

Pine afforestation decreases the long-term performance of understorey shrubs in a semi-arid Mediterranean ecosystem: a stable isotope approach

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Summary

1. Plant–plant interactions shape the structure and composition of plant communities, but shifts in interaction outcomes might occur in the face of ongoing climate change.
2. We assessed the influence of *Pinus halepensis* plantations on the long-term ecophysiological performance of understorey vegetation, by conducting a retrospective comparison (1989–2007) of growth-ring widths, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between *Rhamnus lycioides* shrubs from two contrasting vegetation types: *P. halepensis* plantations vs. open woodlands. We also measured the leaf $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}$, and nutrient concentrations of understorey *R. lycioides* shrubs growing at varying distances from planted trees within pine plantations.
3. Dendroecological and stable isotope data revealed strong competitive effects of planted *P. halepensis* trees on *R. lycioides*. Shrubs in pine plantations showed lower radial growth and higher growth ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ than those in open woodlands, indicating lower stomatal conductance and photosynthesis in the former. The strong competitive effects of *P. halepensis* on understorey *R. lycioides* were most evident in wet, productive years. Conversely, in very dry years, there were indications of a facilitative effect of planted *P. halepensis* canopies on understorey shrubs.
4. Within pine plantations, understorey *R. lycioides* shrubs growing at shorter distances from planted trees were forced to rely on more superficial and ephemeral soil water sources, which reduced their stomatal conductance (higher leaf $\delta^{18}\text{O}$) and interfered with nutrient uptake (lower leaf N and P concentrations and more negative $\delta^{15}\text{N}$).
5. The intrinsic water use efficiency of *R. lycioides* shrubs growing in open woodlands has increased during recent decades as a result of their ability to adjust their stomatal conductance in response to increasing temperature and atmospheric CO_2 . However, this adaptive response was much weaker or absent in the severely drought-stressed shrubs from pine plantations.
6. Pine afforestation strongly reduces water and nutrient availability for understorey shrubs in drylands, with potential long-term consequences for ecosystem biodiversity, structure and functioning. Competition by *P. halepensis* on *R. lycioides* clearly outweighed facilitation in the long-term, thus compromising the ability of understorey shrubs in semi-arid pine plantations to cope with climate change.

Key-words: carbon isotopic composition, competition, dual stable isotope, intrinsic water use efficiency, *Pinus halepensis*, plant–plant interactions, radial growth rings, *Rhamnus lycioides*, water stress

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Introduction

Plant–plant interactions are key forces structuring vegetation assemblages and determining the composition of plant communities (Fowler 1986). Competition and facilitation can occur simultaneously, giving rise to complex interactions that may have variable outcomes depending among other factors on plants life stage and density, on the severity of climatic conditions and on indirect interactions with other species (Callaway & Walker 1997).

In semi-arid environments, below-ground competition for limiting resources like water and nutrients is particularly intense (Fowler 1986). However, according to the stress gradient hypothesis (Bertness & Callaway 1994), there may be a shift towards increasing frequency of positive plant–plant interactions in environments with severe abiotic stress. In water-limited environments, facilitative interactions involving water may occur through hydraulic lift and canopy shading (Prieto, Armas & Pugnaire 2012). Other facilitative interactions include positive impacts of plant species on soil nutrient availability (Temperton *et al.* 2007). Facilitative processes can prevail over the negative effects of competition in semi-arid environments (e.g. Pugnaire, Armas & Valladares 2004), but interactions can shift from facilitation to competition within the same plant community due to variations in water availability across years (Tielbörger & Kadmon 2000), which can be very extreme in arid and semi-arid regions. Resource availability determines the outcome of plant–plant interactions as reported by many studies along abiotic stress gradients (Pugnaire & Luque 2001). The outcome of plant–plant interactions can also change along the different life stages of plant species (Soliveres *et al.* 2010). As a consequence, there is a need to understand trends in the net balance between positive and negative plant–plant interactions over long time periods that cover different climatic conditions (and their associated resources availability), and during different plant life stages (Butterfield *et al.* 2010).

This is especially important in the light of climate change, as the perception of environmental severity can be species-specific, and thus, environmental changes can produce shifts in the outcome of plant–plant interactions. Further, filling this knowledge gap has become a priority in semi-arid Mediterranean ecosystems, which are highly vulnerable to predicted increases in temperature and decreases in precipitation (Millennium-Ecosystem-Assessment 2005) that could produce dramatic changes in the composition and biodiversity of plant communities (McCluney *et al.* 2012). A deeper understanding of the physiological processes affected by plant–plant interactions in the long term can help to formulate better predictions about future shifts in plant community composition and structure under changing environmental conditions and associated variations in resources availability.

To do so, stable isotope techniques can be very useful, as the carbon and oxygen isotopic composition of leaves ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively) provides time-integrated

information on leaf gas exchange processes during the growing season, whereas the isotopic composition of radial growth rings can provide long-term records of plant physiological processes. In C_3 plants, $\delta^{13}\text{C}$ is a good proxy of leaf-level intrinsic water use efficiency (WUE_i), which is given by the ratio between net photosynthetic rate (A) and stomatal conductance (Farquhar, Ehleringer & Hubick 1989; Dawson *et al.* 2002). Plant $\delta^{18}\text{O}$ is influenced by source water $\delta^{18}\text{O}$, but is also inversely related to the ratio of atmospheric to leaf intercellular water vapour pressure (e_a/e_i), and can thus provide a time-integrated indication of leaf stomatal conductance (g_s) during the growing season (Barbour 2007). Measuring plant $\delta^{18}\text{O}$ can thus help to separate the independent effects of A and g_s on $\delta^{13}\text{C}$ (Scheidegger *et al.* 2000; Ramírez, Querejeta & Bellot 2009; Roden & Farquhar 2012). Few studies have used long-term records of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in growth rings to disentangle the ecophysiological processes underlying the outcome of interspecific competitive or facilitative interactions involving water.

The nitrogen isotope composition of plant material ($\delta^{15}\text{N}$) can also provide insight into plant–plant interactions, as $\delta^{15}\text{N}$ is related to nutrient and water availability (Handley *et al.* 1999). The interpretation of plant $\delta^{15}\text{N}$ is not straightforward, as it can be influenced by a wide array of factors and processes (utilization of different N-sources, mycorrhizal associations, spatial and temporal variations in N availability and demand, among others; Höggberg 1997). However, several studies have reported consistently positive relationships between leaf $\delta^{15}\text{N}$ and leaf N concentration in non- N_2 -fixing plant species at local, regional and global scales (BassiriRad *et al.* 2003; Craine *et al.* 2009).

