Drought Treated Seedlings of *Quercus petraea* (Matt.) Liebl., *Q. robur* L. and Their Morphological Intermediates Show Differential Radial Growth and Wood Anatomical Traits

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Abstract: Background and Objectives: Studying responses in woody plants upon water limitation is gaining importance due to the predicted increase in frequency and intensity of droughts in Europe. We studied the variation in radial growth and in wood anatomical traits caused by water limited growth conditions in offspring from *Quercus petraea* (Matt.) Liebl., *Q. robur* L. and their morphological intermediates grown in the same environment. Materials and Methods: Cross sections were prepared from the stems of 210 three-year-old potted seedlings, comprising control plants and seedlings that experienced from late spring until early autumn of the first growing season two sequential periods of water with-holding each followed by plentiful re-watering. Pith radius, ring width of the three growing seasons and latewood vessel diameter in second and third growing season were measured. Presence of intra-annual density fluctuations, dendritic patterns of latewood vessels and the level of ring closure of earlywood vessels were observed. The traits were modelled to examine the explanatory power of the taxon of the mother tree and the drought treatment. Results: Most of the traits displayed significant differences between offspring from *Q. petraea* and *Q. robur* and offspring from the morphological intermediates behaved inconsistent among the traits. Most of the traits were significantly affected by the drought stress in the first growing season. Apart from radial growth, also latewood vessel size was reduced in the two growing seasons following the year in which drought was imposed on the seedlings, suggesting an adaptation to improve the tolerance to drought stress. We also found an indication for a compensation growth mechanism, counteracting the lost growing time during the drought stress, as the level of ring closure of the earlywood vessels in the year following the drought treatment was further advanced in the treated seedlings, an effect that disappeared in the subsequent year. Conclusion: Oaks exposed to drought adapt their growth and xylem structure to improve drought resistance. While youth growth of *Q. robur* is more competition-oriented, with a faster juvenile growth, *Q. petraea* seems to invest more in a precautious growth, being more prepared for stressful conditions. It is therefore possible that *Q. robur* seedlings may suffer more from intensified droughts than *Q. petraea* seedlings.

1. Introduction

The success of seedling growth is the key driver of natural regeneration in forest ecosystems [1]. The frequency and intensity of extreme weather events such as heat waves, drought periods, and late spring frosts is expected to increase, leading to extra risks in forestry [2]. To broaden our understanding of the effects of droughts on forest dynamics, it is therefore important to investigate the responses of seedlings upon water limiting conditions.

Woody plants collect and transport water to the leaves, where the photosynthesis takes place [3]. Functional vessels in the secondary xylem of broad-leaved tree species support this water need. A larger diameter of a functional vessel contributes to a higher hydraulic conductivity [4]. For broad leaved tree species growing in temperate forests, the earlywood vessels are formed first at the branches and upper stem before the buds burst, and they become functional when the new leaves reach a mature stage [5,6]. In *Q. petraea* and *Q. robur*, the earlywood vessel area increases with the age and size of the tree, supporting the higher water demand in the crown [7]. However, ring-porous tree species such as *Quercus* spp. are operating within narrow hydraulic safety margins [8]. The chance for vessel dysfunction during water limiting conditions enlarges with the diameter of the vessels [9], and higher hydraulic conductivity raises the risk of mortality during eventual severe drought events [10]. The large vessels of ring-porous trees account for more than 90% of the water conductivity [11] and remain functional during one growing season [12].

The genus *Quercus* is characterized by a high species diversity, ecological dominance and economic value at the Northern hemisphere [13]. Oaks are present at a broad variety of habitats, such as temperate deciduous forests, temperate and subtropical evergreen forests, subtropical and tropical savannah and subtropical woodlands [14,15]. *Q. robur* (pedunculate oak) and *Q. petraea* (sessile oak) are the two most common deciduous forest oak species in western and central Europe (Brasier et al. 1996). Many natural oak populations consist of a variable proportion of the interfertile sessile and pedunculate oak, including spontaneous natural hybrids, which contributes to a strong variability within the genus [16,17]. Although sympatric, both species deviate to some extent in their ecological niches. *Q. robur* is more a pioneer species with better seed dispersal capacities compared with *Q. petraea* [18]. On the other hand, *Q. petraea* can more easily colonize areas that are already occupied by *Q. robur*. Especially sessile oak (*Q. petraea*) is characterized by a higher adaptation capacity for dry soil conditions [19–21]. Therefore, sessile oak plays a more dominant role in dryer and warmer forests in Europe, whereas pedunculate oak (*Q. robur*) grows better in lowlands on nutrient rich, humid soils [22].

The structure of the secondary xylem varies among the oak species. An overview of the wood anatomical differentiation between adult *Q. robur* and *Q. petraea* trees was elaborated by Feuillat et al. [23]. Differences were found mainly in number and area of earlywood vessel rows (lower in *Q. petraea*), the dendritic patterns of latewood vessels (more narrow in *Q. petraea*), the transition between earlywood and latewood (sharper in *Q. petraea*) and the fiber zone size and area (larger in *Q. petraea*) [7,23]. Few studies dealt with wood anatomical responses induced by changes in the hydrological site conditions of oak seedlings [24]. The response of earlywood vessels of *Quercus robur* to a changing environment has been studied in a dendrochronological context [25]. Drought-exposed sapling of *Q. petraea*, *Q. robur* and *Q. pubescens* were found to adjust their xylem structure to water limited conditions. Water stress reduced the vessel area and increased the abundance of parenchyma cells in the secondary xylem [24], and net photosynthesis lessened more in *Q. robur* under drought conditions compared with the other two studied species [24]. However, *Q. robur* has also been shown to recover quicker after stress than *Q. petreae* [26]. Sessile oak is known to be more shade tolerant [27].

Several studies have already monitored effects of water limitation in oak seedlings [28–31]. Here, we aim to improve our knowledge on the radial growth and the wood anatomical variation in the stems of seedlings from the interfertile *Q. petraea* and *Q. robur* and from their morphological

intermediates, after water limiting treatments. The novelty of this study resides in the comparison between offspring from *Q. petraea*, *Q. robur* and their morphological intermediates all originating from the same natural population, excluding putative provenance effects. We treated part of the seedlings with drought stress in the first growing season and monitored their growth in the following two years. The design of the experiment, growth and phenological responses has been described [32]. In short, germinated seedlings in pot were subjected to two successive water with-holding periods, one in spring and one in summer, each followed by a plentiful re-watering. The experiment was performed in frost-free greenhouse conditions. After the drought treatment, control plants and treated seedlings were intermingled and normally watered. Survival and phenological responses were taxon-independent, whereas growth responses were taxon-dependent. In the part of the experiment described here, we questioned whether the seedlings displayed variability in the pith diameter, the radial growth and in several wood anatomical traits of the secondary xylem. We examined whether the variability in these traits was dependent on the taxon of the mother tree and on the water limiting conditions experienced in the first growing season. We specifically hypothesized that the xylem structure is adapted in the years following the drought treatment as an improvement of the drought tolerance, putatively influencing the dendritic patterns and the level of ring closure of earlywood vessels. We hypothesized that drought stress would result in smaller latewood vessel diameters, reducing the chance on drought induced cavitation. In addition, we hypothesized that the species-specific differences in the studied traits can be ascribed to the known differences in drought tolerance between *Q. robur* and *Q. petraea* and that offspring from the intermediate forms shows intermediary traits.

