Shifts of irrigation in Aleppo pine under semi-arid conditions reveal uncoupled growth and carbon storage and legacy effects on wood anatomy

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A B S T R A C T

Climate models forecast progressive aridification in the Mediterranean region. These warmer and drier conditions will probably lead to a decrease in carbon fixation and growth rates of drought-prone forests. To improve our knowledge on how water availability drives radial growth, wood anatomy and carbon storage we compared changes during a 40-year period in tree-ring width, wood-anatomical traits (tracheid lumen area, cell-wall thickness and parenchyma area), and stem sapwood non-structural carbohydrates (NSC) concentrations. We studied the drought-tolerant Aleppo pine (*Pinus halepensis*) comparing trees subjected to three irrigation regimes (control trees, no irrigation; IR trees, irrigated trees; TI trees, temporarily irrigated trees). Tree-ring width of TI trees widely overtook growth rates shown by IR trees during the irrigation treatment (1980s), whereas transversal lumen area and cell-wall thickness presented similar values in both groups of trees. Non-irrigated trees showed the lowest growth rates and also formed tracheids with the smallest lumen area and the narrowest cell walls. After the irrigation period, TI and trees without irrigation showed similar year-to-year growth variability. Lumen area of TI trees took 4–5 years to show values similar to those of the non-irrigated group. The TI trees had lower ray parenchyma area percentages during the irrigation period; afterwards both TI and non-irrigated trees formed similar amounts of ray parenchyma which were always higher than in IR trees. NSC concentrations were lower for IR trees suggesting either a dilution effect due to their significantly larger biomass or a higher demand for NSC by other carbon sinks such as primary growth. Aleppo pine may coordinate carbon supply and demand (growth) under changing conditions of water availability but long-term modification in soil moisture lead to legacy effects on wood anatomy.

1. Introduction

Drought is one of the major constraints to forest growth and productivity and is expected to become increasingly crucial in many regions because of the ongoing climate warming (Vicente-Serrano et al., 2013). Recent droughts have severely impacted forests worldwide causing: die-off events and tree mortality (Allen et al., 2010; Camarero et al., 2015; Sarris et al., 2007), transient declines in forest productivity (Baldocchi, 1997; Ciais et al., 2005; Dorman et al., 2013; Leuzinger et al., 2005) and higher vulnerability to attacks by biotic agents (Bréda et al., 2006). Improving our knowledge on the factors controlling tree growth is thus a critical step to assess and predict the impact of a drier climate on forests. This is specially compelling for Mediterranean areas where climate models predict warming and aridification (IPCC et al., 2013), which may threaten the survival of some pine stands and tree species at very xeric sites (Sánchez-Salguero et al., 2017, 2012).

There is evidence supporting drought-induced sink limitation in

trees because low water availability restricts carbon sinks (growth) more than sources (photosynthesis) (Lempereur et al., 2015), leading to an accumulation of non-structural carbohydrates (NSC hereafter) and often uncoupling carbon use (growth) from carbon uptake (photosynthesis) (Körner, 2003; Muller et al., 2011). Consequently, drought-triggered growth reduction can lead to increases in the concentrations of NSC (Sala and Hoch, 2009). However, NSC accumulation may also be explained by an active growth suppression to avoid carbon starvation under prolonged water shortage (McDowell, 2011; Wiley and Helliker, 2012). In that case, carbon starvation could be adaptive and driven by other factors such as phloem malfunction (Sala et al., 2012; Sevanto, 2014). This agrees with the observations that drought-tolerant tree species increase NSC content in response to water shortage (Piper, 2011) or with the conversion of stored NSC (e.g. starch) into mobile osmolytes (e.g. soluble sugars) during drought (Salleo et al., 2009). Such controversial views on how water availability drives growth dynamics and NSC storage require further insight (Hartmann and

Trumbore, 2016).Aleppo pine (*Pinus halepensis* Mill.) is one of the most common conifers in the Mediterranean Basin and presents a high growth plasticity and drought tolerance in response to changing water availability, which makes it an ideal subject for studying the effects of drought on tree performance in this region (Girard et al., 2012; Gazol et al., 2017). According to Borghetti et al. (1998) mature Aleppo pines subjected to long-term drought (≥ 12 months) showed significant reduction in most growth parameters followed by a quick recovery of the overall plant hydraulic resistance a few months after the drought treatment. The low wood density in Aleppo pine roots and its vulnerability to xylem embolism has been proposed as a way to protect needles or twigs more exposed to xylem cavitation (Oliveras et al., 2003). At stem level, tracheid lumen area in earlywood can be up to 30% smaller in trees growing in dry areas which allow them to better cope with severe droughts (Pacheco et al., 2017). These strategies for withstanding drought may lead trees to adjust their water conducting tissues to a more negative hydraulic pressure (Bréda et al., 2006). This is a cumulative long-lasting process, as the sapwood consists of multiple annual rings, and such long-term adjustments can be analyzed retrospectively combining growth responses and changes in wood anatomical traits that are proxies of hydraulic functioning and resilience to drought (Montwé et al., 2014).