Pinus halepensis plantations cover extensive areas of the western Mediterranean Region, and are often characterized by a monospecific tree overstorey with a sparse, depauperate and species-poor shrub understorey (Maestre & Cortina 2004). *Rhamnus lycioides* is one of the few woody shrub species capable of colonizing *P. halepensis* plantations in the drier parts of the region. *Rhamnus lycioides* is a medium to large shrub that is dominant in many semi-arid shrublands and open woodlands of the Iberian Peninsula. The presence of *R. lycioides* in the understorey enhances the structural complexity and functional diversity of semi-arid pine plantations, and is also important for vegetation recovery after fire thanks to its resprouting habit. Further, the presence of this shrub species in semi-arid pine plantations can foster forest diversification and understorey development, as *R. lycioides* is a fruit-bearing shrub that is attractive to seed-dispersing birds and other wildlife.

The main goal of this study was to assess the long-term net effects of *P. halepensis* plantations on the performance of the understorey shrub *R. lycioides* L., and to gain insight into the ecophysiological processes involved in this interaction. We also wanted to evaluate how the sign and strength of the interaction are modulated by the high inter-annual climate variability of this semi-arid ecosystem.

Finally, we aimed to investigate whether the physiological adjustment of understorey vegetation to rising atmospheric CO₂ concentration and climate warming might be compromised by the intense competition for soil resources (water and nutrients) in pine plantations. To do so, we compared the growth-rings widths (GRW) and growth-ring oxygen and carbon isotopic composition ($\delta^{13}\text{C}_R$ and $\delta^{18}\text{O}_R$, respectively) of understorey *R. lycioides* shrubs growing in a dense plantation of *P. halepensis* vs. those of shrubs growing in a nearby open woodland with widely scattered *P. halepensis* trees. Within the pine plantation, we also measured leaf isotopic composition (C, O, N) and nutrient (N, P) concentration, as well as stem water content and isotopic composition in *R. lycioides* shrubs growing at varying distances from the planted trees, in 2 years with contrasting rainfall. Based on previous studies (Bellot *et al.* 2004), we hypothesized that competition by *P. halepensis* would exert negative net effects on the long-term water and nutrient status and radial growth of understorey *R. lycioides* shrubs in the forest plantation. However, we also hypothesized that facilitative interactions would prevail in drought years due to the beneficial effects of overstorey canopy shading on shrub water relations. The novelty of this paper is that the temporal depth provided by our combined dendrochronological and isotopic approach allowed us to examine how this trade-off between competition and facilitation has been affected by interannual climate variability and climate change over recent decades.

Materials and methods

STUDY SITES

The climate is semi-arid Mediterranean, with mean annual precipitation of 288 mm and an average annual temperature of 19 °C. The area is characterized by low hills (140–170 m.a.s.l., <20% slopes) and soils are mostly haplic calcisols, with some lithic leptosols (FAO classification).

The experimental area comprises two *P. halepensis* stand types with sharply contrasting structure and Enhanced Vegetation Index (EVI) values (Moreno-Gutiérrez *et al.* 2012a): a dense 60-year-old *P. halepensis* Mill. plantation (with a density of approximately 1150 trees ha⁻¹) and a nearby (<1 km) open woodland with scattered *P. halepensis* trees and a density of less than 20 trees ha⁻¹. Photosynthetically active radiation (PAR) was measured at noon in both stand types during the peak of the growing season (April) with an AccuPAR LP-80 Ceptometer (Decagon Devices Inc., Pullman, WA, USA). PAR was lower in the understorey of the pine plantation stands ($695 \pm 107 \mu\text{mol m}^{-2} \text{s}^{-1}$; $n = 17$) than in the open woodland stands ($2035 \pm 22 \mu\text{mol m}^{-2} \text{s}^{-1}$; $n = 6$).

DENDROECOLOGICAL ANALYSIS

In May 2008, 10 adult individuals of *R. lycioides* L. were sampled in each vegetation type (20 in total). For each vegetation type, shrubs from separate stands (located in different small catchments) were selected for sampling. Stem cross-sections from the main trunk were collected at ground height. Dating was difficult as is commonly found in Mediterranean plant species due to the high presence of missing rings and intra-annual density fluctuations

(Cherubini *et al.* 2003), and it was possible only in eight individuals from open woodland stands and in seven individuals from pine plantation stands. In each stem cross-section, annual growth-ring widths were measured along three radii with a precision of 0.01 mm using 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 slopes intervals with equal sign in two time series) and COFECHA (Holmes 1983). Single-radii ring-width series were cross-dated with the mean of all individual growth series from the same stand type. GLK values were always significant ($P < 0.05$) and higher than 60%. In each stand type, individual growth series were detrended with ARSTAN (Holmes 2001) using a cubic smoothing spline with a 50% frequency response over 25 years and stabilized variance ('Briffa/Osborn' variance adjusted version, computed in ARSTAN; Osborn, Briffa & Jones 1997). 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: Expressed Population Signal (EPS; indicates the level of coherence of the constructed chronology and how it portrays the hypothetical perfect population chronology), r -bar (mean correlation among all possible pairings of individual series within a chronology) and *mean sensitivity* (indicates the degree to which annual ring width changes from year to year and how it is influenced by high-frequency climatic variations). Standardized residual values (GRW_{res}) were used for assessing correlations with climatic data.

In five individuals per stand type, α -cellulose was extracted separately from each annual growth ring formed between 1989 and 2007 to analyse its stable isotope composition. The α -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; Battipaglia *et al.* 2008).

The carbon and oxygen isotopic analyses of cellulose were conducted at the IACT (Granada, Spain) on a Thermo Finnigan Delta Plus XL isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). For oxygen isotopic analysis, cellulose samples were pyrolyzed to CO at 1450 °C in a Thermo Finnigan TC/EA. The precision of the analyses was better than 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{18}\text{O}$. Isotope ratios are expressed in delta notation (‰) relative to the reference standard: V-PDB for $\delta^{13}\text{C}$ and V-SMOW for $\delta^{18}\text{O}$.

Annual intrinsic water use efficiency values (WUE_i) were calculated from the carbon isotopic composition of individual radial growth rings ($\delta^{13}\text{C}_R$) using the model described by Farquhar, O'Leary & Berry (1982):

$$\text{WUE}_i = \frac{c_a(b - \Delta^{13}\text{C})}{1.6(b - a)} \quad \text{eqn 1}$$

with

$$\Delta^{13}\text{C} = \frac{(\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_R)}{(1 + \delta^{13}\text{C}_R)}$$

where $\Delta^{13}\text{C}$ is the photosynthetic discrimination against ¹³C in the atmosphere (‰), $\delta^{13}\text{C}_{\text{atm}}$ is the carbon isotope composition of atmospheric CO₂, c_a is the mean annual atmospheric CO₂ concentration, a is the fractionation during CO₂ diffusion through stomata (4.4‰), and b is the fractionation during carboxylation (27‰). Annual values of c_a and $\delta^{13}\text{C}_{\text{atm}}$ were obtained from Francey *et al.* (1999), McCarroll & Loader (2004) and McCarroll *et al.* (2009). The calculation of WUE_i values removes the effects of

the decline in the $\delta^{13}\text{C}$ of atmospheric CO_2 and the increase in atmospheric CO_2 concentration due to fossil fuel emissions on the carbon isotope ratios of annual growth rings. Thus, WUE_i values were used in all the statistical analyses.

