Tree-ring wood anatomy and stable isotopes show structural and functional adjustments in olive trees under different water availability

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Abstract

Background and Aims Olive tree (*Olea europaea* L.) is a drought-tolerant tree species cultivated in Mediterranean-type environments. Although it is tolerant to drought, dry conditions decrease its productivity. A thorough analysis of the hydraulic architecture and wood anatomical plasticity, as well as of their physiological significance, is needed to understand how olive trees will adapt to the predicted increase in frequency and severity of drought in the Mediterranean region.

Methods Dendrochronological, stable isotopic ($\delta^{13}C$, $\delta^{18}O$) and wood anatomical analyses were applied to understand how different water availability can affect wood stem structure and function, in rainfed and irrigated at 100 % of crop evapotranspiration (ETc) olive trees in an experimental orchard close to Benevento (Italy) from 1992 to 2009.

Results Dendrochronological data indicate that cross-dating and synchronization of ring-width time series in olive tree is possible. After the start of irrigation, significantly more negative $\delta^{13}C$ and lower $\delta^{18}O$ values were recorded in irrigated trees indicating higher stomatal conductance and transpiration rates. Increased water balance induced the formation of a higher number of vessels with higher diameter.

Conclusions Water balance variations affected wood anatomy and isotopic composition. Anatomical analyses detected structural and functional adjustments in rainfed trees that produced more vessels with lower diameter to prevent cavitation. Isotopic analyses

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confirmed that irrigated trees continuously showed enhanced transpiration rates.

Introduction

Olea europaea L. (olive tree) is a drought-tolerant tree species widely cultivated around the Mediterranean basin. It is very important for its typical landscaping characteristics, as well as for its fruit and oil production. Although it is drought-tolerant (Connor and Fereres 2005; Tognetti et al. 2008), dry conditions decrease its productivity and, therefore, irrigation systems have been developed to increase yield (d'Andria et al. 2009; Correa-Tedesco et al. 2010). The economic benefits induced by irrigation techniques has led to an increase in irrigated olive orchards and 20 to 30 % of the olive orchards supplying fruit to the oil extraction industry are irrigated (Lavee 2011). Although several studies have demonstrated the physiological adjustment of olive in response to drought, e.g., tree water consumption (Giorio et al. 1999; Tognetti et al. 2004, 2005, 2007, 2009), we still lack information on the fundamental processes controlling the long-term effect of these short-term adjustments (e.g. vessels whit lower diameter). A thorough understanding of adaptive changes in wood anatomical structure and hydraulic architecture would help explain their functional significance (Fernandéz et al. 2011; Martín-Vertedor et al. 2011a, b).

Water transport capacity through the xylem tissue is closely connected to water use strategy and net primary productivity of the plant in a given environment (Fonti and Jansen 2012). Wood anatomical studies report that the frequent appearance of vessels with short narrow elements, small intervessel pits, and helical thickenings in semiarid-zone species is the result of a strategy for conductive safety (Christman et al. 2011). Narrow vessels can then take over water transport, albeit at much reduced rate because of their higher resistance to flow. Xylem hydraulic architecture, such as the arrangement of conduits, their frequency, length, diameter, wall thickness, and pit characteristics, not only regulates hydraulic resistance but also safeguards against hydraulic failure (Hoffmann et al. 2011; Lens et al. 2011). If xylem physiology can be useful in interpreting long-distance water transport in the short-term, dendrochronological analyses can reveal olive tree response to drought, over the past.

Dendrochronological studies in the Mediterranean region are scarce because tree rings do not always form everywhere. Because of mild winter conditions and summer droughts, false rings or intra-annual density fluctuations are often formed, and the identification of tree-ring boundaries is difficult (Cherubini et al. 2003; Battipaglia et al. 2010; Gea-Izquierdo et al. 2012). However, the study of tree rings can facilitate the understanding of tree response to water availability (Sánchez-Salguero et al. 2012) and this can help design better irrigation protocols for modern olive tree plantations.

Tree-ring stable isotopes can be used to identify long-term adjustments in tree gas-exchange processes (Roden and Farquhar 2012). Carbon stable isotopes, usually reported as δ^{13}C, have been used successfully to estimate past stomatal activities, photosynthetic rates, and growing-season mean water-use efficiency (WUE) of C_3 plants (Farquhar et al. 1989; Cabrera-Bosquet et al. 2009; Di Matteo et al. 2010), whereas δ^{18}O stable isotopes give information on transpiration rates and water resources (Scheidegger et al. 2000; Cabrera-Bosquet et al. 2011). While several studies have clearly shown that the isotopic composition of tree rings can be a valuable source of information for the reconstruction of carbon balance and water relations of plants and environmental variability, most investigations to date have been based on the independent analysis of δ^{13}C or δ^{18}O (McCarroll and Loader 2004). A combined use of δ^{13}C and δ^{18}O can provide useful information on past leaf physiology and xylem functioning.

Our first hypothesis was that tree-ring traits would match water transport processes affected by irrigation practices, and that long-term adjustments to varying water availability in olive trees would be revealed by wood anatomy. We made dendrochronological and wood-anatomical analyses that confirmed adjustments in ring-width and vessels density in the first years after irrigation treatments were imposed. Our second hypothesis was that changes in stress physiology with time, as affected by varying water availability to plants, would be revealed by isotope signature in tree rings. In particular, we anticipated that cellulose δ^{18}O and δ^{13}C might have been a result of changes in leaf functions (such as changing stomatal conductance and photosynthetic rate). Even though many studies have investigated anatomical traits and stable isotopes in tree rings, the combination of these

proxies has not been sufficiently explored, especially in olive tree plantations. These combinations could, indeed, clarify important key yet unanswered ecophysiological questions and would likely help interpreting the often ambiguous physiological data obtained at the same plantation over the years. We therefore applied dendrochronological, stable isotopes and wood anatomical analyses to study the long-term responses of rainfed and irrigated olive trees over 18 years.

