



Site conditions influence the climate signal of intra-annual density fluctuations in tree rings of *Q. ilex* L.

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Abstract

• **Key Message** The investigation of the relations between climate and intra-annual density fluctuation (IADF) traits (e.g. frequency, width and functional anatomical traits) in *Quercus ilex* tree rings of stands living on two opposite slopes, highlighted the influence of local site conditions on the climate signal contained in tree rings. Moreover it allowed to identify the main site-specific environmental drivers for IADF formation.

• **Context** Wood functional traits such as IADFs result from the adaptation of tree physiological processes to seasonal climate variability. They could be used to reconstruct tree response to intra-annual variations in environmental conditions.

• **Aims** We investigated the relationship between IADF features and climate in *Quercus ilex* L. populations living on opposite slopes, aiming to evaluate the influence of local site conditions on wood plasticity in response to seasonal climate variability.

• **Methods** Dominant trees were sampled and dated. IADF frequency and width, non-lumen fraction, vessel size, and frequency were measured. Time series analyses using precipitation and temperature data were performed.

• **Results** A decrease in IADF frequency over time influenced by summer and autumn temperature was observed at both sites. IADFs were more frequent, wider, with smaller vessels and a higher non-lumen fraction in trees growing at the north slope compared to the south slope site. IADF anatomical parameters of south slope tree rings were mainly influenced by precipitation, while the ones of north-slope tree rings by temperature.

• **Conclusion** Temperature-limited IADF occurrence in *Q. ilex* tree rings, probably leading to an earlier stop in cambial activity, and IADF anatomical traits were influenced by site conditions, suggesting them as indicators of site-specific responses to climate.

Keywords Dendrochronology · Quantitative wood anatomy · Holm oak · Mediterranean forests

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Contribution of the co-authors EZ, GB and VDM contributed to the conception and design of the study. EZ was in charge for sampling, sample preparation and analysis, and statistical elaboration of data. GB and VDM gave substantial contribution to data analysis. All authors contributed to the overall interpretation of data. EZ wrote the main part of the manuscript. All authors performed the critical revision of the work and contributed to manuscript revision, read and approved the submitted version.

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1 Introduction

Projections of climate changes for the Mediterranean region forecast high irregularities in seasonal precipitation patterns leading to an increase in the frequency and duration of drought events as well as a shift in time of their occurrence, accompanied by an overall increasing mean annual temperature (IPCC 2017). These variations in climatic conditions will likely induce plastic adaptive response in trees, thus affecting tree growth and forest productivity (Lindner et al. 2010; Lindner and Calama 2013). The study of tree adaptive traits to past climatic conditions may provide information about their ability to adapt and their vulnerability to predicted climate changes, thus being useful for forest management. Tree response to the changing environmental conditions has often been investigated through dendrochronological methods to reconstruct past tree behaviour at annual resolution (Fritts 2001). Insights about vulnerability to peculiar intra-annual stresses, such as summer drought in Mediterranean environments, have also been given by the analysis of the impact of climatic conditions and intra-annual variability of functional wood anatomical traits, such as intra-annual density fluctuations (IADFs) (De Micco et al. 2016a). IADFs are regions within a tree ring where abrupt changes in wood density occur (De Micco et al. 2016a). In Mediterranean areas, IADF frequency, although related to tree age, size and tree ring width, strictly depends on droughts, being the result of the adjustments of wood functional traits induced by the Mediterranean ‘double stress’; i.e. summer drought and winter cold, in the pursuit of preserving hydraulic conductivity while reducing embolism risk (Cherubini et al. 2003; De Micco et al. 2016a). Most of the studies about IADFs within the Mediterranean region have been conducted on conifer species, showing that tree plasticity can lead to high altitude to form IADFs as a reaction to climate fluctuations (Campelo et al. 2015; Carvalho et al. 2015; de Luis et al. 2011; De Micco et al. 2007; DeSoto et al. 2011; Nabais et al. 2014; Novak et al. 2013b; Olivar et al. 2015; Rozas et al. 2011; Vieira et al. 2010, 2015; Zalloni et al. 2016). IADF frequency in tree rings is found to be dependent on tree age, size and/or ring width (Campelo et al. 2015; Nabais et al. 2014; Novak et al. 2013b; Vieira et al. 2009), while patterns of tracheid lumen size at an intra-annual level were detected as indicators of seasonal water stress (Carvalho et al. 2015; DeSoto et al. 2011). IADF frequency has been increasing from the early 1990s to the present in *Pinus halepensis* Mill. and *Pinus pinaster* Aiton in Spain (Bogino and Bravo 2009; Olivar et al. 2012) and seems to be higher in younger than older trees, and in wider than narrower rings in *Pinus* spp. (Bogino and Bravo 2009; Bräuning 1999; Campelo et al. 2013; Copenheaver et al. 2006; Novak et al. 2013b; Olivar et al. 2012; Rigling et al. 2001, 2002; Wimmer et al. 2000; Vieira et al. 2009; Zalloni et al. 2016). Furthermore, in *P. halepensis*, *P. pinaster*, *Pinus pinea* L. and *Juniperus thurifera* L. growing