METEOROLOGICAL DATA

Meteorological data were provided by the Spanish National Meteorological Agency (AEMET). Monthly values of mean temperature and precipitation from 1985 to 2007 were obtained from the 'Embalse de Santomera' meteorological station (38°05' N, 1°05' W, 90 m.a.s.l.), 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.a.s.l.) and 'Murcia-Alfonso X' (37°59' N, 1°07' W, 90 m.a.s.l.). Monthly values of relative humidity were obtained from the 'Murcia' meteorological station (38°00' N, 1°10' W, 62 m.a.s.l.). Monthly values of atmospheric vapour pressure deficit (VPD) were calculated as the difference between mean saturation vapour pressure (calculated from air temperature) and actual vapour pressure (derived from relative humidity). We calculated seasonal values (3 months periods: January to March, April to June, July to September, October to December), annual values (January to 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, P, VPD).

INTERSPECIFIC COMPETITION ANALYSIS

Within the *P. halepensis* plantation, sun-oriented leaves from understorey *R. lycioides* shrubs growing at varying distances (10–300 cm) from the nearest pine were sampled at the end of May in 2 years with contrasting meteorological conditions: 2007 ($n = 13$; total precipitation amount and mean temperature from March to May were 125.5 mm and 17.4 °C, respectively) and 2008 ($n = 18$; total precipitation amount and mean temperature from March to May were 78.7 mm and 17.9 °C, respectively). Distance to the nearest pine was recorded as a measure of competition intensity by *P. halepensis* on *R. lycioides*. Bulk leaf samples were oven-dried at 60 °C, finely ground with a ball mill and encapsulated in tin for stable isotope analyses.

In both sampling campaigns, twig sections (approximately 10 mm in diameter and 20 mm long) were collected from adult shrubs for water extraction with a cryogenic vacuum distillation line. Stem water content was calculated gravimetrically.

In May 2007, May 2008 and September 2008, twig sections were collected from a large number of adult *R. lycioides* shrubs (13–21 individuals per stand type), to compare the oxygen isotope composition of stem water between pine plantation and open woodland stands.

Isotope analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California-Berkeley (USA). The carbon and nitrogen isotope ratios of leaf material ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) were analysed using elemental analyzer/continuous flow isotope ratio mass spectrometry (ANCA/SL elemental analyzer coupled with a Finnigan MAT Delta PlusXL IRMS). The oxygen isotope ratio of leaf material ($\delta^{18}\text{O}$) was determined with a Finnigan MAT Delta Plus XL IRMS (Finnigan MAT, Bremen, Germany) following the method described in Farquhar, Henry & Styles (1997) with some adaptations (Moreno-Gutiérrez et al. 2011). For $\delta^{18}\text{O}$ analyses of stem water, 0.2 mL of water samples was equilibrated with an atmosphere of 0.2% of CO_2 for 48 h at room temperature (21–23 °C). The $\delta^{18}\text{O}$ of equilibrated samples was measured using a continuous flow isotope ratio mass spectrometer (Finnigan MAT Delta Plus XL; ThermoFinnigan,

Bremen, Germany) connected to a GasBench II interface (GB, ThermoFinnigan). Isotope ratios are expressed in delta notation (‰) relative to an accepted reference standard: V-PDB for $\delta^{13}\text{C}$, atmospheric N_2 for $\delta^{15}\text{N}$ and V-SMOW for $\delta^{18}\text{O}$. Long-term (3+ year) external precisions for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of leaf material are 0.14, 0.15 and 0.23‰, respectively, and for oxygen isotope analyses of water are 0.12‰.

Foliar phosphorus concentrations were measured at CEBAS-CSIC (Murcia, Spain) by ICP-OES (Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with $\text{HNO}_3:\text{H}_2\text{O}_2$ (4:1, v:v). Foliar nitrogen concentrations were determined with a Thermo Finnigan Flash 1112 elemental analyzer (Franklin, MA, USA).

During each sampling campaign, plant height and crown diameter (measured at two directions) were recorded and plant biovolume was estimated as the volume of a half ellipsoid with those height and diameters.

STATISTICAL ANALYSES

All statistical analyses were performed with SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). The responses of annual growth rings (from 1983 – when all plants were at least 5 year old – to 2007) and of $\delta^{13}\text{C}_R$ and $\delta^{18}\text{O}_R$ (from 1989 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 a first-order autoregressive covariance structure. The significance of the fixed effect term was assessed with Wald test and likelihood-ratio test. Individual trees were considered subjects. Simple Pearson correlations were used to examine the relationships between pairs of measured variables (meteorological variables and GRW_{res} , $\delta^{13}\text{C}_R$ and $\delta^{18}\text{O}_R$ chronologies) for each stand type separately. The relationships between variables measured in understorey *R. lycioides* shrubs and distance to *P. halepensis* were assessed with simple linear regressions.

Results

SHRUB RADIAL GROWTH ANALYSIS

There was no significant relationship between the age of the *R. lycioides* shrubs and their mean growth-ring width from 1983 to 2007, either within or across stand types. The dendrochronological characteristics of the residual mean chronologies standardized with ARSTAN are shown for each stand type in Table 1. The EPS value of the open woodland chronology was very high (0.95), but the EPS value of the radial growth chronology from pine plantation stands was lower (0.77; Table 1). However, the detrended radial growth chronologies from both stand types showed a low, but significant correlation with one another from 1983 to 2007 ($r^2 = 0.20$, $P = 0.025$; see Fig. 1a).

The growth rings of *R. lycioides* shrubs were significantly wider in open woodland stands than in pine plantation stands from 1983 to 2007 ($P < 0.001$; Fig. 1a). Mean growth-ring width for that period was much greater in open woodland stands (0.56 ± 0.03 mm) than in pine plantation stands (0.35 ± 0.03 mm). Stem width differences between stand types increased with time (Fig. 2), especially during favourable wet periods when *P. halepensis* trees in plantation stands also showed high radial increments.

Table 1. Dendrochronological characteristics of the residual mean chronology (calculated with ARSTAN; Holmes 2001) of *Rhamnus lycioides* in open woodland and pine plantation stands

Characteristics	Open woodland stands	Pine plantation stands
Mean shrub age and SE (years) in 2007	49.4 (3.3)	41.3 (3.3)
Mean growth-ring width and SE (mm) 1983–2007	0.56 (0.03)	0.35 (0.03)
Mean Expressed Population Signal (residual)	0.955	0.773
Mean R -bar (residual)	0.513	0.287
Mean sensitivity (residual)	0.299	0.282
Standard deviation (residual)	0.296	0.272
First order partial autocorrelation (residual)	0.110	0.112

CARBON AND OXYGEN STABLE ISOTOPE COMPOSITION OF GROWTH RINGS

The intrinsic water use efficiency values (WUE_i) of *R. lycioides* shrubs (calculated from $\delta^{13}C_R$), were significantly higher in pine plantation stands than in open woodland stands for the period 1989–2007 ($P = 0.026$, Fig. 1b). Mean WUE_i values for that period were $126.7 \pm 3.0 \mu\text{mol mol}^{-1}$ in pine plantation stands, vs. $116.6 \pm 3.0 \mu\text{mol mol}^{-1}$ in open woodland stands.