Materials and methods

Study area

The study was conducted at the experimental olive orchard of the Consiglio Nazionale delle Ricerche (CNR), Istituto per i Sistemi Agricoli e Forestali del Meridione (ISAFoM), located near the city of Benevento (Southern Italy), in a typical olive growing area (Lat. 41° 06′ N, Long. 14° 43′ E; 250 m a.s.l.). The soil type at the study site is sandy loam (1.76 % organic matter; 1 % $CaCO_3$; 0.15 % N; pH 7.2), characterized by 35.6 % volumetric water content (m m^{-3}) at field capacity (soil matric potential of −0.03 MPa) and 21.2 % at wilting point (soil matric potential −1.5 MPa), and an apparent bulk density of 1.25 t m^{-3} (d'Andria et al. 2009).

Olive trees (Olea europaea L., cultivar 'Nocellara del Belice') were planted in rows 6 m apart with tree spacing of 3 m with a plantation density of 555 plants ha^{-1}. The orchard was established in 1992 with 1-year-old cuttings, and plants were pruned every year according to the central leader system in which the trunk forms a central axis with branches distributed laterally up and down and around the main stem (Fontanazza 1994). For the first 2 years the plants were irrigated equally to ensure uniform development. Six olive trees similar in crown and stem size were selected and assigned to two irrigation levels: a non-irrigated control (rainfed) and an irrigation treatment that received seasonal water amount equivalent to 100 % of maximum evapotranspiration (ETc) when the 'available water' was below 50 %.

Irrigation started in 1995 and it was delivered daily using a drip irrigation system with four drip nozzles per plant, connected to a single drip line at a distance of 0.5 and 1 m from the stem, from the beginning of pit hardening to early fruit veraison. Irrigation volumes were estimated from Class A evaporation pan (Doorenbos and Pruitt 1977), placed in the proximity of a standard

meteorological station adjacent to the experimental field, which recorded half-hourly averages of global radiation, air temperature, relative humidity, wind speed and rainfall. Pan evaporation (ETP, mm) was corrected with a pan coefficient (Kp) to obtain reference crop evapotranspiration (ETo), a crop coefficient (Kc) and a tree ground cover coefficient (Kr), according to Fereres et al. (1981). Coefficients changed over the years and they can be found in the literature of studies on the same plantation (Giorio et al. 1999; Tognetti et al. 2004, 2005, 2009). Treatments were arranged in a completely randomized design and replicated three times.

The groundwater used for irrigation had a pH of 7.40 and electrical conductivity of 0.68 dS m^{-1} (Na$^+$ 1.82 mM, K$^+$ 0.30 mM, Ca^{2+} 3.30 mM, Mg^{2+} 0.35 mM and Cl$^-$ 0.54 mM).

Meteorological data

Temperature, precipitation and class A pan evaporation were estimated from a standard meteorological station adjacent to the experimental field which recorded half-hourly averages of global radiation, air temperature, relative humidity, wind speed and rainfall. Data were computed from April 1st to October 31st each year (from 1992 to 2009) to create Climate Water Balance (CWB = ETP – P, where P is the total precipitation, in mm) and Degree-Days (DD) threshold minimum 10 °C and threshold maximum 30 °C. The total amount of heat required, between the lower and upper thresholds, for the olive plants to develop from one point to another in its vegetative period, is calculated in units called degree-days (°D). Calculation of Degree-Days, by the single triangulation method, was based on Zalom et al. (1983).

Sampling and sample preparation

In November 2009 three rainfed and three irrigated olive trees, were felled and sampled. One cross section was taken at 0.5 m of height from each tree. In the laboratory, the cross sections selected for the ring-width analyses were dried at room temperature and sanded with progressively finer abrasive paper to recognize the annual rings and detect the ring borders under the stereoscope.

Dendrochronological analyses

Tree-ring chronologies were developed using standard dendrochronological procedures (Stokes and Smiley

1968). The tree rings of each section were dated, and for each tree ring the exact year of formation was recorded. Tree rings were identified and dated by counting them from bark to pith with the help of a stereomicroscope (Wild M3Z, Leica, Germany). Ring-width measurements were made to the nearest 0.01 mm using the Time Series Analysis and Presentation (TSAP) software package and the LINTAB measuring table (Rinntech, Heidelberg, Germany).

The tree-ring series were visually synchronized to identify the locally absent rings and the false rings often found in Mediterranean species (Cherubini et al. 2003), and to check for errors (Fritts 1976). Dendroclimatical analyses were used to evaluate the effects of monthly precipitation and temperature on tree-ring growth.

