

# Are wood fibres as sensitive to environmental conditions as vessels in tree rings with intra-annual density fluctuations (IADFs) in Mediterranean species?

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## Abstract

**Key message** Wood fibres are as sensitive to environmental conditions as vessels in tree rings with intra-annual density fluctuations (IADFs) in Mediterranean species.

**Abstract** Forecasted environmental changes are likely to increase the frequency of intra-annual density fluctuations (IADFs) in Mediterranean tree rings. The interpretation of intra-annual anatomical variability of tree rings with IADFs can be useful to understand plant-growth response to environmental changes with seasonal resolution. We analysed the intra-annual variability of quantitative traits of both vessels and fibres in the woods of *Arbutus unedo* L. and *Erica arborea* L. to compare the sensitivity of different cell types to environmental variations. We applied digital image analysis on microphotographs of semi-thin sections of tree rings formed at sites with different soil water availability. Wood of both species showed good

adaptability that allows anatomical traits of vessels and fibres to be harmonised by changing the water transport capacity and wood strength, promoting the efficiency or safety of water transport according to water availability during wood formation. The size of fibres showed trends of variation similar to vessels. Not all parameters of vessels were accurate indicators of the IADF presence. In conclusion, parameters of fibres, which offer advantages during automatic measurement, showed the same sensitivity to environmental fluctuations as vessels. Thus, they could be good indicators of summer drought to describe and interpret the ecological meaning of IADFs in tree rings.

**Keywords** Fibre · Intra-annual density fluctuations (IADFs) · Mediterranean ecosystems · Vessel · Water-conducting cells · Wood anatomy

## Introduction

Forecasted environmental changes are likely to lead to longer periods of drought and an increase in intensity and frequency of extreme events in Mediterranean ecosystems (IPCC 2001, 2007; Giorgi and Lionello 2008). To cope with drought conditions, Mediterranean species show morphological adaptations mainly related to water saving and mechanical reinforcement of tissues (Shields 1950; Fahn 1964; De Micco and Aronne 2007, 2012).

The high plasticity of Mediterranean woody plants to fluctuating environmental conditions is responsible for the intra-annual variability of anatomical traits in tree rings, which form intra-annual density fluctuations (IADFs) in response to specific patterns of cambial activity (Tingley 1937; De Luis et al. 2007). Forecasted climate change will probably result in more pronounced periods of summer

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drought (Gibelin and Déqué 2003; Hertig and Jacobbeit 2008), which may have a severe impact on IADF occurrence and features in Mediterranean woods.

The analysis of anatomical and isotopic features of IADFs can help in understanding past ecological processes, such as those related to species-specific sensitivity to drought, at intra-annual scale (Cherubini et al. 2003; De Micco et al. 2007; Hoffer and Tardif 2009; Battipaglia et al. 2010; Camarero et al. 2010; Griffin et al. 2011).

IADFs have been studied mostly in conifers and their formation has been related to late-summer or autumn rainfall, or drought events (Rigling et al. 2002; Wimmer 2002; Masiokas and Villalba 2004; Campelo et al. 2007; De Luis et al. 2009, 2011; Vieira et al. 2009, 2014; Camarero et al. 2010; Rozas et al. 2011; Campelo et al. 2007, 2013). IADFs in some Mediterranean hardwoods have been recently identified and classified according to their position in tree rings (Battipaglia et al. 2010; De Micco et al. 2012, 2014; Battipaglia et al. 2014a). Early- and Middle-IADFs were latewood-like cells at the beginning or in the middle of the annual ring, respectively, while late-IADFs were earlywood-like cells in latewood (Battipaglia et al. 2010). In *Arbutus unedo*, Battipaglia et al. (2010) verified that the type and position of IADFs along the tree rings mostly depend on water availability at the site where plants are growing, whereas in plants of *Erica arborea* co-occurring at the same sites, IADFs were mainly located in the middle of the ring (Battipaglia et al. 2014a).

Various wood anatomical features measured in tree rings have been used to gain insights into the complexity of wood formation and IADF occurrence also in response to specific environmental events (e.g., insect infestation, fires, soil erosion, landslides, flooding and drought) (e.g., Schweingruber 2007; De Micco et al. 2013; Gea-Izquierdo et al. 2013; Battipaglia et al. 2014b; Vieira et al. 2014).

In comparison to softwoods, few studies have been carried out on hardwoods, although such studies would be very useful, especially in Mediterranean shrubs, whose peculiar anatomical traits are responsible for species ability to endure severe dry conditions where trees usually do not survive (Carlquist 1975; Baas et al. 2004; De Micco et al. 2006, 2008). In such hardwoods, a balance between efficient conductivity, when water is available, and safety against embolism, during water shortage, is regulated through the harmonisation of many wood anatomical traits (e.g., vessel size and frequency, cell-wall thickness) (Carlquist 1989; Martínez-Vilalta et al. 2002; Jansen et al. 2003; Baas et al. 2004; McCulloh and Sperry 2005; De Micco et al. 2006, 2008; Sperry et al. 2006; Jacobsen et al. 2007; Pratt et al. 2007; De Micco and Aronne 2009). Wood density and size of water-conducting cells (vessels and tracheids) have been the most widely explored wood anatomical parameters as indicators of environmental and

mainly climatic information recorded in tree rings, because they are strongly and directly related to water conductivity and wood mechanical strength (García-González and Eckstein 2003; Abrantes et al. 2013; Scholz et al. 2013). However, the relations between various parameters can play a significant role in the control of xylem efficiency and safety. For example, the low water conductivity of narrow vessels is generally compensated by the co-occurrence of higher vessel frequency; vessel redundancy is a way to increase the probability that at least part of vessels remain active in water transport when embolism occurs (Fahn et al. 1986). Moreover, resistance to drought-induced embolism is positively correlated to parameters indicating wood strength (e.g., wood density, stem and vessel strength, fibre traits) (Hacke et al. 2001; Baas et al. 2004). Increased wood strength, achieved also through the ground tissue of fibres, would reduce the risk for air seeding through pit membranes by avoiding the stretching of pit membranes under extreme negative pressures (Baas et al. 2004).