The WUE_i chronologies from both stand types showed similar fluctuations in the short-term (Fig. 1b) and were significantly correlated with one another from 1989 to 2007 ($P = 0.005$, $r^2 = 0.38$). The WUE_i values of shrubs in the open woodland stands followed an increasing trend with time (Fig. 1b). In the pine plantation stands this increasing trend was only observed during the early years of the series, but disappeared afterwards. As a consequence, the difference between the WUE_i values from both stand types diminished with time (Fig. 1b).

The oxygen isotope composition of growth rings ($\delta^{18}O_R$) in *R. lycioides* showed significantly higher values in the pine plantation stands than in the open woodland stands for the period 1989–2007 ($P < 0.001$, Fig. 1d). Mean $\delta^{18}O_R$ values for that period were $29.15 \pm 0.13\text{‰}$ in the pine plantation stands vs. $28.04 \pm 0.18\text{‰}$ in the open woodland stands. The $\delta^{18}O_R$ chronologies from both stand types were not significantly correlated with one another, and showed an increasing trend with time which was more pronounced in shrubs from open woodland stands.

Annual $\delta^{18}O_R$ values in *R. lycioides* shrubs were significantly positively correlated with annual WUE_i values from 1989 to 2007 in the open woodland stands ($P = 0.005$, $r^2 = 0.38$), but not in the pine plantation stands. Conversely, the WUE_i chronology was negatively correlated with the residual radial growth chronology in the pine plantation stands (from 1989 to 2007, $P = 0.019$, $r^2 = 0.28$), but not in the open woodland stands.

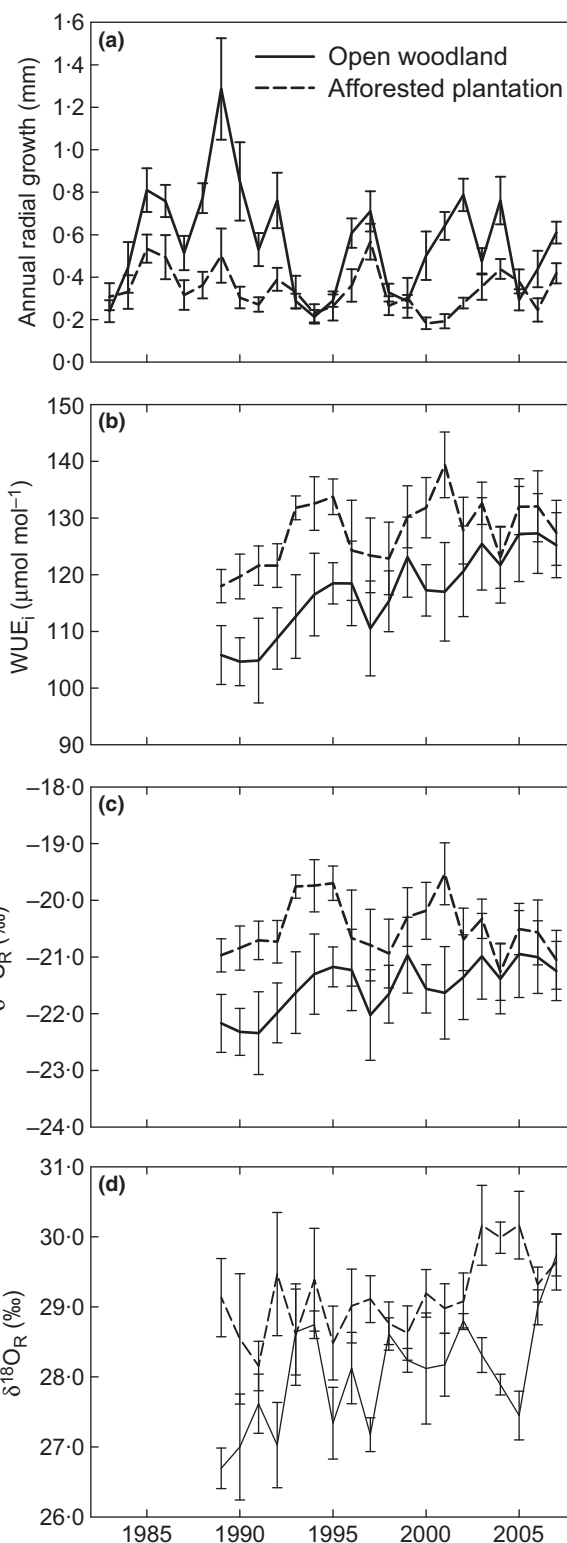


Fig. 1. Open woodland and pine plantation stands mean chronologies of (a) annual radial growth of *Rhamnus lycioides* shrubs from 1983 to 2007 ($n = 8$ in open woodlands and $n = 7$ in plantation stands) and (b) calculated intrinsic water use efficiency, (c) carbon isotopic composition of wood ($\delta^{13}C_R$) and (d) oxygen isotopic composition of wood ($\delta^{18}O_R$) from 1989 to 2007 ($n = 5$ in open woodland stands and $n = 5$ in plantation stands). $\delta^{13}C_R$ values were corrected according to Francey *et al.* (1999) and McCarroll & Loader (2004). Error bars represent ± 1 SE.

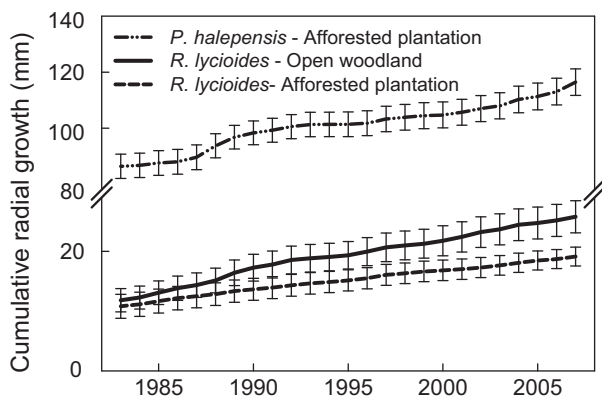


Fig. 2. Average cumulative radial growth of *Rhamnus lycioides* shrubs in open woodland and pine plantation stands. The mean cumulative radial growth of *Pinus halepensis* in plantation stands is also shown (data from Moreno-Gutiérrez *et al.* 2012a). Error bars represent ± 1 SE ($n = 8$ for *R. lycioides* in open woodlands, $n = 7$ for *R. lycioides* in plantation stands, $n = 10$ for *P. halepensis* in afforested stands).

