

## Article

# Wood Density and Ring Width in *Quercus rotundifolia* Trees in Southern Portugal

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**Abstract:** *Quercus rotundifolia* Lam., known as holm oak or evergreen oak, occurs naturally in the western Mediterranean region, mainly as part of the agroforestry or agrosilvopastoral systems in Portugal (“montado”) and Spain (“dehesa”), and is economically important for acorn production. Less attention has been given to *Q. rotundifolia* wood, and its density variability is not known, namely related to tree growth. The wood density of 20 *Q. rotundifolia* trees was measured along the radial direction by X-ray densitometry and the factors responsible for ring width and wood density variation within and between trees were investigated at two sites located within the main species region in southern Portugal. Ring width was significantly different between sites, with an average of 1.81 mm and 1.55 mm. Wood density was very high and averaged between 888 kg/m<sup>3</sup> and 914 kg/m<sup>3</sup> but not significantly different between sites. Ring width and wood density showed a positive and significant correlation at both sites. Cambial age was the main source of variation for ring width and wood density, while between-tree effects accounted for a considerable proportion of wood density variation. The results are an important contribution for the species valorisation aiming at high-value wood products, also adding knowledge on the species growth of interest for tree selection and sustainable management.

**Keywords:** holm oak; Mediterranean oaks; wood density; ring width; variability; microdensitometry



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## 1. Introduction

*Quercus rotundifolia* Lam., known as holm oak but mostly referred to as a subspecies of *Quercus ilex* L. (*Quercus ilex* subsp. *rotundifolia*; *Q. ilex* subsp. *ballota*), is a Mediterranean evergreen oak mainly and naturally distributed in the western Mediterranean region: Portugal (ca. 349,400 ha), south Spain (ca. 2,794,450 ha) and Morocco (ca. 1,415,201 ha) [1–3]. Its economic, ecological, and social relevance is well acknowledged due to its presence in several ecosystems, and especially as part of the agrosilvopastoral system found mainly in Portugal (“montado”) and Spain (“dehesa”). In France, Italy, and Greece, *Q. rotundifolia* is replaced by *Q. ilex* (*Q. ilex* subsp. *ilex*, if considered a subspecies) [1,4]. The different foliar morphology and geographic distribution of these subspecies (or species) compared to *Q. ilex* subsp. *ilex* (or *Q. ilex* L.) are acknowledged by different authors [5–7].

Holm oak, once covering most of the Mediterranean region, is a sclerophyllous species, well adapted to both summer drought and light winter frost which are characteristic of the Mediterranean climate, and occurring from a semiarid (with markedly continental conditions) to per-humid environments [8]. However, some tree decline or dieback in montado is being reported due to climate change, land desertification, and biotic pathogens threats, with concerns related to their different impacts on this management system and species’ future distribution [9,10]. In this context, physiological and growth traits have been studied aiming at species regeneration mainly for *Q. ilex* (or *Q. ilex* subsp. *ilex* if mentioned as subspecies) (e.g., [4,11–13]). However, it is reported that *Q. rotundifolia* is

adapted to more xeric conditions when compared to *Q. ilex* [14], showing also similar or better adaptation than deciduous oak species such as *Quercus faginea* Lam. [13] and *Quercus douglasii* (Fagaceae) [15].

The main economic outcome from holm oak is related to acorn production for Iberian black pigs feeding due to its beneficial effects on the famous dry ham quality and more recently for high-value human food products due to its sweetness, nutritional, and gluten free composition as well as a source of bioactive compounds [16]. Less attention is given to holm oak wood with the exception of the traditional firewood use and charcoal production, obtained from thinnings in coppice management (e.g., France, Italy, and Spain) or pruning operations performed to increase acorn production or to provide shadow for livestock [2,17]. It is well known that holm oak wood has a high density, and it is valued for some wood applications with high wear, such as flooring. Small diameter biomass from *Q. ilex* may also be used for particleboards [18,19]. However, studies are lacking namely on wood density and its variation factors.

Wood density is considered one important property to assess the wood quality and suitability for different products due to its correlation with technological properties. However, wood density shows a large variation, being tree age- and growth-related, and differing among species and wood structure [20]. Moreover, its variation namely in high density woods [21] as well as in different bioclimatic gradients [22] such as in the Mediterranean oak woodlands is still not very well known.

Accurate analyses of wood density variation, within and between trees, should therefore be given at the growth ring level. In the ring-porous (deciduous) or semi-ring porous (evergreen) *Quercus* species, wood density shows a decrease with cambial age (i.e., ring number from the pith, or age of the tree when the ring was formed) (e.g., [23–26]). This age decrease effect is mainly explained by the cellular differences between earlywood and latewood, e.g., latewood is characterised by thick cells and fewer vessels, and therefore the earlywood and latewood within the annual ring show low and high wood density, respectively, and by the variation of latewood proportion. Considering that in *Quercus* species earlywood is generally more constant along the years, a positive correlation between ring width and wood density is expected. However, both strong and weak correlations have been found between these two variables within trees (e.g., [23,26,27]). Overall, wood density and ring width in *Quercus* studies are mostly explained by between-tree variability and cambial age (e.g., [23,28–30]). The environmental influence in ring formation related to site and silviculture accounts more for the ring width variation than for the wood density variation (e.g., [29,31]), even if these are complex effects and controversial results have been reported (e.g., [23,32]). Some *Q. ilex* studies reported that environmental effects result in multi or bimodal growth patterns, meaning that a second growth period may occur after the spring, leading to false ring occurrence, vessel disturbances, and within-ring density fluctuations (e.g., [33–35]).