Improvements in image analysis techniques have attracted more research interests towards the analysis of various wood anatomical traits in both softwoods and hardwoods (von Arx et al. 2013). The position of tracheids in exact and ordered rows makes softwoods suitable for image analysis tools, such as ROXAS, which automatically recognise conduits and build centuries-long chronologies of the tracheid lumen area (Brunel et al. 2014; von Arx and Carrer 2014). Automatic measurement of various vessel traits in tree-ring chronologies has also been successfully achieved in diffuse-porous hardwoods with ROXAS (Fonti et al. 2010; von Arx et al. 2013; Wegner et al. 2013). Even if there is evidence that fibre traits can affect hydraulic resistance to cavitation and, in turn, are affected by water availability, fibres have often been neglected probably because of technical constraints in their measurement due to the narrower lumen if compared with vessels (Hacke et al. 2001; Arend and Fromm 2007; Fonti et al. 2013).

This study analyses the intra-annual variability of different parameters of vessels and fibres in the wood of two species of the Mediterranean maquis, namely *Arbutus unedo* L. and *Erica arborea* L. We compare the different sensitiveness of various wood anatomical traits to the intra-annual variability of environmental conditions by analysing tree rings with and without IADFs, in plants growing at sites characterised by different water availability. The final aim of the study is to evaluate whether anatomical traits of non-conducting cells show similar sensitivity to the variability of environmental conditions compared to conducting cells. Indeed, different anatomical parameters are often highly intercorrelated (Wimmer 2002) and the choice of which parameter to use in ecological studies depends not only on the ease of measurement but also on the potential

use of the data obtained. The use of fibres as indicators of environmental fluctuations in tree rings in hardwoods would help overcome technical difficulties in applying image analysis tools on those hardwoods where vessels are not uniformly distributed along the ring.

## Materials and methods

### Study site and plant material

The study was conducted on plants of *Erica arborea* L. and *Arbutus unedo* L. growing on Elba, an island in the Tyrrhenian sea (Central Italy). The climate is Mediterranean, with a mean annual temperature of 16.4 °C and mean annual precipitation of 375 mm. Two sampling sites with different amounts of soil moisture were selected: a xeric and a mesic site. The xeric site, located on Monte Perone at 420 m a.s.l. (42°46'N, 10°12'E), was characterised by more open, scattered vegetation with a higher frequency of xeric species and shrubs than the mesic site, located in the Nivera Valley at 460 m a.s.l. (42°46'N, 10°11'E). Details on site characteristics are given in Battipaglia et al. (2010).

### Tree-ring sampling, sectioning and microscopy

At both sites, five plants of *E. arborea* L. (2–3 m tall, 4–8 cm in diameter) and five of *A. unedo* L. (3–5 m tall, 5–10 cm in diameter) were randomly selected and sampled. Three cross-sections were taken from the largest main stem per plant. Semi-thin sections (15 µm thick) were obtained from each sample with a sliding microtome: each section corresponded to a tree-ring series from one plant. The sections were double-stained with safranin O (1 g in 65 ml of 100 % ethanol and 30 ml of distilled water) and counterstained with astra blue (1 g in 100 ml of ethanol at 100 % and 5 ml of distilled water) according to Vasquez-Cooz and Meyer (2002). The sections were then dehydrated, immersed in xylene and mounted on glass slides with Canada balsam (Schweingruber 1978; Gartner et al. 2001). The sections were analysed under a light microscope (BX60, Olympus, Germany) in order to identify tree-ring boundaries and IADFs.

### Identification and classification of IADFs, and quantitative wood anatomy

We identified IADFs in the sections using reference tree-ring chronologies to which cross-dating techniques were applied (Battipaglia et al. 2010, 2014a). The most represented types of IADFs were selected in each species and site. More specifically, in the tree-ring chronologies of *E.*

*arborea* from both sites and *A. unedo* from the xeric site, we considered earlywood IADFs (EW-IADFs, accounting in mean for more than 80 % of total IADFs) as those occurring as latewood-like cells in earlywood in the middle of the tree ring. In *A. unedo* plants growing at the mesic site, we considered latewood IADFs (LW-IADFs accounting for almost 90 % of total IADFs) as those occurring as earlywood cells in latewood. In both species, we considered rings without IADFs as control rings.

In brief, the following tree rings were considered: (a) 15 rings (from 1992 to 2006) in *E. arborea* per plant at both sites; (b) 13 rings (from 1994 to 2006) in *A. unedo* per plant at both sites. The variability of the anatomical features along the width of each ring was quantified with digital image analysis techniques. For each ring, microphotographs, at a magnification of 200× for *E. arborea* and 100× for *A. unedo*, were acquired with a digital camera (CAMEDIA C4040, Olympus) in specific regions selected in critical areas of each ring, as reported in De Micco et al. (2014). For *E. arborea*, in rings with IADFs from both sites, four regions were selected, proceeding from the beginning towards the end of each ring: (1) Region 1, selected in earlywood (EW); (2) Region 2, selected in the area where IADF begins, in correspondence with the dark band made of latewood-like cells (FL1); (3) Region 3, selected in the area of the fluctuation in correspondence with the light band (FL2); (4) Region 4, selected in latewood (LW).

For control rings, the same four regions were selected: EW and LW regions were selected, respectively, at the beginning and ending of the ring, while FL1 and FL2 were selected as two consecutive regions in the middle of EW. Since data collected in FL1 and FL2 were not significantly different, they were pooled to consider FL1 and FL2 as a single central region of the ring defined as potential fluctuation (PFL), the region of the ring where the fluctuation could have been found.

As regards *A. unedo*, in tree-ring chronologies of the xeric site, the same regions as in *E. arborea* were selected in both rings with and without IADFs. For sections from the mesic site, according to De Micco et al. (2012), only three regions were identified for the rings with IADFs proceeding from the beginning towards the end of the ring: (1) Region 1, in earlywood (EW); (2) Region 2, in latewood (LW); (3) Region 3, in the fluctuation zone which appears as a light band of earlywood-like cells in the latewood (FL). For control rings, the same three regions were selected, called EW, LW, and PFL at the end of the ring in the area where IADF should potentially occur.

The anatomical parameters were analysed in each region using Analysis 3.2 software (Olympus). The following parameters, related to the characteristics of water transport efficiency and safety, were analysed: vessel and fibre size

measured as maximum, mean and minimum Feret diameters (distance between the parallel lines tangent to the perimeter of the particle); wood density (measured as the percentage of cell walls over total xylem area); vessel frequency [the number of vessels per mm<sup>2</sup>, determined by counting the vessels present in a known area, according to Wheeler et al. (1989)]; thickness of fibre cell walls (avoiding cell corners).