around the Mediterranean basin, the presence of IADFs has been mostly related to autumn and early summer precipitation, depending on the type and position within ring width (i.e. earlywood-like cells in latewood or latewood-like cells in earlywood) (Camarero et al. 2010; Campelo et al. 2007b, 2013; de Luis et al. 2007; Vieira et al. 2009; Zalloni et al. 2016). In the last decade, the interest in studying IADFs in angiosperms is increased because of their potential to be used as proxies for intra-annual climate reconstructions (Battipaglia et al. 2010, 2014; Campelo et al. 2007a; Cherubini et al. 2003; Copenheaver et al. 2010; De Micco and Aronne 2009; Zhang and Romane 1991). Among Mediterranean broadleaves, *Quercus ilex* L. trees growing in Spain and France formed IADFs with a higher frequency in wider than in narrower rings; such IADFs were considered the result of reactivation of wood growth due to the occurrence of precipitation after summer drought periods (Campelo et al. 2007a; Zhang and Romane 1991). A relationship between IADF width in *Q. ilex* and the extent of precipitation during the second part of the growing season has been reported, suggesting IADFs in this species as indicators of xylem phenotypic plasticity (Campelo et al. 2007a). *Q. ilex* is a widespread Mediterranean diffuse-porous broadleaved species, native to southern Europe. It is widely distributed throughout the Mediterranean basin as the dominant species in a transition zone between temperate forests and shrublands, and as sclerophyllous drought-resistant species, it is representative of a key Mediterranean vegetation functional type (Terradas 1999). Long chronologies of vessel traits in *Q. ilex* provide information about the climatic signals held in tree rings (Campelo et al. 2010).

In this paper, intra-annual variation of functional wood traits in tree rings with IADFs is analysed and data are interpreted in relation to the seasonal variation of climate parameters in order to give insights into the ability of a widespread Mediterranean hardwood to cope with the foreseen climate changes. Moreover, we considered different stand structures and microclimates to evaluate whether and to what extent tree response to climate factors is site-specific. In this study, we investigated (1) the growth response of *Q. ilex* populations living on two opposite slopes of the Somma-Vesuvio volcanic complex by analysing the climate signal in tree ring width and wood anatomical traits in tree ring chronologies and (2) the relationships between IADF occurrence, width and wood anatomical traits and temperature and precipitation. We hypothesize that (i) tree growth is positively influenced by the two main Mediterranean precipitation periods in spring and in autumn, and negatively by summer dry period; (ii) anatomical functional traits, e.g. mean annual vessel area, non-lumen fraction and vessel frequency, are more influenced by climatic conditions rather than tree ring width; (iii) IADF frequency is higher at the southern slope site, where the higher exposure to solar radiation should increase drought intensity and frequency during summer; (iv) IADFs are wider at the

northern slope site because of higher humidity during autumn if compared with the southern slope site; (v) anatomical traits of the IADFs are related to the climate of the warmest months of the season and are influenced by temperature or precipitation depending on the site.

2 Material and methods

2.1 Study sites

The study sites are located on two opposite slopes of the Somma-Vesuvio volcanic complex, at about 15 km SE of Naples (Italy). The two sites are characterized by different aspects and vegetation: the SW-faced site (SW, lat 40.49050 N, lon 14.24124 E), located in the ‘Tirone Alto-Vesuvio’ Forest State Reserve, is covered by Mediterranean maquis and sclerophyll forests, whereas the NE-faced site (NE, lat 40.49902 N, lon 14.27067 E), located on the northern slopes of Mount Somma, is mostly characterized by broadleaved mesophilous forests. Both sites are dominated by *Q. ilex* L. with a *Q. ilex* understory and the sporadic presence of *Robinia pseudoacacia* L., a non-native invasive tree species. Mean diameter and height of sampled trees were, respectively, 33.72 ± 0.81 cm (mean value \pm SE) and 16 ± 0.49 m at SW, while 24.36 ± 1.64 cm and 13 ± 0.82 m at NE. Stand density was 11,000 and 33,000 trees/ha at SW and NE, respectively.

Temperature and precipitation data from the nearest meteorological stations were fragmented and did not cover the whole study period; therefore, they were interpolated and compared to the CRU TS3.23 gridded dataset at 0.5° resolution data for the period 1951–2014 (Harris et al. 2014). Since the correlation between the two data series was significant (supplementary material, Fig. S1, Table S1), we considered the climate data from the CRU to be representative for the study sites. The climate is Mediterranean with mild winters and warm summers with a mean annual temperature of 16.1°C . Precipitation is mainly concentrated in autumn and winter and at the beginning of spring, with an average of 766 mm per year (reference period 1951–2014).

Percent gravimetric soil water content, available water capacity and water holding capacity of the two sites were determined by taking three samples per site and following standard procedures (USDA, Natural Resources Conservation Service, National Soil Survey Center 1996).

2.2 Tree ring data

Only dominant *Q. ilex* trees were sampled in the autumn of 2016 (21 at SW and 14 at NE), taking two incremental cores per tree at breast height (1.3 m), with a 0.5-cm diameter increment borer (Haglöf, Sweden), following standard methods (Schweingruber 1988). The number of cored trees at each site was different

because no more than 14 dominant trees were available at NE. The methods used for sample preparation and tree ring width measurements are explained in the supplementary material.

2.3 Intra-annual density fluctuation data

IADF occurrence was detected within the rings of each core by observing them under a stereomicroscope. IADFs were identified following the description of Campelo et al. (2007a), by detecting variations in cell lumen area, frequency and wall density different from the common transition from earlywood to latewood of *Q. ilex* as described in Wheeler (2011). Relative annual IADF frequency chronologies of the sites were calculated as the ratio between the number of cores with an IADF and the total number of cores per year. Stabilized annual IADF frequency chronologies were then calculated according to Osborn et al. (1997) as $f = Fn^{0.5}$, where F is the relative IADF frequency value and n is the total number of cores per year, in order to stabilize the variance and to overcome the issue of the changing sample depth over time. To investigate age- and ring width-related growth trends, age- and ring width-based IADF frequency chronologies were calculated per each site following the same procedure, but by aligning the individual series of IADF presence and absence by cambial age (considering pith-offset estimations) and by classes of ring width, respectively (Zalloni et al. 2016). Polynomial curves were then fitted to the data points to explore the relationship between age and ring width and IADF frequency. The age trend was then removed by dividing the best fitted estimated polynomial curves for the age-based chronologies from the original observed values in order to perform correlations with climate.