The aim of this paper is to study the wood density and ring width variation of *Q. rotundifolia* trees at two sites in southern Portugal, a drought-sensitive region to climate change and land desertification. This evaluation brings new data on *Q. rotundifolia* and will contribute to the species valorisation, adding valuable information to be used in future tree selection and forest sustainable management.

## 2. Materials and Methods

### 2.1. Study Area

The study area is in southern Portugal, a provenance region representative of *Q. rotundifolia* natural range distribution, which occurs mainly at basal (<400 m) and less at submontane levels (400–700 m), corresponding to 91% of the species total country coverage. According to the most recent forest inventory [3], the *Q. rotundifolia* stands are unevenly aged, characterised by sparse forest (occupation 10%–30%) with pasture (80%), shrubland (15%), agricultural and other uses (<6%); mostly (93%) are pure stands (38 trees/ha, 3.57 m<sup>2</sup>/ha, 34.5 cm mean diameter) and the remaining comprises dominant (34 trees/ha, 3.06 m<sup>2</sup>/ha,

33.8 cm mean diameter) and dominated (19 trees/ha, 1.28 m<sup>2</sup>/ha, 29.7 cm mean diameter) mixed stands. The standing volume is 19.05 m<sup>3</sup>/ha, 16.25 m<sup>3</sup>/ha, and 6.74 m<sup>3</sup>/ha, and the annual acorn production is 374.6 kg/ha, 330.3 kg/ha, and 144.3 kg/ha in pure, dominant, and dominated mixed stands, respectively.

In terms of conservation, *Q. rotundifolia* is a protected species with 40% of total country coverage found in natural or semi-natural habitats within 92/43/CEE directives (20% in montado with evergreen *Quercus* species and 15% as *Q. ilex* and *Q. rotundifolia* forests). Regarding vitality, about 9% of the *Q. rotundifolia* stands are classified in a bad state and 2% in dieback, while defoliation and related processes affect 86% and 3% of the trees lightly or severely, respectively.

## 2.2. Sites and Sampling

Sampling was carried out at two sites: Beja, Contenda (site 1; 38°03' N, 07°06' W; 450 m altitude) and Évora, Mora (site 2; 38°56' N, 8°7' W; 130 m altitude). The stands resulted from natural regeneration, with uneven-aged trees mixed with *Quercus suber* L. dominant trees and managed as montado, including wild and domestic cattle livestock mostly at site 1.

Ten dominant or co-dominant trees were randomly selected in each site and harvested. From each tree, a disc was taken at 1.3 m of stem height (breast height, b.h.) as representative for the whole tree [20]. Tree and site characteristics are shown in Table 1. The diameter at b.h. was on average 21.5 cm and 24.7 cm, and tree height 6.1 m and 6.1 m at site 1 and site 2, respectively. As a protected species, the selection and felling were supervised by the responsible public institutes, and it was only possible to collect samples from trees showing decline or dieback.

**Table 1.** Description of sites and *Quercus rotundifolia* Lam. trees sampled in each site. Average of ten trees and standard deviation. Average annual values for the climatic data (period 1971–2000).

	Site 1	Site 2
Latitude	38°03' N	38°56' N
Longitude	07°06' W	08°07' W
Altitude (m)	450	130
Soil	Dystric leptosols	Cambisols and vertic luvisols
Annual temperature (°C)	15.9	16.0
July–August temperature (°C)	24.8	23.5
January temperature (°C)	9.4	9.1
Annual precipitation (mm)	614	555
Tree height (m)	6.1 ± 1.1	6.1 ± 1.7
Diameter (cm) *	21.5 ± 6.8	24.7 ± 6.6
Crown height (m) **	5.2 ± 1.3	4.3 ± 1.7
Radius crown (m)	2.8 ± 0.7	2.7 ± 1.0
Crown area (m) ***	25.2 ± 11.5	25.8 ± 16.8

\* Over bark diameter at 1.3 m of tree height; \*\* Crown height = Total tree height—branch-free stem height; \*\*\* Crown area = Pi (mean crown radius)<sup>2</sup>.

The climate is of Mediterranean type (Csa, according to Köppen-Geiger classification) at both sites, characterised by dry and hot summers and rainy winters (Table 1). The average annual temperature and temperatures for the hottest (July–August) and coldest (January) months are similar at both sites. The average annual precipitation is 614 mm at site 1 and 555 mm at site 2, concentrated between October and March (70%) with only 7% in the dry months (July to September) at both sites.

The soils are classified at site 1 as dystric leptosols and at site 2 as cambisols and vertic luvisols. These soils are susceptible to erosion and only suitable for marginal pastures and forests, with higher limitations at site 1. Both sites are reported as sensitive to land desertification. *Q. rotundifolia* occurs in different types of soils, with the exception of sandy soils when average annual precipitation is less than 600–550 mm, and in rocky soils [5].

Nevertheless, 53% of *Q. rotundifolia* trees are found in medium to high rocky soils and 29% in too high rocky soils [3].

