


# Intra-annual leaf phenology, radial growth and structure of xylem and phloem in different tree parts of *Quercus pubescens*

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**Abstract** Basic knowledge of the intra-annual timings of leaf development and radial growth (including the phloem part) in different tree parts is generally missing although such rudimentary data are crucial to link the structure and function of vascular tissues at the whole tree level. To understand better the time course of leaf development and radial growth patterns in different tree parts, we studied leaf phenology and intra-annual xylem and phloem formation and structure in the stem and at two locations in branches of sub-Mediterranean *Quercus pubescens* in 2015. Onset and end dates of cambial cell production were synchronized at the two locations in branches, but were different at the stem base. The period of cell production was thus a month longer in the stem, resulting in 82.8 and 45.1% wider xylem and phloem increments, respectively. In addition, the xylem ring was wider than the phloem ring in all three parts. Thus, phloem ring widths in stem represented 24.8% and in branches 79.4% of the xylem ring width. Earlywood occupied 52.9% (stem) and 74.9% (branches) of the xylem ring, and early phloem 53.7% (stem) and 43.3% (branches) of the phloem ring. Most of the annual radial increment (i.e. xylem and phloem increments) in stem and branches was formed prior to full leaf

development. Latewood and late phloem were formed in the period of full leaf unfolding. Our study confirmed that the temporal sequence of leaf development and radial growth are not contemporary in *Q. pubescens*. Different intra-annual patterns of radial growth in different tree parts result in different structures of xylem and phloem, which is in line with different roles of stem and branches in terms of tree functioning.

**Keywords** Pubescent oak · Sub-Mediterranean climate · Cambium · Stem · Branch · Earlywood vessel

## Introduction

Changes in the length and timing of the growing season, and shifts in the habitat ranges of plant species, can have substantial effects on ecosystem dynamics such as carbon and water cycling and plant–animal interactions (Polgar and Primack 2011). The impact of global warming on terrestrial ecosystems is well documented by leaf phenological observations (Menzel et al. 2006) because leaf development is highly sensitive to temperature (Polgar and Primack 2011). In addition to shift in leaf phenology (Menzel et al. 2006), tree response to climate change encompasses alterations in growth rates (Rossi et al. 2011). However, since leaf and xylem phenologies are not linearly related and are under different environmental controls, the variability in leaf phenology may have no significant effect on variations in intra-annual radial growth (Rossi et al. 2009; Kraus et al. 2016) and xylem anatomy (Čufar et al. 2015). Therefore, the understanding of current and future tree growth dynamics requires deeper investigation of leaf development and cambial activity as well as their relationship with the main climatic factors.

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In temperate climates, the radial growth of deciduous trees stops in autumn and resumes in spring but is not necessarily synchronized with leaf unfolding. The time courses of intra-annual leaf phenology and secondary growth are species specific, leading to differences in temporal variation of carbon allocation (e.g. Suzuki et al. 1996; Takahashi et al. 2013). In ring-porous species, large earlywood vessels are formed before full leaf expansion (e.g. Zweifel et al. 2006; Sass-Klaassen et al. 2011) to overcome reductions in hydraulic conductivity caused by tylose formation during the winter months in earlywood vessels of the previous years (Cochard and Tyree 1990; Bréda and Granier 1996). The newly formed hydraulic pathway in the spring, built prior to the photosynthetic period, is thus necessary to provide a water supply to the crown (Essiamah and Eschrich 1986; Barbaroux and Bréda 2002). In *Quercus pubescens*, a substantial amount of current xylem increment (about 40%) is formed at the time of full leaf unfolding, confirming the importance of fully formed initial earlywood vessels to provide hydraulic conductivity during leaf development (Zweifel et al. 2006). This pattern demonstrates that tissues formed before full leaf development rely on carbohydrate resources stored in bark and wood parenchyma cells at the end of the previous growing season (Dickson et al. 2000; Barbaroux and Bréda 2002).

To elucidate the relationship between leaf phenology and vessel formation from the ecophysiological viewpoint, both traits need to be examined concurrently to determine how they relate to photosynthesis and water transport (Takahashi et al. 2013). However, with few exceptions (e.g. Zasada and Zahner 1969; Takahashi et al. 2013), studies comparing leaf and earlywood development have mainly been designed in such a way that developing wood has been sampled at the base of the tree, i.e. 1.0–1.7 above the ground, ignoring the fact that the time course of xylem development and wood structure may differ along the tree (Anfodillo et al. 2013; Jyske and Hölttä 2015). Certain physiological measurements, such as leaf gas exchange measurements of stomatal conductivity, transpiration, net photosynthesis and respiration, on the other hand, are usually limited to the level of individual leaves/branches or to young plants or saplings (e.g. Damesin and Rambal 1995; Zweifel et al. 2007; Juárez-López et al. 2008), making anatomical data of xylem growth from the stem base and ecophysiological measurements from the crown often difficult to link and interpret.

Hence, basic knowledge of the intra-annual timings of leaf development and radial growth (including the phloem part) in different tree parts is generally missing, although it is known from older literature that cambial reactivation in different tree parts varies among coniferous, diffuse- and ring-porous trees (Ladefoged 1952; Panshin and de Zeeuw 1980; Takahashi et al. 2013). In deciduous ring-porous

trees, the cambium presumably reactivates before bud break or at the early stage of bud swelling and extends rapidly down the branches and trunk, giving the appearance of simultaneous initiation of cambial cell division throughout the stem (Wareing 1950; Ladefoged 1952). In *Quercus robur*, cambial reactivation is reported to proceed basipetally in first-year shoots, but occurs almost simultaneously in the trunk and branches that are more than 1 year old (Lachaud et al. 1999). However, considerable variations exist in the spread of cambial activity throughout the trunk, depending on the age of the tree, its vigour and the exposure (Panshin and de Zeeuw 1980).