OXYGEN STABLE ISOTOPE COMPOSITION OF STEM WATER IN OPEN WOODLAND VS. PLANTATION STANDS

The oxygen isotopic composition of xylem water in *R. lycioides* was similar between understorey shrubs growing in pine plantations ($-4.67 \pm 0.11\text{‰}$, $n = 17$) and shrubs growing in open woodland areas ($-4.79 \pm 0.24\text{‰}$, $n = 16$) in May 2007. Mean stem water $\delta^{18}\text{O}$ values in *R. lycioides* shrubs were also similar between pine plantation stands ($-4.04 \pm 0.23\text{‰}$, $n = 13$) and open woodland stands ($-4.21 \pm 0.24\text{‰}$, $n = 15$) in September 2008. In contrast, understorey shrubs in dense plantation stands showed more enriched stem water $\delta^{18}\text{O}$ values ($-3.24 \pm 0.16\text{‰}$, $n = 21$) than shrubs growing in neighbouring open woodlands ($-3.99 \pm 0.14\text{‰}$, $n = 20$) in May 2008 ($P < 0.001$).

RELATIONSHIPS OF GROWTH-RING WIDTH AND ISOTOPIC DATA WITH CLIMATIC VARIABLES

The annual radial growth chronologies of *R. lycioides* shrubs in both stand types were similarly correlated with seasonal and annual climatic variables from 1985 to 2007. Relationships with rainfall amounts were of positive sign, whereas relationships with temperature and VPD were of negative sign (Fig. 3a,c). In both stand types, annual radial growth was positively correlated with hydrologic year rainfall amount (P Oct–Sept, Fig. 3a,c), although this correlation was stronger in open woodlands than in pine plantation stands. In addition, in open woodland stands annual radial growth was strongly correlated with the spring rainfall amount of each year. Temperature exerted a stronger influence on the annual radial growth of shrubs in the pine plantation stands, where growth was negatively correlated with temperatures of March and July ($r = 0.49$, $P = 0.018$, and $r = 0.53$, $P = 0.009$, respectively;

$n = 23$ in both cases). In both stand types, annual radial growth was negatively correlated with average annual VPD (Fig. 3a,c).

The WUE_i chronologies derived from annual growth ring $\delta^{13}\text{C}$ data in *R. lycioides* shrubs were negatively correlated with annual rainfall amounts and winter (January–March) rainfall amounts in both stand types (Fig. 3b,d). WUE_i was positively correlated with annual VPD and also with VPD during winter and spring (Fig. 3b,d). WUE_i values were positively correlated with mean spring temperatures in both stand types as well. Only in open woodland stands, WUE_i was positively correlated with mean annual temperatures.

In open woodland stands, the oxygen isotopic composition of annual growth rings ($\delta^{18}\text{O}_R$) was negatively associated with rainfall amount in June ($r = -0.446$, $P = 0.056$, $n = 19$) and positively associated with mean VPD of the hydrological year ($r = 0.454$, $P = 0.051$, $n = 19$) and winter VPD ($r = 0.483$, $P = 0.036$, $n = 19$). In contrast, annual growth ring $\delta^{18}\text{O}_R$ was not significantly correlated with any climatic variable in plantation understorey shrubs.

We calculated the ratio between the annual radial growth of shrubs in pine plantation stands and that in open woodland stands ($\text{GRW}_{\text{plantation}}/\text{GRW}_{\text{open woodland}}$), which provides an index of relative growth differences between vegetation types. This annual growth ratio was negatively related to annual rainfall amount of the hydrological year from 1983 to 2007 (Fig. 4a). Similarly, we calculated the ratio between the $\delta^{18}\text{O}_R$ values of shrubs in the plantation stands and those in the open woodland stands ($\delta^{18}\text{O}_R \text{ plantation}/\delta^{18}\text{O}_R \text{ open woodland}$). This ratio was negatively correlated with mean VPD of the hydrological year from 1989 to 2007 (Fig. 4b).

EFFECTS OF PINE TREE COMPETITION ON THE PERFORMANCE OF UNDERSTOREY RHAMNUS LYCIOIDES SHRUBS WITHIN PLANTATION STANDS

The stem water $\delta^{18}\text{O}$ values of *R. lycioides* shrubs became more enriched with decreasing distance to the nearest pine in both May 2007 and May 2008 (Fig. 5a). During a dry spring (2008), this relationship showed a steeper slope (Fig. 5a) than during a wetter spring (2007). In contrast, stem water content in understorey *R. lycioides* shrubs increased with increasing distance from the nearest pine ($P = 0.006$, $r^2 = 0.52$, $n = 13$) in May 2007. Thus, stem water content and stem water $\delta^{18}\text{O}$ values were negatively associated with each other in May 2007 ($P = 0.034$, $r^2 = 0.35$, $n = 13$). During the dry spring of 2008, all understorey shrubs showed similarly low stem water contents (below 39%), regardless of their distance to the nearest pine.

Leaf P concentration in understorey *R. lycioides* was strongly positively correlated with distance to the nearest pine in May 2007 ($P = 0.002$, $r^2 = 0.61$, $n = 13$). When considering only shrubs growing at less than