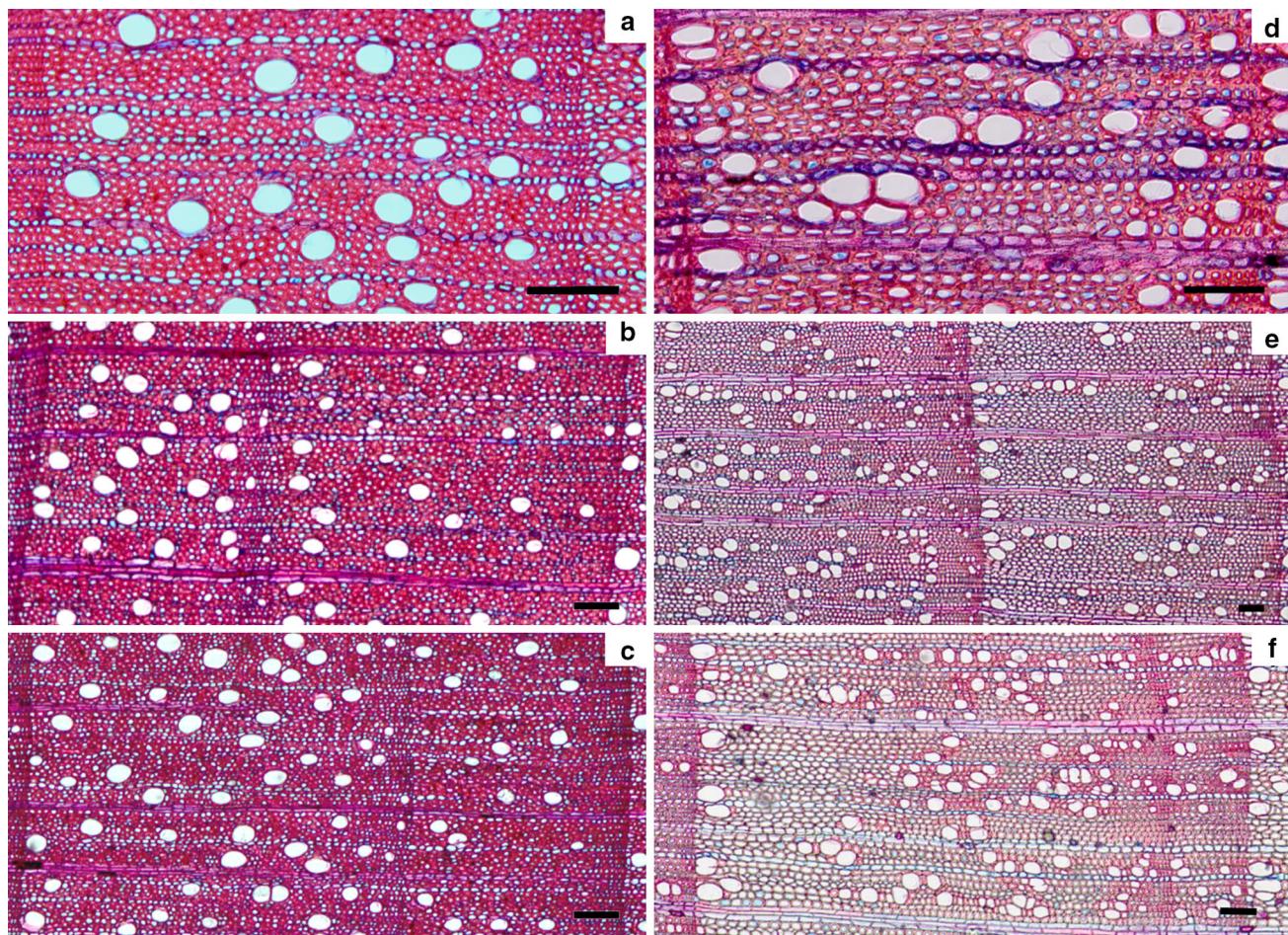
These anatomical parameters were measured in at least 10 elements per region. All abnormal vessels and fibres or collapsed cell walls were excluded from the analysis.

For the statistical analysis, the two sites were kept separated since *A. unedo* wood in the mesic site mainly formed a different type of IADFs if compared to the same species growing at the xeric site and to *E. arborea* at both sites. In the case of *E. arborea*, data were firstly processed with a two-way ANOVA using the site and the tree-ring region as independent factors also showing their interactions. Then, to highlight the intra-annual trend of variations in the two species at the two sites, data were a priori separated

according to the site and species, and processed with a one-way ANOVA, using Student–Newman–Keuls coefficient for multiple comparison tests ( $p < 0.05$ ). The SPSS® statistical package was used (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov and Shapiro–Wilk tests were performed to check for normality. Percent data were transformed through arcsine function before statistical analysis.

## Results

In rings without IADFs, the wood of *E. arborea* L. was characterised at both sites by diffuse porosity, prevalence of solitary vessels and gradual transition from earlywood, with wide vessel lumen area, towards latewood, often reduced in width, with narrower vessels (Fig. 1a). At both sites, tree rings showed a higher occurrence of EW-IADFs, appearing as a dark band formed by latewood-like cells, corresponding to the beginning of fluctuation (FL1), which is followed by a clear band, with earlywood-like cells (FL2) (Fig. 1b, c).



**Fig. 1** Light-microscopy views of cross-sections of *E. arborea* (a–c) and *A. unedo* (d–f) showing rings with and without IADFs. **a, d** rings without IADFs; **b, c, e** rings with EW-IADFs; **f** ring with LW-IADF. Rings are oriented with earlywood on the left. Bars = 100 µm

**Table 1** *F*-values and significance of the two-way ANOVA with site (S) and tree-ring region (R) as main factors and their interaction (S × R), for all anatomical traits measured in tree rings with IADFs in *E. arborea*

	S		R		S × R	
	F	P	F	P	F	P
Wood density	7.01	0.009	44.2	0.000	2.195	0.089
Vessel maximum Feret diameter	8.15	0.005	193.0	0.000	0.172	0.915
Vessel maximum Feret diameter	5.83	0.016	183.0	0.000	0.363	0.780
Vessel maximum Feret diameter	2.37	0.125	128.0	0.000	0.755	0.520
Fibre maximum Feret diameter	13.50	0.000	948.0	0.000	2.195	0.886
Fibre maximum Feret diameter	14.50	0.000	1059.0	0.000	0.122	0.947
Fibre maximum Feret diameter	15.09	0.000	890.0	0.000	0.005	1.000
Fibre wall thickness	1.29	0.258	191.4	0.000	1.346	0.260
Vessel frequency	40.38	0.000	47.9	0.000	1.171	0.320

Given that there were missing rings in some plants and that tree rings with more than one IADF were avoided, in summary we analysed: (a) 47 rings with EW-IADFs and 25 control rings (without IADFs) in *E. arborea* at the xeric site, and (b) 46 rings with EW-IADFs and 29 control rings in *E. arborea* at the mesic site.