### 2.3. X-Ray Microdensitometry

A radial sample with 5 mm width (tangential) and 2 mm thickness (axial) was cut from pith to bark from each disc, avoiding knots and tension wood, and then conditioned at 20 °C and 65% RH to 12% moisture content. Each sample was X-rayed perpendicularly to the transverse section. The images were scanned by microdensitometric analysis and wood density was recorded sequentially within a 100 × 455 µm (radial × tangential) frame. Detailed descriptions of the method are given in [30,36].

A mirror strip of each radial strip was taken and sanded with successively finer grades of sandpaper until optimal surface resolution allowed annual rings to be distinguished under magnification (×10). The observation and identification of growth ring boundaries were performed under a stereomicroscope (Leica MZ6) based on the following anatomical characteristics: the gradual pore size variation within the growth ring, as well as the pore absence zone at the end of the growing season [17]. The cross-examination with the X-ray radial profiles was difficult due to the existence of only small variations. Differences in earlywood and latewood density within the ring were not sufficiently large to allow a good differentiation between them.

Wood density or mean ring density (RD), minimum ring density (MND), maximum ring density (MXD), and ring width (RW) were obtained for each growth ring.

Wood ring structure terminology followed the IAWA list of microscopic features for hardwood identification [37].

### 2.4. Data Analysis

Analyses of variance were performed for wood density and ring width to assess the effects of the site (S), trees in the site (T/S), cambial age or rings (R), and their interactions (R × S), according to the model presented in Table 2. Correlation analysis was also performed between the studied variables. Pearson correlation analysis was performed between wood density components and ring width. Regression analysis was applied to wood density and ring width models as a function of cambial age. The statistical analyses were performed using the JMP Statistical Software (SAS Institute Inc., Cary, NC, USA).

**Table 2.** Model used for analysis of variance for the ring width and wood density components.

Source of Variation	Degrees of Freedom	Expected Variance	Error Term
(1) Sites (S)	$s - 1$	$\sigma^2_\epsilon + r \sigma^2_{T/S} + rt \sigma^2_S$	(2)
(2) Trees/S (T/S)	$(t - 1) s$	$\sigma^2_\epsilon + r \sigma^2_{T/S}$	(5)
(3) Rings (R)	$r - 1$	$\sigma^2_\epsilon + st \sigma^2_R$	(5)
(4) Rings × Sites (R × S)	$(r - 1) (s - 1)$	$\sigma^2_\epsilon + t \sigma^2_{RS}$	(5)
(5) Residual (R × T/S)	$(r - 1) (t - 1) s$	$\sigma^2_\epsilon$	

s = sites (2); t = trees/site (10); r = rings/level/tree (60).  $\sigma^2_S$ ,  $\sigma^2_{T/S}$ ,  $\sigma^2_R$ ,  $\sigma^2_{RS}$ ,  $\sigma^2_{RT/S}$  and  $\sigma^2_\epsilon$  are variance components due to sites, trees/sites, rings, rings × sites, and residual.

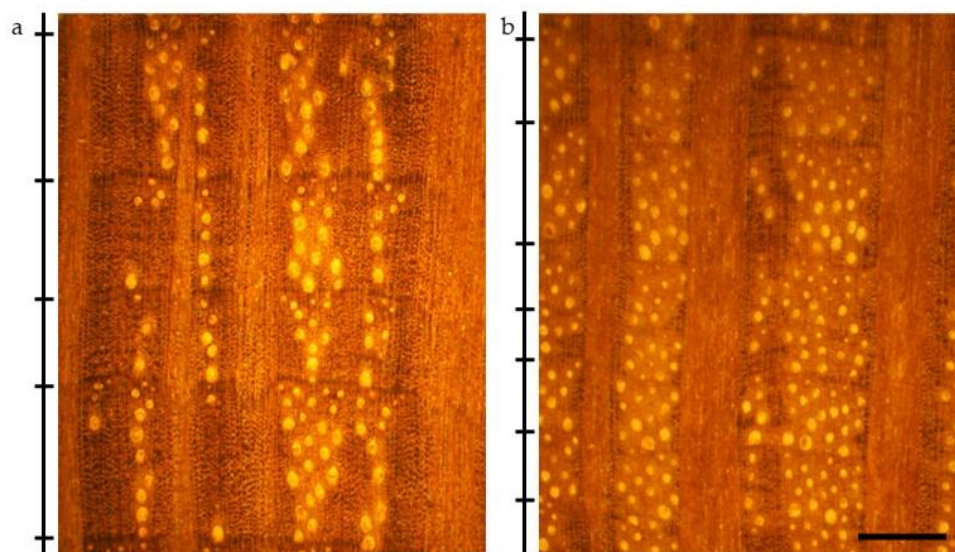
## 3. Results and Discussions

### 3.1. Within-Ring Structure

*Quercus rotundifolia* (in the literature also to referred as *Q. ilex* subsp. *rotundifolia*; *Q. ilex* subsp. *ballota*) is characterised as a diffuse or semi-ring porous species [14,17] while *Q. ilex* (hereafter referred to the studies mentioning *Q. ilex* L. subsp. *ilex* or no subspecies distinction) is described as a presenting semi-ring, diffuse or intermediate [13,38,39]. The pores in *Q. rotundifolia* wood are arranged in a diagonal and/or dendritic pattern showing a gradual decrease of size (with diameter ca. 160 to 50 µm) within the ring and with the growth ring boundaries delimited by a narrow line of radially flattened fibres between the conspicuous rays (4.3 to 12.5 mm in height and wider than 1.5 mm) [17].