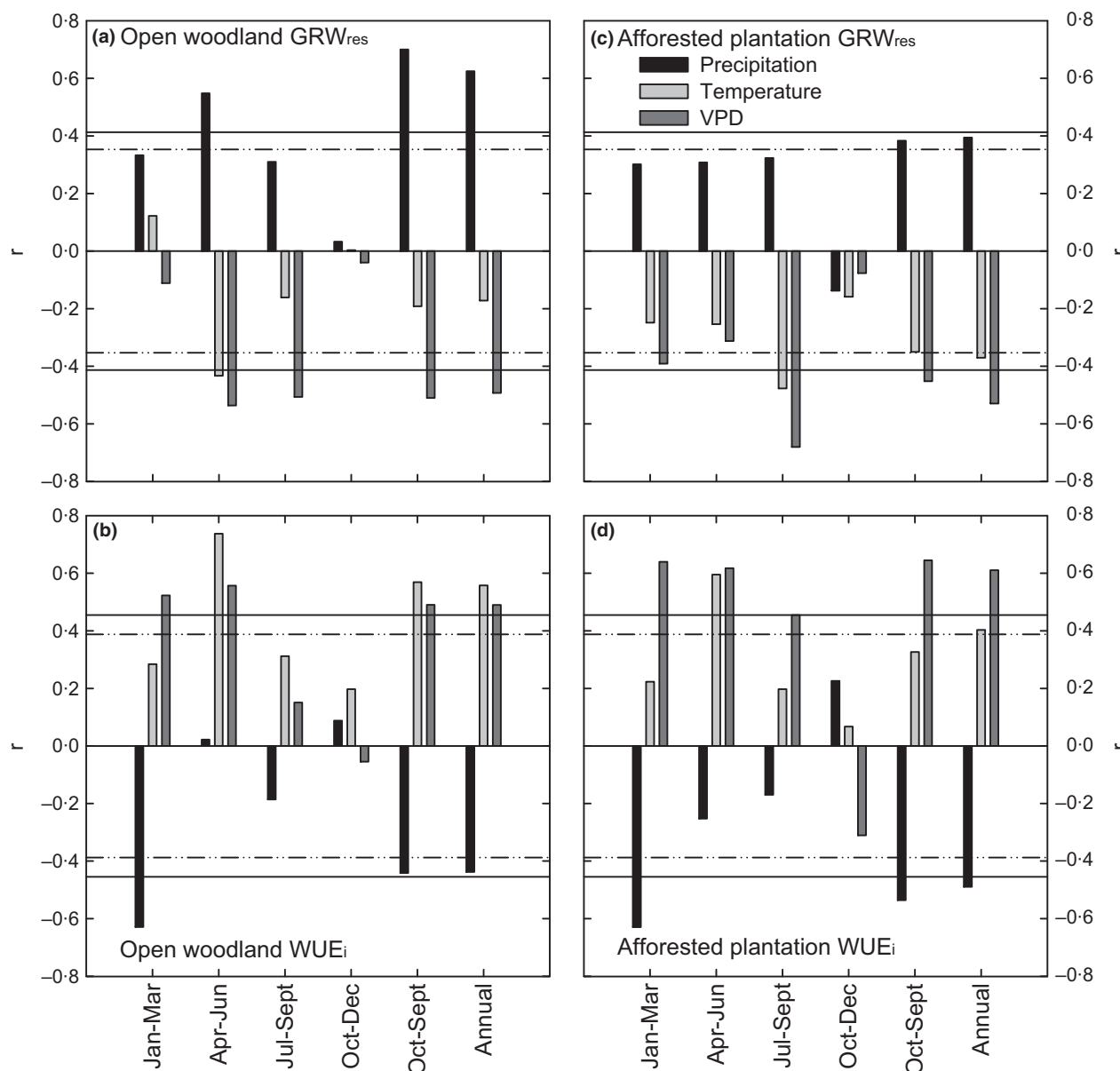


Fig. 3. Pearson's correlation coefficients (r) for the relationship in open woodland stands (a, b) and in pine plantation stands (c, d) between seasonal/annual climatic variables and chronologies of residual growth-ring widths (GRW_{res} ; a, c) and intrinsic water use efficiency (WUE_i ; b, d). 'Oct-Sept' represents the hydrological year, from October of the previous year to September of the current year. Bars over straight lines indicate significant correlations at $\alpha = 0.05$ and bars over dotted lines indicate significant correlations at $\alpha = 0.10$.

200 cm from the nearest pine, leaf N concentration also increased with distance to the nearest pine in May 2007 ($P = 0.015$, $r^2 = 0.59$, $n = 9$). Leaf $\delta^{15}N$ was also positively correlated with distance to the nearest pine (Fig. 5b) and leaf P concentration ($P = 0.004$, $r^2 = 0.55$, $n = 13$ in May 2007 and $P = 0.079$, $r^2 = 0.20$, $n = 16$ in May 2008). In addition, leaf $\delta^{15}N$ was positively associated with leaf N concentration in May 2007 ($P = 0.068$, $r^2 = 0.27$, $n = 13$). Stem water content was positively correlated with leaf $\delta^{15}N$ ($P = 0.001$, $r^2 = 0.65$, $n = 13$) and leaf P ($P = 0.009$, $r^2 = 0.48$, $n = 13$) and N concentrations ($P = 0.034$, $r^2 = 0.29$, $n = 13$) in May 2007.

The leaf $\delta^{18}O$ of *R. lycioides* shrubs was negatively correlated with distance to the nearest *P. halepensis* tree in May 2007 ($P = 0.067$, $r^2 = 0.30$, $n = 12$), but leaf $\delta^{13}C$ was not. Leaf $\delta^{18}O$ was unrelated to stem water $\delta^{18}O$, but was negatively related to leaf P concentration ($P = 0.047$, $r^2 = 0.34$, $n = 12$). In May 2008, leaf $\delta^{13}C$ and $\delta^{18}O$ of *R. lycioides* shrubs did not change with distance to the nearest pine, but leaf $\delta^{13}C$ was strongly related to plant nutrient status, as it correlated positively with both leaf $\delta^{15}N$ ($P = 0.016$, $r^2 = 0.35$, $n = 16$) and leaf P concentration ($P = 0.011$, $r^2 = 0.34$, $n = 18$).

The size (canopy bio-volume) of understorey *R. lycioides* adult shrubs was positively correlated with distance to the

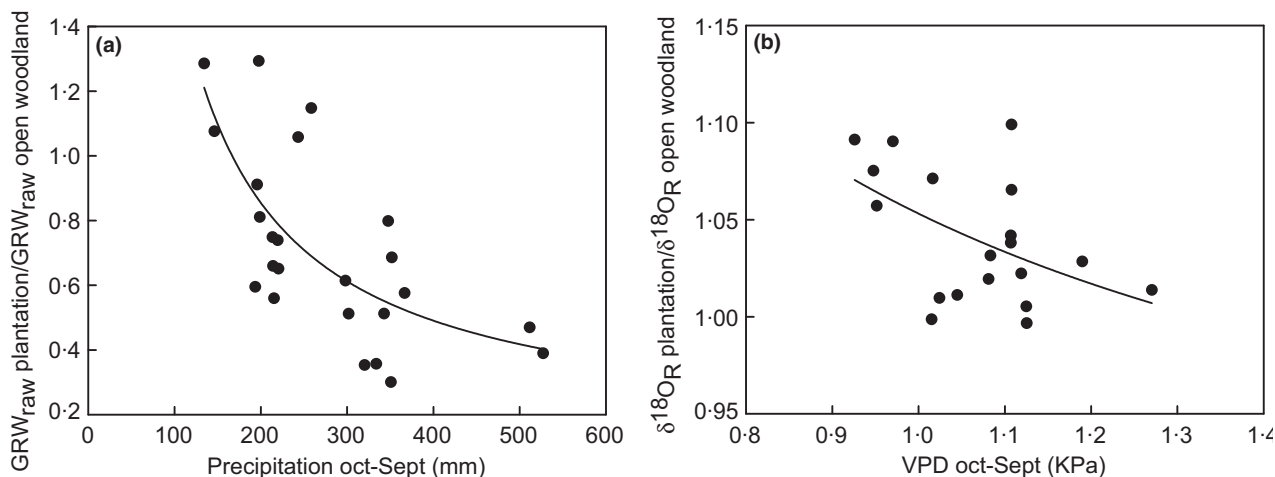


Fig. 4. Inverse first-order relationship between (a) the ratio of mean annual radial growth of *Rhamnus lycioides* shrubs in pine plantation stands to that in open woodland stands, and precipitation of the hydrological year (from October of the previous year to September of the current year) ($P < 0.001$, $r^2 = 0.50$, $n = 24$, 1 outlier excluded) and (b) the ratio of mean annual $\delta^{18}\text{O}_R$ of *R. lycioides* shrubs in plantation stands to that in open woodland stands, and atmospheric vapour pressure deficit (VPD) of the hydrological year ($P = 0.030$, $r^2 = 0.25$, $n = 18$).