The widths of the occurring IADFs were measured with a resolution of 0.01 mm using the measuring system used for measuring ring widths. Mean IADF width chronologies of the sites were built. IADF width values were then converted in IADF width and tree ring width percentage values in order to avoid tree ring width-related biases. A mean IADF width chronology based on the new percentage values was then calculated per each site in order to perform correlations with climate.

2.4 Quantitative wood anatomy

Tree ring microphotographs of all the rings of five cores per site relative to five trees per site (314 rings for SW, about 60 rings per tree, and 182 rings for NE, about 40 rings per tree) were taken under a dissection microscope (SZX16, Olympus, Germany), equipped with a XM10 camera (Olympus), after filling the vessels with chalk in order to maximize the contrast and increase the visibility (Gärtner and Schweingruber 2013). An example of tree ring microphotographs of cross-sections used for the measurements are shown in Fig. 1. Intra-annual anatomical parameters were quantified with the digital image

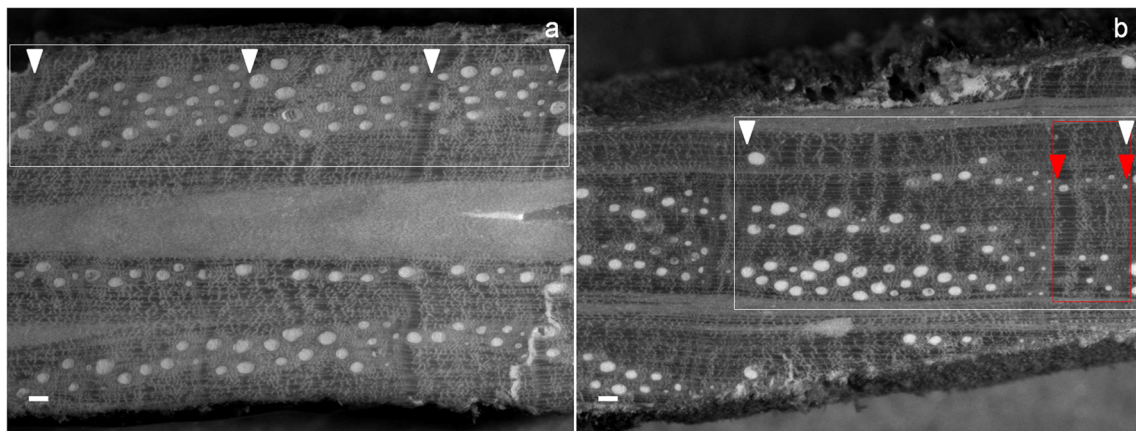


Fig. 1 Microphotographs of *Quercus ilex* tree rings used for anatomical parameter measurements. Vessels are filled with chalk. White arrows point the boundary of tree rings; red arrows delimit the IADF.

Measurement areas are shown by white rectangles; the red rectangle shows the measurement area of an IADF. **a** Years 2013–2015, core n.3b, SW; **b** Year 1968, core n.8a, SW. Bars = 200 μm

analysis software Analysis 3.2 (Olympus). Vessel lumen area was quantified *in continuum* from the beginning to the end of each ring along a transect between two rays (with an area between 378.87 and 9944.156 $10^3 \mu\text{m}^2$ for SW and between 2724.28 and 26,535.23 $10^3 \mu\text{m}^2$ for NE) (De Micco et al. 2014). For the quantification of the percentage of non-lumen fraction and of the frequency of vessels, the same transects were divided into four equal regions (with each region corresponding to the 25% of total ring width). Non-lumen fraction was quantified as the percentage of the area of cell walls over total xylem area per each region (De Micco et al. 2016b), while vessel frequency was calculated as the number of vessels per 10^3 square micrometre, determined by counting vessels in a known area according to Wheeler et al. (1989). Tree ring mean annual values of each anatomical parameter were calculated and mean chronologies were built. Age and low-frequency growth trends were removed in order to calculate climate correlations with the same method applied to tree ring width series to avoid differences associated with detrending procedures (Campelo et al. 2010). Chronology quality was assessed as for tree ring data. Mean annual values of vessel lumen area, non-lumen fraction and vessel frequency within the quarter of IADF appearance within each ring were calculated and mean chronologies were built in order to perform climate correlations.

2.5 Climate signal

The climate signal of all the series was investigated with a bootstrapped correlation function analysis (Pearson's linear correlation, $P < 0.05$) with seasonally grouped monthly total precipitation and mean temperature data from December of the previous year to December of the current year, for the different growth time spans covered by the trees of the two sites: (a) 1952–2014 for tree ring width, IADF frequency, IADF width and anatomical parameters series of SW and (b) 1966–2014 for tree ring width, IADF frequency, IADF width

and anatomical parameters series of NE. All the correlations were computed with the software R using the treeclim package (Zang and Biondi 2015).

Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

3 Results

3.1 Soil characteristics

The soil of the two sites differed regarding the available water and the water holding capacities, but had a similar water content. Indeed, the soil at SW had higher available water capacity (41.08%) and water holding capacity (28.79%) compared to the soil at NE (18.17 and 15.23%, respectively) but both sites had a similar soil water content of 11.67% at SW and 10.51% at NE.

3.2 Tree ring data and anatomical traits

Descriptive statistics and quality assessment indexes of tree ring mean chronologies are shown in Table 1. Mean chronologies at NE covered a shorter period compared to the SW ones: NE trees were about 20 years younger than SW trees. Mean chronologies of the anatomical traits covered a shorter period compared to tree ring width mean chronologies because the five cores per site chosen for quantitative wood anatomy analysis did not reach the pith and/or the very first growth rings of the cores did not have clearly visible and analysable anatomical features. Raw and detrended mean annual chronologies of tree ring width and anatomical parameters are shown in supplementary material (Fig. S2). NE trees showed wider rings with smaller vessels, denser wood and similar vessel frequency compared to SW trees (Table 1).