For all analysed parameters, the interaction between the site and the tree-ring region was not significant, while both factors showed a significant influence as main effects in most cases (Table 1).

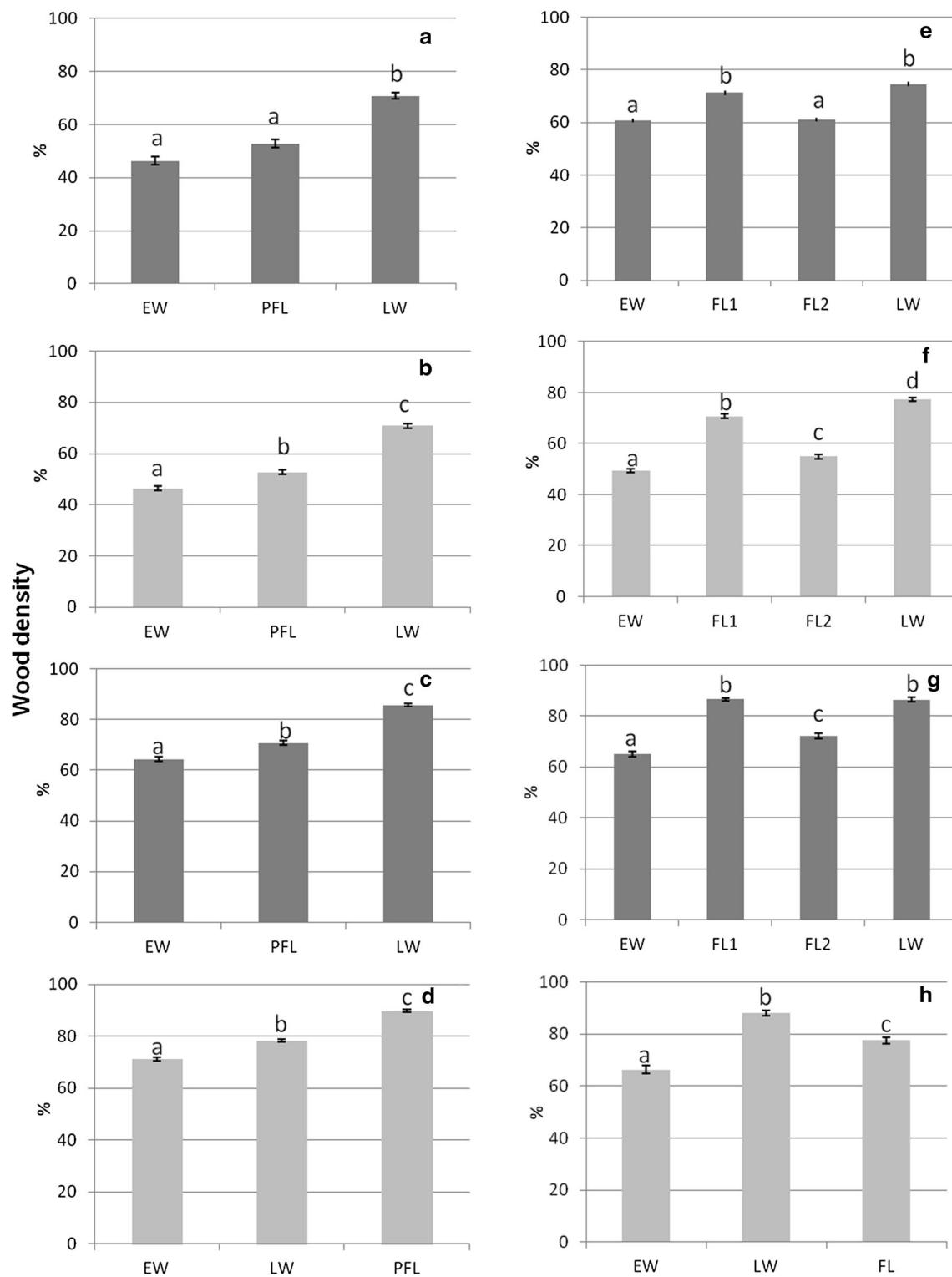
Wood was denser at the beginning of the fluctuation (FL1) and in latewood as compared with other tree-ring regions (FL2 and earlywood). Contrary to tree rings without IADFs, where density (percentage of cell walls over total xylem area) increased towards latewood (Fig. 2a, b), tree rings with IADFs showed a higher density in FL1 and LW than EW and FL2 (Fig. 2e, f). In the presence of IADFs, wood density in EW and FL1 was significantly higher in the xeric than in the mesic site.

In *A. unedo* rings without IADFs, xylem was characterised in both sites by typical diffuse porosity, vessels often arranged in radial rows of four or more elements, vessel lumen with angular boundaries and size gradually decreasing from earlywood to latewood (Fig. 1d). Predominant IADF type varied as a function of the site. Tree rings with IADFs from the xeric site presented the same appearance as EW-IADFs described in *E. arborea* (Fig. 1e). On the contrary, in wood of the wet site, LW-IADFs were the most represented. This type of IADF was characterised by a gradual decrease in vessel lumen from earlywood to latewood, followed by a sudden increase in vessel lumen appearing as earlywood-like cells in latewood (Fig. 1f). A new reduction in vessel lumen size established the real boundary of the ring. As for *E. arborea*, because of the occurrence of missing rings and rings with more than one IADF, in *A. unedo* we analysed: (a) 22 rings with EW-IADFs and 34 control rings at the xeric site, and (b) 13 rings with LW-IADFs and 39 control rings at the mesic site.

At the xeric site, the trends of wood density variation in tree rings with and without IADFs were the same as those found in *E. arborea* (Fig. 2c, g). At the mesic site, in tree rings with LW-IADFs, wood density increased significantly from EW to LW, but wood was significantly lighter in the IADF zone as compared to control rings (Fig. 2d, h).