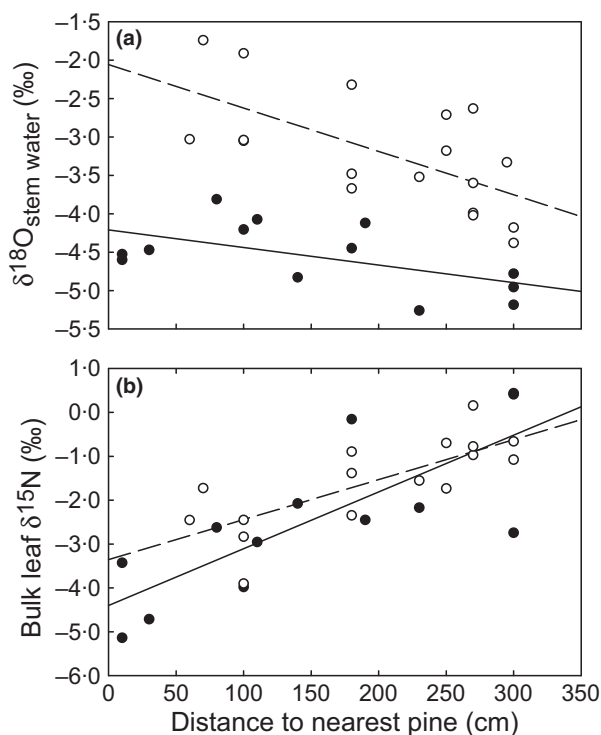


Fig. 5. Relationship between distance to the nearest pine of understorey *Rhamnus lycioides* shrubs within pine plantation stands and their (a) stem water $\delta^{18}\text{O}$ ($P = 0.045$, $r^2 = 0.32$, $n = 13$ in 2007 and $P = 0.004$, $r^2 = 0.41$, $n = 18$ in 2008) and (b) bulk leaf $\delta^{15}\text{N}$ ($P = 0.002$, $r^2 = 0.61$, $n = 13$ in 2007 and $P = 0.001$, $r^2 = 0.58$, $n = 16$ in 2008) in years 2007 (closed symbols, wet spring) and 2008 (open symbols, drier spring).

nearest *P. halepensis* tree in the pine plantation stands ($P = 0.034$, $r^2 = 0.38$, $n = 12$, in May 2007 and $P = 0.018$, $r^2 = 0.30$, $n = 18$, in May 2008).

Discussion

LONG-TERM COMPARISON OF *RHAMNUS LYCIOIDES* PERFORMANCE IN *PINUS HALEPENSIS* PLANTATIONS VS. OPEN WOODLANDS

Light availability was much lower in the shady understorey of pine plantation stands than in adjacent open woodland areas, which should lead to more negative $\delta^{13}\text{C}$ values and lower WUE_i in understorey *R. lycioides* shrubs due to the adverse effects of shade on photosynthetic activity (Franczy *et al.* 1985; Ehleringer *et al.* 1986). However, contrary to expectations, annual growth ring $\delta^{13}\text{C}$ and WUE_i were consistently higher in plantation understorey shrubs than in shrubs growing in nearby open woodlands, which suggests an overriding influence of stomatal effects (rather than light availability) upon the $\delta^{13}\text{C}$ and WUE_i of understorey shrubs. Across stand types, annual radial growth and WUE_i in *R. lycioides* shrubs were tightly controlled by water availability, as indicated by the strong positive and negative correlations of these parameters with annual precipitation amount, respectively (Fig. 3). Throughout the studied period, *R. lycioides* shrubs growing in pine plantation stands consistently showed thinner annual growth-rings and higher $\delta^{13}\text{C}$ and WUE_i than those in open woodland stands (Fig. 1a,b), thus indicating that understorey shrubs were much more severely water stressed. In addition, understorey shrubs in pine plantation stands showed higher growth ring $\delta^{18}\text{O}_R$ values than those in open woodland stands, which further supports the notion that understorey shrubs in pine plantations had lower stomatal conductance associated to lower water availability. These differences in $\delta^{18}\text{O}_R$ cannot be attributed to between-site differences in microclimatic conditions (e.g. VPD), as the open vegetation structure of woodlands

creates more xeric microclimatic conditions that should lead to higher (rather than lower) $\delta^{18}\text{O}_R$ values than in shaded, mesic pine plantations (Aussenac 2000). However, stem water $\delta^{18}\text{O}$ values were sometimes higher in plantation understorey shrubs than in shrubs from nearby open woodlands (e.g. in May 2008), which may have influenced $\delta^{18}\text{O}_R$ as well (in addition to stomatal effects on $\delta^{18}\text{O}_R$). The occasional use of a shallower, more isotopically enriched water source by the understorey shrubs in the pine plantation (relative to the open woodland), may thus have also contributed to their higher $\delta^{18}\text{O}_R$ values (Barbour 2007; Roden & Siegwolf 2012).

Overall, the high $\delta^{13}\text{C}$, WUE_i and $\delta^{18}\text{O}_R$ and low radial growth of understorey shrubs in pine plantation stands throughout the studied period indicate that they had low stomatal conductance, which strongly constrained their photosynthetic activity and growth compared with shrubs in open woodlands. High tree density in pine plantation stands translated into greater intensity of interspecific competition for water and lower moisture availability for *R. lycioides*. Differences in *R. lycioides* cumulative radial growth between stand types became more evident from around 1990 onwards, coinciding with a period of high annual radial growth increments in the pine trees from plantation stands (Fig. 2).

Annual radial growth differences between stand types were greatest during rainy years (Fig. 4a), indicating that water availability was much higher for shrubs in open woodlands during wet periods. Shrubs in plantation stands were exposed to both overstorey canopy interception and intense competition for soil water by neighbouring pine trees, which strongly limited their growth during wet periods. However, in very dry years (annual rainfall <200 mm), the ratio of mean radial growth in plantation stands to that in open woodland stands reached values around or above 1 (Fig. 4), suggesting a facilitative effect of the pine overstorey on understorey shrubs during severe drought. Similarly, differences in $\delta^{18}\text{O}_R$ between stand types were smaller in years with high mean VPD, indicating that shrubs from both stand types reached similar levels of stomatal conductance during the driest years. Closed forest canopies moderate temperature and maintain humidity in the forest understorey (Aussenac 2000), which may buffer *R. lycioides* shrubs in pine plantations against climate extremes. However, competition by *P. halepensis* on *R. lycioides* clearly outweighed facilitation in the long term in this semi-arid pine plantation.