Drought effects on growth and NSC have mostly been studied on saplings or seedlings under imposed drought treatments or different irrigation regimes, indicating a reduction in stem biomass and growth rate, an accumulation of shoot starch in drought-exposed individuals, and the development of carbon starvation under extreme drought conditions (Villar-Salvador et al., 1999; de Luis et al., 2011; Klein et al., 2011). However, there is a lack of similar approaches on mature trees under field conditions.

In this study, we analyze three groups of mature Aleppo pine trees growing in semi-arid conditions at the same site. Throughout 40 years one group was permanently irrigated, another was irrigated only during a 10-year period, and the third was growing without any additional watering. Following this experimental design, the objective of our research is to describe and quantify the long-term effects of irrigation on radial growth, wood anatomical traits (lumen area, cell wall thickness, and parenchyma area percentage) and NSC storage in the sapwood. According to the current knowledge on water and carbohydrate balance formerly presented and using the experimental design proposed, we hypothesize that irrigation will cause an uncoupling between growth, wood anatomy and NSC storage leading to the formation of: wide rings, tracheids with ample lumen areas and thick walls, wood with less parenchyma area and sapwood with lower NSC concentrations. Consequently, we expect that drought would lead to the opposite features.

2. Materials and methods

2.1. Tree species, study area and climate

Aleppo pine (*Pinus halepensis* Mill.) is a drought-tolerant evergreen conifer species, widely distributed across the Western Mediterranean Basin, and covering areas subjected to seasonal water deficit, mainly lowlands under dry to semi-arid conditions (Ne'eman and Trabaud, 2000). In northeastern Spain, this species presents a growing season that extends from early April to early November (Camarero et al., 2010).

Our study sites are Aleppo pine plantations located in the southeastern sub-urban area of Zaragoza (northeastern Spain) at 340 m. a.s.l. and on an almost flat topography (Table 1). Two of the sites (irrigated and non-irrigated trees) are located on the same stand but separated by a ground level difference, with the non-irrigated being few meters higher. The irrigated plot borders a residential area with permanent lawn water irrigation which indirectly maintains high water availability for the irrigated trees. The temporarily irrigated plot is 3 km to the west

on a similar terrain and soil features, and was artificially irrigated from 1980 to 1989 with sprinklers. Irrigation was done at early morning from March to October and corresponded to a supplementary rainfall of about 500 mm per year. In the currently-irrigated plot, irrigation corresponds to a supplementary rainfall of about 400 mm per year. The density and basal area of the Aleppo pine plantations of this area range between 162 and 542 stems ha^{-1}, and 4.12 and 20.82 m^2 ha^{-1}, respectively. The understory vegetation is dominated by shrubs (*Genista scorpius*, *Thymus vulgaris* and *Rosmarinus officinalis*). The soils are cambisols poorly developed over gypsum outcrops. More details on sampling sites are available in Sangüesa-Barreda et al. (2015).

According to data from the nearby Zaragoza-airport weather station (41° 40′ N, 1° 00′ W, 263 m a.s.l.), 10 km from the study sites, the climate in the area is Mediterranean of semi-arid type. During the 1950–2012 period, mean annual temperature was 14.8 °C (the coldest and warmest months were January and July, respectively) and total annual precipitation was 325 mm (the wettest and driest months were May and July, respectively) (Supplementary material, Fig. S1).

2.2. Field sampling and tree-ring data

During the winter of 2016, a total of 30 dominant and mature trees were sampled (10 per class considering control, IR and TI trees). We took two radial cores per tree at 1.3 m with 5-mm Pressler increment borers. Sampled trees were randomly distributed across the plantation and field sampling followed standard dendrochronological methods (Fritts, 2001). Wood samples were air-dried and polished with a series of successively finer grits sand-paper until rings were clearly visible. Tree rings were measured to the nearest 0.01 mm using a binocular scope and a LINTAB measuring device (Rinntech, Heidelberg, Germany). Cross-dating of the tree rings was checked using the COFECHA program (Holmes, 1983). Tree age at 1.3 m was determined by counting rings in the oldest core. With off-center cores we used a geometric pith locator to estimate the number of missing rings (Duncan, 1989).

