Research paper

Reliance on deep soil water in the tree species *Argania spinosa*

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In South-western Morocco, water scarcity and high temperature are the main factors determining species survival. *Argania spinosa* (L.) Skeels is a tree species, endemic to Morocco, which is suffering from ongoing habitat shrinkage. Argan trees play essential local ecological and economic roles: protecting soils from erosion, shading different types of crops, helping maintain soil fertility and, even more importantly, its seeds are used by the local population for oil production, with valuable nutritional, medicinal and cosmetic purposes. The main objective of this study was to identify the sources of water used by this species and to assess the effect of water availability on the photosynthetic rate and stem water potential in two populations: one growing on the coast and a second one 10 km inland. Stem water potential, photosynthetic rate and xylem water isotopic composition ($\delta^{18}O$) were seasonally monitored during 2 years. Trees from both populations showed a similar strategy in the use of the available water sources, which was strongly dependent on deep soil water throughout the year. Nevertheless, during the wet season or under low precipitation a more complex water uptake pattern was found with a mixture of water sources, including precipitation and soil at different depths. No evidence was found of the use of either groundwater or atmospheric water in this species. Despite the similar water-use strategy, the results indicate that *Argania* trees from the inland population explored deeper layers than coastal ones as suggested by more depleted $\delta^{18}O$ values recorded in the inland trees and better photosynthetic performance, hence suggesting that the coastal population of *A. spinosa* could be subjected to higher stress.

Keywords: MixSIAR, photosynthesis, rooting depth, stable oxygen isotopes, tree water use, water sources.

Introduction

Root water uptake plays an important role in plant physiological performance. Knowledge on how plants use different water resources and respond to changes in water availability provides critical understanding about plant growth and survival. How spatial and temporal changes in hydrology can define the type of vegetation that an ecosystem can support has been evidenced by different authors (Flanagan et al. 1992, Stratton et al. 2000, Drake and Franks 2003 among many others). The combination of plants growing in a particular ecosystem typically encompasses a range of properties associated with water acquisition and use (hydraulic architecture, rooting depth and stomatal function) (Drake and Franks 2003).

Argania spinosa (L.) Skeels is a Moroccan endemic tree species which is suffering from ongoing habitat shrinkage. The argan tree is the most important species in Moroccan vegetation not only because it represents the second species in coverage (only surpassed by the cork oak) (Lybbert et al. 2010), but also because of its indispensable ecological functions. Its roots are the principal stabilizing element in these arid and semi-arid ecosystems, providing the final barrier against the encroaching deserts (Morton and Voss 1987), while tree canopies shade different types of crops and contribute to maintaining soil fertility. However, the most economically significant aspect of this species is to be found in its oily seeds, which are appreciated for their valuable nutritional and medicinal attributes and are particularly

very esteemed in the cosmetic industry. Nowadays, argan forest covers around 900,000 ha and sustains 2.5 million people (Nouaïm 2005, Michon et al. 2013). For all these reasons, it is crucial to protect and promote the regeneration and expansion of this species whose forests benefit rural households (Lybbert et al. 2010) and have had a historical involvement in the rural economy of the region (Benchekroun 1990).

In spite of the fact that the argan forest region was declared a UNESCO Biosphere Reserve in 1998, and its importance, during the 20th century almost half of the argan forest disappeared and the average density in the remaining half dropped from 100 trees per hectare to fewer than 30 (Lybbert et al. 2010). Forest degradation is currently happening all over the world in most of the arid and semi-arid forests and can be explained by the overuse of resources, climate change, urbanization and the expansion of agriculture and rangelands (de Waroux and Lambin 2012). In light of this, studying how *A. spinosa* responds to differences in water availability and survives in this arid environment maintaining a positive carbon balance and producing the most expensive edible oil in the world (Lybbert et al. 2010) can provide tools for the protection and management of this useful species.

Díaz Barradas et al. (2013) evidence that this tree species is able to maintain an elevated productivity even in the dry season, by a high assimilating rate of carbon during the early hours of the day. Accordingly, it seems obvious that argan trees are adapted to arid conditions and to an irregular precipitation regime (severe water stress, high radiation and hot temperatures). Nonetheless, a detailed knowledge of the plant water-use strategies is necessary for understanding the particular ability of *A. spinosa* to cope with the harsh climate conditions of its biogeographical area so as to improve management tools for preventing a future decline in argan forest.

In a previous work using electrical resistivity imaging, Ain-Lhout et al. (2016) described that argan trees probably extracted water from underneath the soil zone, from the weathered layers to depths of 3–6 m. This result agrees with numerous observations in various forest types which assume that plants subjected to drought explore deeper soil layers to compensate for the low moisture in the topsoil layers (David et al. 2007, Prechsl et al. 2015, Grossiord et al. 2017). What is more, different authors have pointed out that in dry climates some tree species depend on access to groundwater to withstand periods without precipitation (Dawson and Pate 1996, Barbeta et al. 2015). However, most of the argan forests are located in areas where the groundwater level is deeper than maximum rooting depth. According to Canadell et al. (1996), on a global scale, tree maximum rooting depth has been estimated as ~7 m. Ain-Lhout et al. (2016) described 4 m as the maximum rooting depth of argan in the core of the species' main distribution area, but also in populations located in the mountainous regions of Argana or Beni Snassen, located in fractured rock formations (Hssaisoune et al. 2016), where groundwater is located

10–80 m deep, and thus hardly reachable for tree roots. This is not an exceptional situation, as it is estimated that in 44.8% of terrestrial ecosystems, water tables are deeper than 10 m (Fan et al. 2013, Barbeta et al. 2015).

The question of what are the water sources of argan forests remains unanswered. Considering that *A. spinosa* is the only tree species capable to survive under the severe summer water deficit of these agroforestry ecosystems of Southwest of Morocco, knowing the water-use strategies of this emblematic species will help to understand its physiological performance under water stress and will improve the particular management tools for argan woodlands.

Stable isotopes have been shown to be effective tracers for determining plant water uptake patterns (White et al. 1985, Sternberg and Swart 1987, Dawson 1993, Asbjornsen et al., 2007, Wang et al., 2010, Ma and Song 2016), as different water sources often have different isotopic signatures due to differences in origins and physical processes (Dawson et al. 2002, Ewe et al. 2007), and because during water uptake by roots, isotopic fractionation does not occur (Ehleringer and Dawson 1992); therefore, water sources used by plants can be identified by comparing the isotopic signatures of xylem sap with those of the available water sources at a given moment (soil, rain and groundwater). Additionally, this technique has also provided new insights into the possible relationships of xylem sap isotopic signature to rooting depth (Dawson and Ehleringer 1991, Jackson et al. 1995, Prechsl et al. 2015, Grossiord et al. 2017), water-use efficiency (Flanagan et al. 1992, Moreno-Gutiérrez et al. 2012) and seasonal interspecific-variation (Voltas et al. 2015).