Table 1 Descriptive features and quality assessment indexes of tree ring mean annual chronologies

	TRW (mm)		MVA (10 ³ μm ²)		NLF (%)		VF (N vessels/10 ³ μm ²)	
	SW	NE	SW	NE	SW	NE	SW	NE
Timespan	1948–2015	1966–2015	1950–2015	1971–2015	1950–2015	1971–2015	1950–2015	1971–2015
Length	68	50	66	45	66	45	66	45
N cores	42	28	5	5	5	5	5	5
N trees	21	14	5	5	5	5	5	5
Mean value ±SE	2.38 ± 0.16	2.47 ± 0.12	33.07 ± 0.98	24.45 ± 0.93	93.35 ± 0.22	94.63 ± 0.2	0.0021 ± 0.00008	0.0022 ± 0.00005
EPS	0.86	0.91	0.69	0.85	0.79	0.65	0.37	0.26
R _{bt}	0.81	0.4	0.31	0.58	0.44	0.31	0.11	0.05
MS	0.35	0.33	0.21	0.17	0.02	0.01	0.24	0.22

TRW tree ring width, MVA mean vessel area, NLF non-lumen fraction, VF vessel frequency, SW south-west site, NE northeast site

Tree ring width chronologies (TRW) showed the highest expressed population signal (EPS) and mean sensitivity (MS) values. EPS values of the anatomical parameters chronologies were lower, reaching the lowest values for vessel frequency chronologies (VF). VF also showed the lowest values of R_{bt} , while non-lumen fraction chronologies (WD) were very compliant with consistently low MS values.

3.3 IADF data

IADFs were more frequent and wider in SW tree rings than NE ones, even considering the common period aligned by cambial age (Table 2). IADFs of SW tree rings showed larger and more sparse vessels with a less dense wood than NE IADFs. High values of standard errors for all the mean parameters indicate a high variability among years. An age trend was found: IADFs were more frequently formed when trees were younger at both sites (Fig. 2). Trees from both sites showed the same tendency, with the occurrence of more IADFs in wide rings than in very narrow or very large rings, which is observable in the bell-shaped polynomial curves in Fig. 2. The highest IADF frequency was found between 3 and 4 mm for SW tree ring widths while between 2 and 3 mm for NE ones (Fig. 2). Both sites showed an IADF frequency decrease

during the most recent growth years in the stabilized IADF frequency chronologies, while NE tree rings showed the occurrence of IADFs during periods of no occurrence for SW ones (Fig. 3). Stabilized IADF frequency chronologies of the two sites did not show a high peak coincidence of their occurrence, which was instead found between the IADF width chronologies of the two sites (Fig. 3).

3.4 Climate signal

Tree ring width was positively influenced by spring (SPR—from current March to current June) precipitation at both sites ($r = 0.317$ for SW, 0.265 for NE, $P < 0.05$), while only SW tree ring width showed a significant positive correlation with autumn (AUT—from current September to current December) precipitation ($r = 0.230$, $P < 0.05$) (Fig. 4). Also, NE tree rings showed a positive correlation with autumn precipitation, although not a significant one ($r = 0.178$, $P < 0.05$) (Fig. 4). SW tree ring width showed a negative significant correlation with summer (SUM—from current June to current September) temperature ($r = -0.257$, $P < 0.05$) (Fig. 4). Tree ring mean vessel area presented no significant correlation with climate, except for SW mean vessel area which was negatively correlated with summer precipitation ($r = -0.249$, $P < 0.05$)

Table 2 IADF-related parameter values

	SW	NE
IADF frequency % (Nr rings with IADF/Nr rings tot)	21 ($CP_{CA} = 27$)	17
Mean stabilized IADF frequency ($f = Fn^{0.5} \pm SE$)	1.35 ± 0.24 ($CP_{CA} = 1.76 \pm 0.24$)	0.85 ± 0.12
Mean IADF width (mm ± SE)	0.90 ± 0.08 ($CP_{CA} = 0.6 \pm 0.06$)	0.86 ± 0.08
Mean vessel area (10 ³ μm ² ± SE)	9.89 ± 0.57 ($CP_{CA} = 9.85 \pm 0.57$)	8.39 ± 0.9
Mean non-lumen fraction (% ± SE)	98.09 ± 0.14 ($CP_{CA} = 98.07 \pm 0.15$)	98.15 ± 0.17
Mean vessel frequency (Nr vessels/10 ³ μm ² ± SE)	0.0016 ± 0.0001 ($CP_{CA} = 0.0016 \pm 0.0001$)	0.0020 ± 0.0002

CP_{CA} referred to the common period aligned by cambial age, SW south-west site, NE northeast site

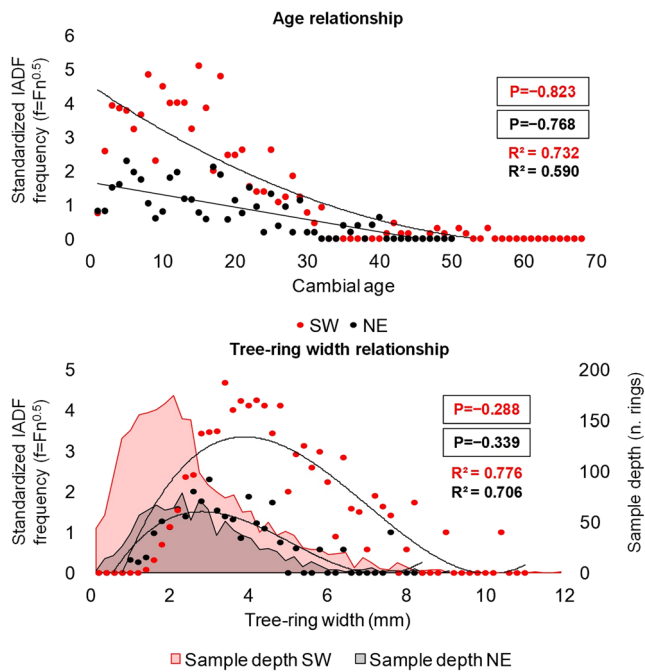


Fig. 2 IADF frequency chronologies aligned by cambial age and tree ring width classes (SW in red, NE in black). P = Pearson product-moment correlation coefficient between each series and cambial age/tree ring width, and R^2 of the fitted polynomial curves ($P < 0.001$)