Chronologies of tree-ring width (as well as wood anatomy traits) were produced for each site using ARSTAN, a software developed to remove age-size related growth trends, which are also present in wood-anatomical data (Carrer et al., 2015), and potential disturbance pulses (Cook, 1985). Individual series were fitted using a cubic smoothing spline function with 50% frequency–response cutoff of 30 years. Subsequently, we divided observed by expected values to obtain detrended and dimensionless indices, which were then subjected to autoregressive modelling to remove the remaining serial autocorrelation. These residual or pre-whitened indices were finally averaged using the biweight robust mean to create the site chronology of each variable for each irrigation plot.

2.3. Wood-anatomy analyses

A third 10-mm core was obtained from each tree for wood-anatomy analyses. In the laboratory, these cores were divided into 4-cm long pieces, boiled in water to soften the wood and remove the resin, and sliced into 10–12 μm thick transversal sections using a rotary microtome (Leica RM 2025, Heidelberg, Germany). The samples were then stained using a water-diluted mixture of safranin (1%) and astrablue (0.5%), and rinsed with water and ethanol. Afterwards sections were fixed on permanent slides with Eukitt (BiOptica, Milan, Italy) and scanned using a D-sight 2.0 System (Menarini Diagnostics, Florence, Italy) at 100× magnification, with a resolution of 1.99 pixels μm^{-1}. The images were then processed with the image analysis software ROXAS v3 (von Arx and Carrer, 2014; Prendin et al., 2017) that provided the lumen area (LA), cell-wall thickness (CWT), and relative position within the dated annual ring for each of the > 3.5 million measured tracheids. Tree-ring borders were manually drawn on the images and cross-dating was checked using the reference curves obtained in the previous tree-ring measurements. Information related to

Table 1
Geographical features and characterization of the sampled *Pinus halepensis* trees from the control (non-irrigated trees) and two treatments (IR: irrigated plot; TI: temporarily irrigated plot). DBH is diameter at breast height (measured at 1.3 m). Values are means ± SE. (Data collected on February 2016).

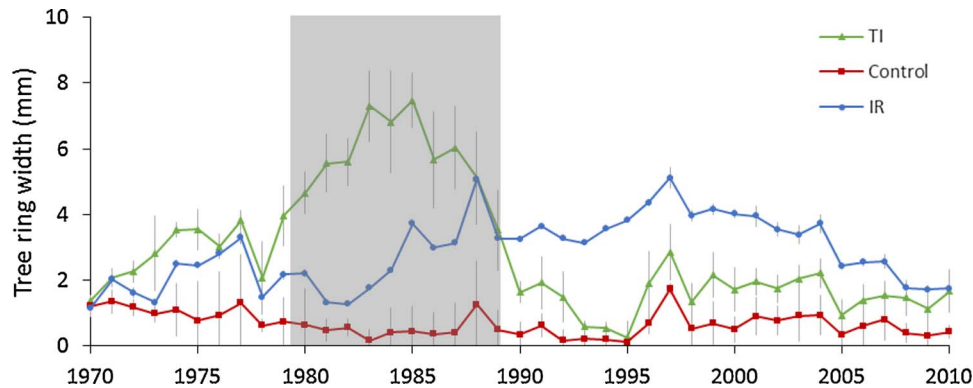|	Lat.	Long.	Altitude (m.a.s.l)	Tree characteristics						
				Plots	No. Trees	Age at 1.3 m (years)	DBH (cm)	Height (m)	Density (stems ha⁻¹)	Basal area (m² ha⁻¹)
Site characteristics	41° 47′ N	00° 43′ W	340	IR	10	48 ± 4	46.3 ± 2.5	13.3 ± 0.2	522	18.82
				Control	10	55 ± 7	16.4 ± 0.8	6.3 ± 0.2	182	6.12
				TI	10	51 ± 4	35.9 ± 1.2	14.1 ± 0.4	415	15.24

Fig. 1. Tree ring width (means ± SE) of the three Aleppo pine treatments (TI: temporarily irrigated trees; control: non-irrigated trees; IR: irrigated trees). Data correspond to the common period of 1970–2010, considering six trees per treatment. Shadowed area indicates temporary irrigation period.

cell position was used to better define the intra-annual profile of LA and CWT. For this, rings were divided in ten sectors following Castagneri et al. (2017). Lastly, we performed a supplementary manual analysis of the images with ROXAS to extract the values of the percentage area of transversal ray parenchyma in three separate years per decade (1972, 1975, 1978…, 2002, 2005 and 2008). The analysis was performed on the rings corresponding to the period from 1970 to 2015 (Supplementary material, Fig. S2).