This study aims to compare and to assess the seasonal variations in water uptake patterns of *A. spinosa* and their influence on the trees' ecophysiological status in different locations during two growing cycles in Agadir (Morocco). We proposed three primary objectives: (i) to compare the seasonal variations in water uptake patterns in trees growing in an inland population and those in a coastal location with different soil types; (ii) to quantify the contribution of soil water at different depths, and precipitation to the water uptake by *A. spinosa*; and (iii) to evaluate the relationships between water sources and the ecophysiological status. We used oxygen stable isotopes to determine the proportional contributions of different water sources throughout the year. Oxygen stable isotopes can be used to estimate the proportion of water that plants use from various sources or different depths, provided that the isotopic signatures of the water sources and different soils depths are distinct.

Díaz Barradas et al. (2010) have already shown that *A. spinosa* mountain populations, with limestone type soil, face greater physiological stress than populations near the coast, with alluvial deposits soils. In this study, we hypothesized that due to the geological and microclimatic conditions, the two populations might use different water sources and experience different

ecophysiological responses, in spite of the short geographical distance between them. Overall, we addressed the following hypothesis: (i) argan trees present an opportunistic strategy in the use of water resources, with plants exploring deep soil layers for water uptake during summer, but during the rest of the year taking advantage of each rainy event and multiscale soil water depths. (ii) According to contrasted environmental conditions between populations, the contribution of the available water sources would present differences that might result in different water status and photosynthetic performance of argan trees.

Materials and methods

Study sites

Field measurements were conducted during 2010 and 2011 growing cycles (seasonality from winter 2010 to autumn 2011: February 2010, April 2010, 2011, September 2010, 2011 and November 2010, 2011) in two argan forests close to Agadir city, Morocco. One population was located near the coast (N30°36′07.72′, W9°46′33.49′, Coastal site hereafter) and the other one in an inner area (N30°20′15.65′, W9°28′08.68′, Inland site hereafter). Natural vegetation in both study sites is

very scarce; apart from the dominant *A. spinosa*, it is composed of spiny shrubs such as *Acacia gummifera* or *Ziziphus lotus* and by cactus-like plants such as *Euphorbia beaumeriana* or *Senecio anteuphorbium*. The climate is temperate thanks to the marine influence, with minimum temperatures never below 7 °C and maximum temperatures never surpassing 30 °C. Annual rainfall is scarce (302 mm in Coastal site and 241 mm in Inland site) with a long summer drought (Figure 1). Climatic data recorded daily were obtained from the Souss-Massa-Drâa hydraulic Agency and from the National Centers for Environmental Prediction (NCEP) website (http://globalweather.tamu.edu/).

The study site in the Agadir area is considered as the ecological optimum for the species and the center of its distribution area (Benabid 1982, El Yousfi 1988, M'Hirit et al. 1998, Tarrier and Benzyane 2003). This region, located in the low valleys of the Souss and Massa Rivers, is separated from the rest of the country by high mountains. *Argania spinosa* has little specificity for any particular soil type, having a great acclimation capacity to different types of soils such as schists, quarzitic rocks, limestone or alluvial, and even being found on salty soils (Nouaïm 2005). The Inland population was located in Admine Forest in the suburbs of Agadir city, with our study site 10 km inland. This area is positioned on
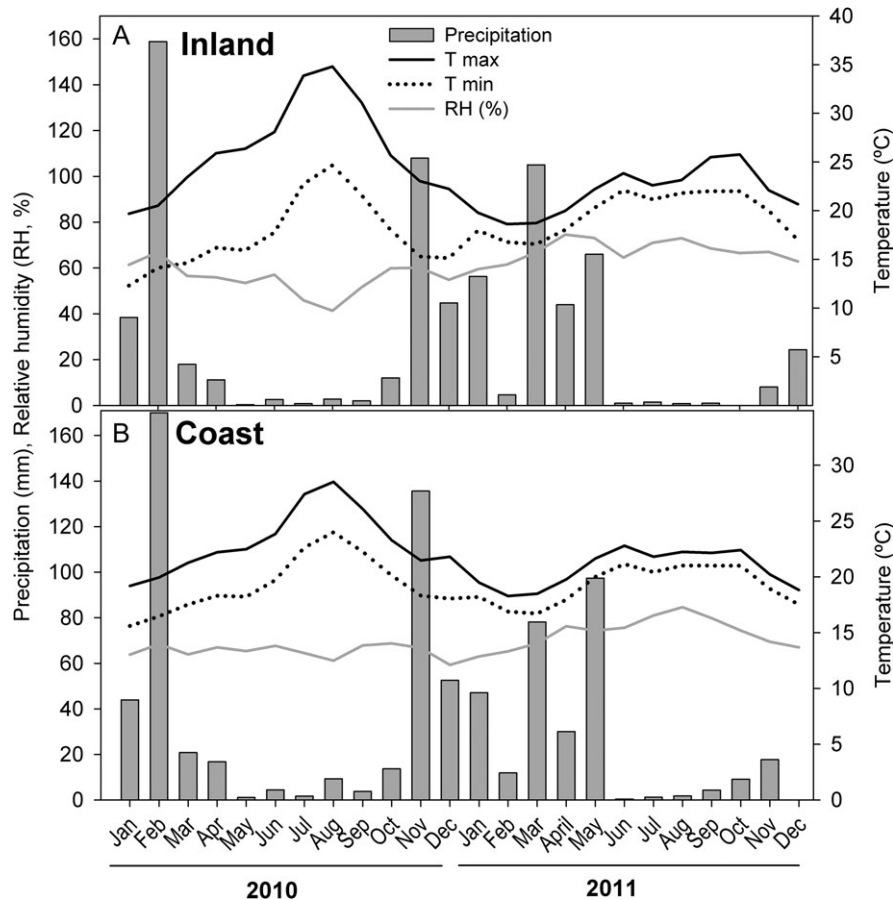

Figure 1. Monthly precipitation, and daily maximum and minimum mean temperatures and air relative humidity during the study period (2010–11) in Inland (A) and Coastal sites (B).