Representative growth ring boundaries of the studied *Q. rotundifolia* trees are shown in Figure 1. Rings are identified macroscopically mainly by the vessel distribution pattern in ring-porous or semi-ring porous *Quercus* species (e.g., [40,41]). However, this criterion did not always allow unambiguous ring distinction in *Q. rotundifolia* due to the presence of an intermediate porosity between diffuse to semi-ring porosity within some rings (data not published). In fact, these ambiguous patterns have been discussed in relation to mesic to xeric environmental conditions [42]. For example, vessel climatic sensitivity was reported for *Q. ilex* (e.g., [43,44]), i.e., within ring variation of vessel features seemingly related to monthly precipitation and temperature [35], and relevant for false ring identification [33], as well as for several *Quercus* ring-porous species (e.g., [45,46]).



**Figure 1.** Growth ring boundaries of the studied *Quercus rotundifolia* Lam. trees shown in a transverse section: (a) distinctly marked by the occurrence of fibre tissue without pores and rings showing a gradual pore size variation; (b) less distinct and narrower rings resembling diffuse porosity due to a more gradual pore size variation. Ring boundaries are represented by ticks at the left of each image. Scale bar = 1 mm.

Therefore, other anatomical features were relevant to distinguish growth annual rings in the studied *Q. rotundifolia* trees, such as the dense zone characterised by the absence of pores [17]. These non-vessel anatomical characteristics allowed also growth ring boundaries distinctiveness in *Q. ilex* and other diffuse-porous oaks [47,48] as well as in other diffuse species such as *Acacia melanoxylon* R.B. [49], *Eucalyptus tereticornis* Smith. and *Eucalyptus propinqua* Maiden & Deane [50]. The differences regarding the annual growth structure (namely of vessel and fibre tissue distribution) were observed in some of the studied trees, suggesting a multi or bimodal growth pattern as reported and previously introduced for *Q. ilex*. However, the annual resolution (e.g., the occurrence of false rings) with preliminary cross-dating is not required to analyse age-related trends related to wood density [24].

The macroscopic anatomical features are also valuable for microscopic wood identification [51] and particularly for the wood density studies when there is no sharp difference between the earlywood and latewood density, as observed in the present case. This smooth difference is not typical in temperate species, and in *Q. rotundifolia* may be related to the effect on the wood density of other cellular tissues, namely the presence of large rays and frequent eccentric variation (data not published).

### 3.2. Ring Width and Wood Density

The average ring width and wood density components for each tree at each site are shown at Table 3.

**Table 3.** Ring width (RW), ring density (RD), minimum (MND) and maximum ring density (MXD) for the studied *Quercus rotundifolia* trees at site 1 (S1) and site 2 (S2). Mean values of ten trees and standard deviation.

Tree	Rings		RW (mm)		RD (g/cm <sup>3</sup> )		MND (g/cm <sup>3</sup> )		MXD(g/cm <sup>3</sup> )	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
#	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
1	52	60	1.74 ± 0.81	1.37 ± 1.30	0.879 ± 0.147	0.841 ± 0.158	0.799 ± 0.142	0.794 ± 0.157	0.960 ± 0.167	0.887 ± 0.170
2	45	68	1.40 ± 0.82	1.81 ± 1.20	0.878 ± 0.079	0.901 ± 0.133	0.807 ± 0.084	0.832 ± 0.155	0.959 ± 0.102	0.959 ± 0.129
3	33	73	2.89 ± 1.14	1.94 ± 1.44	0.873 ± 0.062	0.948 ± 0.185	0.797 ± 0.063	0.866 ± 0.182	0.963 ± 0.086	1.019 ± 0.198
4	35	83	1.77 ± 0.93	1.13 ± 1.14	0.983 ± 0.188	0.883 ± 0.230	0.922 ± 0.197	0.810 ± 0.217	1.046 ± 0.186	0.945 ± 0.246
5	57	58	1.81 ± 1.06	1.23 ± 1.06	0.881 ± 0.120	1.139 ± 0.183	0.814 ± 0.108	1.069 ± 0.207	0.952 ± 0.142	1.204 ± 0.167
6	31	78	2.18 ± 1.31	1.42 ± 0.86	0.944 ± 0.073	0.797 ± 0.143	0.871 ± 0.079	0.749 ± 0.145	1.034 ± 0.078	0.846 ± 0.146
7	70	84	2.05 ± 1.17	1.70 ± 1.37	0.880 ± 0.185	0.864 ± 0.193	0.777 ± 0.197	0.790 ± 0.203	0.973 ± 0.179	0.929 ± 0.194
8	46	61	1.27 ± 0.89	1.86 ± 1.49	1.069 ± 0.179	0.893 ± 0.239	0.986 ± 0.187	0.829 ± 0.237	1.143 ± 0.173	0.959 ± 0.251
9	81	59	1.57 ± 1.39	1.53 ± 1.27	0.834 ± 0.209	0.785 ± 0.159	0.769 ± 0.210	0.741 ± 0.150	0.905 ± 0.210	0.833 ± 0.178
10	61	59	1.84 ± 0.91	1.47 ± 0.96	0.997 ± 0.245	0.857 ± 0.248	0.941 ± 0.258	0.753 ± 0.239	1.055 ± 0.230	0.956 ± 0.267
Mean	51	68	1.81 ± 1.13	1.55 ± 1.24	0.914 ± 0.183	0.888 ± 0.210	0.840 ± 0.188	0.820 ± 0.209	0.991 ± 0.183	0.951 ± 0.219

On average, ring width was 1.69 mm, with trees at site 1 showing on average wider rings compared to site 2 (1.81 mm vs. 1.55 mm) (Table 3). The highest annual growth rate was 2.89 mm in a young tree at site 1, and the lowest was 1.13 mm in an old tree at site 2. The mean annual growth rate was  $1.9 \pm 0.4$  mm/yr at site 1 and  $1.5 \pm 0.3$  mm/yr at site 2.