2.4. Non-structural carbohydrates in stem sapwood

We quantified the mass-based concentrations of non-structural carbohydrates (NSC) in stem sapwood to compare the carbon status of trees subjected to different irrigation regimes (Hoch et al., 2003; Hoch, 2015). We selected ten individuals in each of the three pine classes (control, IR and TI trees). Sampling was conducted in February 2016 before growth started. In each individual, we sampled the stem sapwood by taking two radial cores at 0.5 m using a Pressler increment borer. All samples were collected between 10 and 12 a.m. Samples were transported to the laboratory in a portable cooler and then dried in an oven for 72 h at 60 °C. The outer sapwood portion (ca. 5 cm) was visually identified and separated from each core using a razor blade. In the case of TI trees, we considered sapwood rings corresponding to the irrigation period during the 1980s and those corresponding to the post-irrigation period (1990–2010) separately. Wood samples were subsequently frozen and stored at −20 °C until freeze dried. All dried samples were weighed and milled to a fine powder in a ball mill (Retsch Mixer MM301, Leeds, UK), and powder from both cores of each tree was mixed. Soluble sugars (SS) were extracted with 80% (v/v) ethanol and their concentration determined colorimetrically using the phenol-sulfuric method (Buysse and Merckx, 1993). Starch and complex sugars remaining after ethanol extractions were reduced enzymatically. NSC measured after ethanol extraction are referred to as SS (soluble sugars) and carbohydrates measured after enzymatic digestion are referred to as starch. The sum of SS and starch are NSC.

2.5. Data analyses

Growth and anatomical characteristic were compared using standard errors and to facilitate the interpretation of the figures considering the clear differences that they presented. Afterwards, assumptions of normality were tested by Kolmogorov-Smirnov and differences among the treatments in regard to growth and anatomical characteristics were analyzed by one-way ANOVA, considering the final irrigation treatments as main factors. Post-hoc comparison of the means was made using the Bonferroni test using $p < 0.05$ as the level of probability. Mann-Whitney U tests were performed to evaluate whether significant differences arose when comparing wood anatomical traits (LA and CWT) and sapwood NSC values among all treatments.

3. Results

3.1. Growth and wood anatomical features

Tree-ring width was significantly and constantly lower ($F = 40.32$; $p < 0.001$) in the non-irrigated trees (control plot, mean 0.6 mm) if compared to the irrigated trees (IR plot, mean 3.2 mm) during most of the common period from 1970 to 2010 (Fig. 1). During the 1980s, when irrigation was provided to the temporarily irrigated trees (TI plot), their mean ring-width almost doubled (mean 5.7 mm) that of the IR trees. From 1990 onwards, with no more irrigation, growth of TI trees decreased to values similar to those of the control plot, being slightly wider (2.1 mm) but not significantly different compared to control trees (0.4 mm).

The quantitative description of wood anatomy was based on rings formed between 1970 and 2010 and included the measurement of almost 3 million tracheids between the three groups of trees (Control:0.49 10⁶ tracheids, TI:1.20 10⁶ tracheids, IR:1.24 10⁶ tracheids) (Fig. 2). Non-irrigated trees showed smaller tracheid dimensions derived from both reduced lumen area (LA = 445.7 ± 24.1 µm²) ($F = 54.86$; $p < 0.001$) and cell wall thickness (CWT = 4.66 ± 0.08 µm) ($F = 182$; $p < 0.001$) when compared with the other two plots. While the IR trees showed significantly higher values for both wood traits
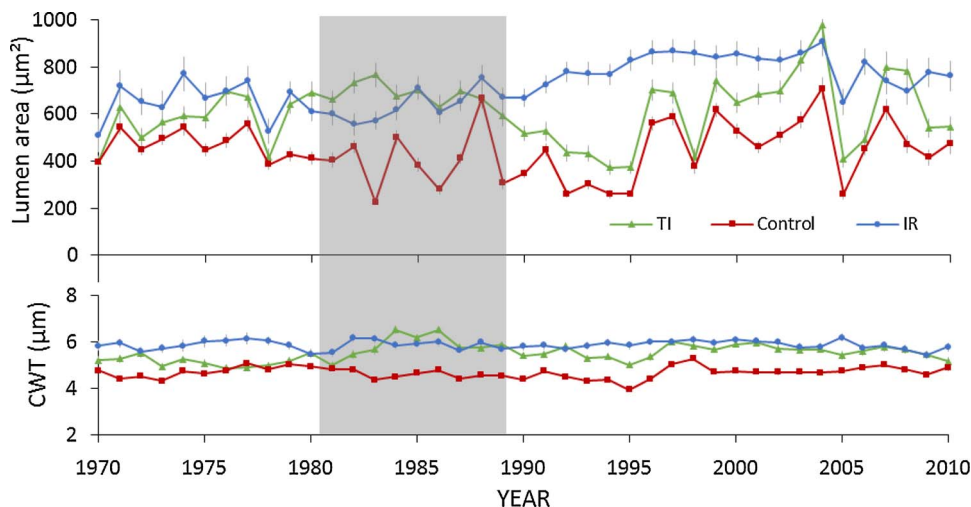
Fig. 2. Wood anatomy series (means ± SE) of lumen area and cell wall thickness (CWT) for the common period of 1970–2010 in the three Aleppo pine treatments (TI: temporarily irrigated trees; control: non-irrigated trees; IR: irrigated trees). Shadowed area indicates irrigation period in the TI plot.