Differences in growth may be misleading on the basis of changes in ring width alone. The conversion of ring width to basal area increment (BAI) overcomes this problem. Measurements of BAI are closely related to annual aboveground wood production and, unlike tree ring width, temporal BAI trends are positive and can be maintained for many decades after trees reach maturity, until senescence is reached, or environmental stress is applied (Silva and Anand 2013; Gómez-Guerrero et al. 2013). The conversion of ring width into BAI is usually expressed in annual time step resolution (1 year = 1 full ring, late and early wood) using a simple equation: $BAI = \pi (R_n^2 - R_{n-1}^2)$. Where R is the tree radius and n is the year of tree ring formation

Stable isotopes analyses

Samples were taken for the isotopic analyses from the lowest not sanded cross-section. Earlywood and late-wood from each single tree-ring were split with a razor and each wood was ground using a centrifugal mill (Mixer Mills, MM 200, Retsch, Haan, Germany). Each section was milled and an aliquot of a few mg (approximately 1.5 mg) was packed in porous bags and used for cellulose extraction, as described in Battipaglia et al. (2008). Wood isotopic ratio was analysed by combustion of the samples in an elemental analyzer (EA-1108, Carlo Erba, Milan, Italy) coupled with an isotopic ratio mass spectrometer (S delta, Finnigan MAT, Bremen, Germany) via a variable open split interface (Conflo II, Finnigan Mat, Bremen, Germany). The results were expressed as delta (δ^{13}C) in ‰ relative to the Vienna Pee Dee Belemnite (VPDB) standard where: [δ^{13}C=(R sample/R standard −1)×1000], where R sample and R standard represent

the ^{13}C/^{12}C molar ratios of the sample and the standard respectively. Analysis precision was higher than ±0.11‰.

For δ^{18}O, in a separate measurement, an aliquot (1.1–1.3 mg) of bulk organic material was decomposed to carbon monoxide (CO) by thermal pyrolysis at 1080 °C, according to Saurer et al. (1998) in a different elemental analyzer (EA-1108, Carlo Erba, Milano, Italy), which was connected to a continuous flow mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). The results are presented in the same notation of the carbon: δ^{18}O=(R sample/R standard)−1)×1000(‰), relative to the international Vienna Standard Mean Ocean Water (VSMOW) for oxygen. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was better than ±0.3‰ for oxygen.

The calibration versus VPDB was done by measuring IAEA USGS-24 (graphite) and IAEA-CH7 (polyethylene), while the calibration versus VSMOW was done using IAEA-C3 cellulose and other intercomparison standards (Saurer et al. 1998).

The raw δ^{13}C chronology exhibited a decline in δ^{13}C mainly in the twentieth century, which may be attributed to the lowering of δ^{13}C of air through anthropogenic-related increases in CO_2 concentration (the "^{13}C Suess effect"), see Cullen and Grierson (2007). We removed this trend in the carbon isotope chronology using the annual records of past atmospheric δ^{13}C obtained from ice cores (Francey et al. 1999).

Wood anatomical analyses

Wood anatomical analyses were performed on all tree rings of all trees, but only in 1995, 1996 and 1997 variations in wood anatomy could be observed. Thin sections for wood-anatomical analyses were prepared using the cross sections previously used for dendrochronological analyses. Radial sections were subdivided into approximately 1.5–2 cm long pieces in order to fit into the microtome. Thin sections (10 μm thick) were then cut from each piece with a sliding microtome (Reichert, Germany), stained with safranin (1 %) and astrablue (2 %), dehydrated with ethanol (70, 95 and 100 %) and xylol, and fixed on microscope slides with Canada balsam (Schweingruber 2001; Fonti et al. 2009). Images (RGB, color 24 bit) were captured ring-by-ring using a digital video camera (EC3, Leica Microsystems, Wetzlar, Germany) connected to a transmitted light microscope (Orthoplan, Ernst Leitz, Wetzlar, Germany). Vessel diameters were measured using the ImageJ

Fig. 1 Weather trends: DD (Degree-Day, threshold minimum 10 °C, threshold maximum 30 °C) and CWB (Climate Water Balance, CWB = ETP – precipitations). Data were recorded daily by a weather station located directly into the experimental farm. Meteorological data were selected each year by April 1st until October 31st (only the vegetative season was considered)
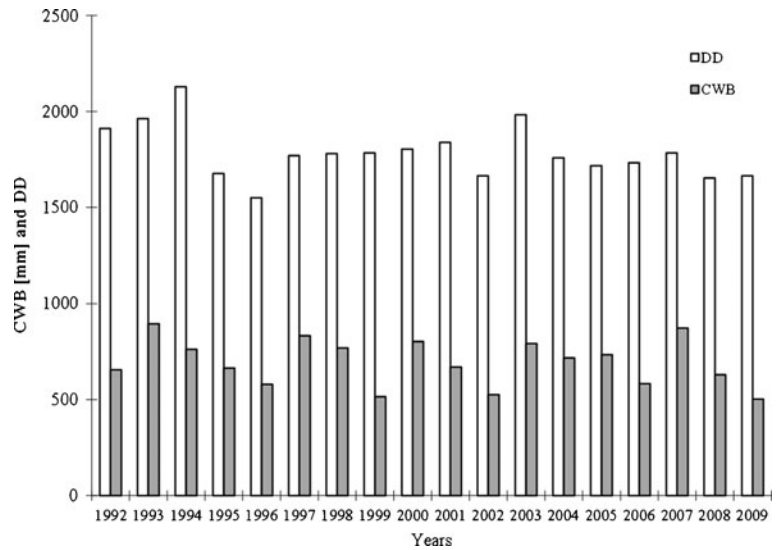
program (version 1.46, National Institutes of Health, Bethesda, MD, USA).