In *E. arborea*, Feret diameters of vessels and fibres presented the same trends of intra-annual variation which varied according to the presence/absence of IADFs (Fig. 3a, b, e, f). In the absence of fluctuation, Feret diameters of both conducting and non-conducting cells significantly decreased from EW to LW with no differences between sites (Fig. 3a, b). In the presence of IADFs, intra-annual variability of Feret diameters of both conducting and non-conducting cells was similar for the two sites, with a strong decrease from EW to the region where the fluctuation starts (FL1), followed by a significant increase in the region FL2. In LW, diameters of both conducting and non-conducting cells decreased again, reaching values almost comparable to those found in the region FL1 (Fig. 3e, f). In the presence of IADFs, vessel size in FL2 was significantly higher in the xeric than in the mesic site.

In *A. unedo*, Feret diameters of vessels and fibres presented the same trends of intra-annual variation which varied according to the presence/absence of IADFs and to the site. In the absence of IADFs, Feret diameters of both conducting and non-conducting cells decreased significantly from the beginning to the end of the ring in both sites (Fig. 3c, d). In the presence of IADFs, the trend was different in the two sites. At the xeric site, we observed a severe decrease from EW to FL1, followed by a subsequent significant increase in FL2. In LW, diameters of both conducting and non-conducting cells decreased again, reaching values not significantly different from those found in region FL1 (Fig. 3g). At the mesic site, we observed a significant decrease in diameters of both conducting and non-conducting cells from EW to LW, followed by a



**Fig. 2** Wood density (measured as percentage of cell walls over total xylem area) in tree rings of *E. arborea* (**a**, **b**, **e**, **f**) without (**a**, **b**) and with (**e**, **f**) IADFs at the xeric (**a**, **e**) and mesic sites (**b**, **f**), and of *A. unedo* (**c**, **d**, **g**, **h**) without (**c**, **d**) and with (**g**, **h**) IADFs in xeric (**c**,

**g**) and mesic sites (**d**, **h**). Mean values and standard errors are shown. Different letters indicate significantly different values ( $p < 0.05$ ) between different regions

significant increase at the end of the ring in the FL region (Fig. 3h).

Cell-wall thickness of fibres showed the same trends of intra-annual variation as Feret diameters (Fig. 4). For *E. arborea* and *A. unedo*, in the rings without IADFs at both sites, wall thickness of fibres decreased gradually from EW to LW with the most evident decrease in LW of *E. arborea* (Fig. 4a–d). In the rings with fluctuations of *E. arborea*, high fibre wall thicknesses were found in regions EW and FL2; besides, there was a significant decrease in FL1 and LW regions (Fig. 4e, f). The same trend occurred in *A. unedo* tree rings with EW-IADFs (Fig. 4g), while in the presence of LW-IADFs, fibre wall thickness decreased from EW to LW, and increased significantly in region FL (Fig. 4h). In rings without IADFs, in most cases, cell-wall thickness was significantly higher in the wood formed in the xeric than in the mesic site.

Vessel frequency showed a general increase, which was not always significant, in the middle of the ring in both species, at the two sites, irrespective of the presence of IADFs (Fig. 5). More specifically, in *E. arborea*, vessel frequency showed no significant changes between the various regions of the rings with and without IADFs for plants at the xeric site, except in LW where it underwent a significant decrease in the presence of IADFs (Fig. 5e). For samples from the mesic site, in the rings without IADFs, there was a significant increase in vessel frequency in the PFL region and a subsequent marked decrease in the LW region (Fig. 5b). Instead, in rings with IADFs this increase occurred in FL1 with a subsequent significant decrease first in region FL2 and then in LW (Fig. 5f).

For *A. unedo* at the xeric site, vessel frequency increased in regions PFL and FL1 of tree rings with and without IADFs, respectively (Fig. 5c, g). In the same species growing at the mesic site, vessel frequency significantly increased in LW of both rings with and without IADFs (Fig. 5d, h).