(LA = 724.3 ± 27.1 μm²; CWT = 5.88 ± 0.11 μm), the TI trees presented intermediate values (LA = 613.9 ± 34.2 μm²; CWT = 5.54 ± 0.12 μm). In the case of LA, TI and IR trees showed similar inter-annual variability since the 1990s. This last result mirrors year-to-year changes in tree-ring width presented in Fig. 1.

3.2. Intra-annual wood anatomical patterns

During the 1980s, IR and TI trees showed no significant differences in their intra-annual wood-anatomy profiles ($F = 3.20$; $p = 0.08$) (Fig. 3). During the other decades, tracheid lumens were wider in IR trees but smaller in control trees. This difference magnified in warm and dry periods such as the early 1990s when radial growth was very low (0.1–2.0 mm) in control and TI trees (Fig. 1). CWT values did not differ between TI and IR trees during the 1980s, but differences appeared in the other decades and particularly in the latewood (6th–10th sectors) ($F = 54.94$; $p < 0.001$). Sector by sector values are available for consultation in Table S1, Supplementary material.

3.3. Sapwood non-structural carbohydrates and ray parenchyma

Only the IR trees showed a significantly lower percentage of non-structural carbohydrates (NSC), especially the starch component (Fig. 4). Soluble sugar concentrations showed no differences among the treatments.

Analyses of the NSC of TI trees in rings formed during the irrigation period (1980s) and post-irrigation period (1990–2010) resulted in non-significant differences ($p = 0.028$) showing NSC concentrations of 3.27 ± 0.13% and 3.37 ± 0.13%, respectively. In contrast, and considering the 1980–2010 period, the average values of wood anatomical traits were significantly lower for control trees, while tree-ring width values differed between all treatments (Table 2).

Overall, IR trees formed significantly ($p < 0.05$) less ray parenchyma area (3.9% on average) than control (5.2%) and TI (5.4%) trees, but differences between the two last groups were not significant for most of the years. IR and TI trees had similar percentages of ray parenchyma area during the 1980s, after which IR trees featured a rather constant percentage (ca. 4%) while TI trees showed an increase
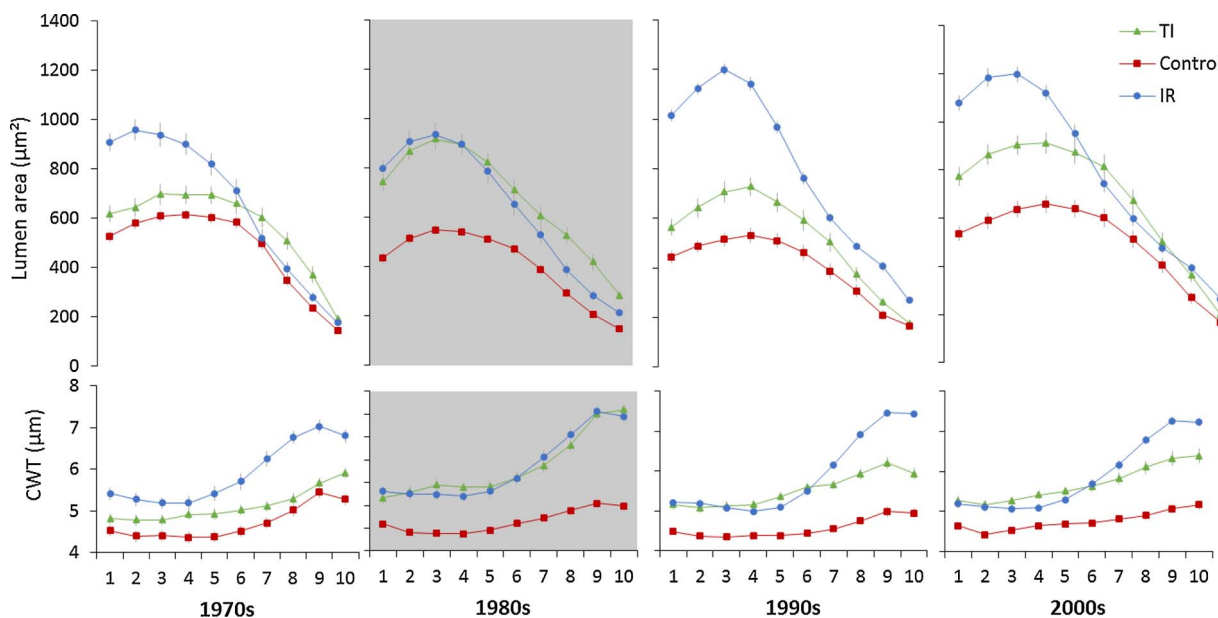