the Souss plain, formed by recent alluvial deposits of the Souss and Massa Rivers and, their tributaries. Soil thickness covering the silty-limestone formation is low, with a mean soil profile depth of 0.8 m (Watteeu 1964). The water table depth in the area is on average 50 m below the soil surface (Hssaisoune et al. 2016). The Coastal population was located 24 km North of Agadir city, near the Atlantic Ocean. This area corresponds to the border between the High-Atlas formation and the Souss basin. The soil is a shallow limestone type (Hssaisoune et al. 2016) with a mean soil profile depth of 0.5 m (Watteeu 1964). The water table is located in alluvium formation and the average depth of water is about 15 m. Due to the atmospheric high-pressure belt of the area, fogs are common across the coastline, from Essaouira to Agadir, and frequently extend over our Coastal site even during the summer period. The distance between the two populations was 40 km. These two populations were selected to test whether their differences in oceanic influence, depth and nature of soil lead to differences in the use of water sources and ecophysiological response.

Ecophysiological measurements

Net photosynthetic rate (A_n, µmol CO_2 m^{-2}s^{-1}), transpiration rate (E, mmol H_2O m^{-2}s^{-1}) and stomatal conductance (g_s, mol H_2O m^{-2}s^{-1}) were measured on mature leaves of three terminal shoots per plant (3 shoot × 10 trees × 2 populations) during clear sunny days. Measurements were taken between 9:00 and 11:00 h solar time, when maximum photosynthetic rate values are recorded, by means of an open system compact infrared gas exchange analyzer (LCi-portable photosynthesis System, ADC, Hertfordshire, UK). In order to avoid pseudoreplication, mean values per tree were calculated for statistical analysis. Intrinsic water-use efficiency (WUE$_i$ µmol CO_2 mol^{-1} H_2O) defined as carbon gain in relation to the potential water loss was calculated as the ratio A/g_s. The WUE of plants is a key aspect of their water economy and of their performance and survival in arid sites (Damesin et al. 1997, Valladares et al. 2008). All leaves used for photosynthetic activity measurements were scanned and leaf area was calculated using Midebmp software (Ordiales-Plaza 2000) to express the results on a projected leaf area basis. Due to technical problems, photosynthetic activity could not be measured on the 2011 spring campaign.

Stem water potential was measured in terminal shoots of every selected tree (1 shoot × 10 trees × 2 populations) before dawn (Ψ_{pd}) and at midday (Ψ_{md}) using a Scholander pressure chamber (Manofrigido, Lisboa, Portugal). Measurements were performed in the field, immediately after the shoot was cut.

Xylem water and water sources sampling for isotopic analysis

Seasonal water sources were determined in adult trees ($n = 7$ per location and season) through analyses of the oxygen isotopic composition of sap xylem water ($\delta^{18}O$), carried out seasonally from winter 2010 to autumn 2011 (February 2010,

April 2010, 2011, September 2010, 2011 and November 2010, 2011). We compared xylem sap values to those of soil water at four different depths (0.25, 0.5, 1 and 1.5 m), rainwater (RW), atmospheric water (AW) and groundwater (GW). In the 2010 campaign, soil samples were not collected; however, when the study was repeated in 2011 we included the soil samples at different depths. From now on soil water sources are named SW$_{0.25}$, SW$_{0.5}$, SW$_1$ and SW$_{1.5}$. We assumed that tree water uptake occurred mainly within this gradient of depths, where soil cores were collected.

Leafless, lignified and mature stem sections were collected the same day as ecophysiological measurements and transferred in the field to glass vials with silicone septa tops. The vials were sealed with Parafilm and stored in a portable cooler to prevent evaporation. Precipitation samples were collected from pluviometers placed near to the study sites and provided with a 5 mm layer of liquid paraffin to avoid evaporation. Groundwater samples were obtained by means of a borehole in each study site. Atmospheric water vapor was collected cryogenically following Helliker et al. (2002) where the air was pulled through a system of glass tubes and ultra-torr adapters attached to a collecting tube which was placed in a dry-ice-vacuum flask such that water vapor condensed on the collecting tube. Soil samples were collected in the morning in screw-cap glass vials, sealed with Parafilm and kept refrigerated (three replicates per site). To prevent evaporation and isotopic fractionation, the water samples collected were refrigerated in double cap polyethylene containers sealed with Parafilm until processing and analysis. Plant and soil samples were stored frozen until the water was extracted by means of a custom-made cryogenic vacuum distillation system (Ehleringer and Osmond 1989) housed in the Department of Plant Biology and Ecology at the Universidad de Sevilla (Sevilla, Spain). The extraction system consisted of four independent extraction glass arms connected to a glass manifold evacuated under 60 mTorr of negative pressure (vacuum). Attached to each arm were a collecting and an extraction Pyrex tube. The complete vacuum system was connected to a vacuum pump. Stem or soil samples were frozen with a liquid nitrogen bath (-176 °C), connected to a distillation arm and the system was evacuated until the working pressure was reached. After evacuation, the distillation arm was isolated from the vacuum manifold closing the valve. The extraction tube containing the sample vessels was submerged in a pot containing boiling water whilst the collection tube was immersed in liquid nitrogen to capture the extracted water vapor for isotopic analysis. After the extraction, water samples were transferred into cap-crimp 5-ml vials and kept frozen at -15 °C until analysis. Extraction timing ranged between 60 and 120 min for stems, and 60–90 min for soil samples, a time considered sufficient to achieve full recovery of sample water following West et al. (2006). Our soil samples were limestone and silty-limestone types, which have shown not to be problematic for water extraction, and in any case, several

studies have found 60 min to be sufficient time to extract gravi-metric water from a soil sample (see West et al. 2006, Oshun et al. 2016).

The isotopic abundance was expressed in delta notation (δ) in parts per thousand (‰) as

$$\delta^{18}\text{O or } \delta D_{sample} (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the molar ratios of heavy to light isotope of the sample and the international standard (Vienna standard mean ocean water, VSMOW). Previous stud-ies have shown that δ^2H in xylem water samples may fraction-ate during plant water uptake in species adapted to saline or xeric environments (Ellsworth and Williams 2007); for this reason, only δ^{18}O was used to determine water sources in plants. Nevertheless, δ^2H was analyzed in meteoric water and groundwater in order to compare with the global meteoric water line (see Supplementary Data available at *Tree Physiology* Online). Water isotope content was measured by CO_2 equilibration using a Gas Bench coupled in a continuous flow to a Delta V Advantage IRMS (Thermo Fisher Scientific Inc., Bremen, Germany) at the Laboratorio de Isótopos Estables de la Universidad Autónoma de Madrid. Average precision of the isotopic analyses was 0.2‰ (standard deviation).