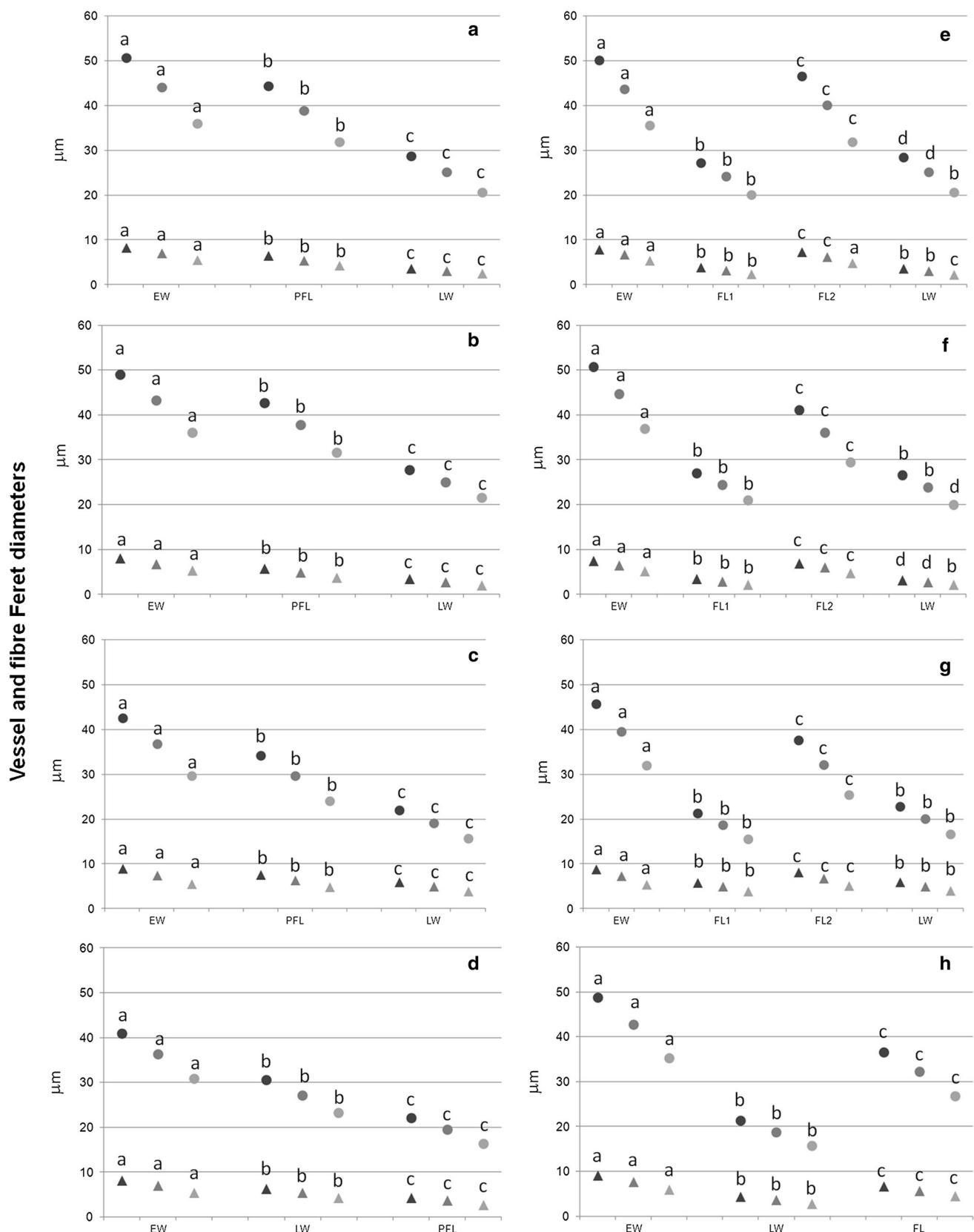
## Discussion

In this paper, we showed that parameters of non-conducting cells can be used to understand the effect of water availability on tree-ring growth, hence to describe and interpret the ecological significance of IADFs. Indeed, fibres can provide the same ecological information as gained from the analysis of vessel size.

IADFs can be used as tools to gain insights into the effect of environmental fluctuations on wood, provided that the physiological and ecological meaning of anatomical “signals” are correctly elucidated. Until now, the analysis of intra-annual variability of tree rings has been mainly based on the measurement of wood density and tracheid

size in softwoods that are ideal models for the application of image analysis tools, such as ROXAS, which automatically detect and measure tracheids in ordered rows along tree rings (von Arx and Carrer 2014). Based on the assumption that water availability triggering IADFs would mostly affect water-conducting cells, studies on intra-annual variability of hardwood tree rings have mainly focused on the analysis of vessel features (Battipaglia et al. 2010, 2014a). By establishing a specific lower vessel area threshold, ROXAS is used for automatic measurement of vessel size chronologies in ring porous woods (Wegner et al. 2013). Our results indicate that the size and cell-wall thickness of fibres could be used instead of vessel size to analyse the intra-annual variability of tree rings in relation to environmental fluctuations, especially drought occurrence. The use of fibre parameters in automatic measurements can help in overcoming technical constraints due to different vessel arrangement in hardwoods, and would furnish a more continuous picture of the cell size variation along tree-ring width in woods where vessels are not frequent. Indeed, non-conducting cells in hardwoods form the ground tissue in which vessels are spread with various aggregations. Although fibres in hardwoods are not always precisely arranged in ordered rows as tracheids in softwoods, it is still possible to follow the variability of their traits by tracing continuous transects along the ring width. This helps cell detection and automatic measurement, which could be applied by establishing a specific higher fibre lumen area threshold to avoid the automatic measurement of vessels.

The wood of *E. arborea* and *A. unedo* is very plastic and often forms IADFs: the mechanism triggering their appearance has been recently hypothesised by applying a multidisciplinary approach combining dendro-ecological, wood anatomical and isotopic analyses (Battipaglia et al. 2010, 2014a). Quantitative wood anatomy allowed fine characterisation of wood traits of the IADFs occurring in tree rings formed in two different conditions of water availability in the two species. The intra-annual variability of wood density and of vessel and fibre size follows the same species- and site-specific trends of variation. Indeed, the values of wood density and of Feret diameters of both vessels and fibres in the tree rings with EW-IADFs encounter a significant increase or decrease, respectively, in the middle of the ring that marks the beginning of IADFs. The beginning of EW-IADFs is characterised by high safety against embolism due not only to reduced vessel lumen size but also to reduced fibre size and increased density that improve wood strength. In the same region, the decrease in fibre cell-wall thickness indicates that there is more volume available for cell elements, leading to the formation of a stiffer wood made of more numerous and smaller elements: such characteristics are