Statistics

Statistical tests were performed to evaluate the variability of the results and, in particular, their significance ($P \leq 0.05$). The CoStat program (version 6.203, Cohort Software, Monterey, CA, USA) was used to perform the analysis. We carried student's t-test to evaluate the variability of growth trends, diameter and vessel density in wood anatomical analyses and carbon ($\delta^{13}C$) and

oxygen ($\delta^{18}O$) stable isotope results. We also carried out χ^2-test to evaluate the variability on vessel diametric classes. As growth trends, diameter and vessel density, and carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes have a normal distribution, Student's t-test was used to determine if our two sets of data (Rainfed and Irrigated) were significantly different from each other. However, wood anatomical experimental data were composed of frequencies distributed in discrete categories, therefore, χ^2-test was performed in order to determine significance of differences between the two independent groups (Rainfed and Irrigated). With the aim to obtain a measure

Fig. 2 Ring-width growth (mm) trends. Water availability was modified starting in 1995. Student's t-test was performed and significant difference ($P \leq 0.05$) have an asterisk (*). Bars indicate ± SE. Triangles (▲) indicate years in with water availability is the same for both rainfed and irrigated trees
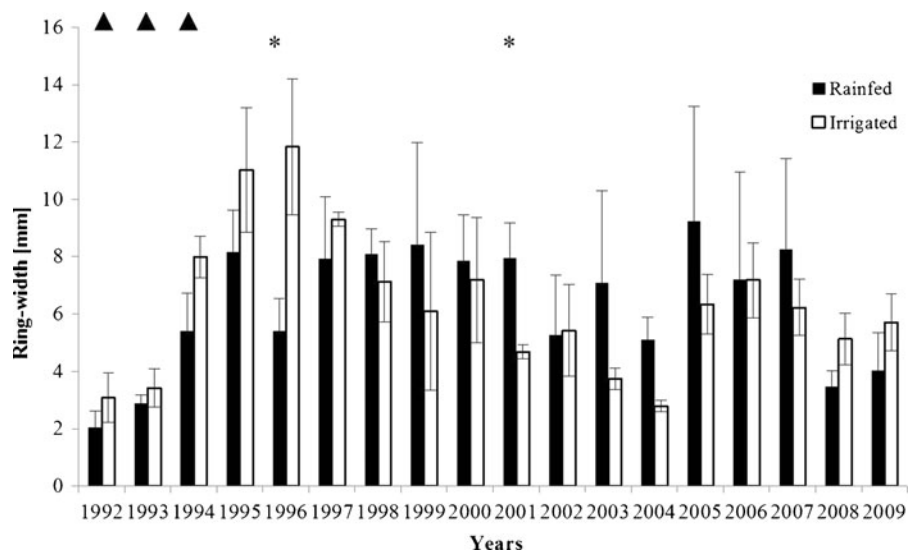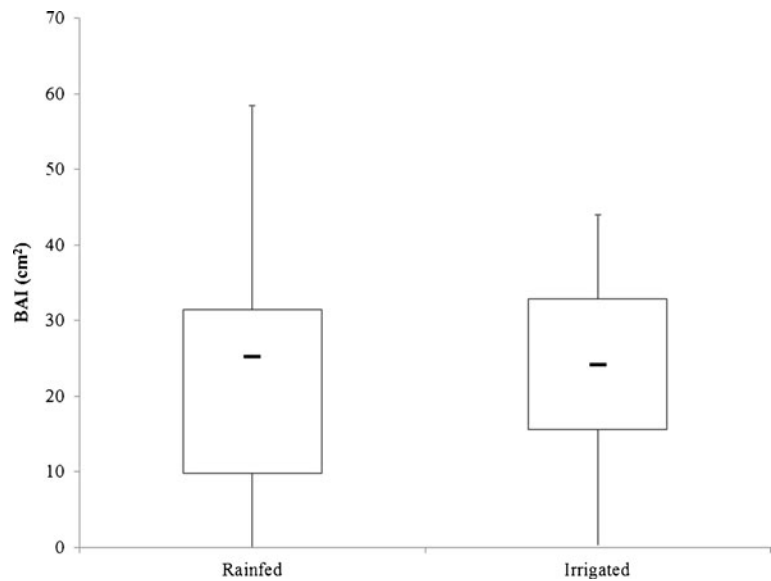
Fig. 3 BAI (Basal Area In-
dex) express in box plot
form. Data were calculated
by the equation BAI=
BAI=$\pi(R_n^2 - R_{n-1}^2)$, where
R is the tree radius and n is
the year of tree ring
formation

Fig. 3 BAI (Basal Area Index) express in box plot form. Data were calculated by the equation BAI= BAI=$\pi(R_n^2 - R_{n-1}^2)$, where R is the tree radius and n is the year of tree ring formation

of the degree to which one variable is related to another, correlation analyses were also performed among all the data.

Results

Climate analysis

The CWB (Climate Water Balance) from 1992 until to 2009 shows a maximum value of 894 mm in 1993 and a minimum value of 502 mm in 2009 (Fig. 1). The DD (Degree-Days) was generally constant during this period, with a minimum value of 1550 in 1996, and a maximum value of 2128 in 1994 (Fig. 1).

Ring-width analyses

The trees were planted in spring 1992 and similar growth rates were recorded in 1993 and 1994 (Fig. 2). The average ring-width growth of 1992, 1993 and 1994 was 2.05, 2.90 and 5.41 mm for rainfed olive trees and 3.08, 3.42 and 7.98 mm for irrigated trees. Irrigation started in 1995, and in 1996 it was reported that the irrigated olive trees showed higher tree-ring width (11.38 mm) compared to the rainfed ones (5.42 mm). Thereafter, a significant positive effect of irrigation was no longer detected (e.g., tree ring width in rainfed and irrigated trees was similar in 1998, i.e., 8.08 mm for rainfed and 7.12 mm for irrigated). An inversion in

growth trends has been noticed since 1998 and rainfed olive trees have shown higher, though not significant, ring-width growth than irrigated trees. A significant difference was only found in 2001, when the rainfed trees registered a ring-width of 7.94 mm, which was much higher than the irrigated trees (4.69 mm). Overall, no age-trend was noticed. BAI measurements are reported in boxplot form and show that irrigated plant tend to reduce their basal areal (Fig. 3). These data confirm previously results on the same olive plantation (Tognetti et al. 2006).