Overall ring width in *Q. rotundifolia* is consistent with previous results obtained for *Q. ilex* in different Mediterranean regions: 0.93 mm, 1.48 mm, 1.54 mm [52], and 1.19 mm [53] in Spain, and 2.62 mm in Italy [35]. This comparison should be made with caution because the mentioned studies were mostly related to the sensitivity of *Q. ilex* radial growth to climate and, therefore, the analysed rings (29 to 65) did not correspond to the total cambial age at d.b.h., and tree age was usually unknown. In wood density studies, similar values (d.b.h. measurements) are also found in deciduous oak species such as *Q. faginea* (1.18 mm, mature over averaged 125-year-old trees) [25] and *Quercus petraea* (Matt.) Liebl. (1.65 mm, mature 61 to 224-year-old trees) [32]. Higher values are found in younger (40 year old) trees of *Quercus acutissima* (Fagaceae) (2.63 mm) and *Quercus variabilis* Bl. (2.60 mm) [54], and in mature trees of *Q. petraea* (2.34 mm) [32], growing in plantations or coppice stands, respectively, as well as in *Quercus robur* L. (2.21 mm, average 92-year-old) trees growing in unfavourable site conditions [28].

Wood density of *Q. rotundifolia* is very high, with an overall average of 0.906 g/cm<sup>3</sup> for the 20 studied trees and with site averages of 0.914 g/cm<sup>3</sup> and 0.888 g/cm<sup>3</sup> at site 1 and site 2, respectively (Table 3). The maximum ring density values were very high, especially in the trees at site 1, with an average of 0.991 g/cm<sup>3</sup>, while at site 2, the average value was 0.951 g/cm<sup>3</sup>. The minimum ring density values were also high, with an average of 0.840 g/cm<sup>3</sup> and 0.820 g/cm<sup>3</sup> at site 1 and site 2, respectively.

The few available wood density studies are mostly based on standard methods (i.e., basic density) referring high values of 0.900 g/cm<sup>3</sup> for *Q. rotundifolia* [17] and 0.750 g/cm<sup>3</sup> [52], 0.900 g/cm<sup>3</sup> [55] to 0.960 g/cm<sup>3</sup> [19] for *Q. ilex*. Other oaks with high wood density are *Q. faginea* (0.932 g/cm<sup>3</sup>) [25] and *Q. suber* (0.750 to 1.070 g/cm<sup>3</sup>) [26,56], showing ca. 40% higher density than the most valued oaks *Q. robur* and *Q. petraea* (e.g., [29,57]) as measured by X-ray densitometry. Wood density values determined by X-ray densitometry are generally higher than those obtained from standard methods [58].

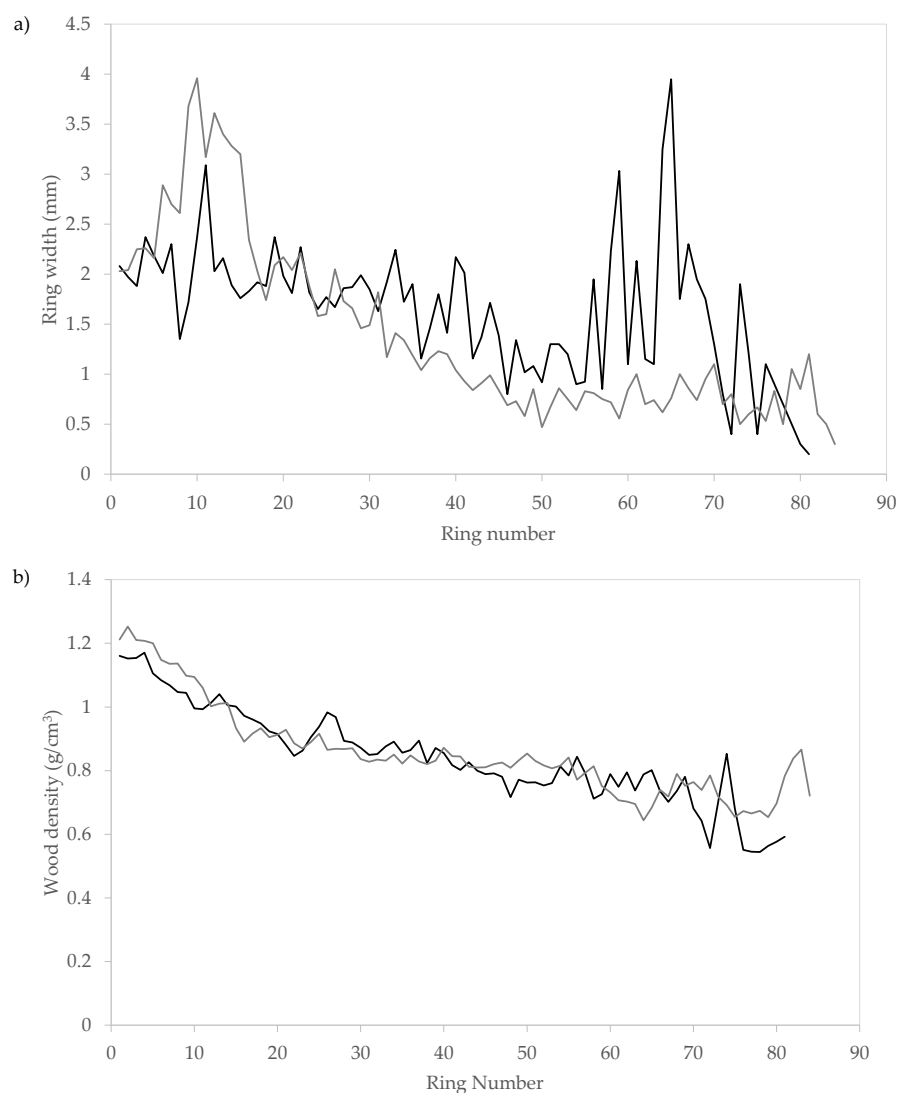
The high wood density of *Q. rotundifolia* compared to the other co-occurring evergreen *Quercus* spp. may be explained due to its multi or bimodal growth and ring porosity patterns as previously discussed for the within-ring structure. High wood density in *Q. rotundifolia* may be related to the presence of thicker fibres and large rays, as common characteristics in *Quercus* spp. and also suggested for *Q. faginea* high wood density [25].