(Fig. 4). Mean annual non-lumen fraction and vessel frequency did not show significant correlations (data not shown). IADF frequency and width were influenced by mean temperature rather than total precipitation: mean temperatures from spring to autumn were negatively correlated with IADF frequency ($r = -0.376$ (SPR), 0.315 (SUM), 0.226 (AUT), $P < 0.05$) (Fig. 5). Summer ($r = -0.384$, $P < 0.05$) and autumn ($r = -0.433$, $P < 0.05$) temperatures were negatively correlated with IADF width in SW trees (Fig. 5). IADF frequency was

negatively influenced by autumn temperature (-0.133 , $P < 0.05$) and IADF width by spring (-0.454 , $P < 0.05$) and autumn ($r = -0.433$, $P < 0.05$) temperature in NE trees (Fig. 5). IADF frequency was also negatively influenced by spring precipitation in NE trees ($r = -0.166$, $P < 0.05$) (Fig. 5). Precipitation seemed to have no effect on IADF width at both sites. IADF anatomical parameters of SW tree rings were influenced by precipitation, while the ones of NE tree rings were mainly affected by temperature (Fig. 6). Summer autumnal precipitation positively influenced IADF mean vessel area in SW tree rings ($r = 0.469$ (SUM), $r = 0.379$ (AUT), $P < 0.05$) as well as negatively affected IADF non-lumen fraction and positively affected vessel frequency ($r = -0.449$, $r = 0.448$, $P < 0.05$), respectively. Spring and summer mean temperatures were positively correlated with IADF non-lumen fraction ($r = 0.344$ (SPR), $r = 0.430$ (SUM), $P < 0.05$) while summer temperature was negatively correlated with IADF vessel frequency in NE tree rings ($r = -0.366$, $P < 0.05$) (Fig. 6).

4 Discussion

An age trend was found in the IADF frequency chronologies which is in agreement with other findings in Mediterranean hardwoods (De Micco et al. 2016a), as well as in Mediterranean pines in Italy, Portugal and Spain (Zalloni et al. 2016). Younger trees are more prone to form IADFs than older ones because of a higher sensitivity to environmental variability, due to the longer growing season of young trees (Rossi et al. 2008), as well as to the tree’s morphology and physiology (e.g. the shallower root system which does not allow to reach deep soil water sources) (Battipaglia et al. 2014; De Micco et al. 2016a, b; Zalloni et al. 2016). A higher frequency of IADF occurrence in wider rings in *Q. ilex* is also in agreement with findings by

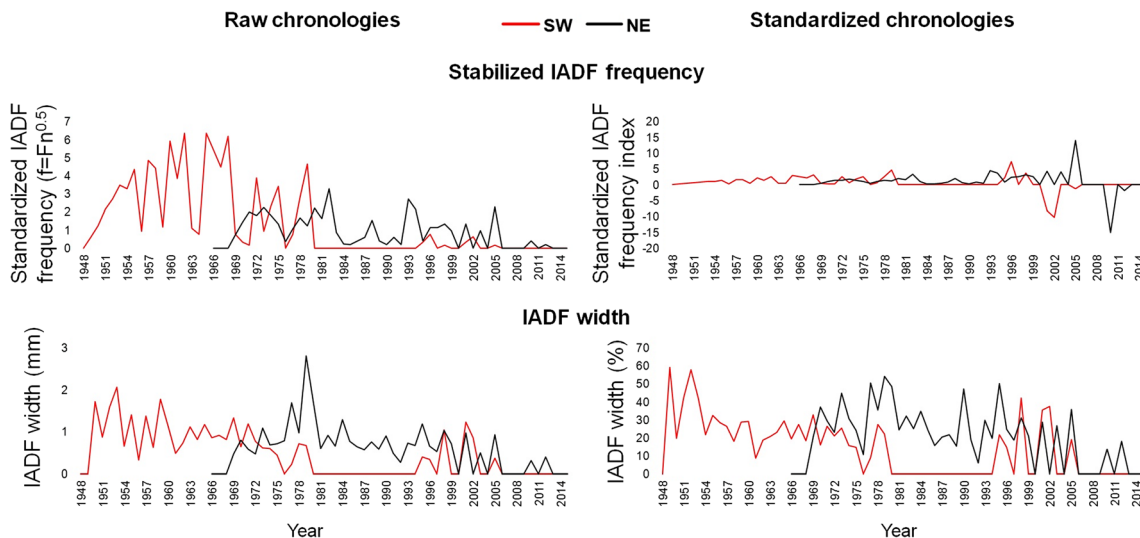


Fig. 3 Raw and standardized mean annual chronologies of stabilized IADF frequency and IADF width of the two sites (red line, SW; black line, NE)