The WUE_i of *R. lycioides* shrubs in open woodland stands showed a steady increasing trend throughout the study period. This increasing trend was also apparent in shrubs from pine plantation stands until the late 1990s, but not thereafter. Other studies have reported similar increments in the intrinsic water use efficiency of trees during recent decades in response to increasing temperatures and atmospheric CO_2 concentration (Saurer, Siegwolf & Schweingruber 2004; Linares *et al.* 2009; Maseyk *et al.* 2011). This increasing trend in WUE_i may indicate that

plants maintain constant internal CO_2 concentration inside their leaves (constant c_i) in the face of rising atmospheric CO_2 . This is achieved by decreasing stomatal conductance, thus increasing WUE_i without necessarily increasing photosynthetic rate or growth (Battipaglia *et al.* 2010, 2013). In open woodland stands, annual $\delta^{18}\text{O}_R$ was correlated with annual WUE_i , and both variables showed a similar increasing trend with time, further supporting the notion that decreasing stomatal conductance led to increases in WUE_i in response to higher temperatures and increasing atmospheric CO_2 concentration (but without changes in radial growth).

In contrast to open woodland stands, the lack of an increasing trend in $\delta^{13}\text{C}$ -derived WUE_i values and the highly enriched growth ring $\delta^{18}\text{O}_R$ values found in pine plantations during recent decades indicate that these understorey shrubs are severely water stressed and operate at low stomatal conductance. This may have allowed little margin for additional increases in WUE_i (through stomatal adjustment) in response to increasing temperature and atmospheric CO_2 (Linares *et al.* 2009). Tight stomatal limitation of photosynthesis in these understorey shrubs is further supported by the negative relationship found between WUE_i and radial growth in pine plantation stands. In addition to intense competition for water, other factors (e.g. competition for nutrients) may be co-limiting photosynthetic activity and stem growth in these understorey shrubs and may thus prevent any additional increases in intrinsic water use efficiency in response to rising atmospheric CO_2 concentration (e.g. Tognetti, Cherubini & Innes 2000).

INTERSPECIFIC PLANT-PLANT INTERACTIONS WITHIN *PINUS HALEPENSIS* PLANTATIONS

Understorey *R. lycioides* shrubs growing in close vicinity of *P. halepensis* trees showed higher values of stem water $\delta^{18}\text{O}$ than those shrubs growing further away from trees (Fig. 5). Stem water $\delta^{18}\text{O}$ accurately reflects the isotope ratio of soil water used by plants, as no isotopic fractionation occurs during soil water uptake by roots (Barbour 2007). Steep vertical gradients in soil water $\delta^{18}\text{O}$ develop during rainless periods in semi-arid environments, due to intense evaporative isotopic enrichment of water stored in upper soil layers (Barnes & Allison 1988). Moreno-Gutiérrez *et al.* (2012b) found that *R. lycioides* shrubs and *P. halepensis* trees exploit the same or very similar pools of water (i.e. they use water stored at same or similar soil depths) when they coexist in open woodlands where interspecific competition for soil resources is lower than in dense pine plantations. However, in plantation stands, we found significantly higher stem water $\delta^{18}\text{O}$ values in those *R. lycioides* shrubs growing at shorter distances from *P. halepensis* trees, which indicates utilization of shallower soil water sources. These results suggest that there is strong below-ground competition for water between coexisting species, with *P. halepensis* trees out-competing neighbouring *R. lycioides* shrubs and

forcing them to rely on more superficial soil water sources (a highly fluctuating and less abundant resource pool).

The negative effect that competition by *P. halepensis* exerted on the water relations of understorey *R. lycioides* shrubs was most evident during the wet spring of 2007, when shrubs growing at shorter distance from planted pines had lower stem water contents and higher leaf $\delta^{18}\text{O}$ values (indicating lower stomatal conductance) than those growing further away from pine trees. Interestingly, bulk leaf $\delta^{13}\text{C}$ values did not change with distance to the nearest pine, which overall indicates that reduced stomatal conductance caused a parallel reduction in photosynthetic activity with no change in WUE_i in *R. lycioides* shrubs growing near pine trees (Scheidegger *et al.* 2000).

The intensity of competition by *P. halepensis* trees also influenced the nutrient status of understorey *R. lycioides* shrubs in plantation stands, with lower leaf N and P concentrations in shrubs growing at shorter distances from pines in May 2007. During this wet spring, we found a tight coupling between the nutrient and water status of understorey *R. lycioides* shrubs in plantation stands, as stem water content was positively correlated with leaf $\delta^{15}\text{N}$, P and N concentrations. During the drier spring of 2008, all shrubs in plantation stands were similarly water stressed regardless of their distance from neighbouring pines, but changes in nutrient status with competition intensity were still evident. In 2008, bulk leaf $\delta^{13}\text{C}$ in *R. lycioides* shrubs was strongly positively correlated with foliar nutrient concentrations, which suggests enhanced photosynthetic activity in high-nutrient-status plants during dry years.

Leaf $\delta^{15}\text{N}$ values are positively related to plant nutrient status in non-leguminous plants (Craine *et al.* 2009). Leaf $\delta^{15}\text{N}$ in understorey shrubs was indeed correlated with leaf N and P concentrations, and increased with distance to the nearest pine (Fig. 5). This could also reflect shifts in the N source used by *R. lycioides* shrubs (nitrate vs. ammonium) due to changes in the strength of plant–plant competitive interactions (Kahmen, Wanek & Buchmann 2008), or shifts in the depth of N uptake, as soil $\delta^{15}\text{N}$ increases with depth in ectomycorrhizal-dominated ecosystems (Hobbie & Ouimette 2009).

Overall, isotopic and stoichiometric data indicate that understorey *R. lycioides* shrubs growing at shorter distances from planted pine trees in afforested stands were more severely water and nutrient stressed, which eventually led to a smaller shrub canopy size.

In conclusion, the combination of dendroecological and stable isotope data provided insight into the short- and long-term outcomes of plant–plant interactions in a water-limited ecosystem undergoing climate change. We found that the intensity of competition by overstorey *P. halepensis* trees strongly affects the water and nutrient status of understorey *R. lycioides* shrubs in semi-arid forest plantations. The sign and strength of this interaction are strongly modulated by the high interannual climate variability of this dryland ecosystem, with the negative effects of pine competition on *R. lycioides* performance being most evi-

dent during wet years. Understorey shrubs in pine plantations showed an impaired ability to increase their intrinsic water use efficiency as an adaptive response to rising temperature and atmospheric CO_2 concentration, which has led to parallel reductions in their stomatal conductance and photosynthetic activity during recent decades. The physiological adjustment of understorey vegetation to climate change is thus severely compromised by intense competition for soil resources in semi-arid forest plantations, which may have important long-term negative consequences for the functional diversity, structural complexity and resilience to disturbance of these man-made ecosystems. Intensive thinning of semi-arid forest plantations may become necessary in the coming decades to maintain understorey plant diversity and ecosystem services and multifunctionality (Isbell *et al.* 2011) under a warmer and drier climate.

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Data accessibility

Data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.m2db0> (Moreno-Gutiérrez *et al.* 2015).

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