To understand better the time course of leaf development and radial growth patterns in different tree parts, also in the light of tree functioning, we (a) compared seasonal leaf development and dynamics of xylem and phloem formation in three positions in adult *Q. pubescens* trees, i.e. at two locations in branches and one at the stem base and (b) analysed xylem and phloem hydraulic architecture in fully formed increments of 2015 in order to characterize an effective structure of vascular tissues crucial for the functioning of deciduous *Q. pubescens* in the sub-Mediterranean region. We hypothesized that seasonal dynamics of xylem and phloem processes are not synchronized in different tree parts, which is also reflected in their structure. In terms of linking the structure and function of vascular tissues, such rudimentary data from different tree parts are crucial because discussion on translocation pathways and water transport in a tree is based on these findings.

## Materials and methods

### Study site characteristics

The study site was located in karst grassland at Podgorški Kras (45°32′56.3″N, 13°54′36.1″E, 429 m a.s.l.) in Slovenia, which was abandoned ca. 30 years ago. It has since been encroached by various woody plant species, among which pubescent oak dominates, growing in patches or solitary with an average height of 7 m and average age ca. 30–40 years.

The site is influenced by a sub-Mediterranean climate, characterized by fairly harsh winters and frequently occurring dry and hot summer drought periods. The average annual temperature in the period 1981–2010 measured at the nearby Ilirska Bistrica weather station is 10.4 °C. The lowest monthly average temperature was recorded in January (1.3 °C) and the highest in July (20.1 °C). The average annual precipitation is 1170 mm, with two annual rainfall peaks, in autumn and in late spring. A more detailed description of the measured environmental parameters at the study site in 2015 is given in Table 1.

**Table 1** Detailed description of the measured environmental parameters at the study site in 2015

Air temperature (°C)	Yearly average	13.8
	Average during growing season	18.2
	Average during non-growing season	7.1
	Absolute maximum	36.9 (July)
	Absolute minimum	−6.2 (January)
Precipitation (mm)	Yearly sum	1038
	Sum during growing season	624
	Sum during non-growing season	414
Soil water content (m <sup>3</sup> m <sup>−3</sup> )	Average annual	0.22
	Average during growing season	0.19
	Average during non-growing season	0.27

Growing season = period April–September (DOY 91–273); non-growing season = period January–March (DOY 1–90) and October–December (DOY 274–365)

The dominant soil type is Rendzic Leptosol with uneven soil depth and a rocky surface. The soil has a mainly clay texture with a low amount of nutrients, 12–15% proportion of organic matter in the top layer and pH value 6.9. The sub-Mediterranean climate accompanied by shallow soil and frequent wind diminishes the impact of high precipitation, so drought stress frequently occurs in the growing season (March–October) (Ferlan et al. 2011).

### Tree selection and leaf phenological observations

At the beginning of the 2015 growing season, we selected five solitary healthy pubescent oaks (*Q. pubescens* Wild.) without any visible injuries on stems, branches and roots. The trees were  $35 \pm 5$  years old and  $7 \pm 0.5$  m high, with diameters at breast height (DBH) of  $0.23 \pm 0.01$  m.

Leaf phenological observations were carried out on all oaks at 7- to 10-day intervals from March until December. We monitored leaf phenology phases from leaf unfolding until leaf senescence according to a 10-stage scale: 1—dormant buds, 2—swollen buds, 3—bud break, 4—leaf emergence, 5—leaf development, 6—full leaf unfolding, 7—flowering (beginning of opening of male flowers), 8—initiation of autumn colouring (first leaves start to change colour from green to yellow, red and orange), 9—abundant autumn colouring (>50% leaves of the observed crown change colour from green to yellow, red, orange and brown) and 10—leaf fall (adapted from Bréda and Granier 1996; Vilhar et al. 2013). On each sampling date, we took images of a selected part in the crown with a digital camera to document leaf development.

### Microcore collection, section preparation and tissue analysis

Microcores were collected at weekly intervals from mid-March until mid-October 2015, using a Trephor tool (Rossi

et al. 2006). The samples were taken from three different tree parts: from a branch 1.3–1.5 m from the branch apex; from a branch 1.9–2.1 from the branch apex, and from the stem 1.0–1.3 m above the ground. The sampled branches, with diameters at the sampling locations of  $6.0 \pm 1.1$  cm, were located approximately 3 m above the ground. Samples were collected in a helical pattern and separated by 3 cm in order to avoid wound effects. Each microcore contained phloem (non-collapsed and collapsed), cambium and at least two of the last-formed xylem rings. Immediately after removal from the trees, the samples were fixed in ethanol–formalin–acetic acid solution (FAA) (Gričar et al. 2007). After 1 week, the samples were dehydrated in a graded series of ethanol, infiltrated with D-limonene (Bio Clear, Bio Optica, Milano, Italy) and embedded in paraffin blocks (Paraplast plus, ROTH, Karlsruhe, Germany) (Rossi et al. 2006). Transverse sections of 14  $\mu$ m in thickness were cut with a Leica RM 2245 rotary microtome (Leica Microsystems, Wetzlar, Germany). The sections were stained with a safranin (Merck, Darmstadt, Germany) (0.04%) and astra blue (Sigma-Aldrich, Steinheim, Germany) (0.15%) water mixture (van der Werf et al. 2007) and mounted in Euparal (Waldeck, Münster, Germany). They were observed under an Olympus BX51 light microscope (Tokyo, Japan) using transmission and polarized light modes. Histometrical analyses were performed with the Nikon NIS-Elements Basic Research version 2.3 image analysis system (Tokyo, Japan).

On the sections, the number of cells in the cambium was counted. The widths of currently formed xylem, and phloem increments along three radial files were measured and then averaged. Additionally, the following phenological phases of cambial activity were assessed: (1) onset of cambial cell production, (2) maximum rate of xylem and phloem cell production, (3) cessation of cambial cell production, (4) cessation of the differentiation process in terminal latewood cells and (5) transition from earlywood to

latewood and from early to late phloem. Phases were assessed for each tree and were computed in days of the year (DOY). Tension wood was often present in branches; however, in such cases, samples were excluded from further analysis.