2. Materials and Methods

2.1. Source Material and Experimental Design

This study was part of an experiment that examined the responses of seedlings from *Q. petraea*, *Q. robur* and their morphological intermediates upon water limitation. The sourcing of the acorns and the experimental setup are described in [32]. In brief, acorns were collected at the end of September 2013 from a natural oak population in the northern part of Belgium (51°0′57.8556″N 5°31′57.0384″E). A cpDNA analysis reported a uniform haplotype in this population, which was in line with the reconstructed postglacial migration routes [33,34]. The acorns were collected directly from the mother trees, when the seeds were ripened but still hanging on the branches. The taxa of the mother trees were identified in the field, as described in [35]. *Q. robur* showed a leaf stalk smaller than 1 cm and an infructescence stalk larger than 2 cm (abbreviated as r). *Q. petraea* was characterized by a leaf stalk larger than 1 cm and an infructescence stalk smaller than 2 cm (abbreviated as p). Any other combination of these two measures was considered to belong to the mother trees with intermediate morphological traits, further called intermediates. Two types of intermediates were present: the very rare trees with long leaf stalks (>1 cm) and long infructescence stalks (>2 cm), further called the long stalked intermediates (abbreviated as pr) and the more common trees with short leaf stalks (<1 cm) and short infructescence stalks (<2 cm), further called the short-stalked intermediates (abbreviated as rp). Seedlings germinated in 2014. The number of seedlings described in this paper and the corresponding taxa of the mother trees are presented in Table 1.

Table 1. Number of oak seedlings (n) according to the drought treatment in 2014 and the taxon of the mother tree.

Treatment	Taxon Mother Tree*	*n*
control	p	42
	pr	10
	r	35
	rp	27
drought	p	30
	pr	10
	r	27
	rp	29

* p: *Quercus petraea*, pr: long stalked intermediate, r: *Q. robur*, rp: short stalked intermediate.

In April 2014 the germinated seeds were transferred to one-liter pots (12 × 11 × 11 cm) using standard nursery soil (organic matter 20%, pH 5.0–6.5, Electrical Conductivity (EC): 450 μS/cm, dry matter 25%, fertilization: 1.5 kg/m^3 powdered compound fertilizer NPK 12 + 14 + 24).

The whole experiment was performed in a frost-free greenhouse. The seedlings were divided in two groups. The control group was watered manually by experienced greenhouse technicians during the whole experiment. The seedlings from the water limitation group were exposed to two succeeding drought periods in the first growing season, (starting at DOY (day of the year) 134 and DOY 217, and ending at DOY 183 and DOY 291, respectively), in which plants did not receive any water. The first drought period during early summer lasted until a few plants showed first visual symptoms of wilting and/or curling of the leaves. This period was followed by plentiful re-watering. The seedlings were subsequently exposed to a second and longer lasting drought period during late summer until plants started dying off. This period was again ended by plentiful re-watering of the plants.

After the drought treatments, all seedlings were kept in optimal growing conditions. The pots of the control and the treated group of plants were individually mingled after the second drought period. At the end of 2015, the seedlings were transferred to a two-liter pot, using standard nursery potting soil. The potted plants were then transferred to an open field with automatic irrigation. At the end of 2016, all the stems were cut at 2 cm above soil level.

2.2. Plant Measurements and Preparation of the Microsectioning

The pots were weighted on a regular basis during the drought periods in the first growing season. As described in Vander Mijnsbrugge et al. [32], we estimated the soil water reserve with an approximation which was related to the relative extractable water (REW). To approach the water limitation experienced by the seedlings during the second drought period, we used an adjusted REW. For a given day (j):

REWj = (weight DOY j – mean weight DOY 290)/(mean weight DOY218 – mean weight DOY 290).

Mean weight DOY (day of the year) 290 was the mean weight on DOY 290 of the pots with plants that had died off at the end of the second drought period and did not recover anymore afterwards, thus approximating the permanent wilting point. Mean weight DOY 218 was the mean weight of all the pots on DOY 218 and approximated field capacity. The calculation did not take into account possible weight gain by plant growth. The calculation of the adjusted REW was easily applicable to a larger amount of potted plants.

At the end of each growing season, from 2014 until 2016, the height of the plants was measured (Figure S1). The relative growth rate for 2015 and 2016 was calculated by dividing the height increment in the growing season (increment for 2015 = height at the end of 2015 – height at the end of 2014; increment for 2016 = height at the end of 2016 – height at the end of 2015) by the initial height of the seedlings at the beginning of the respective growing season. After harvesting the stems at the end of 2016, microsections (thickness of 16 μm) were taken at 5 cm stem height above the soil level using a sliding microtome (Reichert, Germany). Wood samples were softened in a Copenhagen Mix (99% ethanol : DIwater : glycerol (70:28:2)). The cross-sections were kept in distillate water and soaked in a staining solution (mix of safranin and alcian blue (1:2)) for 5 minutes, followed by dehydration with ethanol (50%, 75%, 96% and 100%). The colored cross-sections were fixed with a coverslip on a slide with Euparal glue. Images were taken using a digital video camera that was connected to a transmitted light microscope (Olympus CX31 transmitted light microscope equipped with SC30 camera and analysis getIT software) using a 4X objective.

2.3. Wood Anatomical Traits

The radial growth of the stems was measured on the cross sections in three succeeding years from 2014 until 2016, with 2 measures for the pith width in two perpendicular directions and 4 measures for each growth ring in the 4 cardinal directions using imagJ software [36]. The pith radius was calculated by averaging the 2 diameter measurements and dividing the average by 2. The year

ring widths were calculated by taking the mean of the four measures per ring. The diameter of 25 random vessels in the latewood of the separate rings were measured using imageJ software [36]. This number of vessels resulted in stable values for mean and variance that were characteristic for the rings.

In addition, other wood anatomical traits were observed. As the cross-sections involved three growing seasons, the presence of an extra growth zone in one of the three years was documented as a separate trait. The extra growth zone, also called intra-annual density fluctuation (IADF), concerned well visible rows of radially flattened fiber cells, which are normally only formed at the end of the growing season (Figure 1a). Next, the presence/absence of grouped latewood vessels in a dendritic pattern [37], which is in the form of flame-like structures, in the second and third growing season were observed. The distribution pattern of the latewood vessels is determined from cross sections at low microscopic magnification in each individual ring. The large earlywood vessels form a clear ring in the typical ring-porous wood of many of the *Quercus* species [37]. This ring is not complete in the juvenile wood, being interrupted by other tissues. We evaluated the level of vessel ring closure at the onset of the earlywood in the second and third growth ring. The percentage of achieved ring closure was assessed in classes of 10%, which resulted in a variable consisting of a lowest level of 10% and a highest level of 90% (total absence, 0%, and total ring formation, 100%, were absent in the cross-sections).
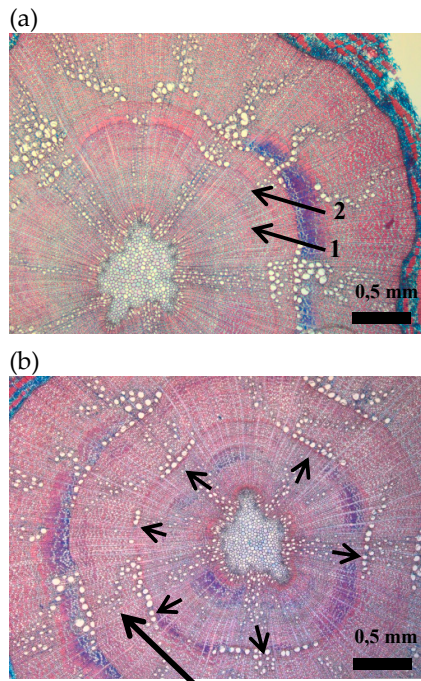
(a)

(b)

Figure 1. Cross sections of oak seedlings: (**a**) intra-annual density fluctuation (arrow n°1) in the secondary xylem of the first growing season. Arrow n° 2 indicates the year ring border between the first and the second growing season. (**b**) Cross section indicating a dendritic pattern of latewood vessels in the second growing season (long arrow) and a 50% of vessel ring closure at the onset of the earlywood in the second growing season (short arrows).