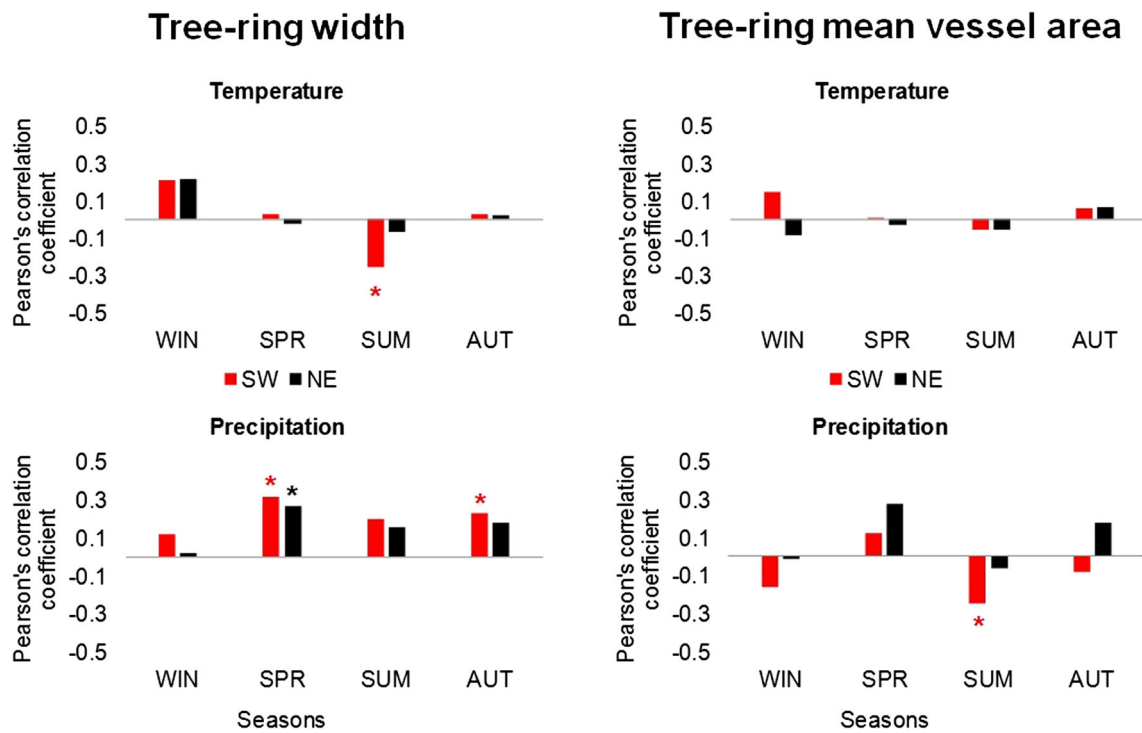


Fig. 4 Correlation function analysis between seasonally grouped mean temperature and total precipitation (WIN = from December of the previous year to March of the current year; SPR = from current March

to current June; SUM = from current June to current September; AUT = from current September to current December) and tree ring width and tree ring mean vessel area with $P < 0.05$ (SW in red, NE in black)

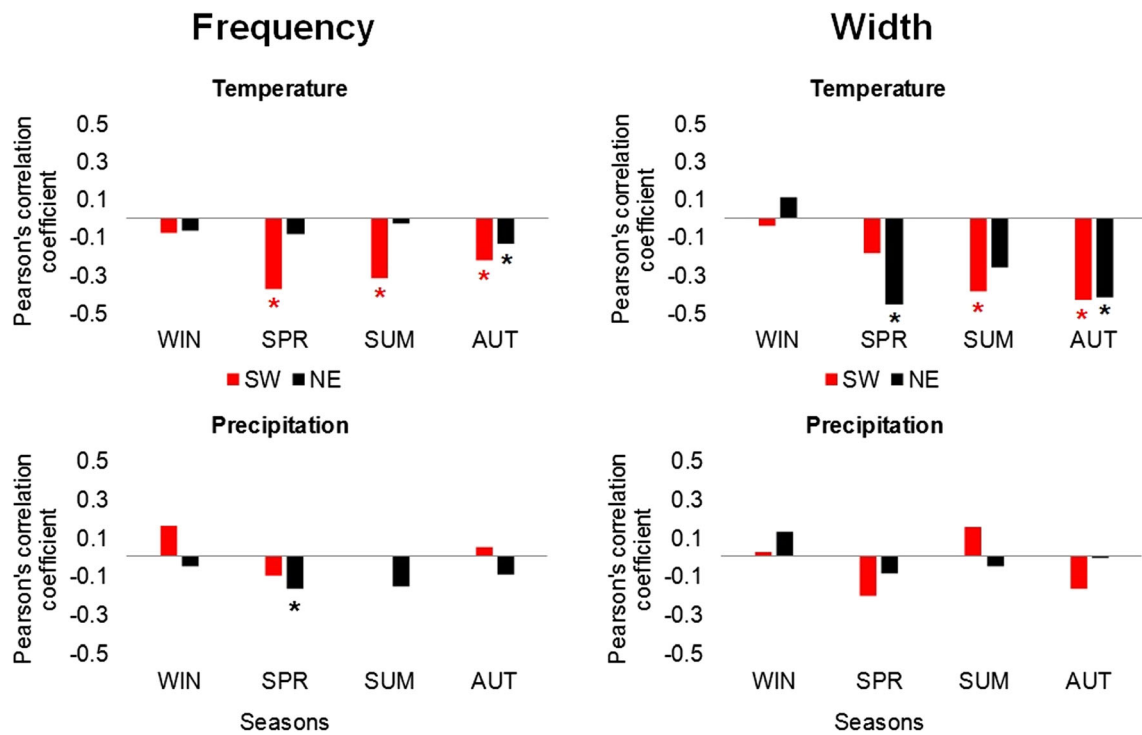


Fig. 5 Correlation function analysis between seasonally grouped mean temperature and total precipitation (WIN = from December of the previous year to March of the current year; SPR = from current March to current June; SUM = from current June to current September; AUT =

from current September to current December) and IADF frequency and IADF width (named as frequency and width in the plot titles) with $P < 0.05$ (red line, SW; black line, NE)