To explore the seasonal effect on water sources used, the dif-ferences between the most enriched and most depleted sea-sonal δ^{18}O values were calculated ($\Delta^{18}O_{soil}$ = summer soil water δ^{18}O – spring soil water δ^{18}O; $\Delta^{18}O_{xylem}$ = summer xylem water δ^{18}O – autumn xylem water δ^{18}O).

Statistical analysis

Three-way ANOVAs were used to test for effects of season, year and site on argan tree xylem water δ^{18}O values and ecophysio-logical variables. The analyses were carried out independently for the study variables due to the fact that the number of samples in isotope analysis was not the same as the ecophysiological variables. We contrasted stem water potential, gas exchange and δ^{18}O values searching for seasonal differences between sites using Student's *t*-tests. All values conformed normality assumptions. Homogeneity of variances was checked using Levene´s test. Data analyses were performed with SPSS v.23.0 software (SPSS Inc., Chicago, IL, USA).

We used MixSIAR for R (Stock and Semmens 2013), a Bayesian mixing model, to estimate the most likely proportions of water source contributions to the trees, based on their δ^{18}O on xylem sap values. The MixSIAR is a model framework in R that allows one to create and run Bayesian mixing models to ana-lyze the uncertainties in biotracer data (stable isotopes in our case) and addresses hierarchical random or fixed effects in the analysis (Stock and Semmens 2013). These Bayesian mixing models use as input three files of isotope values: consumers (mixture), sources and a correction file for potential fractionation

(a discrimination factor to refer to the differences between iso-tope values found in the mixture and sources). In our study the model used individual δ^{18}O values of argan tree xylem water ('mixture', raw data), all water sources described previously (mean plus standard deviation) and the discrimination factor, which was set as 0. We calculated the water-use proportion in the three seasons separately using site as a fixed factor. We set the Markov Chain Monte Carlo (MCMC) to 200,000 or 1,000,000 burn-in size and the number of iterations to 300,000 ('long' model run), until the diagnoses diagnostic tests Gelman–Rubin, and Geweke were satisfied.

As in our study there were more than $n + 1$ sources, we com-bined some sources to obtain less diffuse solutions (Phillips et al. 2005, 2014). We decided to follow an a priori aggregation approach (Phillips et al. 2005) so that the sources considered were similar but, also that the combined source had some bio-logical meaning. After a first evaluation, atmospheric water and groundwater sources were dismissed, since δ^{18}O values were considerably different from the δ^{18}O of argan trees and of groundwater because in both populations the water table was probably deeper than maximum rooting capacity. On the other hand, when soil water sources at near depths were not signifi-cantly different in their isotopic composition in both populations, they were represented as a single set computed as the means of the individual sources. The spring mixing model was run with four sources, rainwater and soil water at three depths (RW, $SW_{0.25 + 0.5}$, SW_1 and $SW_{1.5}$). In summer no rain was collected so the mixing model was run with only three sources, soil water at three depths ($SW_{0.25 + 0.5}$, SW_1 and $SW_{1.5}$); in autumn the mixing model was run with four sources (RW, $SW_{0.25}$, $SW_{0.5 + 1}$ and $SW_{1.5}$).

Results

Rainfall seasonality throughout the 2 years was typical for this area, with rains in autumn, winter and spring, and a summer drought that lasted 4 months (Figure 1). The first study year was wetter than the second, as the hydrological years 2009/2010 and 2010/2011 were over the mean values (proxy 494 and 473 mm in Coastal site and 377 and 452 mm in Inland site), while 2011/2012 was extremely dry (71.4 mm in Coastal site and 34 mm in Inland site, 86% lower than annual mean values in Inland site) and consequently 2011 autumn was very dry.

Ecophysiological measurements

When comparing gas exchange and stem water potential vari-ables, multivariate analysis showed significant differences (P = 0.001) between the two argan populations throughout the year. It also showed differences between years (season F_{24} = 12.9, site F_8 = 3.97, year F_8 = 22.0), with interaction between both factors (year * site: F_8 = 5.3 , P = 0.001; season * site: F_{24} = 5.0, P = 0.001).

Inland argan trees presented consistently higher photosynthetic rates (A_n) than coastal ones (Table 1, Figure 2). On the contrary, g_s and WUE_i showed great variability, with significant differences between sites, though not always with the same pattern, showing little apparent influence of the water source used (Table 1).

Significantly lower Ψ_{pd} were observed in coastal trees in 2010 summer and both springs and in Ψ_{md} in 2010 summer, with coastal trees displaying lower midday water potentials than the inland ones (Figure 3). Differences between sites were also found In 2011 autumn and summer Ψ_{md} and in 2011 summer Ψ_{pd}, though with opposite sign, inland argan trees displaying lower values than the coastal ones as shown by the interaction between sites and seasonal factors ($F = 3.29$, $P < 0.05$). Both variables Ψ_{pd} and Ψ_{md} differed significantly across seasons

(Table 1, $P < 0.001$), becoming more negative at the end of the summer in both years (Figure 3).

Isotopic signature

The oxygen isotopic signal in argan trees varied annually and seasonally throughout the study period (Figure 4A and B, Tables 1 and 2). The most $\delta^{18}O$-enriched values were registered in autumn (-1.13‰ for inland trees and 0.24‰ for coastal trees) and the most depleted ones in winter (-4.18‰ for inland trees and -5.83 in coastal trees), but also in summer in the case of the inland population (-4.49‰). The depletion in $\delta^{18}O$ was positively associated with a decrease in Ψ_{pd} in the two study sites (Figure 5, $P < 0.001$, $R^2_{coast} = 0.70$ and $R^2_{inland} = 0.645$).

Table 1. Multivariate ANOVA for the effects of site, year and season on $\delta^{18}O$ xylem sap, stem water potential at predawn (Ψ_{pd}) and midday (Ψ_{md}), assimilation of CO_2 (A_n), intrinsic water use efficiency ($WUE_i = A/g_s$), stomatal conductance (g_s) and seasonal range of $\delta^{18}O$ xylem sap ($\Delta^{18}O$) of argan trees. Degrees of freedom in parenthesis. Significant values ($\alpha=0.05$) are shown in bold.