2.4. Data Analysis

The open source software R 3.1.2 (R development Core Team, Vienna, AT.) was used for all statistical analyses.

The four size traits (pith radius and three ring widths) were examined as four response variables using linear models. The 25 latewood vessel diameter measurements in each of the three-year rings of each seedling were modelled with linear mixed models. Three response variables were analyzed with logistic regression: the presence/absence of an intra-annual density fluctuation in the first year

and the presence/absence of grouped latewood vessels in the form of flame-like structures (dendritic patterns) in the second and the third-year ring. Finally, two response variables were studied with cumulative logistic regression: the level of vessel ring closure at the onset of the earlywood in the second and the third growing year.

The three year ring width variables (*R*) were modelled using as explanatory variables the taxon of the mother tree (*T*, a categorical variable with the levels p: *Q. petraea*, pr: long stalked intermediate, r: *Q. robur*, rp: short stalked intermediate), the adjusted relative extractable water in the second water limitation period in the summer of 2014 (*W*) as a proxy for the drought experienced by the seedlings, and the seedling height (first growing season in 2014) or the relative growth rate in height (second and third growing seasons) (*H* and *RGR*, respectively). Interaction terms between the three explanatory variables were added in the models. Here, we show the full model for the first growing season:

$$R = \alpha + \beta_T.T + \beta_W.W + \beta_H.H + \beta_{TW}.T.W + \beta_{TH}.T.H + \beta_{WH}.W.H \tag{1}$$

with α as the estimated intercept and the β's as the estimated parameters of the fitted model. For the second and the third growing season, height (*H*) was replaced by the respective relative growth rates (*RGR*).

In the model of the response variable pith radius, the relative weight loss of the pots (*W*) was omitted as the pith was formed before the onset of the water limitation periods in the first growing year, resulting in the following full model:

$$R = \alpha + \beta_T.T + \beta_H.H + \beta_{TH}.T.H \tag{2}$$

All linear models were reduced up to only significant explanatory variables using the command drop1.

The diameter of the latewood vessels (*D*) in the three separate growth rings of each seedling were modelled using linear mixed models in the package lme4 [38]. The same three explanatory variables as for the radial growth modelling were examined in the fixed part of the models. Because of the repeated vessel measurements for one growth ring in one plant (25 vessels), a unique plant identity code was added in the random part of the model. Here we show the full model

$$D = \alpha + \beta_T.T + \beta_W.W + \beta_H.H + \beta_{TW}.T.W + \beta_{TH}.T.H + \beta_{WH}.W.H + plantID \text{ (random)} \tag{3}$$

with α as the estimated intercept and the β's as the estimated parameters of the fitted model. For the second and the third growing season, height (*H*) was replaced by the respective relative growth rates (*RGR*). All linear models were reduced up to only significant explanatory variables using the command drop1.

The probability of having formed an extra ring in the first growing season (p) and the probability of having formed dendritic vessel patterns (flame-like groupings) in the latewood of the second and third growing seasons (p) were modelled applying logistic regression. The same explanatory variables were examined as for the linear models of the measured traits. Here, the full model is shown:

$$\log(p/1 - p) = \alpha + \beta_T.T + \beta_W.W + \beta_H.H + \beta_{TW}.T.W + \beta_{TH}.T.H + \beta_{WH}.W.H \tag{4}$$

with α as the estimated intercept and the β's as the estimated parameters of the fitted model. For the second and the third growing season, height (*H*) was replaced by the respective relative growth rates (*RGR*). Models were reduced up to only significant explanatory variables using the command drop1.

The percentage of achieved vessel ring closure at the onset of the earlywood of the second and third growing season was modelled using cumulative logistic regression [39]. Cumulative logistic regression models the probability of having reached maximally a certain level of the ordinal response variable. The percentage of vessel ring closure at the onset of the earlywood was assessed in classes of 10%. This ordinal response variable was ordered in decreasing order from 90% up to 10%. In this way, the probability was modelled to have reached maximally for instance 50% of ring closure, which

included all levels of ring closure of 50% and higher (90%, 80%, 70%, 60% and 50%). This can be interpreted as having formed at least 50% of a vessel ring closure. The full model is shown:

$$\log(p/1 - p) = \alpha_{tr} - \beta_T.T - \beta_W.W - \beta_{RGR}.RGR - \beta_{TW}.T.W - \beta_{TRGR}.T.RGR - \beta_{WRGR}.W.RGR \tag{5}$$

α_{tr} is the threshold value indicating an intercept for each transition from one level of the ordinal response variable to the next. Models were reduced up to only significant explanatory variables using the command drop1.

3. Results

3.1. Pith Radius

Modelling the pith radius revealed that this trait was dependent on the interaction between the height growth of the seedlings in the first growing season and the taxon of the mother trees (Figure 2). When considering seedlings with comparable heights, the pith radius differed among the sampled taxa of the mother trees. Whereas small seedlings from *Q. petraea* (<9 cm height) displayed a smaller pith radius compared with seedlings from *Q. robur* of the same height, the larger seedlings (>9 cm height) had developed a larger sized pith compared to seedlings from *Q. robur* of the same height (significant interaction term between height and taxon of the mother tree for *Q. robur* with p-value = 0.004 in Table 2). Seedlings from the short-stalked intermediate formed a smaller pith radius with increasing seedling height (significant interaction term between height and taxon of the mother tree for short stalked intermediate with *p*-value < 0.001).
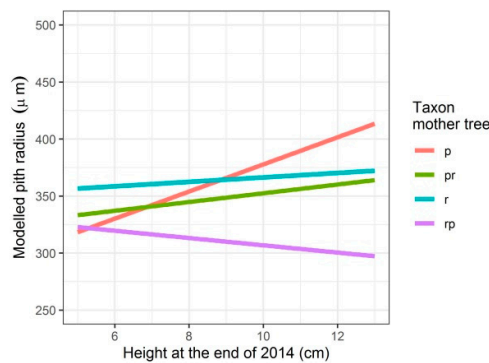

Figure 2. Modelled pith radius depending on the height the seedlings attained in the first growing season and on the taxon of the mother tree. p: *Q. petraea*, pr: long stalked intermediate, r: *Q. robur*, rp: short-stalked intermediate.

Table 2. Model statistics for the response variables pith radius and ring widths in 2014, 2015 and 2016. The standard level of the categorical variable taxon of the mother tree (*T*) was *Q. petraea*, to which the other taxa were compared with. T_{pr}: long stalked intermediate form, T_r: *Q. robur*, T_{rp}: short-stalked intermediate form. H_{2014}: height at the end of the first growing season. RGR_{2015} and RGR_{2016}: relative growth rates in height for 2015 and 2016, respectively. *W*: adjusted relative extractable water in 2014.