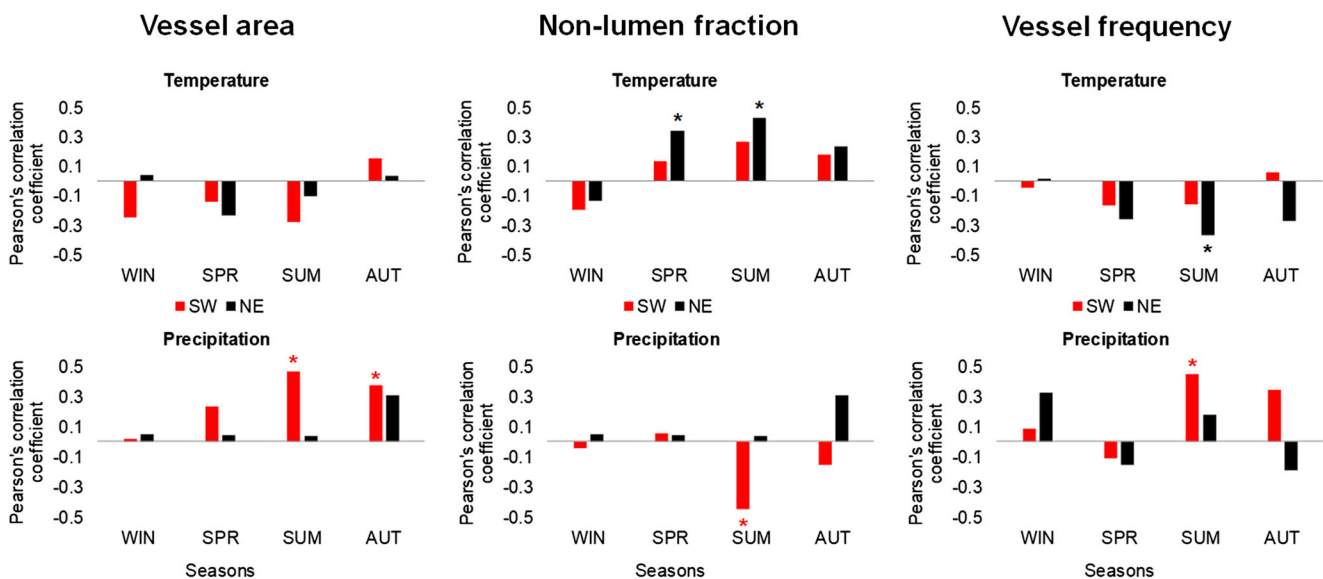


Fig. 6 Correlation function analysis between seasonally grouped mean temperature and total precipitation (WIN = from December of the previous year to March of the current year; SPR = from current March to current June; SUM = from current June to current September; AUT =

from current September to current December) and IADF anatomical parameters (named as Vessel area, non-lumen fraction and vessel frequency in the plot titles) with $P < 0.05$ (red line, SW; black line, NE)

Campelo et al. (2007a) in northeast Spain and by Zhang and Romane (1991) in Southern France. The occurrence of more IADFs in wide rings than in very narrow or very large rings was found in pine species throughout the Mediterranean basin, relating very wide rings to years without marked variability in environmental conditions, and very narrow years to yearly unfavourable conditions of growth which could not allow the resumption of cambial activity responsible for the formation of IADFs (Zalloni et al. 2016). Our results support our first working hypothesis: tree ring width was mainly driven by spring precipitation at both sites but with a stronger influence at SW. Autumn precipitation was the other driving factor and summer temperature also seemed to play a significant negative role. Tree ring growth was found to be positively influenced by spring precipitation in previous studies of *Q. ilex* (Campelo et al. 2009; Gea-Izquierdo et al. 2009, 2011) and of other Mediterranean tree species (Campelo et al. 2006; de Luis et al. 2009, 2013; Di Filippo et al. 2010; Novak et al. 2013a; Piraino et al. 2013; Vieira et al. 2010), indicating the importance of an adequate water availability at the beginning of the growing season, in order to cope with the water shortage of the following dry period during summer. The significant negative correlation between ring width and summer temperature that we found only at one site, suggests a stronger influence of the drought period on tree ring growth at SW than at NE. South-facing slopes have a longer photoperiod but evapotranspiration is also higher, determining more severe summer drought stress (Gratani et al. 2008) than at north-facing slopes. A decline in tree ring growth with increasing summer drought has been reported in Mediterranean oaks (Corcuera et al. 2004; Di Filippo et al. 2010; Gea-Izquierdo et al. 2009, 2011; Colangelo et al. 2017), as well as in other species

(Fernández-de-Uña et al. 2017; Linares et al. 2009; Peñuelas et al. 2008; Piovesan et al. 2008; Sarris et al. 2011). At the same time, the positive relationship between tree ring width at SW and autumn precipitation could reflect a marked autumnal period of growth, which could be explained by the formation of IADFs (Battipaglia et al. 2010; Gea-Izquierdo et al. 2011, 2013). Indeed, IADFs were more frequent, wider and with larger vessels in trees at SW. Marked seasonal variability in environmental conditions as the succession of dry summer and wet autumn, is considered to be the main requirement for IADF formation (Carvalho et al. 2015; Zalloni et al. 2016). The absence of significant relationships between tree ring growth and summer and autumn climate at NE suggests that cambial activity here ceased earlier (Cherubini et al. 2003) and cell differentiation led to a lower IADF frequency. On the other hand, the lower IADF frequency and the wider rings which were found in NE compared to SW, suggest that growth increases where no or less severe summer drought occurs. Moreover, the IADFs at the NE site were narrower, which leads us to partly reject our fourth hypothesis of higher humidity triggering wider IADFs. The soil of NE, with lower available water and water holding capacities compared to SW, could not assure enough water reserves after the period of summer drought if autumn precipitation was not enough for soil recharge (Pumo et al. 2008), leading to the formation of a narrower growth band. Moreover, the younger trees of NE growing in a denser stand, may have shallow root systems and be less able to access the deep soil layers, besides competing more for water resources of the top soil layers, thus having a lower capability to profit from the autumnal precipitation period (Battipaglia et al. 2014). Indeed, IADF frequency was negatively related to autumn temperature at NE, suggesting that soil