Fig. 3. Intra-annual values (means ± SE) of lumen area and cell wall thickness (CWT) of the three Aleppo pine treatments (TI: temporarily irrigated trees; control: non-irrigated trees; IR: irrigated trees). Values are shown separately for each decade of the common period (1970– 2010). The shadowed area indicates the decade under temporary irrigation.
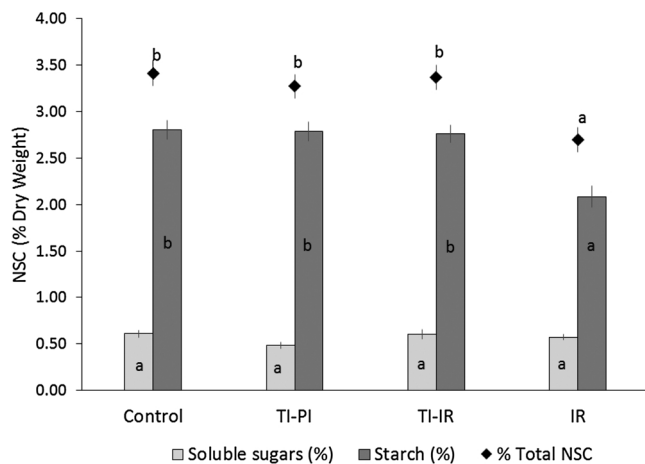
Fig. 4. Sapwood non-structural carbohydrate (NSC) concentrations obtained in TI-IP (temporarily irrigated trees, tree rings formed during the irrigation period in the 1980s), TI-PI (temporarily irrigated trees and rings corresponding to the post-irrigation period, 1990–2010), control (non-irrigated trees) and IR (irrigated trees). NSC was divided into starch and soluble sugars represented as bars with different fills, while black diamonds are the summed amount of all NSC. Error bars show standard errors and different letters represent significant ($p < 0.05$) differences between groups according to Mann-Whitney U tests.

in ray area which stayed close to that of the control group (ca. 6%). It is possible to appreciate a convergence in percentage ray area (4–5%) among the three groups of trees, likely in response to wet conditions in 1988, 2002 and 2008 (Fig. 5).

4. Discussion

As hypothesized, irrigation enhanced both primary (tree height; Table 1) and secondary growth (ring width; Fig. 1), allowed the formation of tracheids with wider lumen areas and thicker cell walls (Figs. 2 and 3), and reduced the sapwood NSC concentration (Fig. 4) and ray parenchyma area (Fig. 5).

Most previous studies aiming to explore the growth and carbon balance response of trees to different regimes of water availability focused on simulating different levels of short-term drought (Irvine et al., 1998; Jyske et al., 2010; Klein et al., 2011, 2014; Montwé et al., 2014). In our research, as in a few others (Dobbertin et al., 2010; Martin-Benito et al., 2017; Timofeeva et al., 2017; von Arx et al., 2017), we instead aimed to investigate the long-term irrigation effects on radial growth and carbon storage in wood, specifically targeting mature Aleppo pines growing in the field and on a xeric site under semi-arid conditions. All trees presented similar year-to-year growth variations (Fig. 1) which parallel the precipitation pattern already reported for the region (Pacheco et al., 2016). This represents the typical response of Aleppo pine growing in water-limited forests (Pasho et al., 2012; Klein et al., 2014; Sarris et al., 2013; Gazol et al., 2017) and supports the evident reaction of the species, even under moderate irrigation, to

changes in soil water availability or precipitation. In the case of the TI trees, their tree-ring growth was double those in the IR plot during the irrigation period. This difference is explained given that IR trees were exposed to indirect irrigation due to their position next to an irrigated area, while TI trees were directly irrigated. After the years of watering, the tree-ring width values of TI trees fell immediately and synchronized with those of the other plots for the rest of the period, tending to maintain higher values than the control trees but not significantly different in most of the years. This supports the idea of a short-term influence of irrigation on the growth performances of the species, without any significant carryover or legacy effect.