Isotopic analyses

Carbon stable isotope composition $\delta^{13}C$ in rainfed and irrigated trees (Fig. 4) was similar in the first 2 years after plantation (e.g., in 1994, −22.38‰ in rainfed trees and −22.35‰ in irrigated trees). Significant differences were recorded after the beginning of irrigation treatment. Irrigated trees always had more negative $\delta^{13}C$ values than rainfed trees in 1998, 2000 and 2001. For the first 2 years, oxygen stable isotopes $\delta^{18}O$ (Fig. 5) reported a similar trend to the $\delta^{13}C$ and the values registered in 1993 and in 1994 were similar. In 1997, irrigated trees show significant lower $\delta^{18}O$ values (33.35‰) than rainfed trees (34.68‰). Later, starting from 1998, stable isotope values inverted their trends. In 2001, irrigated trees reported higher $\delta^{18}O$ values (33.86 ‰) than rainfed trees (32.68‰). Starting from 2001, a very variable trend, including changes in the direction,

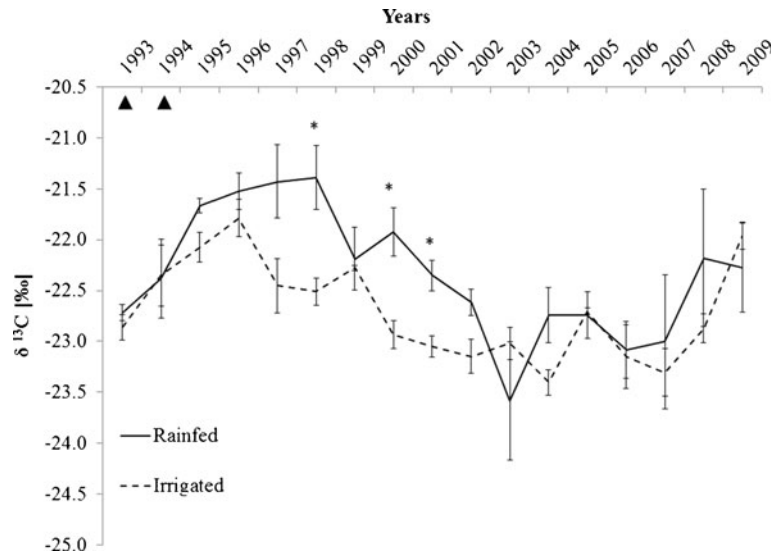**Fig. 4** Carbon $\delta^{13}C$ stable isotopes (‰) trends. The raw $\delta^{13}C$ chronology exhibited a decline in $\delta^{13}C$ mainly in the twentieth century, which may be attributed to the lowering of $\delta^{13}C$ of air through anthropogenic-related increases in CO_2 concentration (the "^{13}C *Suess effect*") this trend has been removed in the carbon isotope chronology using the annual records of past atmospheric $\delta^{13}C$ obtained from ice cores. Student's *t*-test was performed and significant difference ($P \leq 0.05$) have an *asterisk* (*). *Bars* indicate ± SE. *Triangles* (▲) indicate years in with water availability is the same for both rainfed and irrigated trees

was recorded, but no significant difference was detected. The relationship between $\delta^{13}C$ and $\delta^{18}O$ was difficult to interpret across the years and treatments (data not shown). Significant correlation was found between ring-width and $\delta^{18}O$ in the first 3 years after the start of irrigation ($R^2=0.355$, $P \leq 0.0091$)

Anatomical analysis

The frequency of vessel diameter classes in single tree rings in 1995, 1996 and 1997 year, are reported in Fig. 6. The irrigated trees formed fewer vessels with a smaller diameter (<20 μm) than the rainfed trees (Fig. 6a, b, c).