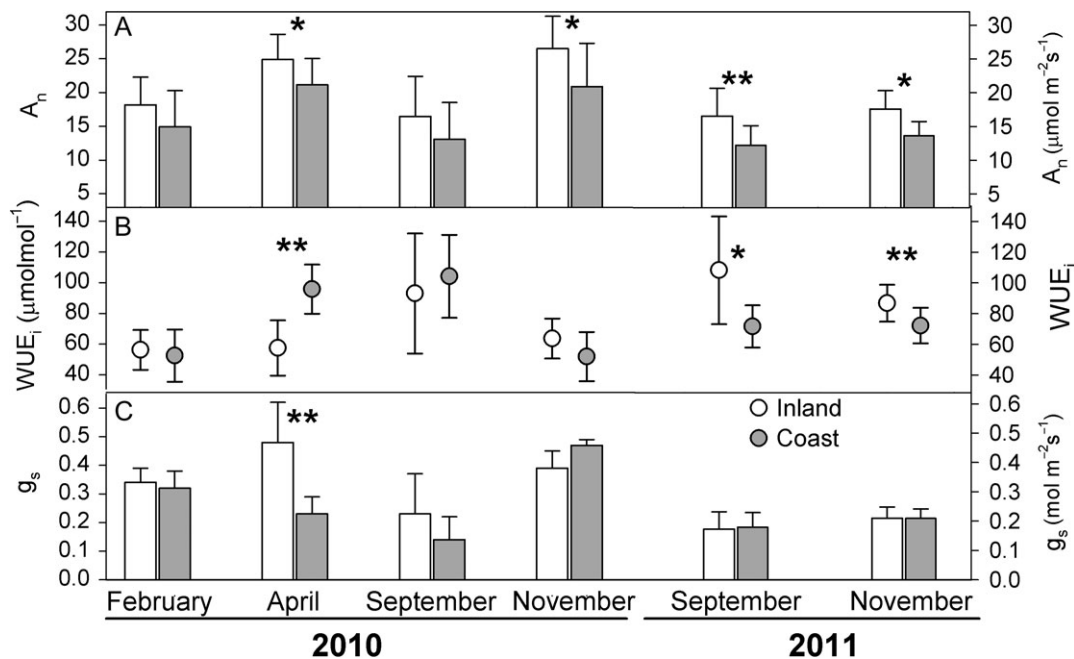| Factor		$\delta^{18}O$	Ψ_{pd}	Ψ_{md}	A_n	WUE_i	g_s	$\Delta^{18}O$
Season (3)	F	103	255.5	91.9	12.6	9.9	9.1	
	P	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	
Site (1)	F	25.8	1.5	1.06	20.1	1.6	5.9	30.27
	P	**0.001**	0.30	0.21	**0.001**	0.215	**0.02**	**0.001**
Year (1)	F	10.7	3.4	23.3	16.8	1.18	23.4	2.1
	P	**0.002**	0.66	**0.001**	**0.001**	0.28	**0.001**	0.160
Site * year (1)	F	4.19	13.7	8.7	0.008	4.54	0.001	1.82
	P	**0.044**	**0.001**	**0.04**	0.93	**0.04**	0.996	0.192
Season * site * year (2)	F	2.3	5.7	0.3	0.6	0.33	1.74	
	P	0.109	**0.018**	0.50	0.44	0.562	0.19	

Figure 2. Mean ($\pm$SD) net photosynthetic rate (A_n, A), intrinsic water use efficiency (WUE_i calculated as the ratio A/g_s, B) and stomatal conductance (g_s, C) for inland and coastal argan populations during the study period. Asterisks denote significance levels for the comparisons between the two populations by Student's t-tests (**$P < 0.001$; *$P < 0.05$).
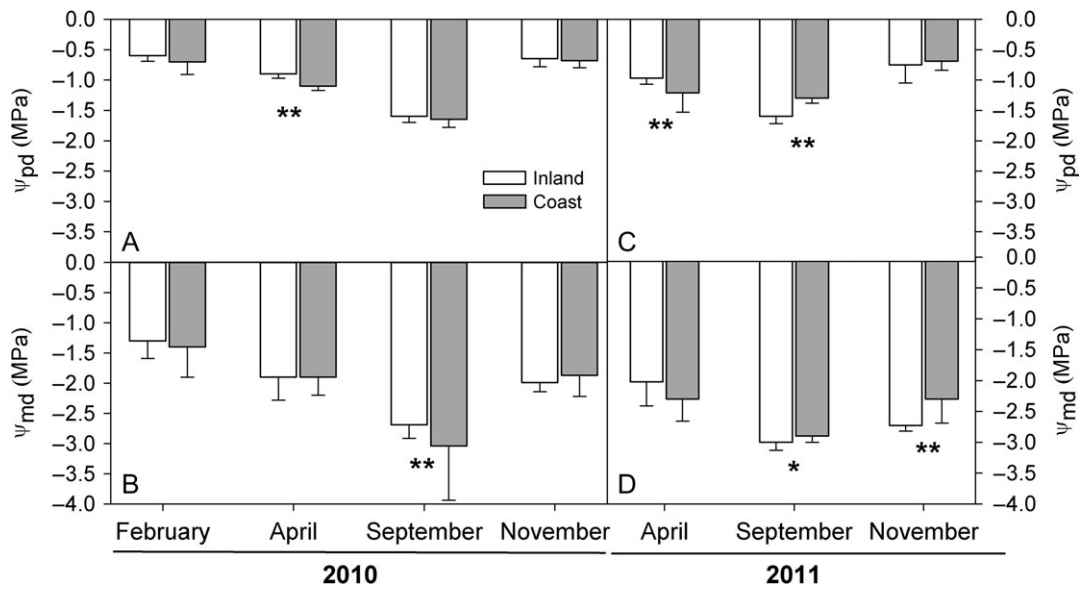
Figure 3. Mean (±SD) predawn (A and C) and midday (B and D) stem water potentials for argan of inland and coastal populations. Asterisks denote significance levels for the comparisons between the two populations by Student's *t*-tests (**$P < 0.001$; *$P < 0.05$).
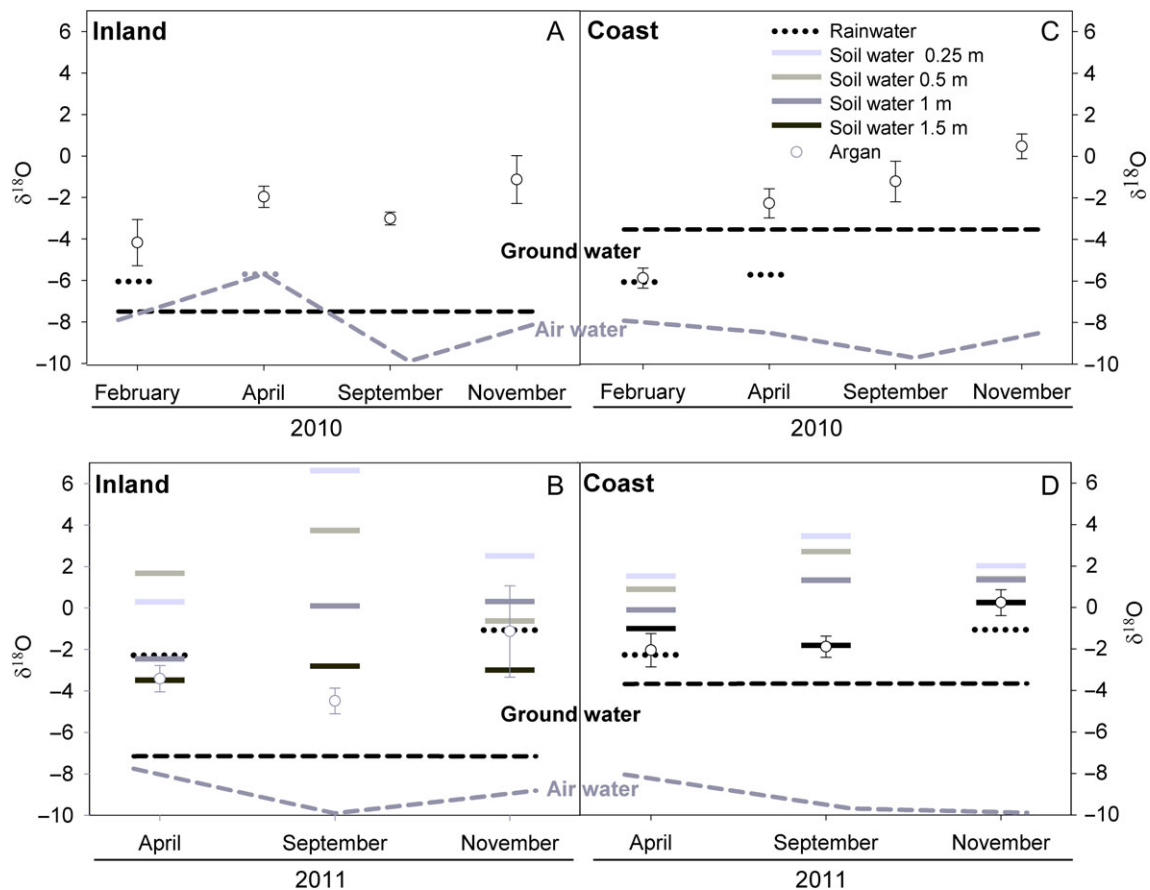