Wood-anatomical traits provide further insight into the long-term effects of irrigation on tree functioning (Fig. 2). TI trees depicted a legacy effect of the irrigation treatment on lumen area, with 4–5 years of consistently higher values before returning to follow the same pattern as control trees. This reduction in lumen area is in line with the expected plastic response of xylem anatomy (tracheid expansion) to increase resistance to cavitation when drought conditions arise (Hacke et al., 2001; Hacke and Sperry, 2001). No significant inter-annual changes were observed in cell wall thickness over the analyzed period, confirming that this trait is less sensitive than lumen area to environmental variability in drought-prone regions (Pacheco et al., 2016, 2017). The decadal averaged ring profiles (Fig. 3) permitted a better assessment in tune with long-term changes of wood-anatomical traits. With irrigation during the 1980s, TI trees showed an increase in both lumen area and cell-wall thickness that presented values similar to IR trees. In contrast to the tree-ring width data, the average ring profiles highlighted how in the two decades following the irrigation treatment, lumen areas of TI trees remained significantly larger than those of control trees. In the case of cell-wall thickness during the post-irrigation decades, TI trees maintained similar values to IR trees, but just along the first six sectors of the profile that matches the earlywood. Considering the tree characteristics shown in Table 1 (similar age and height in IR and TI trees), allometric balance is likely to be the main reason for their similar wood-anatomical traits after the irrigation treatment. Having similar height and therefore similar root-to-leaves path length for water transport, trees will tend to produce cells with similar lumen area in order to optimize their hydraulic architecture and minimize resistance to water flow (Carrer et al., 2015). Nonetheless, while both groups of trees feature similar heights they do have significantly different diameter, suggesting that secondary growth has been more affected by water limitation than primary growth. Stem sapwood NSC showed no significant differences between the rings formed during or after irrigation in the TI trees, nor was there a difference from the control (Fig. 4). The only significant difference we found was with IR trees, where the starch component of total NSC had a smaller percentage than any other group (Fig. 4). This result, where trees in the wettest condition (IR) had a lower NSC concentration, but also less xylem area occupied by ray parenchyma (Fig. 5) than non-irrigated and TI ones, conforms more with the idea of drought-induced growth reductions than with a decline of carbon uptake (Granda and

Table 2

Mean growth (tree-ring width), wood-anatomical traits (lumen area, cell-wall thickness) and stem sapwood non-structural carbohydrate (NSC) concentrations in Aleppo pine trees. Trees are classified as irrigated (IR), non-irrigated (control), and temporarily irrigated (TI) trees. Rings of TI tree are analyzed separately for the irrigation- (TI-IP) and post-irrigation (TI-PI) periods. Growth and anatomy data correspond to the common period 1980–2010. NSC values are shown considering soluble sugars (SS), starch and total NSC concentrations. Different letters indicate significant ($p < 0.05$) differences according to Mann-Whitney U tests. Values are means ± SE.

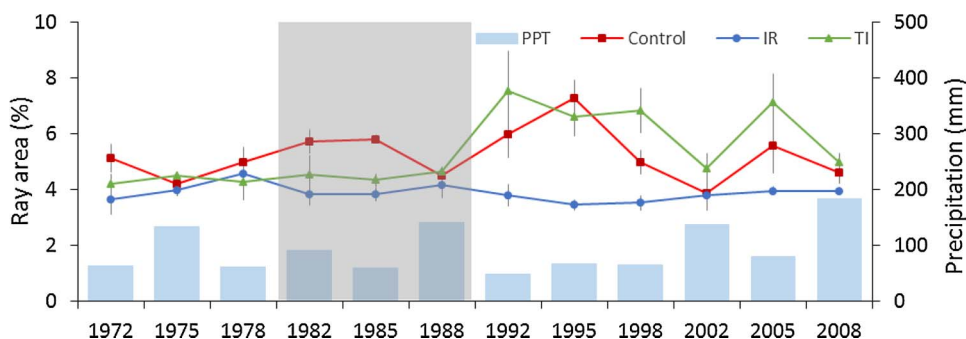| | Tree-ring width (mm) | Wood anatomy | | Sapwood NSC concentration | | |
		Lumen area (µm²)	Cell-wall thickness (µm)	SS (%)	Starch (%)	Total NSC (%)
IR	3.23 ± 0.13	750.2 ± 28.5 b	5.88 ± 0.08 b	0.57 ± 0.03 a	2.09 ± 0.12 a	2.70 ± 0.13 a
Control	0.57 ± 0.04	435.8 ± 19.0 a	4.66 ± 0.05 a	0.61 ± 0.04 a	2.80 ± 0.10 b	3.41 ± 0.14 b
TI-IP	5.78 ± 0.42	678.4 ± 45.6 b	5.86 ± 0.16 b	0.48 ± 0.04 a	2.79 ± 0.10 b	3.27 ± 0.13 b
TI-PI	1.55 ± 0.14	598.8 ± 32.4 b	5.58 ± 0.09 b	0.60 ± 0.05 a	2.76 ± 0.09 b	3.37 ± 0.13 b

Fig. 5. Percentage of parenchyma ray area for the three Aleppo pine groups (TI: temporarily irrigated trees; control: non-irrigated trees; IR: irrigated trees) measured from transversal anatomical sections. Values are means ± SE for each depicted year and bars show the precipitation sum from March to June of each year. Shadowed area indicates temporary irrigation during the 1980s.