Fig. 5 Oxygen $\delta^{18}O$ stable isotopes (‰) trends. Student's *t*-test was performed and significant difference ($P \leq 0.05$) have an *asterisk* (*), *bars* indicate ± SE. *Triangles* (▲) indicate years in with water availability is the same for both rainfed and irrigated trees
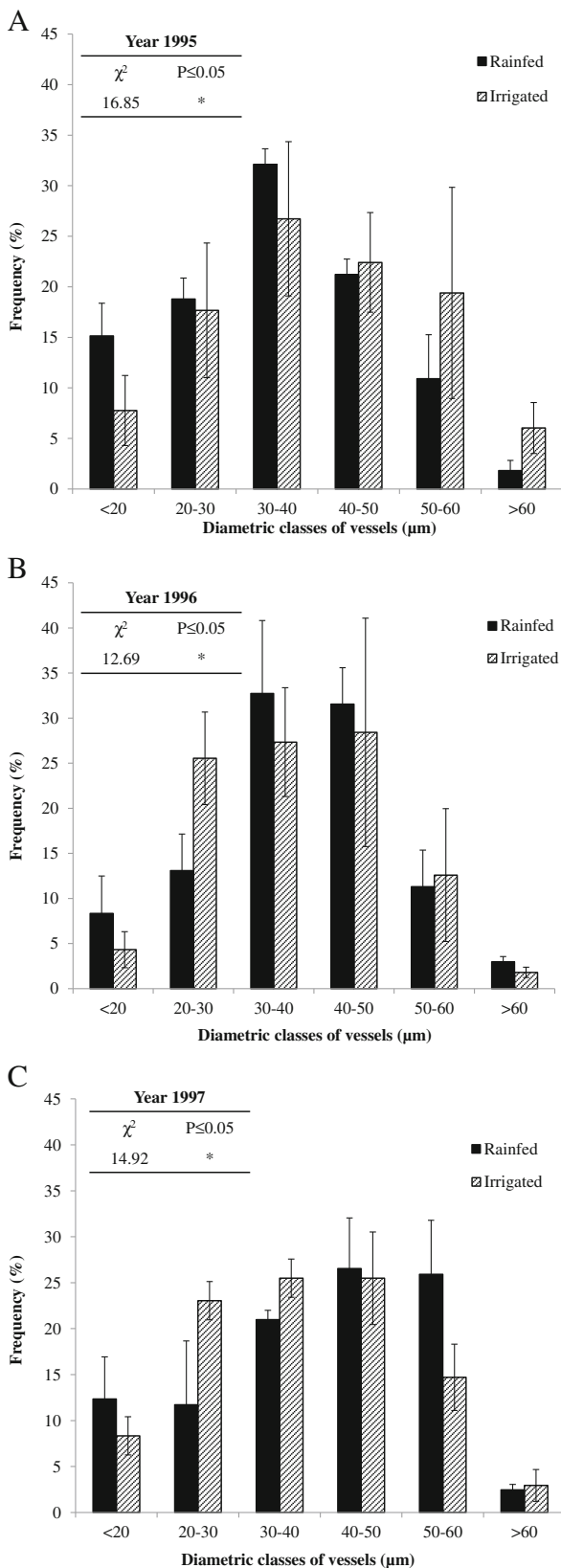
A

B

C
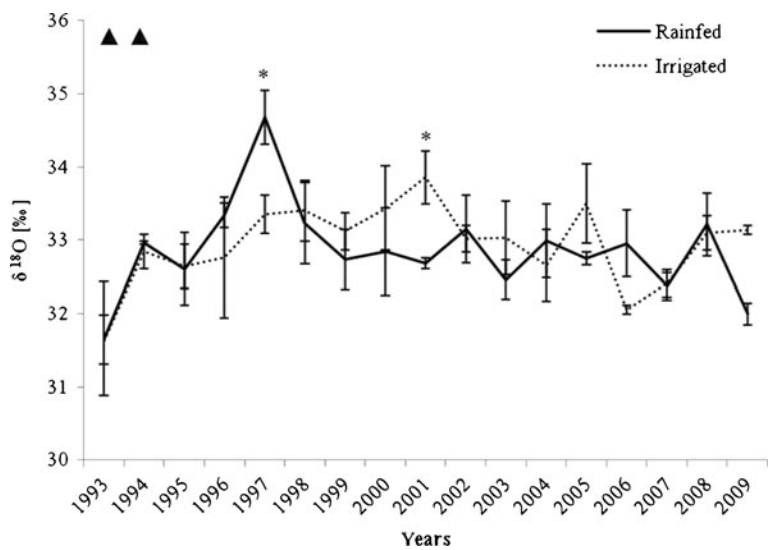
◀ **Fig. 6** Trends in diametric classes of vessels distribution. Figures 6a, b, c show the first 3 year after irrigation level: 1995, 1996 and 1997 respectively. *Bars* indicate ± SE. χ^2-test was performed and significant difference are marked with an *asterisk* (*)

In 1995, rainfed trees formed 15 % of vessels with <20 μm diameter, whereas irrigated trees formed only 7.8 % of vessels with <20 μm diameter. In 1996 and 1997, in rainfed trees, 8.3 % and 12.3 % of vessels had a diameter <20 μm, respectively, while irrigated trees formed, in the same years, 4.3 % and 8.3 % of vessels with <20 μm diameter. Diameter classes higher than 20 μm were more abundant in irrigated trees.

The χ^2-test (Fig. 6) shows significant differences between watering treatments in 1995, 1996 and 1997. Average diameter (μm) and vessel density are presented in Table 1. Significant higher diameter and vessel density values were found in irrigated tree diameters formed in 1995 and in irrigated tree vessel density formed in 1996.

A representative microphotography of the whole growth ring section, formed in 1997, shows wood anatomical differences in earlywood and latewood detected in rainfed trees (Fig. 7a, b). Such differences were not observed in irrigated trees.

The distribution of vessel diameter classes, for both the earlywood and the latewood, in rainfed trees (1995, 1996 and 1997) is shown in Fig. 8. Latewood had more vessels with a smaller diameter than earlywood (e.g., only 1 % of vessels with <20 μm was formed in spring, (i.e., earlywood), whereas 17 % of vessels with the same diameter was formed in summer, i.e., (latewood). The values of diameter (μm) and vessel density (vessel mm^{-2}) in earlywood and latewood are reported in Table 2.