The state of the cambium (activity/dormancy) and cell differentiation phases listed above were identified and interpreted within the context of the multiseriate concept that the vascular cambium comprises both the cambial initial cells and the xylem and phloem mother cells (Plomion et al. 2001). On the cross sections, it was not possible to recognize cytoplasmic changes associated with the seasonal cycle of the cambium to hint at cambial activity/dormancy. This was mainly due to insufficient resolution of the light microscope and fixative used (FAA) adequately to capture and preserve the cytoplasm of the cambial cells. Reactivation of the cambium at the cellular level was therefore histologically defined by an increased number of cambial cells and the occurrence of newly formed xylem and phloem cells in early developmental stages (Prislan et al. 2011). Due to the methodological limitations, we were unable to determine whether initial sieve tubes and earlywood vessels were formed from overwintered cells or newly divided cambial derivatives (Frankenstein et al. 2005).

The observed phases were defined as follows: (1) the onset of cambial cell production was identified as an increased number of thin-walled cambial derivatives (Prislan et al. 2011); (2) the maximum rate of new xylem and phloem cell production was calculated and plotted using a Gompertz function that indicated the maximum growth rate at the inflection point of the curve (Rossi et al. 2003); (3) cessation of cambial cell production was identified as the time at which no new thin-walled cells were observed adjacent to the cambium and the number of cambial cells was comparable to the number before its reactivation in spring; (4) cessation of xylem differentiation was identified by the complete lignification of the terminal latewood cells, as indicated by cell walls stained completely red by the safranin–astra blue procedure; (5) transition from earlywood to latewood was determined when small latewood vessels were no longer arranged in rings, which is characteristic of large earlywood vessels; (6) transition from early to late phloem was identified by the appearance of small, tangentially orientated groups of phloem fibres separating the two parts (Gričar 2010).

In completely developed xylem increments of 2015, the vessel tangential diameter, vessel density (VD: number of cells/1 mm<sup>2</sup>) and percentage water conductive area (WCA) were analysed in earlywood. From all measured vessel tangential diameters, we calculated mean vessel diameter (MVD) and mean vessel area (MVA). The percentage of WCA in earlywood was calculated by the following formula (Stojnic et al. 2013):

$$\text{Water conductive area} = \frac{\text{Total earlywood vessel area}}{\text{Earlywood area}} \times 100 (\%)$$

In phloem increments of 2015, the sieve tube tangential diameter and area of initial sieve tubes of early phloem were measured. We then calculated from all measured values the mean tangential diameter (MSD) and mean area (MSA) of initial sieve tubes.

## Data analysis

The widths of xylem and phloem increments were fitted to the Gompertz function (Rossi et al. 2003) using the *nlme* package in R. Model fits were evaluated by computing mean absolute error and Efron's pseudo- $R^2$  (calculated as one minus the ratio between the sum of squared model residuals and the sum of total variability). The first derivative of the Gompertz function was calculated to determine maximum cambial cell production of xylem and phloem rings during the growing season. To investigate the differences between tree parts in xylem and phloem growth, the joint Gompertz model for combined data of all tree parts was compared with a model with tree parts being the fixed cofactor. The comparison was made using a partial  $F$  test at 0.05 significance level. Differences between tree parts in specific model parameters were tested by constructing models with a common parameter among groups and again testing these models, using a partial  $F$  test, against a model with separate parameters for each group.

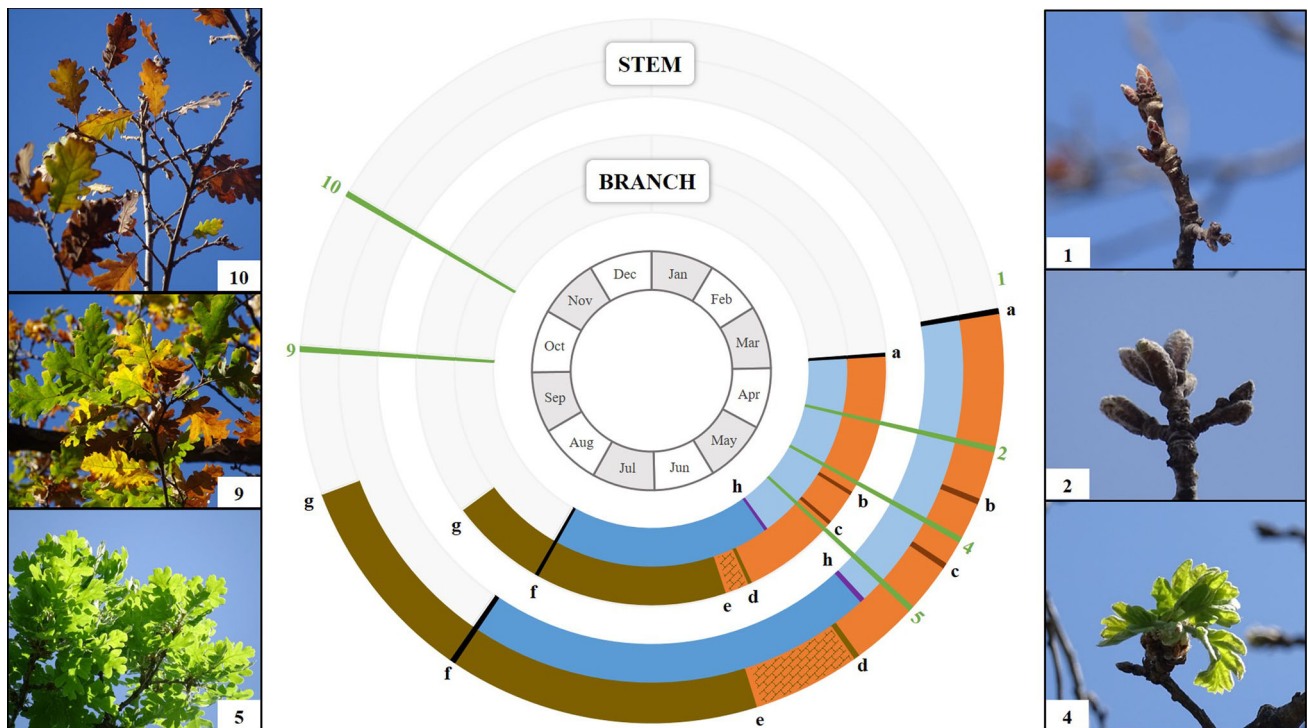
Data of the final samples (earlywood vessel and sieve tube features, and widths of xylem and phloem increments) were analysed using a linear mixed model with the tree part as a fixed factor and tree as a random (blocking) factor. Prior to the analyses, the homogeneity of variances across levels of tree position was compared graphically (box-plots). Tukey's HSD tests were performed to test for significance between levels of tree part. Finally, Pearson correlations were calculated between the meaningful xylem and phloem parameters. The significance of correlations was tested at a 0.05 significance level. Analyses were performed using the statistical software R, version 3.2.3 (R Core Team 2012).