The production of dense wood increases trunk strength which is relevant for the *Q. rotundifolia* trees in montado since these systems are open woodlands with low tree density. Physiological interpretations have highlighted the role of high wood density for protection against physical damage and potential pathogens resistance in tropical trees (e.g., [59,60]), and this could be also extrapolated to *Q. rotundifolia* in montado since its characteristics and frequent silvicultural operations may increase physical and biological risks. The high wood density results for *Q. rotundifolia* seem to be in agreement with previous

findings associating species with dense woods with an early root system development [21] as found in *Q. rotundifolia* [5] and *Q. ilex* [11,61].

### 3.3. Radial Variation of Ring Width

A radial growth decreasing trend from pith to bark was found at both sites (Figure 2a). The widest rings were found near the pith and the narrowest near the bark. The radial decrease of ring width was more pronounced in the inner 20 rings (corresponding to ca. 47% of the radius) and less afterwards for both sites, although with year-to-year fluctuations that were more prominent at site 1. Nevertheless, the values obtained for the wider rings observed at site 1 after the 32nd ring and at site 2 after the 59th ring should take into account that they result from the average of fewer trees (i.e., less than 10 trees): at site 1 the highest values (3.03 mm and 3.95 mm) obtained over 58 years of cambial age are a mean of three or fewer trees. Ring width showed differences between sites and between cambial age. In juvenile years, corresponding to the first 20 rings, the ring width was  $1.93 \pm 0.95$  mm at site 1 and  $2.69 \pm 1.03$  mm at site 2, while in the young adult phase, corresponding to the 21st–40th rings, the ring width was  $1.88 \pm 0.72$  mm at site 1 and  $1.29 \pm 0.56$  mm at site 2; in adult years after the 41st ring onwards, ring width was  $1.39 \pm 0.72$  mm at site 1 and  $0.70 \pm 0.30$  mm at site 2 (Figure 2a).



**Figure 2.** Variation of (a) ring width (RW) and (b) mean ring density (RD) at b.h. for the studied *Quercus rotundifolia* trees at site 1 (black line) and site 2 (grey line). Mean for 10 trees till the 31st ring at site 1 and till the 58th at site 2, decreasing afterwards according the cambial age of trees.

Reports of ring width or average growth rate (i.e., diameter divided by tree age) in *Q. ilex* trees showed also wider rings for the first 20 rings compared to later periods, decreasing from 3.8 mm to 2.1 mm (from the 21st to the 65th ring) [35]. This growth variation was also found in ring-porous oak species such as *Q. faginea* [25], *Quercus garryana* Douglas ex Hook. [62], *Q. acutissima*, and *Q. variabilis* [54]. A similar growth trend with irregular and higher growth during the first 15–20 years followed by a more continuous growth decrease was found in tropical species, for instance, in *Tectona grandis* L. (6.1 mm for the first 20 years and 4.0 mm for the period 30–45 years) [63]. However, comparisons should be made carefully since species growth depends on genetic, site, and climate conditions as well as on silviculture.

The radial decrease of ring width has been also explained by a different growth of earlywood and latewood, namely by a decrease in latewood proportion in some ring-porous *Quercus* species such as *Q. acutissima* and *Q. variabilis* [54], *Quercus rubra* Liebl. [64], *Q. petraea* and *Q. robur* [23,31]. In diffuse or semi-ring porous species this explanation does not apply, and other ring structural features may be important. However, some of the year-to-year fluctuations found in *Q. rotundifolia* could be explained as reported for *Q. ilex*, i.e., the narrow rings may be a result of the drought summer conditions [14,33,65,66], while the wider rings may be a result of a second or multi growth during the year [35,67,68]. Nevertheless, the ring width variation in *Q. ilex* was found as less climate-sensitive compared, for example, to vessel-related features [44].