Camarero, 2017; Pellizzari et al., 2016). These findings depict the long-term extension of what has been described in Aleppo pine by other authors working on the effects of short-term (two years) drought conditions (Klein et al., 2014); i.e. higher NSC levels in slow-growing trees subjected to dry conditions and lower levels in fast-growing unstressed trees. Higher values for both NSC and ray parenchyma area could be a response of non-irrigated trees in order to protect their hydraulic system under dry conditions as sugars probably play an important role in safeguarding the hydraulic system (Granda and Camarero, 2017; von Arx et al., 2017). In parallel, NSC concentrations are generally higher in actively growing tissues such as leaves, fine roots and phloem (Gruber et al., 2013; Hoch et al., 2003; Pruyn et al., 2005), and this could explain why sapwood NSC concentrations are significantly lower in our fast-growing IR trees. These results seem to indicate a dilution effect on NSC when considering the total biomass of IR trees compared to the other treatments. From the total NSC, the starch component is the one accounting for the significantly lower concentrations measured, which reinforces the assumption that the highly active areas of the crown require these rapidly available assimilates as soluble sugars to sustain growth, or that these sugars are used as osmolytes to equilibrate intra- and extra-cellular solute concentrations (Oribe et al., 2003).

Parenchyma in the stem sapwood stores a large proportion (25–40%) of the overall NSC reserves in a tree, which makes it a more important NSC reservoir than the phloem or leaves (Jacquet et al., 2014). Measuring the total percentage of ray area per year (Fig. 5), we tried to elucidate if irrigation affected long-term carbon allocation in the wood and if this anatomical variable could be considered a proxy for sapwood NSC concentrations. The lower percentage of ray parenchyma area in IR trees suggests that irrigation enhanced growth and reduced NSC concentration, however the increase in ray parenchyma was not proportional to the increase in xylem area occupied by tracheids. This result may indicate that higher water availability improves growth and tracheid expansion rather than the formation of ray parenchyma and the corresponding increase in NSC reserves. In addition, the TI trees had lower ray area percentages during the irrigation decade (1980s) and these percentages rose afterwards to match those of the control, which is in line with other researches on Scots pine (von Arx et al., 2017). During the studied period, the three wettest springs (1988, 2002 and 2008) confirm the key role of water availability not just on ring width, but also on parenchyma ray area, as shown in Fig. 5. This convergence of all treatments in those three years coincides with the peak growth rate for Aleppo pine in this location, as observed by Camarero et al. (2010) and Pacheco et al. (2017). Further research focusing on NSC pools could investigate whether the increase of NSC concentrations in non-irrigated trees compensates for their smaller diameter as compared with IR trees.

Growth, wood-anatomical traits and parenchyma measurements, but not NSC concentrations, showed significant differences between the irrigation and post-irrigation phases in TI trees. As NSC concentrations are not evenly distributed in the sapwood, but typically show a decrease from the outer to inner sapwood (Hoch et al., 2003; Saranpää and Höll, 1989), this could explain why no significant differences were found in

this case. Another explanation could be the ability of the trees to mobilize sapwood NSCs of different age (Richardson et al., 2013). However, it is also possible that when TI trees were first exposed to irrigation it allowed them to enhance photosynthesis and allocate relatively more NSC to growth than carbon storage, while still increasing their NSC pool size (von Arx et al., 2017). This could be considered as a strategy to increase NSC storage during good periods to withstand future droughts. Nevertheless, an open question is to discern if NSC pools changed as a function of irrigation, in parallel with the long-term changes to ray parenchyma suggesting an active long-term response. The decrease of NSC concentration in IR trees could in fact also be viewed as a passive "dilution effect".

5. Conclusions

Our 40-year record of tree-ring growth and wood anatomical traits has improved the general understanding of long-term responses to different water availability in the drought-tolerant Aleppo pine. We showed that xylem anatomy was affected by changes in the irrigation regime; that anatomical traits are able to promptly adapt their structure to cope with improved water availability but also that they can maintain a legacy effect. Irrigation enhanced radial growth, favored the formation of tracheids with wider lumen areas and thicker cell walls, reduced sapwood NSC concentration and the xylem area occupied by ray parenchyma. Our findings indicate that under changing environmental conditions drought-adapted tree species are able to coordinate the carbon storage and carbon use in growth.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agrformet.2018.02. 018.

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