moisture supply could be limiting with high temperature, i.e. because of direct evaporation, and thus not allow a substantial re-growth. Temperature was the factor limiting IADF occurrence at SW too, where high mean temperature from spring to autumn would lead to the formation of less IADF within the rings. Cambial activity may slow down or even cease under intense drought (Babst et al. 2016; Battipaglia et al. 2010; de Luis et al. 2011; Vieira et al. 2015), preventing the formation of IADFs, as suggested in Zalloni et al. (2016). Therefore, the decrease in IADF frequency observed through time at both sites could be linked not only to cambial age, but also to the rise of mean annual temperature during the last decades, suggesting that the expected temperature increase in the Mediterranean area may affect cambial growth in *Q. ilex*, most likely inducing an earlier stop, not allowing the autumnal secondary re-growth which would lead to the formation of IADFs. Further studies which include *Q. ilex* trees of different ages would be useful to discern the two influences. This hypothesis is consistent with the stop of primary growth in this species in response to summer water deficit (Gratani 1996; Montserrat-Martí et al. 2009). Furthermore, this finding shows that IADFs in *Q. ilex* tree rings had an opposite relationship with drought compared to Mediterranean pines in Spain, which showed higher frequency in IADFs occurrence in the last 50 years, due to the increase in the frequency of drought events (Bogino and Bravo 2009; Olivar et al. 2012). Therefore, IADF occurrence and its climate signal is species-specific, in addition to the fact that IADFs are of different types between angiosperms and conifers because of the intrinsic wood structure differences (De Micco et al. 2016a). IADF width chronologies showed the same negative correlation with temperature, but with higher correlation coefficient values than for IADF frequency. Therefore, considering also the higher peak coincidence observed between IADF width chronologies of the two sites compared to IADF frequency chronologies, which could be related to the strong common driving climate signal found, we suggest IADF width as a more valuable parameter to compute climate correlations than the mere IADF occurrence.

Our fifth working hypothesis can also be accepted. Wood anatomical traits at IADF level resulted mainly related to summer climatic conditions, showing that temperature and precipitation were the main triggering IADFs factors but with a different role at NE and at SW. Temperature and precipitation as the predominant climatic drivers of wood anatomical traits, respectively, at a northern and southern site were found also in the Mediterranean shrub *Erica arborea* L. by Gea-Izquierdo et al. (2013). In our study, high temperatures during spring and summer would lead to the formation of a conservative wood structure with a denser IADF wood, also due to lower vessel occurrence, in NE compared to SW trees; such a wood promotes the safety of water transport with the increase of wood strength, which is directly related to embolism resistance (Battipaglia et al. 2016; Carlquist 1975, 1989; De Micco et al. 2008). On the contrary, summer and autumn precipitation were

the driving factors of IADF anatomical traits at SW, leading to the formation of larger and more frequent vessels, thus to a lighter wood compared to the NW trees. It seems that the IADFs formed at the SW site promote higher conductivity when water is available, reducing safety (Sperry et al. 2008; Tyree and Zimmermann 2002). As for IADF frequency and width, the anatomical traits of IADFs at NE were affected by high temperature, according to the hypothesis of summer drought limiting soil moisture supply. Contrarily, water availability drives the adaptive plasticity of IADF xylem at SW, where trees seemed to be more sensitive to precipitation seasonal fluctuations. The different wood anatomy structures of the IADFs found at the two sites suggests that summer climatic conditions induce a different reaction in SW and NE trees, when temperature and precipitation conditions are favorable to growth during the following autumn. Further studies are needed to assess the potential link between *Q. ilex* IADF anatomical traits and physiological responses to drought.

Our results reject our second working hypothesis: mean chronologies of anatomical traits did not provide a better climatic signal than tree ring width, disagreeing with Campelo et al. (2010), since they showed no significant correlation except for the one between high mean annual vessel area and drier summer in SW tree rings.

5 Conclusion

The analysis of the climate signal in IADF occurrence and anatomical trait chronologies in *Q. ilex* tree rings provided insights on the relationship between wood structure and growth response to temperature and precipitation at the two different sites. Tree ring growth was strongly influenced by the alternation between dry summer and wet autumn only at SW, leading to more frequent and wider IADFs compared to NE. Age trends were found in IADF frequency of *Q. ilex* at both the study sites. Temperature was the factor limiting IADF occurrence at both sites, suggesting that the expected temperature increase in the Mediterranean area may induce an earlier stop in cambial activity, not allowing the autumnal secondary re-growth leading to IADF formation. Furthermore, IADF width contains a better climatic signal than IADF frequency. Climatic correlations with anatomical traits of the IADFs helped to distinguish between the different predominant climatic factors at the two sites. Summer and autumn precipitation lead to the formation of IADFs with an efficient wood with large and more frequent vessels at SW. On the other hand, spring and summer temperatures influenced the anatomical structure of IADFs at NE, leading to a conservative strategy with less vessels and a denser wood. The differences we found between sites could be ascribed to differences in aspect, soil water holding capacity and stand density. Further research is needed to assess how impacts of climate variability can vary

depending on stand structure and local site conditions and to associate IADF occurrence with the ability of the species to adapt to climate changes. A long-term study of intra-annual xylogenesis under controlled conditions, and the physiological processes behind it, is necessary to provide process-based evidence explaining correlation-based tree ring studies of IADF formation. Improving the knowledge about tree adaptation traits could provide information about their capacity to adapt at different site conditions and their vulnerability to future predicted climate change, thus being useful in forest management choices.

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Compliance with ethical standards

Conflict of interest statement The authors declare that they have no competing interests.

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