combined with lower $\delta^{18}\text{O}_{\text{LW}}$ values in open woodland than in afforested stands indicates greater g_s and A in the former. Again, this interpretation of isotopic data is well supported by both leaf gas exchange and tree radial growth data (Figs 5 & 2c, respectively). Furthermore, the strong positive correlation found between A and g_s across trees from both stand types (Fig. 6) is evidence of tight stomatal control of carbon assimilation rate in *P. halepensis*.

Large differences in the degree of inter-tree competition for water between stand types can explain the lower stem water potential and content, lower stomatal conductance and poorer radial growth of trees in dense afforested stands compared with those in neighbouring open woodland stands. The widely scattered distribution of trees in open woodland stands resulted in lower stand level transpiration compared with dense afforested stands as indicated by lower EVI values in the former (Glenn *et al.* 2008; Contreras *et al.* 2011), thus allowing for a more complete recharge of the soil profile that permitted higher leaf-level stomatal conductance in open woodland stands. Similar results were found in a previous study that compared the physiological performance *P. halepensis* between contrasting stand densities after thinning application (Moreno-Gutiérrez *et al.* 2011), which further supports the notion that differences in tree water status between afforested and open woodland stands are related to differences in tree density.

Similar $\delta^{13}\text{C}_{\text{LW}}$ values between afforested and open woodland stands suggest that *P. halepensis* maintains in the long term a homeostatic control of the ratio c_i/c_a (the intercellular to atmospheric CO_2 concentration, which determines $\delta^{13}\text{C}_{\text{LW}}$ and WUE_i) as an acclimation response to chronic water shortage (McDowell *et al.* 2006; Maseyk *et al.* 2011). In the afforested stands, the ‘setpoint’ for c_i/c_a was accomplished by down-regulation of A through tight stomatal control of transpiration (indicated by higher $\delta^{18}\text{O}_{\text{LW}}$ and lower g_s in pines of the afforested stands; Figs 2a & 5a). However, the complicating effects of density-dependent disparities in light and nutrient availability between contrasting stand types (Dawson *et al.* 2002) might have also contributed to the lack of difference in $\delta^{13}\text{C}_{\text{LW}}$ between them. In April 2010, instantaneous leaf gas exchange measurements showed marginal differences in WUE_i between stand types (Fig. 5), thus revealing the occurrence of transient differences in water use efficiency between stand types that were not detected at the annual time scale of $\delta^{13}\text{C}_{\text{LW}}$ values. Several authors have reported that there is a dampening of the carbon and oxygen isotopic signal of soluble

carbohydrates during phloem loading and transport from the leaves to the trunk and/or during heterotrophic cellulose synthesis (Klein *et al.* 2005; Gessler *et al.* 2009; Offermann *et al.* 2011), which can lead to discrepancies between instantaneous leaf gas exchange measurements and the stable isotope composition of tree rings.

In addition to lower g_s , consistently less enriched $\delta^{18}\text{O}_{\text{LW}}$ values in open woodland compared with afforested stands might also be related in part to more depleted source water $\delta^{18}\text{O}$ in the former. As no fractionation occurs during water uptake by plants (Dawson *et al.* 2002), lower $\delta^{18}\text{O}_{\text{stem water}}$ indicated less evaporatively enriched source water for trees in open woodland stands than in afforested stands. As upper soil layers dry up faster in open woodland than in dense afforested stands (Raz-Yaseef, Rotenberg & Yakir 2010), pines in open woodlands may be forced to rely more heavily on water stored in deeper soil layers, which tends to have lower $\delta^{18}\text{O}$ than water from upper soil because evaporative isotopic fractionation decreases with soil depth (Dawson *et al.* 2002).

In conclusion, tree-ring growth and $\delta^{18}\text{O}_{\text{LW}}$, but not $\delta^{13}\text{C}_{\text{LW}}$, are affected by stand structure in this severely water-limited ecosystem. TRW_{res} , $\delta^{18}\text{O}_{\text{LW}}$ and $\delta^{13}\text{C}_{\text{LW}}$ are strongly correlated with total annual rainfall in both afforested stands and open woodlands. However, tree-ring growth and $\delta^{18}\text{O}_{\text{LW}}$ analysis revealed that widely scattered pines in open woodland stands are consistently less water stressed than those in dense afforested stands. Trees in afforested stands experience more severe water shortage due to intense inter-tree competition for soil moisture, and therefore may be more vulnerable to climatic drought than trees in open woodland stands. In this semiarid ecosystem, the benefits of negligible inter-tree competition for water in open woodland stands clearly outweigh potential benefits that canopy closure might provide to trees in dense afforested stands (e.g. enhanced run-off infiltration and retention, reduced evaporation of soil water due to shading, more mesic microclimate, etc.). In view of the projected increases in the frequency and duration of drought in the Mediterranean basin (Giorgi & Lionello 2008), these results have important implications for the management of *P. halepensis* plantations, which currently cover thousands of hectares in the region. Silvicultural thinning aimed at reducing inter-tree competition for water may alleviate drought stress in the remaining trees, and may help mitigate the adverse impacts of climate aridification on dense conifer plantations. Our results indicate that the influence of stand structure on tree vulnerability to climatic drought should be taken into account when designing afforestation and silvicultural management strategies for drylands, in order to foster the long-term sustainability of semiarid conifer woodlands under projected climate change scenarios.

ACKNOWLEDGMENTS

We would like to thank Martín De Luis, Emilia Gutiérrez, Fritz Schweingruber, Simcha Lev-Yadun, Andrea Seim and María José Espinosa for their help with this project, and

Paul Brooks for isotopic analyses conducted at the University of California-Berkeley. This study was supported by the Spanish Ministry of Science and Innovation (Grants AGL2006-11234 and CSICintramural 200940I146). C.M.-G. and S.C. acknowledge a FPI predoctoral fellowship and a 'Juan de la Cierva' postdoctoral fellowship, respectively, both funded by the Spanish Ministry of Science and Innovation.

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Received 23 September 2011; received in revised form 17 November 2011; accepted for publication 20 November 2011