Pith	Estimate	Std. Error	T-Value	P-Value	Ring 2014	Estimate	Std. Error	T-Value	P-Value
(Intercept)	258.8	26.5	9.77	<0.001***	(Intercept)	290.3	68.0	4.26	<0.001***
T_{pr}	55.2	57.1	0.97	0.335	T_{pr}	−28.6	146.7	−0.20	0.846
T_r	87.9	32.5	2.70	0.007**	T_r	298.2	83.6	3.57	<0.001***
T_{rp}	79.9	36.8	2.17	0.031*	T_{rp}	191.4	94.4	2.03	0.044*
H_{2014}	11.9	3.1	3.78	<0.001***	H_{2014}	48.5	8.1	6.02	<0.001***
$T_{pr} : H_{2014}$	−8.1	6.1	−1.32	0.190	$T_{pr} : H_{2014}$	−2.7	15.7	−0.17	0.862
$T_r : H_{2014}$	−9.9	3.4	−2.96	0.004**	$T_r : H_{2014}$	−25.1	8.6	−2.91	0.004**
$T_{rp} : H_{2014}$	−15.1	4.3	−3.51	<0.001***	$T_{rp} : H_{2014}$	−12.2	11.0	−1.11	0.269
Ring 2015	Estimate	Std. Error	T-Value	P-Value	**Ring 2016**	Estimate	Std. Error	T-Value	P-Value
(Intercept)	197.1	21.9	9.02	<0.001***	(Intercept)	246.2	72.2	3.41	<0.001***

W	93.7	26.3	3.56	<0.001***	W	194.3	97.4	2.00	0.048*
T_{pr}	108.7	37.5	2.90	0.004**	T_{pr}	324.3	162.5	2.00	0.047*
T_r	35.8	25.6	1.40	0.163	T_r	586.3	119.6	4.90	<0.001***
T_{rp}	34.7	26.6	1.31	0.193	T_{rp}	−38.1	115.6	−0.33	0.742
RGR_{2015}	62.0	8.9	6.98	<0.001***	RGR_{2016}	127.5	29.7	4.30	<0.001***
					$W : T_{pr}$	203.6	198.9	1.02	0.307
					$W : T_r$	−85.7	134.1	−0.64	0.524
					$W : T_{rp}$	401.6	146.1	2.75	0.007**
					$T_{pr} : RGR_{2016}$	43.1	76.5	0.56	0.574
					$T_r : RGR_{2016}$	−112.9	44.1	−2.56	0.011*
					$T_{rp} : RGR_{2016}$	28.5	44.9	0.63	0.527

Significance codes: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$.
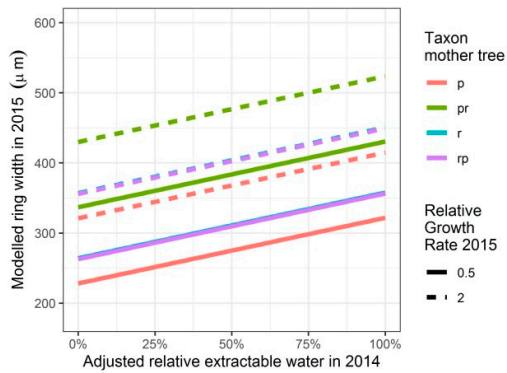
3.2. Radial Growth of the Secondary Xylem in the First Three Growing Seasons

Modelling the radial growth of the secondary xylem in the first growing season showed, quite evidently, an enlargement of the ring width with increasing height of the seedlings (Table 3, Figure 3a). For a given plant height lower than approximately 12 cm (most of the seedlings from *Q. petraea* were smaller than 12 cm, Figure S1) seedlings from *Q. petraea* tended to a smaller xylem radius in the first growing year compared to seedlings from *Q. robur* (Figure 3a).
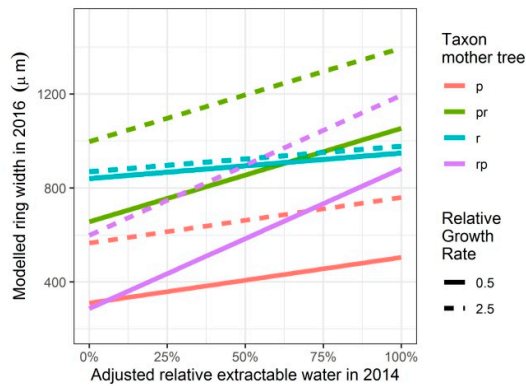
(a)
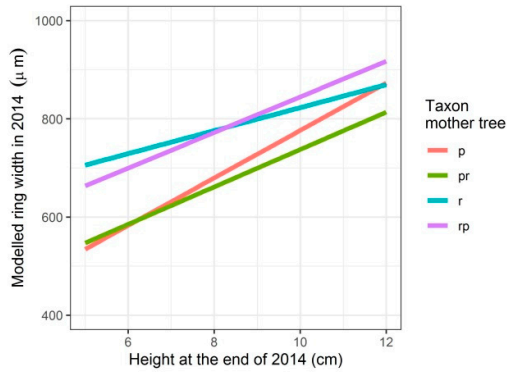
(b)

(c)

Figure 3. Modelled ring widths in the first three growing seasons (2014, 2015 and 2016), depending on the height of the seedlings in 2014 or on the relative growth rate in height (2015 and 2016), on the taxon of the mother trees and, only for 2015 and 2016, on the adjusted relative extractable water in 2014. p: *Q. petraea*, pr: long stalked intermediate, r: *Q. robur*, rp: short-stalked intermediate: (**a**) first growing season 2014; (**b**) second growing season 2015; (**c**) third growing season 2016.

Modelling the radial growth of the secondary xylem in the second year showed that the drought treatment in 2014, and the relative growth rate in height in 2015 mainly explained the ring width (Table 2), with control plants and higher plants, respectively, having quite evidently larger ring widths. Only seedlings from the long-stalked intermediate had larger ring widths compared with the seedlings from *Q. petraea* (T_{pr} with p-value of 0.004 in Table 2).

In the third growing season, the radial growth became more variable (Figure 3c). The relative growth rate in height in 2016 mainly explained the ring width (p-value < 0.001 in Table 2). Still, the effect of the drought treatment in 2014 was detectable in the radial growth of 2016 (p–value = 0.048) for *W* in Table 2). In addition, seedlings from the short stalked intermediate showed a stronger decrease in ring width when having experienced a more severe drought stress (a stronger sloped line in Figure 3c) in comparison with seedlings from *Q. petraea* (significant interaction term between *W* and T_{rp} with p-value = 0.007 in Table 2). Seedlings from *Q. robur* developed significantly larger ring widths than seedlings from *Q. petraea* (p-value < 0.001 for T_r in Table 2). This difference attenuated in seedlings with a higher relative growth rate (significant interaction term between T_r and RGR_{2016} with p-value = 0.011). Additionally, the ring width in seedlings from *Q. robur* differed from seedlings from *Q. petraea* depending on the relative growth rate in height (significant interaction term between T_r and the relative height growth with p-value = 0.011 in Table 2). For a given adjusted relative extractable water value in Figure 3c, the distance between the full and dashed lines for seedlings from *Q. robur* is smaller than the distances between these two lines for the three other taxa, suggesting that the ring width in seedlings from *Q. robur* is less influenced by the relative growth rate in height in comparison with *Q. petraea*.