## Results

### Leaf phenology

At the beginning of the 2015 growing season, buds were closed and remained dormant until mid-April, when they became swollen on all oaks (Fig. 1). In the second half of April, buds broke and first leaves emerged on two oaks. Leaf development subsequently proceeded on all trees until





**Fig. 1** Schematic representation of the time course of leaf phenology and intra-annual xylem (orange-brown colour) and phloem formation (blue colour) in different tree parts of *Quercus pubescens* in the growing season of 2015. The two bigger circles represent the tree parts, stem (outer) and branch (inner). Since the intra-annual dynamics of xylem and phloem formation were synchronized in two locations in the branches, it is presented as one circle. The innermost grey circle represents the year divided into 12 months. The main phenological stages of leaf development (green lines) are presented as numbers according to the 10-stage scale described in

“Materials and methods” section: 1—dormant buds, 2—swollen buds, 4—leaf emergence, 5—full leaf unfolding, 9—abundant autumn colouring and 10—leaf fall. The most important milestones of xylem and phloem formation are indicated with letters: a onset of cambial cell production; b onset of expansion of initial earlywood vessels; c complete development of initial earlywood vessels; d transition from earlywood to latewood; e complete development of earlywood; f end of cambial cell production; g cessation of xylem growth ring formation; and h transition from early phloem to late phloem. (Color figure online)

full leaf unfolding in mid-May, which coincided with flowering and opening of the male flowers. In 2015, therefore, the period from bud opening to full leaf unfolding lasted on average  $14.2 \pm 7.0$  days, and from bud swelling to full leaf unfolding around 31.0 days. Autumn leaf colouring began in the first decade of October. The first leaves started to change from green to yellow, red and orange. The average period in which the trees had green leaves lasted  $160 \pm 7.3$  days. In late autumn, i.e. November, leaves were turning brown and falling off the trees. At the beginning of December, tree canopies of pubescent oak were still partly covered with brown leaves, among which a few green leaves were still present. A small proportion of old leaves from the previous year were still attached to the branches of pubescent oak in the following early spring.

**Seasonal dynamics of xylem and phloem formation in stem and branches**

Xylem and phloem growth patterns, evaluated using the Gompertz function and compared between different

positions along the trees, showed no difference in curves between lower and upper parts of branches ( $p = 0.943$  and  $p = 0.886$  for xylem and phloem growth, respectively). Consequently, we merged the branches data and only compared them with stem data in the subsequent analyses (Table 2). Cell division in the cambium started about 1 week earlier in the stem (mid-March) than in the branches (end of March) ( $p < 0.05$ ). In all samples, the number of cambial cells was generally larger in the stem than in the branches ( $p < 0.05$ ) (stem: max =  $8.7 \pm 1.5$  cell layers, min =  $6.5 \pm 1.8$  cell layers; branch: max =  $7.6 \pm 1.6$  cell layers, min =  $5.8 \pm 1.5$  cell layers). Cell production ceased in the third decade of July in the branches and in mid-August in the stem. The period of cell production was thus almost a month longer in the stem than in the branches ( $p < 0.05$ , Table 2).