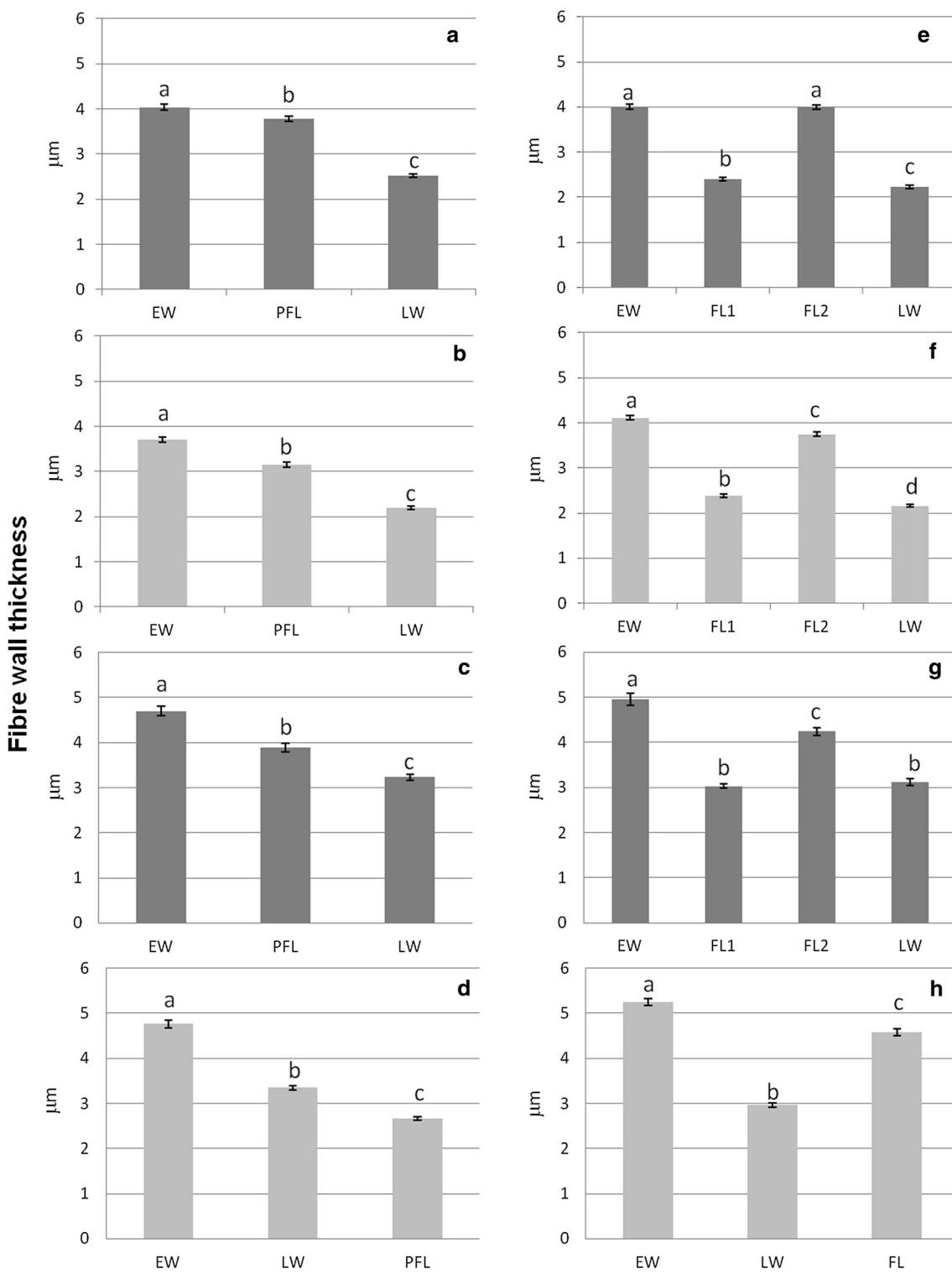
**Fig. 3** Vessel (circle) and fibre (triangle) Feret maximum (dark grey), mean (medium grey) and minimum (light grey) diameters in *E. arborea* (**a**, **b**, **e**, **f**) tree rings without (**a**, **b**) and with (**e**, **f**) IADFs at the xeric (**a**, **e**) and mesic sites (**b**, **f**), and in *A. unedo* (**c**, **d**, **g**, **h**) tree rings without (**c**, **d**) and with (**g**, **h**) IADFs at the xeric (**c**, **g**) and mesic sites (**d**, **h**). Mean values are shown; standard errors are not visualised because they are too small. Different letters indicate significantly different values ( $p < 0.05$ ) between different regions within cell type

the sign of a wood which is less prone to drought-induced embolism (Hacke et al. 2001). The higher values of wood density and fibre wall thickness in the wood of the xeric than mesic site is also in line with the trend of increasing wood strength under stressful conditions. The decrease in vessel and fibre Feret diameters at the beginning of the IADF zone is around 50 % in all cases and seems to be independent of cell type. This could be a mechanical consequence of the reduced turgor-driven cell expansion due to drought (Hsiao et al. 1976; Sperry et al. 2006). Moreover, the decrease in fibre cell-wall thickness suggests a reduction in cell-wall synthesis due to reduced carbon uptake following stomata closure (Chaves et al. 2002). This is in agreement with increased  $\delta^{13}\text{C}$  or water use efficiency found in the same species at the beginning of the IADF zone (Battipaglia et al. 2010, 2014a). The onset of a drought period in the two species leads to the formation of xylem in which safety features are privileged over hydraulic efficiency. In both species, safe water transport is also favoured by the occurrence of tracheids which are still capable of transporting water even when parts of vessels are embolised (Baas et al. 2004). The same phenomenon in *A. unedo* is permitted also by vessel grouping since it is rare that embolism would spread towards all vessels of a group, especially because reduced vessel size also means decreased size of the membrane pores in intervessel pitting (Baas et al. 2004; Wheeler et al. 2005). The substantial increase in size of both vessels and fibres in the second part of the EW-IADFs indicates that the wood of the two species reacts by following the principle that the survival of Mediterranean species after a period of summer water deficit may be strongly influenced by their ability to recover by quickly absorbing water after a rain pulse (Gratani and Varone 2004; Gallé and Feller 2007). The larger cell size in FL2 of tree rings formed in the xeric than mesic site would support the idea of a prompt recovery under favourable conditions following severe stress events.

As regards vessel frequency, in the two species at both sites, the trends of variation in tree rings with IADFs appear to follow the normal pattern found in the rings without IADFs. The lack of control of vessel frequency by water availability suggests that these species do not implement an adaptation strategy in favour of hydraulic safety through the “redundancy” of the conductive