It should be noted that the silvicultural operations carried out on the *Q. rotundifolia* montado stands, e.g., prunings and thinnings, namely related to acorn production may affect tree radial growth and ring width variation [69,70]. A positive response of stem diameter in relation to thinning was obtained in *Q. ilex* coppices, even if growth remained relatively unchanged at the stand level, and conversion to natural or high forest was suggested by the authors [12,71,72]. On the other hand, the effects on reducing water stress are highlighted, and thinnings are considered possible adaptation measures to Mediterranean summer droughts [73], which is in line with ecophysiological findings referring *Q. ilex* and *Q. rotundifolia* adjustment of water transport capacity in parallel to the foliage area [43]. In fact, crown and radial growth-related traits are also being studied in other oaks, such as in *Q. petraea*, showing a water transport improvement to the more vigorous crowns after thinning operations [57], while in diffuse-porous species, the reduction of the crown size induced less wood [64].

### 3.4. Radial Variation of Wood Density

A decrease of wood density from pith to bark was observed in the studied *Q. rotundifolia* trees at both sites (Figure 2b), with a more intense decrease in the first 20–25 years of cambial age. The wood formed during this early period is very dense ( $>1 \text{ g/cm}^3$ ), decreasing afterwards to ca. 30% and 40% (mean of seven trees) from pith to the 45th and 60th ring at site 1 and site 2, respectively. A linear decreasing tendency is observed in spite of the fluctuations observed at both sites.

This decreasing trend is described in the literature for ring-porous *Quercus* species such as *Q. faginea* [25], *Q. mongolica* [74], *Q. garryana* [62], *Q. rubra* [21], *Q. petraea*, and *Q. robur* [23,24], even if some exceptions and between tree variation are reported. Semi-ring porous species such as *Q. suber* also showed a similar pith to bark variation with the innermost rings with higher density [26]. While this pattern is found in other diffuse-porous species such as *Cercidiphyllum japonicum* Sieb. et Zucc. [74] and *Acer saccharum* Marshall [64], the opposite pattern of an increasing tendency from pith to bark is also found in other species such as *A. melanoxylon* [49], *Tilia* spp. and *Betula* spp. [64,74].

The wood density radial variation followed that of ring width (Figure 2a). While in ring-porous species ring width radial variation may be explained by the latewood proportion and with a positive correlation with RD, in diffuse to semi ring-porous woods other ring structural features may be important to explain the wood density variation. For instance, vessel characteristics have been reported to explain RD trends in ring-porous



species such as *Q. faginea* [75], *Q. robur* [76], and *Q. liaotugensis* [77], as well as in semi-ring porous species such as *Q. suber* [78]. Lower ring density was explained by a smaller proportion of dense thick-walled cells and a larger number of pores in diffuse-porous species [64]. These ring structural changes, called by some authors inter-annual density fluctuations, will affect the within-ring density variation and may occur in *Q. rotundifolia* as reported in *Q. ilex* in Spain and Italy as growth and vessel climate responses, related mainly to Mediterranean summer drought conditions [35,44,67,68].

Other physiological interpretations related to tree ageing are involved since the lower density values at older cambial ages are theoretically expected due to competition for resources over the years. Besides, the higher density values in the inner part of the *Q. rotundifolia* stem cross-section (above 1 g/cm<sup>3</sup> in the first 15 rings) in opposition to the low values (ca. 0.600 g/cm<sup>3</sup>) in the outermost rings of the sapwood are also related to the formation of heartwood and the accumulation of extractives (e.g., [20]). This was also found for the radial density variation between the heartwood and sapwood in the deciduous diffuse-porous *Nothofagus pumilio* (Poepp. & Endl.) Krasser [79].

### 3.5. Wood Density and Ring Width Relationships

The correlation between RW and RD was positive and significant ( $p = 0.01$ ) at both sites (Table 4). These values agree with the previous observations of RW and RD radial variations observed in Figure 2. Faster growth rates resulted in higher RD at site 2 ( $r = 0.766$ ) and site 1 ( $r = 0.516$ ).

**Table 4.** Correlation matrix (Pearson bivariate) for ring width and wood density components at site 1 (lower triangle,  $n = 81$ ) and site 2 (upper triangle,  $n = 84$ ). RD, average ring density; MND, minimum ring density; MXD, maximum ring density; RW, ring width.

		Site 2			
		RW	RD	MND	MXD
Site 1	RW	1	0.766	0.729	0.823
	RD	0.516	1	0.961	0.989
	MND	−0.909	−0.583	1	0.945
	MXD	−0.916	−0.495	0.936	1

All correlations are significant at the 0.01 level.

Positive and strong correlations were found at site 2 between RW and both MND and MXD values, with the wider rings showing the highest MND values as well as the highest MXD values. At site 1, an opposite correlation was found between RW and both MND and MXD, meaning that narrow rings showed the highest MND and MXD values.