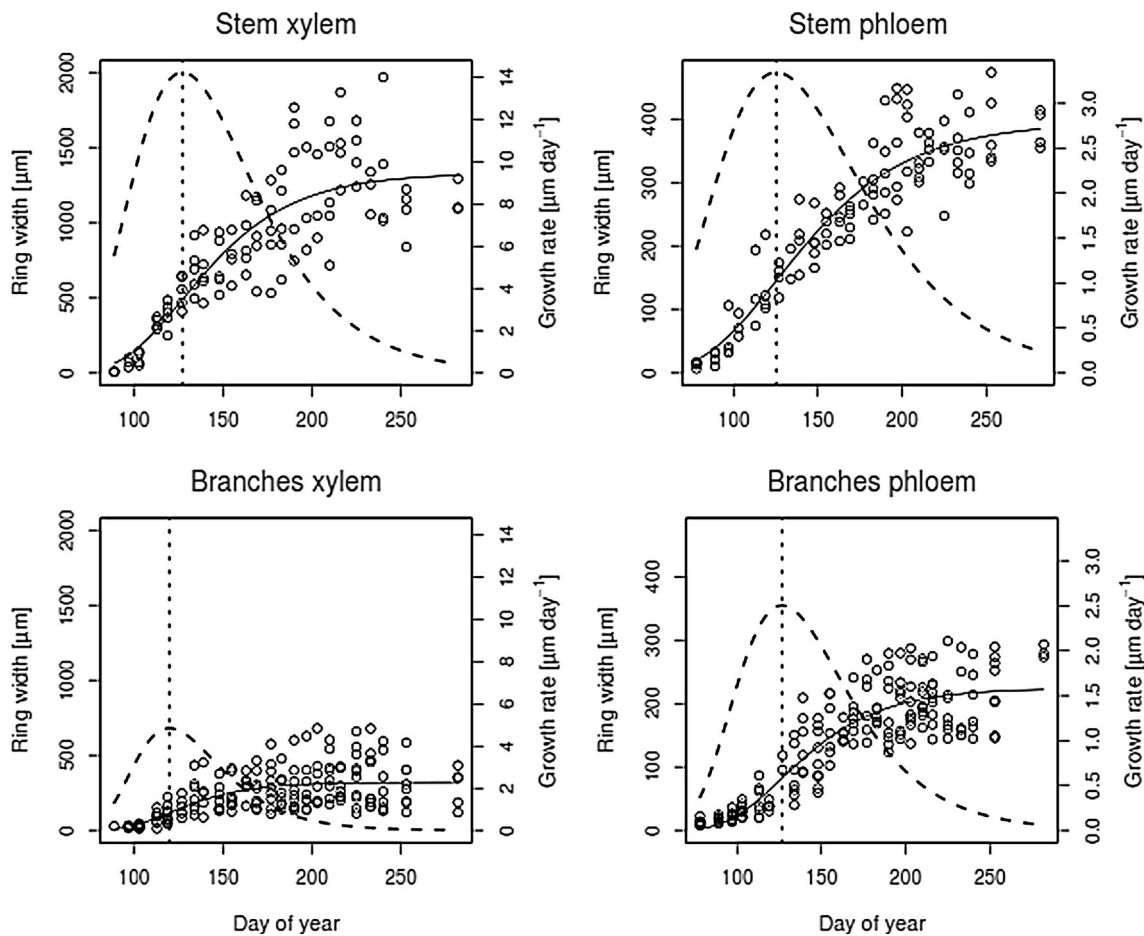
In the first 3 weeks of radial growth (stem: DOY 78–97; branches: DOY 78–103), phloem growth exceeded that of xylem. However, after that, xylem growth surpassed that of phloem until the cessation of cambial cell production (Fig. 2). By the first week of May, earlywood vessels were

**Table 2** Main wood and phloem formation stages in stem and branches of *Quercus pubescens* in 2015

	Stem	Branch	Significance of difference ( <i>p</i> value)
<i>Wood and phloem formation phases (DOY ± standard deviation)</i>			
Onset of cambial cell production	82.4 ± 6.0	87.9 ± 3.5	0.049
Complete development of initial earlywood vessel	126.2 ± 8.2	133.3 ± 8.4	0.108
Transition from earlywood to latewood	147.2 ± 8.0	156.1 ± 4.9	0.018
Transition from early phloem to late phloem	140.2 ± 8.6	146.7 ± 8.3	0.113
End of cell production	228.0 ± 7.5	205.8 ± 3.6	<0.001
End of xylem formation	253.0 ± 5.0	236.5 ± 3.7	<0.001
<i>Duration of different phases (days ± standard deviation)</i>			
Duration of cambial cell production	145.6 ± 6.2	117.9 ± 5.9	<0.001
Duration of initial earlywood vessel enlargement	31.8 ± 4.9	37.1 ± 6.5	0.301
Duration of initial earlywood vessel formation	43.8 ± 11.8	45.4 ± 6.9	0.737

*DOY* day of the year

No significant differences in Gompertz curves were found for upper and lower parts of branches, so the data were merged and compared with stem data using mixed models with tree as a random factor



**Fig. 2** Xylem and phloem development in *Quercus pubescens* in 2015, presented as cumulative growth (solid line) and as weekly increment growth (dashed line). Dots represent microcore data from five individuals sampled in the 2015 growing season

fully created in the stem and 1 week later also in the branches, and the duration of their formation was comparable in the stem and branches (Fig. 1; Table 2). Latewood started to form in the third week of May in the stem and in mid-June in the branches (Fig. 1), while the entire earlywood part was fully formed by mid-June in the stem and by the end of June in the branches. The transition from early to late phloem occurred at the end of May in the stem and 1 week later in the branches, as evident from the formation of the groups of phloem fibres (Fig. 3).