3.3. Latewood Vessel Diameter

The diameter of the latewood vessels in the three growing seasons was modelled. In the first growing season, the vessel diameter was not yet affected by the drought treatment (Figure 4a, Table 3), whereas this was the case in the two succeeding years. The smaller was the value of the adjusted relative extractable water, which was a proxy for the drought experienced by the seedlings in 2014, the smaller were the latewood vessel diameters in both growing seasons 2015 and 2016 (Figure 4b,c). In the first growing season, larger seedlings from *Q. petraea* displayed vessels with larger diameters compared with seedlings from *Q. robur* of the same height (Figure 4a) (significant interaction term between plant height in 2014 and taxon of the mother tree for *Q. robur* with p-value < 0.001 in Table 3), indicating a stronger relationship between seedling height and latewood vessel diameter in *Q. petraea* compared with *Q. robur*.
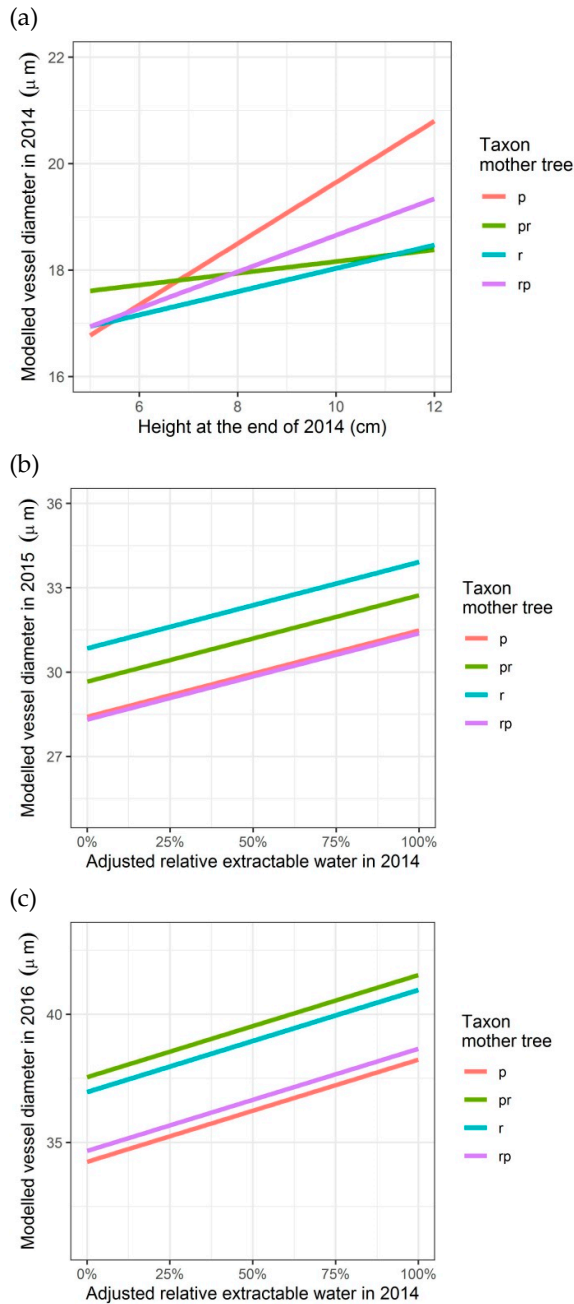
(a)

(b)

(c)

Figure 4. Modelled vessel diameter depending on the height of the seedlings (2014) or on the relative growth rate in height (2015), on the adjusted relative extractable water in 2014 (latewood vessel diameters in rings of 2015 and 2016) and on the taxon of the mother trees (latewood vessel diameters in rings of 2014, 2015 and 2016): (**a**) first growing season 2014; (**b**) second growing season 2015; (**c**) third growing season 2016. p: *Q. petraea*, pr: long stalked intermediate form, r: *Q. robur*, rp: short-stalked intermediate form.

The modelled diameter of the latewood vessels displayed a similar pattern in both 2015 and 2016 (Figure 4b,c; Table 3). The diameter of the vessels depended on the adjusted relative extractable water in 2014 (p-value < 0.001 for both 2015 and 2016 in Table 3). For a given relative extractable water level, seedlings from *Q. robur* tended to larger vessel diameters compared with seedlings from *Q. petraea* (significant taxon of the mother tree for *Q. robur* (T_r) with p-value = 0.004 in 2015 and p-value = 0.006 in 2016 in Table 3), which was opposite to the first growing season.

Table 3. Model statistics for the response variable latewood vessel diameter. The standard level of the categorical variable taxon of the mother tree (*T*) was *Q. petraea*, to which the other taxa were compared with. T_{pr}: long stalked intermediate form, T_r: *Q. robur*, T_{rp}: short-stalked intermediate form. H_{2014}: height at the end of the first growing season. RGR_{2015} and RGR_{2016}: relative growth rates in height for 2015 and 2016, respectively. *W*: adjusted relative extractable water in 2014.

Ring 2014										
	Estimate	Std. Error	df	T-Value	P-Value					
(Intercept)	13.89	0.74	5041	18.79	<0.001***					
H_{2014}	0.58	0.09	201	6.58	<0.001***					
T_{pr}	3.16	1.59	201	1.99	0.048*					
T_r	1.95	0.91	201	2.15	0.033*					
T_{rp}	1.33	1.03	201	1.29	0.197					
$T_{pr} : H_{2014}$	−0.47	0.17	201	−2.73	0.007**					
$T_r : H_{2014}$	−0.36	0.09	201	−3.81	<0.001***					
$T_{rp} : H_{2014}$	−0.23	0.12	201	−1.94	0.054					
Ring 2015						**Ring 2016**				
	Estimate	Std. Error	df	T-Value	P-Value		Estimate	Std. Error	df	T-Value	P-Value
(Intercept)	28.81	0.71	5041	40.56	<0.001***	(Intercept)	34.24	0.81	4896	42.12	<0.001***
RGR_{2015}	−0.55	0.29	203	−1.91	0.057						
W	3.07	0.86	203	3.57	<0.001***	*W*	3.99	0.99	199	4.01	<0.001***
T_{pr}	1.25	1.22	203	1.03	0.306	T_{pr}	3.30	1.40	199	2.36	0.019*
T_r	2.43	0.83	203	2.92	0.004**	T_r	2.72	0.97	199	2.79	0.006**
T_{rp}	−0.10	0.87	203	−0.12	0.742	T_{rp}	0.43	1.00	199	0.43	0.671

Significance codes: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$.

3.4. Formation of Intra-Annual Density Fluctuations in the Xylem Growth Zone

Within the annual xylem growth rings, intra-annual xylem growth zones can be formed, existing of flattened fiber cells [40]. These intra-annual density fluctuations in the latewood xylem of a given growing season were observed in several cross-sections, mainly in the first growth year (Table 4). Therefore, only the probability of having formed intra-annual density fluctuations in the first year was modelled (logistic regression). In the model, only the adjusted relative extractable water in 2014 turned out to be significant, with the probability of forming an intra-annual density fluctuation being higher when seedlings experienced drought followed by plentiful re-watering, independent from the height of the seedlings or from the taxon of the mother tree (Figure 5, Table 5). This effect did not show a legacy in the following years as the number of intra-annual density fluctuations dropped drastically in the following two growing seasons (Table 4).