Figure 4. Tree xylem water $\delta^{18}O$ and potential source water $\delta^{18}O$ compositions, including air-water (gray dashed line), groundwater (black dashed line), soil water at four depths and rainwater during the study period (2010–11) for inland (A, B) and coastal (C, D) populations of argan trees (significant differences are shown in Tables 1 and 2).

Table 2. Analysis of variance for the effects of site, year and season on xylem sap $\delta^{18}O$ of argan trees. The asterisks denote significance differences by the post hoc Tukey test.

Factor	Season	F	P
Site	Winter	18.595	0.001*
	Spring	5.042	0.034*
	Summer	99.006	0.000*
	Autumn	10.731	0.003*
Year	Spring	6.843	0.015*
	Summer	23.079	0.000*
	Autumn	0.069	0.796
Site * year	Spring	12.125	0.002*
	Summer	3.679	0.067
	Autumn	0.074	0.787

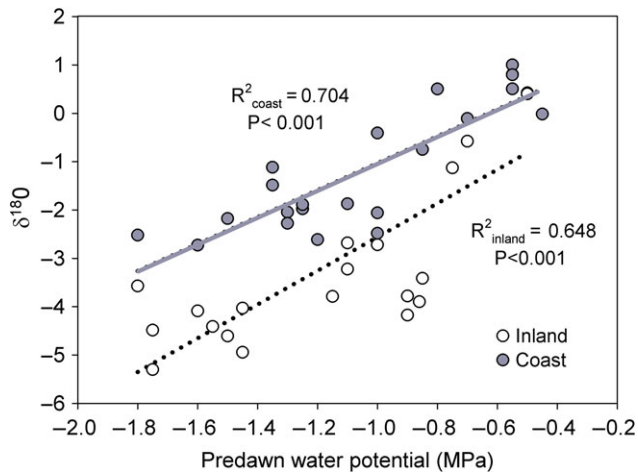

Figure 5. Tree xylem water $\delta^{18}O$ and predawn stem water potential linear regression for inland and coastal argan populations. Points are individual values for the three seasonal measures during 2011.

Figure 6. Seasonal variations in 2010 and 2011 for xylem sap ($\Delta^{18}O_{xylem}$, left) and in 2011 for soil water $\delta^{18}O$ at four depths ($\Delta^{18}O_{soil}$, right) in inland and coastal populations.

When comparing $\Delta^{18}O_{soil}$ patterns in the two experimental sites, variations were always higher in the Inland site than in the Coastal site and accordingly, also $\Delta^{18}O_{xylem}$ showed stronger variation in inland trees than in coastal trees (Figure 6), suggesting a more conservative and stable water-use pattern throughout the year. Soil water ^{18}O signature varied with soil depth and season. The $\Delta^{18}O_{soil}$ were bigger in shallow soil layers compared with deeper layers (Figure 6, right). No seasonal variations were found in deep soil water, which was the main source of water for both populations. Thus, one possible explanation for the variability found in the Inland population it is that trees of this site were using water from more variable sources than those of the Coastal site to support transpiration.