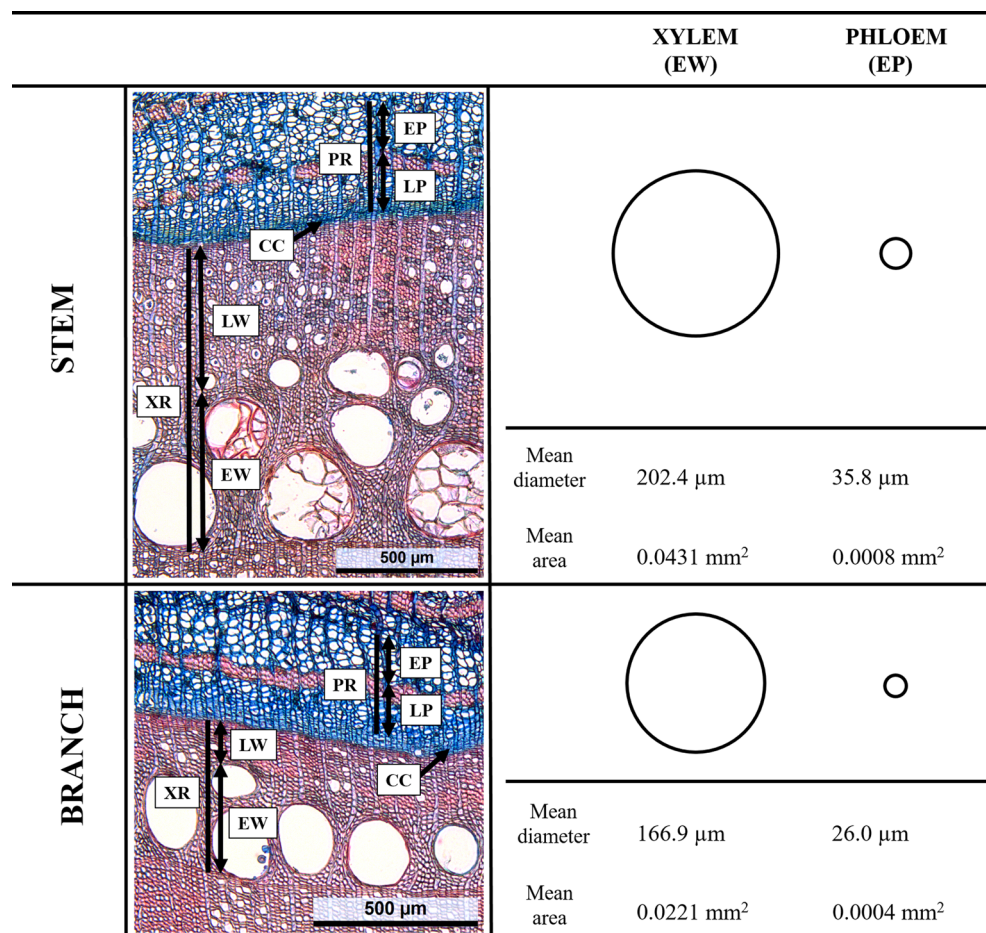
The peak of cambial production of xylem cells occurred 1 week earlier in the branches (DOY 120—end of April) than in the stem (DOY 127—beginning of May) ( $p < 0.05$ ). At that time, on average 14.2 and 4.8  $\mu\text{m}$  of the xylem increment were formed per day in stem and branches, respectively, as calculated from the first derivative of the Gompertz function (Fig. 2). At the height of xylem growth, around one-third of the annual xylem increment was formed in the stem (32.3%) and branches (35.4%). For phloem, maximum increment growth in the stem was 3.3  $\mu\text{m}$  per day and coincided with the peak of xylem growth. In branches, on average 2.5  $\mu\text{m}$  of phloem tissue was formed per day at the height of growth and the peak

was reached 1 week later than in the xylem (Fig. 2). At the height of phloem growth, 36.0 and 46.6% of the annual increments were formed in the stem and branches, respectively.

**Time course of leaf phenology, and intra-annual xylem and phloem formation in stem and branches**

The expansion of the initial earlywood vessels already started at the beginning of April, when buds were still closed (Fig. 1). By mid-April, at the time of bud swelling, about 5.8 and 9.9% of the annual xylem ring of 2015 were formed in the stem and branches, respectively. In addition, 17.7 and 13.6% of the phloem xylem ring were formed in the stem and branches, respectively. At the end of April, buds started to open and the first leaves appeared at the beginning of May. At the same time, initial earlywood vessels in the stem were fully lignified and thus ready for water transport. About 27.7 and 35.2% of the annual increments were created in xylem and phloem, respectively. In branches, initial earlywood vessels were fully lignified 1 week later than in the stem. At that time, about 83.3% of the xylem and 61.3% of the phloem annual

**Fig. 3** Structure of xylem and phloem rings in the stem and branches of *Q. pubescens* with corresponded mean values of earlywood vessel and initial early phloem sieve tube diameter and area. CC—cambium; XR—xylem ring; EW—earlywood; LW—latewood; PR—phloem ring; EP—early phloem; LP—late phloem



increments were created. Full leaf unfolding occurred in mid-May, when about 40.8 and 41.1% of xylem and phloem increments, respectively, were formed in the stem. The transition from early to late phloem took place 1–2 weeks after full leaf development in the stem and branches, respectively (Fig. 1). A week later, transition from earlywood to latewood followed in all three sampled parts.

### Structure of xylem and phloem increments of 2015 in stem and branches

Measured anatomical parameters showed statistically significant differences in the stem and branches, whereas no differences were found in samples taken at two points in the branches (Table 3). Pearson correlation coefficients among the studied anatomical variables are presented in Table 4. In 2015, the oaks had more than five times wider xylem increments in the stem than in the branches. Similarly, phloem annual increment was 45% wider in the stem than in the branches (Fig. 3). The xylem ring was wider than the phloem ring in all tree parts, being 80.1 and 55.8% in stem and branches, respectively, of the annual radial increment. The earlywood part occupied 52.9% (stem) and 74.9% (branches) of the xylem ring, whereas early phloem occupied 53.7% (stem) and 43.3% (branches) of the phloem ring. The variability in phloem and xylem increment widths as indicated by standard deviation values was higher in the branches than in the stem (Table 3).

Correlation coefficients calculated between pairs of xylem (earlywood, latewood) and phloem (early phloem, late phloem) increment widths show that many of these anatomical parameters are correlated with fairly larger number of significant correlations found for the branches than for the stem (Table 4).

Similar to increment widths, variables of conducting elements also showed statistically significant differences in the stem and branches but no differences at the two sampled points of the branches (Table 3). In 2015, *Q. pubescens* developed mostly one to two rows of earlywood vessels in the stem, with the largest ones being mostly located in the first row, i.e. at the growth ring boundary (Fig. 3). In the branches, with markedly narrower xylem rings and consequently also earlywood part, only one ring of vessels was formed in earlywood. In relation to earlywood vessel characteristics, MVD was about 18% wider in the stem than in the branches, which corresponded to a 49% larger MVA. In contrast, VD was about 40% higher in the branches than in the stem, resulting in 17% bigger WCA. In early phloem, MSD was about 27% smaller in the branches than in the stem, corresponding to a 46% smaller MSA (Fig. 3). Calculated Pearson coefficients between increment widths and parameters of conductive elements showed that only xylem ring widths and WCA were correlated in the stem (Table 4). In branches, however, correlations were found between increment width and VD and MVA in xylem. Furthermore, phloem ring and early phloem widths were related to MSD and MSA in branches.