Table 4. Number of seedlings showing an intra-annual density fluctuation in the secondary xylem, depending on the water limiting treatment experienced by the seedlings in 2014.

Growing Season	*n* of Plants with Extra Ring	
	Control	Drought
2014	5	23
2015	2	1
2016	2	1

Table 5. Statistics from the logistic regression model with the absence/presence of an intra-annual density fluctuation in the secondary xylem of the first growing season as response variable. *W*: adjusted relative extractable water in 2014.

	Estimate	**Std. Error**	**Z-Value**	**P-Value**
(Intercept)	−1.49	0.25	−5.93	<0.001***
W	−1.64	0.61	−2.70	0.007**

Significance codes: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$.
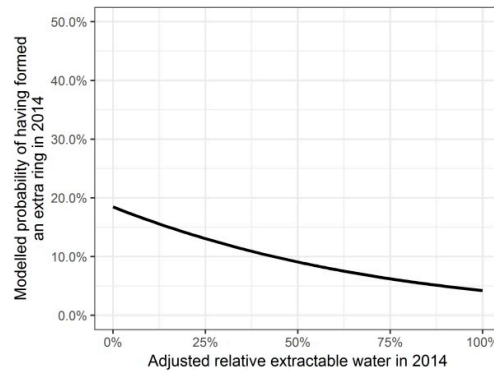
Figure 5. Modelled probability of having formed an intra-annual density fluctuation in the secondary xylem in 2014, depending on the adjusted relative extractable water in 2014.

3.5. Dendritic Vessel Pattern

The probabilities of forming dendritic patterns (seen as flame-like groupings of latewood vessels in cross sections) in the second and third growing season were modelled. In 2015, seedlings with a higher relative growth rate in height displayed higher probabilities of forming dendritic patterns in de latewood (Table 6 and Figure 6a). Moreover, for a given relative growth rate in height, seedlings from *Q. petraea* developed less dendritic patterns, compared with seedlings from *Q. robur* and from the intermediate forms (significant T_{pr}, T_r and T_{rp} with p-values = 0.012, 0.002 and 0.003, respectively, in Table 6, Figure 6a).
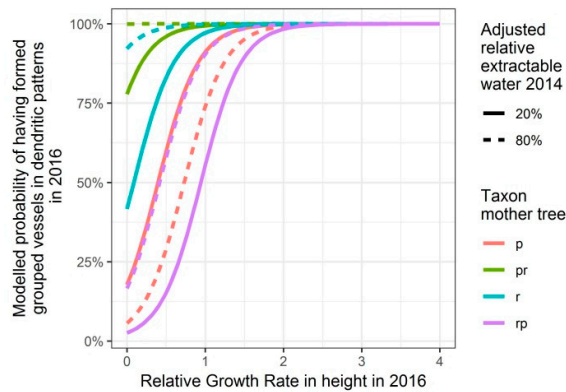
(a)
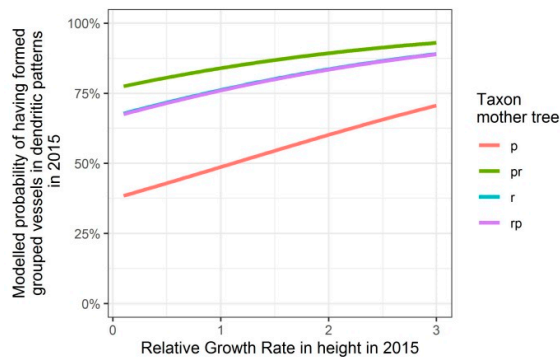
(b)

Figure 6. Modelled probability of having formed dendritic patterns (grouped vessels in flame-like structures) in the latewood xylem, depending on the relative growth rate in height, on the taxon of the mother tree and, only for 2016, on the adjusted relative extractable water in 2014: (**a**) second growing season 2015; (**b**) third growing season 2016. p: *Q. petraea*, pr: long stalked intermediate form, r: *Q. robur*, rp: short-stalked intermediate form.

Table 6. Statistics from the logistic regression models with the absence/presence of dendritic patterns (flame-like structures of grouped latewood vessels) in the secondary xylem in 2015 and 2016 as response variables. The standard level of the categorical variable taxon of the mother tree (*T*) was *Q. petraea*, to which the other taxa were compared with. T_{pr}: long stalked intermediate form, T_r: *Q. robur*, T_{rp}: short-stalked intermediate form. RGR_{2015} and RGR_{2016}: relative growth rate of height in 2015 and 2016, respectively.

Ring 2015					Ring 2016				
	Estimate	Std. Error	Z-Value	P-Value		Estimate	Std. Error	Z-Value	P-Value
(Intercept)	−0.51	0.28	−1.82	0.069	(Intercept)	−1.10	0.78	−1.41	0.159
					W	−2.15	1.27	−1.70	0.089
T_{pr}	1.71	0.68	2.51	0.012*	T_{pr}	−0.12	1.82	−0.07	0.946
T_r	1.21	0.39	3.15	0.002**	T_r	−0.17	1.67	−0.11	0.917
T_{rp}	1.20	0.40	2.98	0.003**	T_{rp}	−3.22	1.19	−2.71	0.007**
RGR_{2015}	0.46	0.22	2.15	0.032*	RGR_{2016}	3.86	0.92	4.19	<0.001***
					$W : T_{pr}$	14.54	41.58	0.35	0.727
					$W : T_r$	6.83	6.57	1.04	0.299
					$W : T_{rp}$	5.54	2.04	2.72	0.006**

Significance codes: *** $p \le 0.001$; ** $0.001 < p \le 0.01$; * $0.01 < p \le 0.05$.

In the third growing season, the presence of flame-like groupings of latewood vessels had become strongly dependent on the relative growth rate in height in 2016 (*p*-value < 0.001 in Table 6, Figure 6b). There was a dependency on the experienced drought in 2014 for seedlings from the short stalked intermediate in comparison with the seedlings from *Q. petraea* (significant T_{rp} with p-value of 0.007 and significant interaction term between T_{rp} and *W* with *p*-value = 0.006 in Table 6).

3.6. Level of Earlywood Vessel Ring Closure

We modelled the level of vessel ring closure at the onset of the earlywood in the second and the third growing season (Figure 7). An exposure to drought in 2014 enlarged the probability of having achieved a higher level of ring closure in 2015, and this effect was stronger for plants with a high relative growth rate in height (significant interaction term between *W* and RGR_{2015}, with a *p*-value < 0.001 in Table 7) (dashed lines showing higher probabilities than the corresponding full lines for a given adjusted relative extractable water in 2014 in Figure 7a). Seedlings from *Q. robur* and from the short stalked intermediate differed significantly from the seedlings from *Q. petraea* and from the long-stalked intermediates (*p*-values < 0.001 for both T_r and T_{rp}, Table 7). This indicated that for a given percentage of vessel ring closure, seedlings from *Q. petraea* and from the long stalked intermediate