Deep soil ($SW_{1.5}$) water was always more depleted in the Inland (with $\delta^{18}O$ values from −3.6 to −2.9‰, Figure 4B) than in the Coastal site (Figure 4D, from −1.9 to 0.11‰). Water from shallow soil layers showed an irregular pattern. Soil water $\delta^{18}O$ from both sites showed higher values in summer (dry season), with topsoil water displaying the higher enrichment among seasons (Figure 6, right).
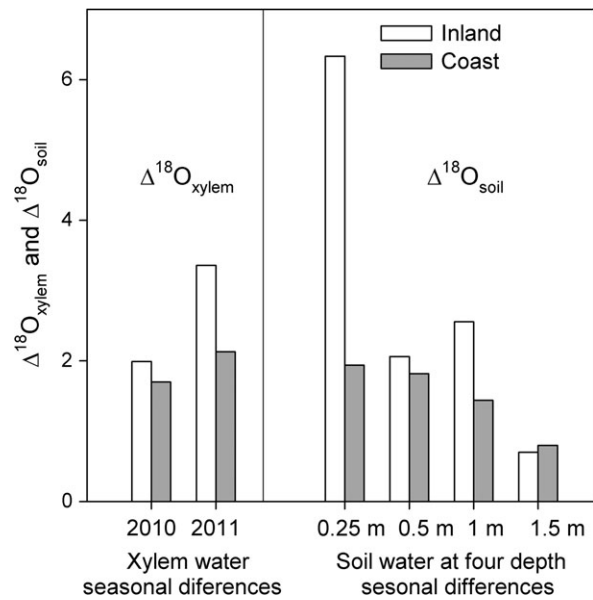
Rainwater $\delta^{18}O$ varied among seasons with a maximum to minimum range of −6.1‰ (winter 2010) to −1.07‰ (autumn 2011), with the more depleted values coinciding with moments when rainfall was more abundant. The mean groundwater $\delta^{18}O$ signature was −7.7‰ in the Inland site and −3.66‰ in the Coastal site (Figure 4A and B). Air-water apparently had no effect on the plant's isotopic composition, ranging from −10.5‰ in autumn to −5.7‰ in spring.

The analysis of the most likely sources of water used for argan trees revealed similar water-use patterns in both sites (Figure 7), but also that the relative contribution of the different water sources to the water balance of trees changed throughout the year. Trees of both sites showed $\delta^{18}O$ xylem sap values different from groundwater and air-water in any season. Deep soil ($SW_{1.5}$) was always the main source of water in the Inland site (92% in summer, 54% in spring and autumn). In contrast, in the Coastal site $SW_{1.5}$ was the main source of water in autumn (81%) and summer (96%), but not in spring, when water uptake was a mixture of rainfall (43%) and soil water at different depths ($SW_{1.5}$, SW_1 and $SW_{0.25}$ + $_{0.5}$; 40%, 17% and 13%, respectively). A noteworthy observation was that in the Inland site xylem $\delta^{18}O$ was more depleted than water from the 1.5 m layer in summer, suggesting water extraction from deeper soil layers (Figure 4B and D).

Discussion

In this study, we found evidence that deep soil layers (up to 150 cm) were the main source of water through the year, although occasionally argan trees could also use a combination of all available water sources. These sources of water appeared to be enough for an optimal performance and water balance of argan trees even

in summer, as indicated by the stem water potential and photosynthetic values recorded in both populations (Figures 2 and 3).

Impact on physiology and implications

The distribution range of this species includes areas with annual precipitation from 400 mm to close to 150 mm (de Waroux and Lambin 2012). Accordingly, although it is evident that argan trees must be adapted to extremely arid conditions and an irregular precipitation regime, the high midday stem water potential and high photosynthetic rates recorded even in the hottest seasons are still surprising. The midday water potential values recorded in the most unfavorable period of the year (−2.9 ± 0.20 MPa, the average of the two study sites and 2 years) after 3 or 4 months without precipitation contrast with those obtained by other authors. Zunzunegui et al. (2011) reported values ranging between −4 and −5 MPa at the end of the summer for six different species of the Mediterranean shrub community. But in this same study, extreme stem water potentials, reaching values as low as −15 MPa, were also found. Furthermore, in different trees species (*Acacia tortilis*, *Acacia aptaneura*, *Prosopis*, *Quercus ilex*, *Quercus douglasii*) from semi-arid or arid ecosystems, summer stem water potential at midday ranged from −4 to −8 MPa (Maranga 1987, Page et al. 2016, Hultine et al. 2006, Serrano et al. 2005, Osuna et al. 2015, respectively). On the other hand, other authors have described in the same area stem water potential values for *A. spinosa* lower than the ones recorded in this study, ranging from −3.5 to −4 MPa, although the study was carried out in a drier year (Díaz Barradas et al. 2010). In any case, the stem water potential values recorded in this study at the end of the dry period reveal the exceptionally good capacity of this species to withstand water scarcity and could help to form a better understanding of root distribution, as discussed below.

In general, summer photosynthetic rate values in the Agadir area obtained by Díaz Barradas et al. (2010) were similar to the results obtained in this study and in the range of 15 ± 4.5 μmol m^{-2}s^{-1}. Previous studies (Díaz Barradas et al. 2010) have described *A. spinosa* as a drought-avoiding, water-saving species, described by Kozlowski and Pallardy (2002) as one with the ability to avoid or postpone the decline of leaf water potential during drought. Our results agree with the drought-avoiding strategy since Argan trees' deep root system evidenced in this work would confer the ability to extract water from the soil to compensate for transpiration water losses and would avoid the decline of leaf water potential during drought enabling a high photosynthetic rate throughout the year. In addition, the data support other water-saving mechanisms such as the closing of stomata in the early hours of the morning, and leaf shedding during summer (personal observation, Díaz Barradas et al. 2010).

Trees from Inland site presented higher photosynthetic rates and higher water-use efficiency than coastal trees, even during summer. These results could be explained by a deeper root system of argan trees from this population, a specific adaptation to the lower precipitation and higher temperature recorded on this site, which would enable a better performance during summer stress. Evaporative enrichment in ^{18}O decreases with depth, with shallow depth presenting the most positive δ^{18}O values, which decrease with increasing depth (Feild and Dawson 1998). Provided that in inland trees xylem water δ^{18}O values were more depleted than soil water from 1.5 m, a deeper water uptake is likely to occur in this population in summer.

Changes in water sources along the growing season

Precipitation δ^{18}O values recorded in our study are consistent with precipitation δ^{18}O values due to temperature and amount of precipitation that falls combined effects (Dansgaard 1964, Flanagan and Ehleringer 1991). The δ^{18}O values recorded also confirmed that groundwater was not a water source for argan trees, just like in many forests in other biomes (Barbeta et al. 2015), where groundwater, as in the study sites, is deeper than 10 m, while the mean maximum rooting depth estimated for trees is ~7 m (Canadell et al. 1996).