**Table 3** Descriptive statistics and the significance of the differences ( $\alpha = 0.05$ ) among the tree parts for the anatomical variables of the xylem and phloem increments of 2015

	Stem			Branch—lower part			Branch—upper part		
	Mean	SD	Hom. group	Mean	SD	Hom. group	Mean	SD	Hom. group
Xylem ring width ( $\mu\text{m}$ )	1684.38	735.13	b	265.77	103.70	a	312.52	103.28	a
Earlywood width ( $\mu\text{m}$ )	890.49	388.28	b	207.70	6.55	a	225.56	81.76	a
Latewood width ( $\mu\text{m}$ )	793.88	376.21	b	130.06	32.20	a	116.24	52.89	a
Phloem ring width ( $\mu\text{m}$ )	417.72	76.07	b	211.53	70.97	a	247.37	67.61	a
Early phloem width ( $\mu\text{m}$ )	224.27	47.96	b	90.03	29.85	a	108.78	33.29	a
Late phloem width ( $\mu\text{m}$ )	193.46	43.72	b	121.50	46.03	a	138.60	38.83	ab
VD (no/mm <sup>2</sup> )	7.60	1.12	a	12.61	3.36	b	12.96	4.36	b
MVD ( $\mu\text{m}$ )	202.45	18.96	b	169.87	17.41	ab	162.67	36.29	a
MVA ( $\mu\text{m}^2$ )	43,144	6492	b	21,820	5430	a	22,301	7707	a
WCA (%)	31.27	5.65	a	26.01	2.60	a	26.10	5.95	a
MSD ( $\mu\text{m}$ )	35.80	2.25	b	27.03	3.23	a	24.97	3.07	a
MSA ( $\mu\text{m}^2$ )	801.41	69.61	b	453.44	121.35	a	413.82	113.83	a

SD standard deviation, VD mean earlywood vessel density, MVD mean earlywood vessel diameter, MVA mean earlywood vessel area, WCA percentage of water conductive area in earlywood, MSD mean initial sieve tube diameter in early phloem, MSA mean initial sieve tube area in early phloem

Different letters (Hom. group after Tukey's HSD test) explain significant differences between sites for a given parameter (row-wise)



**Table 4** Pearson correlation coefficients among the studied anatomical variables

Anatomical variables		Stem	Branch
Xylem ring width	Phloem ring width	0.81 ( $p = 0.000$ )	0.63 ( $p = 0.000$ )
Xylem ring width	Earlywood width	0.96 ( $p = 0.000$ )	0.87 ( $p = 0.000$ )
Xylem ring width	Latewood width	0.95 ( $p = 0.000$ )	0.75 ( $p = 0.000$ )
Earlywood width	Latewood width	0.83 ( $p = 0.000$ )	0.34
Phloem ring width	Early phloem width	0.80 ( $p = 0.000$ )	0.87 ( $p = 0.000$ )
Phloem ring width	Late phloem width	0.75 ( $p = 0.001$ )	0.91 ( $p = 0.000$ )
Early phloem width	Late phloem width	0.19	0.59 ( $p = 0.001$ )
Xylem ring width	VD	−0.33	−0.38 ( $p = 0.041$ )
Earlywood width	VD	−0.33	−0.25
Xylem ring width	MVD	−0.13	0.20
Earlywood width	MVD	−0.17	0.17
VD	MVD	−0.58 ( $p = 0.024$ )	−0.65 ( $p = 0.000$ )
Xylem ring width	MVA	0.00	0.39 ( $p = 0.034$ )
Earlywood width	MVA	0.02	0.28
VD	MVA	−0.67 ( $p = 0.006$ )	−0.64 ( $p = 0.000$ )
Xylem ring width	WCA	−0.54 ( $p = 0.037$ )	0.08
Earlywood width	WCA	−0.50	0.21
Phloem ring width	MSD	−0.24	0.43 ( $p = 0.020$ )
Early phloem width	MSD	−0.44	0.44 ( $p = 0.018$ )
Phloem ring width	MSA	−0.04	0.52 ( $p = 0.004$ )
Early phloem width	MSA	−0.26	0.45 ( $p = 0.015$ )

VD mean earlywood vessel density, MVD mean earlywood vessel diameter, MVA mean earlywood vessel area, WCA percentage of water conductive area in earlywood, MSD mean initial sieve tube diameter in early phloem, MSA mean initial sieve tube area in early phloem

$p$  value of correlation significance added

## Discussion

Linking structure to function is vital for quantifying the impact of environmentally caused changes in the dynamics of radial growth as reflected in xylem and phloem anatomy (Sass-Klaassen et al. 2016). Our observations revealed that the phenologies of leaf development and radial growth are not synchronous, nor are the temporal dynamics of xylem and phloem formation in stem and branches. This is reflected in the different xylem and phloem architectures of *Q. pubescens* in different tree parts, which is further linked with changed proportions of tree tissues and cell types, and by that with tree functioning.

### Synchronicity of xylem and phloem formation in stem and branch

The timing of radial growth in different tree parts can be rather asynchronous, as already previously observed in different tree species (Ladefoged 1952). In *Q. robur*, cambial reactivation occurs almost simultaneously in older trunk and branches. Only in the youngest branches does cambial reactivation spread basipetally and depend on bud growth (Lachaud and Bonnemain 1981; Lachaud et al.

1999). In contrast to these findings, we observed that cambium reactivated and ceased in acropetal direction. The duration of cambial cell production was a month shorter and the rate of xylem and phloem growth lower, resulting in considerably narrower annual increments in branches than in the stem. Thus, xylem increment was 83% and phloem increment 45% narrower in branches. The discrepancy in radial growth patterns within a tree reported by different authors can be ascribed to differences in tree age, species and site specifics. For example, more than 30-year-old branches of *Q. pubescens* were included in our study, whereas young twigs of *Q. robur* were used in the study of Lachaud and Bonnemain (1981).