tended to a lower probability of having achieved this percentage in comparison to seedlings from *Q. robur* and from the short stalked intermediate, or in other words, seedlings from *Q. petraea* and from the long stalked intermediate displayed less ring closure when compared with seedlings from *Q. robur* and the short stalked intermediate independent from the drought treatment in 2014.
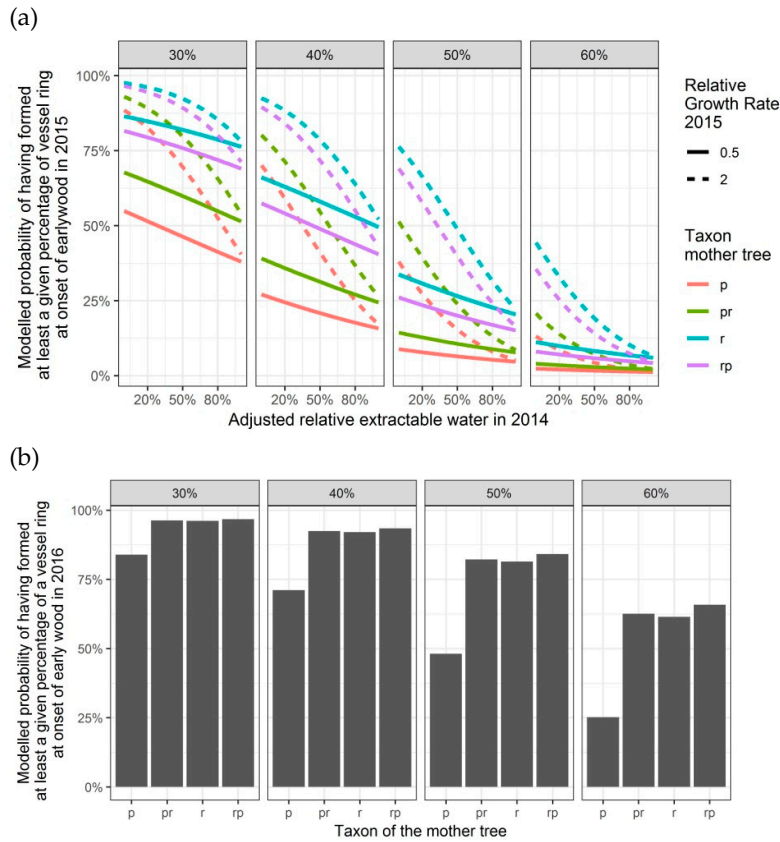
(a)

(b)

Figure 7. Modelled probabilities for a given level of vessel ring closure at the onset of the earlywood in the second and the third growing season, depending on the taxon of the mother tree and, only for 2015, on the adjusted relative extractable water in 2014 and on the relative growth rate in height in 2015: (**a**) probabilities for having formed 30%, 40%, 50% or 60% of the vessel ring in the second growing season 2015; (**b**) probabilities for having formed 30%, 40%, 50% or 60% of the vessel ring in the third growing season 2016. p: *Q. petraea*, pr: long stalked intermediate form, r: *Q. robur*, rp: short-stalked intermediate form.

Table 7. Statistics from the cumulative logistic regression models with the level of vessel ring closure at the onset of the earlywood in the second and third growing season as response variables. The standard level of the categorical variable taxon of the mother tree (*T*) was *Q. petraea*, to which the other taxa were compared with. T_{pr}: long stalked intermediate form, T_r: *Q. robur*, T_{rp}: short-stalked intermediate form. RGR_{2015}: relative growth rate in height in the second growing season. *W*: adjusted relative extractable water in 2014.

Ring 2015						**Ring 2016**			
	Estimate	**Std. error**	**Z-Value**	**P-Value**		**Estimate**	**Std. Error**	**Z-Value**	**P-Value**
W	0.15	0.44	0.35	0.727					
T_{pr}	−0.61	0.46	−1.35	0.177	T_{pr}	−1.61	0.46	−3.46	<0.001***
T_r	−1.64	0.34	−4.80	<0.001***	T_r	−1.55	0.33	−4.78	<0.001***
T_{rp}	−1.28	0.34	−3.80	<0.001***	T_{rp}	−1.75	0.35	−5.05	<0.001***
RGR_{2015}	−1.12	0.26	−4.41	<0.001***					
$W : RGR_{2015}$	1.06	0.31	3.43	<0.001***					

Significance codes: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$.

In the third growing season, the modelling indicated that only the taxon of the mother tree still affected the probability of having formed a certain degree of vessel ring closure at the onset of the earlywood (Figure 7b). For a given percentage of vessel ring closure, the seedlings from *Q. petraea* displayed a significant lower probability of having achieved at least this percentage in comparison to the seedlings from *Q. robur* and from both types of intermediates (Figure 7b, Table 7).

4. Discussion

In broad-leaved tree species, the radial growth of the stem, the vessel size and the structure of the secondary xylem are influenced in variable degrees by the age of the tree and by the prevailing environmental conditions, with primary growth (height growth) principally driven by soil conditions and secondary growth (radial growth), apart from other environmental factors, being strongly determined by light [41–43]. We studied the pith size, the radial growth dynamics, the latewood vessel size, dendritic patterns of latewood vessel arrangement and earlywood vessel ring closure in the secondary xylem of three-year-old oak seedlings that were offspring from *Q. petraea*, from *Q. robur* and from their morphological intermediates and that experienced drought stress in the first growing season.

4.1. Differentiation between Offspring from Q. Robur, Q. Petraea and the Morphological Intermediates

We found growth and wood anatomical differences between seedlings from *Q. petraea*, *Q. robur* and their morphological intermediates. In the first year and for higher plants, seedlings from *Q. petraea* have wider piths than *Q. robur*, whereas the pith radius for seedlings from *Q. robur* and from the long-stalked intermediate are more or less independent from the height growth. Surprisingly, the pith tends to more narrow widths for larger seedlings from the short stalked intermediate. As the pith is composed of soft, spongy parenchyma cells, which can store and transport nutrients throughout the juvenile plant (next to the primary xylem and phloem), the latter finding is counterintuitive as higher seedlings would need comparably more nutrients for growth. Still, pith size is known to have a fluctuating trajectory along the plant axis and tends to be correlated with leaf surface [44]. This response of seedlings from the short-stalked intermediate is situated outside the scope of the parental responses and is likely caused by the more hybridogenic character of the seedlings [45]. For similar height (2014) or relative growth rate in height (2016), seedlings from *Q. petraea* display smaller ring widths than seedlings from *Q. robur*, the difference being bigger for smaller seedlings and most obviously present in the first (2014) and third growing seasons (2016). The second growing season is characterized by lower relative growth rates in height in comparison with the third growing season, most probably because plants were not yet transferred to a larger sized pot in this year, which can have attenuated the differential responses between the taxa (no significant difference between seedlings from *Q. petraea* and from *Q. robur* in this year).