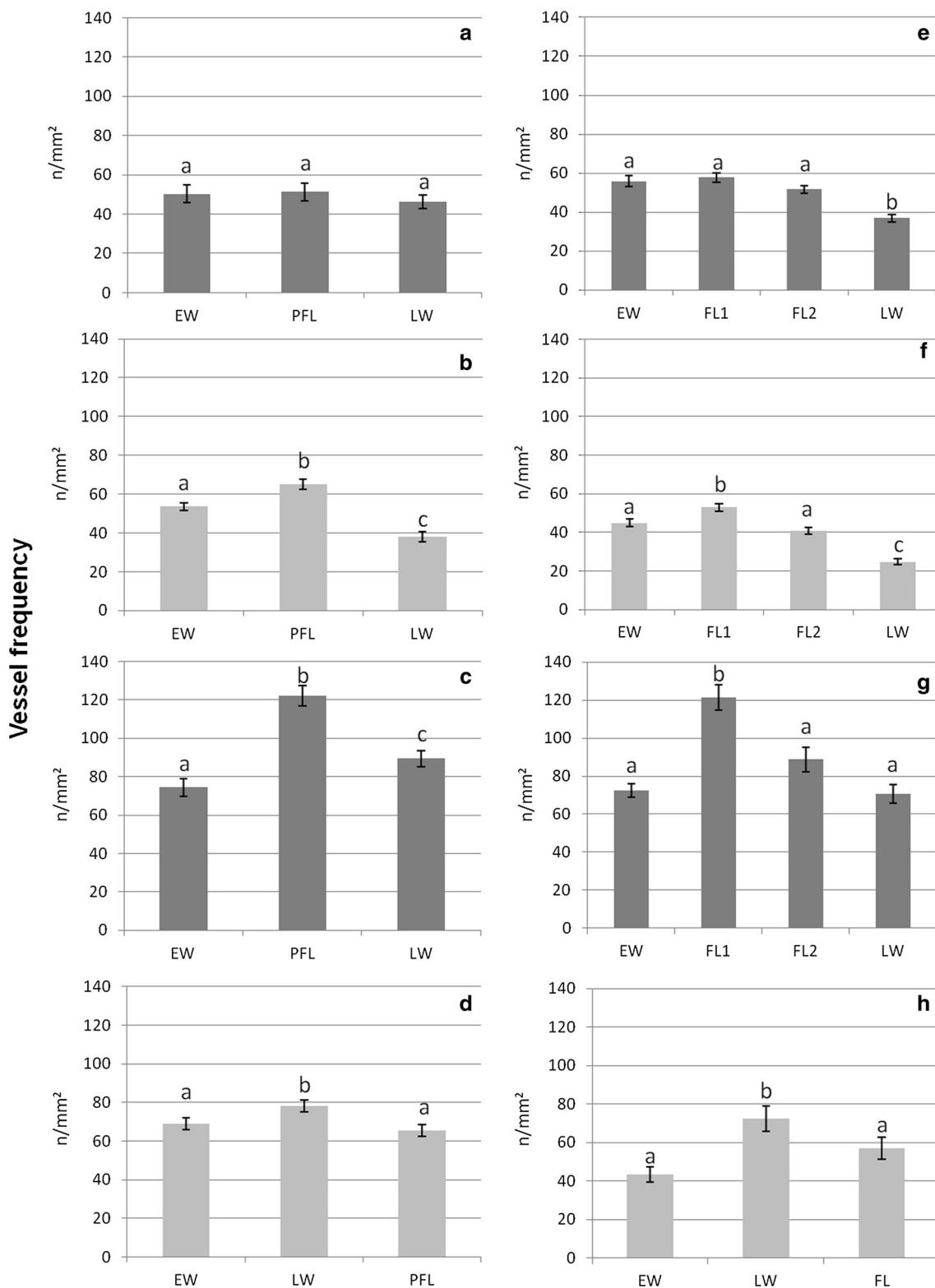
elements. This is in contrast with the typical increment of vessel frequency that occurs with the shift from mesic to xeric conditions (Fahn et al. 1986; Carlquist 1989). This wood trait might be among those features mainly controlled by other factors or interactions between various variables. Indeed, the IADFs are generally produced in response to physiological, genetic and climatic factors (Larson 1960; Wimmer 2002). Many reasons, such as genetic factors or differences at the root level, could explain the lack of EW-IADFs in *A. unedo* tree rings formed at the mesic site. Moreover, in these plants, the occurrence of LW-IADFs might conceivably be ascribed to a longer duration of cambial activity. Whatever the reason, the onset of such IADFs in *A. unedo* at the mesic site seems to be triggered by conditions other than those acting at the xeric site (Battipaglia et al. 2010). Also in this type of IADF, the size of both vessels and fibres as well as fibre cell-wall thickness, follow similar trends of variation opposite to wood density, which suggest a recovery in wood growth soon after latewood formation. This restoration of wood growth, occurring at the same extent in vessels as in fibres, can be ascribed to possible rain events favouring turgor-driven cell enlargement at the end of the growing season after the period of water stress that primed the formation of latewood. Indeed, when in late-summer, rainfall is more abundant than normal seasonal trends, the dormancy induced by water stress in cambial activity could be interrupted and cambium could be reactivated to produce new larger cells (Masiokas and Villalba 2004). This hypothesis is supported by the finding that  $\delta^{13}\text{C}$  was reduced in the zone of the fluctuation, indicating a regrowth of the rings in the last part of the growing process as a response to unexpected rain events (Battipaglia et al. 2010). However, many other factors (including the duration of the day, availability of carbohydrates and hormonal regulators) would ultimately affect the properties of vessels and fibres by also influencing cell enlargement and maturation.

The overall analysis of the two species at the two sites indicates that the tree rings of *E. arborea* and *A. unedo* have good plasticity that allows anatomical traits of both conducting and non-conducting cells to be harmonised by changing the capacity of water transport and wood strength to promote the efficiency of transport when water is available, while favouring the traits of hydraulic safety in conditions of stress. The finding that vessel and fibre size show the same sensitivity to water availability (e.g., similar trends and extent of variation) suggests that no cell type is privileged when water availability is scarce. Consequently, anatomical characteristics of both conducting and non-conducting cells can be used to reconstruct climatic information. From a methodological viewpoint, this finding is interesting because the use of fibre parameters instead of



**Fig. 4** Fibre wall thickness in *E. arborea* (**a**, **b**, **e**, **f**) tree rings without (**a**, **b**) and with (**e**, **f**) IADFs at the xeric (**a**, **e**) and mesic sites (**b**, **f**), and in *A. unedo* (**c**, **d**, **g**, **h**) tree rings without (**c**, **d**) and with (**g**, **h**) IADFs at the xeric (**c**, **g**) and mesic sites (**d**, **h**). Mean values and standard errors are shown. Different letters indicate significantly different values ( $p < 0.05$ ) between different regions

vessels would help overcome technical constraints in automatic measurements of the variation of wood traits in hardwoods when vessel frequency is too low or when vessels are not uniformly distributed along the tree ring. Indeed, the distribution of fibres along tree rings from earlywood to latewood follows more ordered and regular



**Fig. 5** Vessel frequency in *E. arborea* (**a**, **b**, **e**, **f**) tree rings without (**a**, **b**) and with (**e**, **f**) IADFs at the xeric (**a**, **e**) and mesic sites (**b**, **f**), and in *A. unedo* (**c**, **d**, **g**, **h**) tree rings without (**c**, **d**) and with (**g**,

**h)** IADFs at the xeric (**c**, **g**) and mesic sites (**d**, **h**). Mean values and standard errors are shown. *Different letters* indicate significantly different values ( $p < 0.05$ ) between different regions

rows than vessels, in a structure more similar to tracheids in softwoods. This would help achieve more continuous reconstructions of the trends of cell size variation within hardwood tree rings.

**Author contribution statement** VDM, GB and PC designed the study; VDM, GB and PC carried out sampling; VDM and AB performed microscopy and digital image analyses; VDM and GA performed statistical analyses; GA and PC contributed to the analysis tools; VDM and AB wrote the main part of the manuscript; all authors contributed to manuscript revision, read and approved the submitted version.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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