In *Q. ilex* a weak negative correlation ( $r = -0.45$ ,  $p = 0.10$ ) between tree-ring width and RD was reported [52]. Similar variation or little influence on basic density was found in *N. pumilio* [79], *Tilia japonica* (Miq.) Simonk., *Eucalyptus* spp. [20], *Betula* spp. and *Populus* spp. [74,80], in opposition to the general ring-porous species wood density dependence on ring width (e.g., [23,27,81]). However, contrasting results are also reported, e.g., within-tree weak correlations for *Q. faginea* old trees ( $r = 0.432$ ,  $p = 0.05$ ) [25], and at the stand level no correlation for *Q. petraea* ( $r = 0.04$ ) [29]. The lack of significant correlations in many of the species is explained by the strong genetic control of wood density, throughout the formation of the latewood and cell structural characteristics [20].

### 3.6. Ring Width and Wood Density Variability Sources

The variation of RW was mainly explained by the cambial age (R), accounting for 14.4% of the total variation, by the interaction between sites and cambial age ( $S \times R$ ), accounting for 10% of the total variation as well as by the trees within each site (T/S) that accounted for 7.3% of the total variation. The site effect (S) only explained 0.4% of the total variation, even if statistically significant (Table 5). Identical results were obtained for the wood of other *Quercus* species, such as of *Q. robur* [28], for *Q. petraea* [24,81], and for *Q. suber* [26]. The edaphoclimatic characteristics are similar at both sites (Table 1), and

even if high rocky soils are found at site 1 this might not be a constraint to radial growth and corroborates the developed root system known for the species [5]. However, in *Q. ilex* a drought-induced reduced growth was associated with a lack of deep roots development in thin soils [14].

**Table 5.** Summary of the analysis of variance for ring width (RW), mean ring density (RD), minimum ring density (MND) and maximum ring density (MXD), showing their significance (Sig) and percentage of total variation due to each source of variation (%).

Source of Variation	D.f.	RW		RD		MND		MXD	
		Sig.	%	Sig.	%	Sig.	%	Sig.	%
S	1	**	0.4	ns	0.0	ns	0.0	ns	0.0
T/S	18	***	7.3	***	18.5	***	17.0	***	18.2
R	83	***	14.4	***	29.8	***	27.4	***	30.0
S × R	80	***	10.0	ns	0.0	ns	0.0	ns	0.0
R × T/S	1011		67.9		51.7		55.6		51.9

Site (S), Tree/Site (T/S), Ring (R), Ring × Site (R × S) and Residual (R × T/S). \*\*\* ( $p < 0.001$ ); \*\* ( $p < 0.01$ ); ns ( $p > 0.05$ ).

Concerning the wood density components, cambial age (R), which is an important source of environmental and physiological information, accounted for 29.8%, 27.4%, and 30.0% of the total variation for the mean, minimum and maximum wood densities. This cambial age (R) effect is independent of sites since the interaction between sites and rings (S × R) has a null contribution of the mean, minimum, and maximum ring density variation. The cambial age was also a main cause (ca. 30%) of wood density variation in mature trees of *Q. faginea* [25] even if its contribution was small (2.3%) when comparing the two sites [30]. These results agree with the observed linear decrease of wood density as a function of cambial age, which is related to the annual ring structure, as previously suggested.

The between tree variation (T/S) is the second highly significant source of wood density variation, accounting for 18.5%, 17.0%, and 18.2% of the total variation of the mean, minimum and maximum wood density, respectively. Results for *Q. petraea* and *Q. robur* refer to the effect of trees as one of the main causes of variation in density components [28,81,82]. More recently, a study for the same species [32] estimated that the variation between trees ranged from 29% to 31% of the total variation. For *Q. faginea*, values of 9% variation in the mean wood density due to trees were reported [25], i.e., values in the same order of magnitude as those mentioned here for *Q. rotundifolia*.

Site (S) was not a significant source and did not contribute to the total variation of RD components. Other studies with *Q. suber* [26] and *Q. petraea* [32,81] also concluded that the differences between sites were not statistically relevant for the wood density variation. Similar results were found in other semi-ring porous species such as *Juglans nigra*, while contrasting results were found for diffuse-porous *Populus* spp. [64]. A recent study on *Q. rotundifolia* barks structure and chemical composition revealed high phenolic content and excellent antioxidant characteristics of polar extracts similar at both sites [83]. Regarding wood quality, two regional types of *Q. rotundifolia* quality were assigned in Portugal [12], one for more qualified uses (site 1) and the other for current uses (site 2), which could not be confirmed with these results.

Overall, the residual effect (R × T/S) was responsible for most of the total variation of density components explaining about 50% of the variation and 68% of the variation in ring width. High residual effects were also found in *Q. faginea* [30] and *Q. petraea* [32]. This is mainly explained by the non-accounted factors that would be related to e.g., wood extractives content, crown distance, and cross-section directions, the latter two frequently with high effects.

#### 4. Conclusions

The wood of *Quercus rotundifolia* presents narrow rings, with very high mean, minimum, and maximum wood densities.

The variation of ring width and wood density was mainly explained by the cambial age, showing a decreasing trend from pith to bark. The between tree effect accounted more for the wood density than the ring width variation. The site effect was small for the radial growth and null for the wood density variation of the *Q. rotundifolia* studied trees.

The correlation between ring width and wood density was positive and significant at both sites, i.e., the higher radial growth (ring width) is made at the expense of greater accumulation of cell wall material, i.e., of an increase in carbon sequestration. This aspect is important from the point of view of the species conservation as part of the montado system under the future climate change scenario.

Certainly, further studies are necessary to elucidate wood density variation in *Q. rotundifolia* namely related to heartwood formation, within ring vessel size variation, and other anatomical features.

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