In the summer season, when the lowest stem water potentials were measured, contrary to what was expected (heavier isotopes because of isotopic fractionation during evaporation processes), the isotopic signal of argan trees showed the most depleted values. The association of the lowest Ψ$_{pd}$ with the most depleted δ^{18}O shows the water-use strategy of *A. spinosa*, extracting water resources from deeper soil layers when drought conditions exacerbate during summer days, which was consistent with that estimated through the MixSIAR model (Figures 6 and 7).

Our results provide evidence that the contribution of the different water sources was similar in the two sites, with deep soil water being the main source throughout the year (54–92% in inland and 40–96% in coastal). In spite of this, we detected subtle changes in the proportions of their water source preferences related to rainfall distribution. While coastal trees showed higher reliability on rainwater in spring, the opposite pattern was found in autumn, with the coastal population relying on deep soil water and inland populations preferring a mixture of the available water resources (rain and shallow, mid and deep soil layers). This opportunistic strategy is consistent with the presence of dimorphic root systems that enable the trees to extract water from rain and simultaneously uptake water from multiscale depth soil layers, recharged in previous rainy events (Ehleringer and Dawson 1992, Dawson and Pate 1996, Rossatto et al. 2012).

These differences in the water uptake in response to rain might be related to a more rainy spring in the Coastal population, but also to a different rainfall distribution in the Inland site, that promoted a root development in shallow soil depths enabling the trees to take advantage of each rain event, even the limited ones, as in the spring of 2011. Differences were also detected in summer, even if trees from both populations relied on deep soil water for more than 90%. Inland trees explored deeper layers than coastal ones,
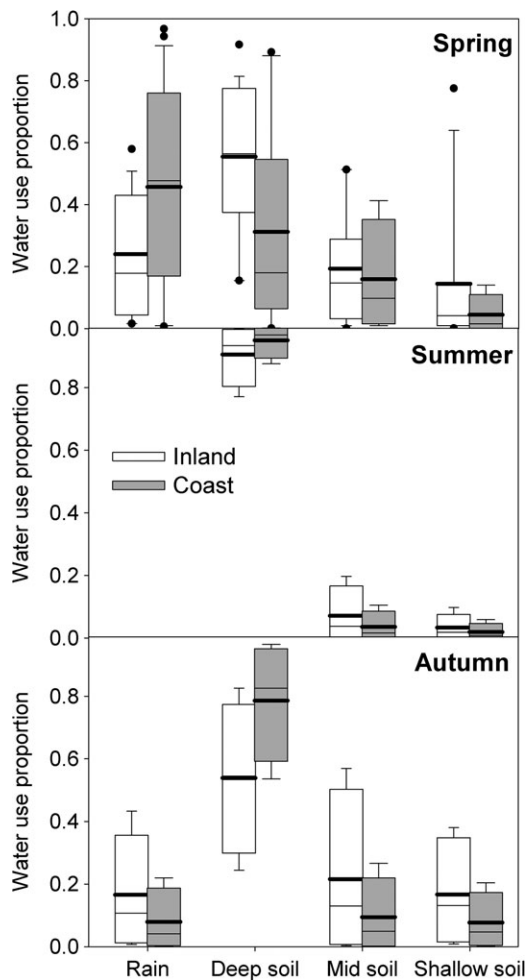
Figure 7. Results of MixSIAR Bayesian mixing models showing mean percentage of contributions to plant water uptake for each season in the coastal and inland populations obtained from the measured water isotope data of plants, rain and three different soil layers (deep, mid and shallow). Upper and lower boundaries of box denote 75th and 25th percentile ranges, respectively, with mean and median at the center line. Error bars show 95th and 5th percentile ranges.

as suggested by the more depleted $\delta^{18}O$ values recorded in the inland trees in both summers (Figure 4B and D).

Water sources and implications

In a previous work using Electrical Resistivity Imaging as a proxy for soil moisture, Ain-Lhout et al. (2016), showed that after a rain event, the moisture profile underneath the argan trees was more stable than outside tree locations. This study brings to light the role of argan roots in regulating the redistribution of soil moisture after a rain event, and that argan trees probably extracted water from the weathered layers to maximum depths of 3–6 m. Even though our study also highlights the importance of a deep root system, the data obtained allow us to conclude that major water extraction occurs at a lesser depth than the one described in Ain-Lhout et al. (2016), around 1.5 m. The physiological data recorded in our study evidence that this root depth is sufficient for providing an

optimal physiological performance of the species. In this same sense, in a global level simulation study, Kleidon and Heimann (1998) found that photosynthetic and transpiration rates increased 16% and 18%, respectively, when root systems incorporated soil water deeper than 1 m. It is noticeable that deeper root systems make the vegetation less vulnerable to seasonal water fluctuations, which has a positive impact on photosynthesis.

Although deep roots have been observed in many water-limited ecosystems in species adapted to water scarcity as the study case (Sternberg et al. 1996, Schenk and Jackson 2002a), the majority of roots are likely to be distributed in the top 30 cm of soil (Jackson et al. 1996, Schenk and Jackson 2002b, Gaines et al. 2016). Nevertheless, a relatively small biomass of deep roots may be disproportionately important for plants' performance (Jackson et al. 1999). As was expected for vegetation in ecosystems where water is limited, argan trees strongly rely on access to deep water stocks to withstand dry periods (Barbeta et al. 2015). Other authors (Msanda et al. 2007) have also emphasized that the ability of this species to survive arid conditions is in part due to its deep-reaching roots. However, so far the depth of the root system had not described as in this study.

Conclusion

Data allowed us to conclude that *A. spinosa* trees are capable of surviving long dry periods using soil water 1 m deep and usually close to 1.5 m, maintaining green leaves and high photosynthetic activity. Although there were slight differences between sites in water dependence, deep soil water was the main source in both sites. Furthermore, as the typical summer drought progresses, deeper soil water use increases. These findings can provide quantitative implications for further studies on argan restoration but they can also be a useful tool for other dryland woodlands. Drylands cover about 41% of Earth's land surface, and it is estimated that between 10% and 20% of the world's drylands endure one or more forms of land degradation (Safriel et al. 2005). Any effort to understand and protect these valuable systems would help to preserve their populations. Argan trees use water mainly up to 1.5 m depth; in order to improve the management of Argan forests in coexistence with a traditional agricultural settlement, other dryland crops using shallow water could be planted together. This association would be beneficial for both species, preventing at the same time soil erosion thanks to root systems.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of interest

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