Discussion

Ring-width analyses in cultivated olive trees are quite uncommon, other than difficult, because of the difficulties to find wood materials with regular and concentric rings, which allow for cross-dating and synchronization of ring-width time series. Data showed that correlations between climatic parameters (Fig. 1) and tree-ring widths (Fig. 2) were unclear in both rainfed and irrigated trees. However, significant differences in tree-ring width were recorded 1 year after the start of the irrigation treatment, as a confirmation of the drought stress suffered from rainfed plants. Irrigated olive trees grew

Table 1 Diameter (μm) and vessel density (vessel mm^{-2}) values refer to the first 3 years after the irrigation level was imposed: 1995, 1996 and 1997

Years	Diameter [μm]			Vessels Density [vessels mm^{-2}]		
	Rainfed	Irrigated	P≤0.05	Rainfed	Irrigated	P≤0.05
1995	34.80	38.83	*	55.00	77.33	ns
1996	37.89	37.66	ns	56.00	92.67	*
1997	40.16	37.29	ns	54.00	68.00	ns

Student's *t*-test was performed (P≤0.05) and significant differences are indicated by an asterisk (*)

faster than rainfed olive trees and BAI measurements (Fig. 3) report that irrigated plants tend to invest more carbon—that comes from photosynthesis—to produce more fruits and leaves (Tognetti et al. 2006). In 2001, a significantly lower ring width in irrigated trees was found, probably because the supra optimal water supply and high water table. Our anatomical and isotopic results show that rainfed plants activate physiological adjustment mechanisms of acclimation (i.e., by regulating stomatal activity and limiting wood formation during droughts), confirming the previous study on tree water relations in the same experimental orchard (Tognetti et al. 2004, 2005, 2009). Long-term hydraulic acclimation allows plants to reduce stomatal conductance and acclimate to water stress (Silva and Horwath 2013). Despite stomatal down-regulation of transpiration rate in response to increasing evaporative demand in rainfed trees, midday leaf water potentials fell below critical values in the driest month (Tognetti et al. 2007, 2009), to levels likely to induce xylem air embolisms. Nevertheless, anisohydric plants sustain longer periods of transpiration and photosynthesis, even under large soil water deficit, and are thought to be more drought-tolerant than isohydric plants (McDowell et al. 2011). The tree-ring stable isotopic series presented here is the first series

for olive tree. The carbon isotope signature ($\delta^{13}C$) is an important tool for identifying long-term adjustments in gas-exchange processes (e.g., De Micco et al. 2012). The oxygen isotope of organic matter ($\delta^{18}O$) is mainly determined by the isotopic composition of the soil water, the leaf water enrichment due to transpiration, and the biochemical fractionations (Chaves et al. 2009; Moreno-Gutierrez et al. 2012; Roden and Farquhar 2012). In this study, we relied on the conceptual model of Scheidegger et al. (2000) to interpret our dual-isotope results where changes in $\delta^{18}O$ cell are primarily due to changes in leaf water enrichment caused by variations in air humidity, while changes in cellulose $\delta^{13}C$ are related to plant water-use efficiency. The model assumed that the $\delta^{18}O$ of source water and water vapour is the same in all the investigation periods and treatments and the relationship between cellulose $\delta^{13}C$ and intercellular CO_2 concentration is negative.

After the start of irrigation significantly higher $\delta^{13}C$ values were recorded in irrigated trees, 1998, 2000 and 2001, which can be explained by a decrease in stomatal activity or an increase in photosynthetic rate, confirming a change in the photosynthetic capacity and stomatal conductance as reported in Tognetti et al. (2009). In fact, photosynthesis is determined by biochemical factors

Fig. 7 Light microphotography of the 1997 annual ring section in a rainfed (**a**) and in an irrigated (**b**) olive tree, captured by an RGB camera connected to a transmitted light microscopy. In **b**, EW represents the earlywood and LW the latewood; in **a**, no earlywood and latewood can be detected
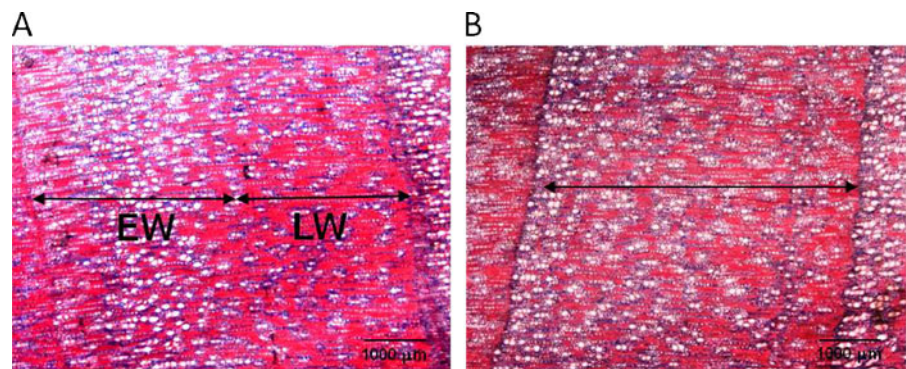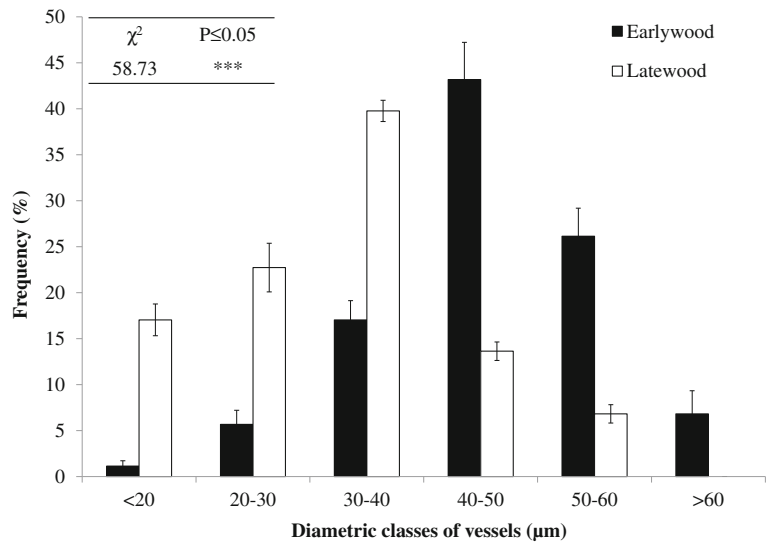
Fig. 8 Average distribution in diametric classes of vessel frequency in latewood and earlywood (1995, 1996 and 1997). Analyses were made on the rainfed olive trees and were based on the first 3 years. *Bars* indicate ± SE. χ^2-test was performed and significant difference are marked with an *asterisk* (*)