The vessel diameter in the latewood diverges significantly between seedlings from *Q. petraea* and *Q. robur*. In the first year and for similar heights, seedlings from *Q. petraea* display larger sized vessels than seedlings from *Q. robur* or from the intermediate forms. This is in contradiction with the smaller vessel sizes in the two succeeding years (for seedlings with similar relative growth rates in height). The larger sized vessel diameters in the first year for the seedlings from *Q. petraea*, still concurring with a smaller ring width compared with *Q. robur*, could indicate a mechanism of plastic response of vessel growth to the favorable soil and growth conditions in our experimental setup specifically for *Q. petraea*. This response disappears after the first season. The response is not reflected in a promotion of radial growth, but it can be suggested to be associated to the wider piths that were observed in seedlings from *Q. petraea*. These results may be related to the finding that seedlings from a Spanish beech provenance originating from a drought-prone site developed the largest vessels compared to nine other European beech provenances in a common garden [46]. The larger sized latewood vessels together with the larger ring widths in the second and third year in offspring from *Q. robur* compared to *Q. petraea* point to the importance of the seedling and sapling stage of a tree in which competition is at stake, with *Q. robur* investing more in a quick youth growth (both height and radial growth, when compared with *Q. petraea*, developing large vessel sizes to manage the high

demand of sap flow. *Q. robur* is also known to display a faster post-stress recovery of the growth rate in comparison with *Q. petraea* [26]. In 2015, seedlings from *Q. robur* formed more latewood dendritic patterns than seedlings from *Q. petraea* for a given relative growth rate, a differentiation that disappeared in 2016. In the latter year, it became clear that this formation of flame-like vessel structures predominantly depends on higher relative growth rates in height. In this year, seedlings from *Q. petraea* and from *Q. robur* did not differ from each other anymore and only seedlings from the short stalked intermediate had a lower probability of forming dendritic patterns for a given relative growth rate in height in the control group of plants (no water limitation in 2014). This response lies outside the scope of the parental species and likely underlines the higher hybridogenic origin of the seedlings [45]. This result is likely related to the finding of Feuillat et al. [23] that *Q. robur* and *Q. petraea* trees displaying an atypical wood anatomy were also less representative in their leaf morphology.

Seedlings from *Q. robur* get closer to full vessel ring closure at the onset of the earlywood in comparison with *Q. petraea*, both in the second and the third growing season. This finding can be related to the significant higher proportion of total ring surface area taken up by earlywood vessels and the higher number of earlywood vessels in adult *Q. robur* compared to adult *Q. petraea* trees [23]. As the function of the earlywood vessel ring is likely to support a quick sap flow at the start of the growing season, the further advanced closure of the vessel ring for offspring from *Q. robur* is most probably linked to the relative higher juvenile height growth in *Q. robur* compared with *Q. petraea* and may explain the stronger water requirements and higher vulnerability to cavitation of this species in comparison with *Q. petraea* [23,47,48]. For the response of the level of vessel ring closure in the second growing season (2015), the seedlings from the two types of intermediate forms follow the seedlings of the oak species with the same leaf stalk trait, i.e., seedlings from the long stalked intermediate follow the response of the seedlings from *Q. petraea* and seedlings from the short stalked intermediate follow the response of the seedlings from *Q. robur* (in the model statistics: seedlings from *Q. robur* and the short-stalked intermediate differ significantly from the standard level *Q. petraea* with *p*-values < 0.001 in Table 7). This type of response was also found for the height growth of these seedlings [32]. In the third growing season, however, seedlings from both types of intermediate forms tend to a response similar to the seedlings from *Q. robur* (seedlings from the three taxa differ from the standard *Q. petraea* with *p*-values < 0.001 in Table 7).

4.2. Drought Responses

It was already described in 1950 that drought can throw back the growth of trees for several years [49]. The drought treatment in the first growing season decreased both the ring widths and the vessel diameters in the two succeeding years. Only for ring width and only in the third growing season (2016) we found a differentiation between the different taxa of the mother trees, with seedlings from the short-stalked intermediate displaying a stronger reduction in radial growth when having experienced stronger drought in the first growing season; also here, this response lies outside the range of the parental species and can likely be attributed to the higher hybridogenic origin of the seedlings [45]. Reduction in vessel diameter upon experienced drought has been observed for 6-year-old saplings of both *Q. robur* and *Q. petraea* and can be interpreted as an adaptation of the tree to protect its vessels against cavitation [24]. A distinct relationship between meteorological variables and vessel diameter was also detected for in situ adult *Q. robur* trees [50]. In our study, the water limiting conditions in the first growing season, followed by plentiful re-watering, also led to a higher probability of forming intra-annual density fluctuations in the secondary xylem of this growing season, independent of the taxon of the mother tree. Intra-annual density fluctuations in hardwood are morphologically the result of variation in different anatomical features, such as lumen size and wall thickness of both vessels and ground tissue [51]. Intra-annual density fluctuations have already been described for several species such as *Tectona grandis* [52], *Q. ilex* [53] or *Arbutus unedo* [54] and correlate with precipitation patterns. Our data suggests that in a restricted number of plants the plentiful re-watering at the end of the drought treatment likely re-activated the radial growth, leading

to a higher probability of forming slightly deviating cell wall sizes of the fibers and thus creating a visible extra ring on the cross sections within the first year ring.

The drought stress experienced in the first growing season affected the level of vessel ring closure at the onset of the earlywood only in 2015, not anymore in 2016. Remarkably, the drought exposed seedlings in 2015 display a further advanced ring closure than the control group, indicating a higher capacity of water transport at the start of the growing season. Re-watering after severe water limiting conditions in oak seedlings can result in a retarded leaf senescence, which can be interpreted as a type of re-activation to compensate for the lost growing time during the drought period itself [30]. Moreover, in beech, a stimulation of the net-photosynthesis after drought and re-watering was suggested to compensate the restraint of photosynthesis during the drought treatment [55]. Such re-activation mechanisms may result not only in a higher probability of forming an extra false ring in the year of the treatment but also in a legacy effect at the onset of the next growing season with a further advanced vessel ring closure to enhance the startup growth. As the latewood vessel sizes were smaller in the drought treated group of plants in 2015, which can be interpreted as an adaptation to potential future drought periods, these results suggest multiple independent signals that are activated upon drought and re-watering that drive earlywood and latewood vessel growth.

5. Conclusions

Radial growth and wood anatomical differences between offspring of the sampled oak taxa in this study can be attributed to genetic differentiation rather than to environmental conditions, apart from the drought treatment, as we sourced the acorns in a naturally mixed population (same provenance) and grew seedlings (same age) in controlled conditions (minimal variation of environment). For a majority of traits, offspring from *Q. petraea* differed significantly from *Q. robur*: (i) for higher seedlings, offspring from *Q. petraea* had a wider pith; (ii) for similar height growth, seedlings from *Q. petraea* formed smaller ring widths; (iii) in the first year, seedlings from *Q. petraea* formed wider latewood vessels, whereas these were smaller in the two succeeding years; (iv) dendritic patterns of latewood vessels appeared later and earlywood ring closure was less in seedlings from *Q. petraea*. Moreover, we found indications for the offspring of the intermediate forms displaying phenotypes outside the scope of the seedlings of *Q. petraea* and *Q. robur*. In addition, we observed two types of responses on the water limiting treatment in the first growing season, followed by plentiful re-watering. On the one hand, drought treatment resulted in a reduction in radial growth and in latewood vessel size in the two succeeding growing seasons, which can be interpreted as an adaptation of the newly formed xylem to improve the tolerance of the seedling to future water limiting conditions. On the other hand, we found indications for a compensation growth mechanism in the treated seedlings that may be attributed to the plentiful re-watering after the drought treatments, allowing the seedling to compensate for the lost growing time. As youth growth of *Q. robur* is more competition-oriented, with a faster juvenile growth and a quicker post-stress recovery, whereas *Q. petraea* seems to invest more in precautious growth, being more prepared for stressful conditions. It is possible that *Q. robur* seedlings may suffer more than seedlings from *Q. petraea* from the more frequent and more severe droughts that are predicted.

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