(activity of RuBisCO and regeneration of RuBP in C_3 plants) directly correlated to environmental changes. Olive trees behave as anisohydric species (Gucci et al. 2002) when in rainfed conditions, and as nearly-isohydric species when irrigated (Torres-Ruiz et al. 2011). Isohydric plants tend to reduce stomatal conductance as soil water potential decreases, maintaining relatively constant midday leaf water potential. Anisohydric plants, by contrast, allow midday leaf water potential to decline as soil water potential declines with drought. Anisohydric behaviour allows olive trees to occupy more drought-prone habitats compared with isohydric species and they have a xylem that is more resistant to negative water potential (Centritto et al. 2011). Significant lower $\delta^{18}O$ values in irrigated trees were recorded in 1997, confirming lower transpiration rates in the rainfed trees.

It might be expected to find variations in the isotopic composition of tree rings between treatments, due to changes in hydraulic conductance with increasing tree

size (Yoder et al. 1994). Variations in isotopic composition could be also related to treatment-specific microclimate, such as gradients in relative humidity (Elias et al. 1989). However, this olive tree orchard tends to be well coupled to the atmosphere and we do not expect large gradients in relative humidity (Tognetti et al. 2009).

Significant differences in carbon and oxygen isotopic composition were detected only until 2001. After the first 6 years of irrigation (from 1995 to 2001) no significant difference between treatments was found in both tree-ring width and vessel diameter. Rainfed trees acclimated to drought by shifting from isohydric to anisohydric mechanisms throughout the seasons. Plants could also vary root depth in search of groundwater resources (Rossatto et al. 2012), which could explain differences in $\delta^{18}O$ also by changes in root development and depth of water uptake. Changing root depth could allow plant to reach soil water reservoirs overcoming the drought stress, explaining differences in ring-width, wood anatomy and isotopic composition results between rainfed and irrigated plants.

In Mediterranean conditions, maintaining hydraulic functions under drought stress is crucial to survive (Fonti and Jansen 2012). In general, the ability to move water to the site of evaporation with minimum investment is a major factor driving the architecture and physiology of Mediterranean woody plants, including the function of stomatal regulation (Tognetti et al. 2004, 2005, 2009). Olive trees have a diffuse-porous structure with vessels that are generally, in short to fairly long radial multiples, occasionally in clusters, rarely solitary. Olive trees respond to different water availability by forming vessels with different diameters as previously

Table 2 Diameter (μm) and vessel density (vessel mm^{-2}) mean values refer to the first 3 years (1995, 1996 and 1997) after irrigation level was imposed on the rainfed olive trees

	Diameter [μm]	Density [vessel mm^{-2}]
Earlywood	43	56
Latewood	29	35
$P \leq 0.05$	*	*

Student's *t*-test was performed ($P \leq 0.05$) and significant differences are indicated by an *asterisk* (*)

shown by Lo Gullo and Salleo (1988). The water status of the olive tree has an influence on the anatomy of the xylem and low water availability in the soil induces the formation of a higher number of vessels with lower diameters as shown in one-year-old shoots (Torres-Ruiz et al. 2011). Higher tensions are needed for vessels with reduced diameter to cavitate. In addition, vessels with reduced diameter have also a reduced collective pit area between vessels, which reduces the incidence of embolism due to air seeding (Hacke et al. 2009). Effects of water stress, both on anatomical and hydraulic characteristics, have been observed in other broadleaved tree species, such as *Cedrus* spp. and *Quercus ilex* L. (Ladjal et al. 2005; Limousin et al. 2010). Results show that the plasticity in vessel diameter induced by changes in water availability is an efficient mechanism to maintain similar hydraulic conductivity in rainfed olive trees (von Arx et al. 2012). Only the smallest vessel diameter classes were influenced by water availability. In fact, in terms of diameter class distribution, a significant higher number of smaller vessels in rainfed trees was found, though mean vessel diameter per year and vessel density per year did not show significant differences. This confirms that water availability modifies xylem plasticity in olive trees, inducing the formation of more vessels with small diameters.

In conclusion, rainfed olive trees produce more vessels with lower diameters. Wood anatomy and isotope signature characterized rainfed and irrigated trees in the first years after differentiating irrigation. After this first period, rainfed trees adapted to low water availability varying root development in function of depth of water uptake, forming vessels with lower diameters, while it was not possible to distinguish between earlywood and latewood in irrigated trees. Dendrochronological, stable isotopic and wood-anatomical analyses on olive trees can be useful tools to interpret and understand the structural and functional responses of olive trees to drought. These systems could also help evaluate the efficiency of irrigation practices and scheduling protocols.

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