Nevertheless, although the timing of radial growth differed in stem and branches, the time course of xylem and phloem formation was similar. In the first 3 weeks of radial growth, corresponding to the formation of early increment components, phloem growth exceeded that of xylem, and vice versa in the second part of the growing season (i.e. the period of late component formation). This pattern confirms our previous hypothesis (Gričar et al. 2014; Lavrič et al. 2017) that phloem formation has priority over that of xylem at the beginning of the growing season. Namely, a rapid build-up of the phloem pathway in spring is

necessary because (a) the developing foliage is a very large sink for carbohydrates but, at the same time, represents a small transpirational area; (b) the hydraulic conductivity and water storage of sapwood developed in preceding years efficiently support the water balance of leaves at this very early stage of development; and (c) sieve tubes function for only one to two growing seasons (Franceschi et al. 2000), so newly formed phloem cells are important for linking the storage tissues to developing leaves, which require photosynthates for respiration and biosynthesis in spring (Loeschner et al. 1990; Barbaroux et al. 2003).

### Xylem and phloem increments and their ratios

Trees rely on the efficiency of xylem to supply water and nutrients to leaves to sustain transpiration and photosynthesis, whereas phloem is important for delivering leaf carbohydrates to living and developing tissues, which are necessary for cell respiration and growth (Petit and Crivellaro 2014). Because of the strong hydraulic link between the two vascular systems through rays, Pfautsch et al. (2015) proposed considering them as a single, highly segregated system.

Different intra-annual patterns of radial growth in stem and branches results in different structures of xylem and phloem. The 2015 xylem rings were in all cases wider than those of phloem; however, the widths of xylem and phloem increments were markedly narrower in the branches than in the stem. Since the width reduction is not linear on xylem and phloem sides, the ratios between these two conducting tissues changed in favour of phloem. This trend supports the findings of Gričar et al. (2014) that, irrespective of tree vitality or growing conditions, which greatly affects the width of increments, a certain amount of phloem increment is formed every year.

In wood, the proportion of earlywood increases with a reduction in annual increment width. This part is formed in spring, usually in more favourable conditions and is presumably more internally regulated, which appears to be a successful strategy for efficient water transport in xylem (Sass and Eckstein 1995). A decrease in latewood proportion would then decrease the mechanical properties of wood, but the need for additional strength becomes less crucial as the stem increases in diameter (Rao et al. 1997). Except for parts in which tension wood was present, the latewood proportion was relatively small in branches, confirming the importance of the conducting function over the mechanical one in narrow xylem in branches of *Q. pubescens*.

Phloem tissue in the stem consists of early and late components in similar proportions, while we found less early than late tissue in the phloem of branches. Since the anatomy of phloem and xylem tissues in branches is generally very modestly investigated compared to the stem, it is difficult to infer whether the proportion can be ascribed

to differences in physical and physiological requirements in different tree parts or is a site-/species-specific phenomenon. However, for a comprehensive discussion on functional differences of secondary tissues within a tree, other bark tissues, in addition to phloem, should be included. Exploring bark anatomical traits associated with different functions would contribute to a better understanding of the ecology and physiology of bark (Rosell et al. 2015).

### Xylem and phloem anatomy

Not only the widths of xylem and phloem increments differ in the stem and branches, but also their anatomy. We found that the diameters of conductive elements in early formed parts of xylem and phloem increments were wider in the stem than in the branches, in agreement with the tapering of conduits along the stem axis (Anfodillo et al. 2012; Petit and Crivellaro 2014; Jyske and Hölttä 2015). The lack of differences in the conduit features in the two locations in branches is probably due to the relatively small difference between the two sampling points (about 0.6 m).

Vessel features strongly influence the amount of water that can be transported in a living tree. Since there is a strong vessel size–conductivity relationship because the hydraulic efficiency of vessel increases proportionally to the fourth power of its radius (Hagen–Poiseuille law), even small differences in vessel size would drastically change water transport efficiency and security (Tyree and Zimmermann 2010). We found an inverse correlation between vessel density and diameter in all sampling parts, which can be explained by hormonal regulation (Aloni 2015). Conduit width and vessel density are controlled by the auxin concentration gradient from young leaves to root tips, which acts as a morphogenetic signal. The steady downward increase in cambium circumference gradually dilutes the auxin concentration and contributes to the decrease in auxin concentrations along a tree (Aloni 2015). This gradient is thought to control the rate of cell differentiation and cell expansion. For example, high auxin concentrations near young leaves induce numerous and narrower vessels because of the rapid differentiation, whereas a decrease in auxin concentrations results in slow differentiation, which permits more cell expansion and thereby results in fewer and wider vessels, and vice versa (Aloni and Zimmermann 1983). A universal vessel diameter–stem length correlation thus exists, which, compared with climate and biomes, is the main driver of global variation in mean vessel diameter in plants (Olson et al. 2014).

### Time course of leaf phenology and seasonal radial growth

Our study confirmed that the temporal sequence of leaf development and radial growth is not contemporaneous in

*Q. pubescens*. At the time of bud swelling (mid-April), the initial parts of xylem and phloem increments were already formed. A large upward remobilization of carbohydrates occurs in the spring, preceding leaf expansion and bud break (Barbaroux et al. 2003), demonstrating that tissues formed before full leaf development rely on carbohydrate resources stored mainly during summer/autumn of the previous growing season (Barbaroux and Bréda 2002). The increased cambium sensitivity to low auxin concentrations in ring-porous trees enables its reactivation before bud break (Aloni 2015). Auxin concentrations also determine whether phloem or xylem is induced. The general developmental pattern in which phloem growth precedes xylem early in spring can be explained by changes in auxin concentrations; phloem formation starts when only extremely low-concentration auxin streams are produced (Aloni 2001). In addition to the auxin radial distribution pattern, steep concentration gradients of soluble carbohydrates (particularly sucrose) across developing vascular tissues in plants suggest a role for sugar signalling in vascular development (Uggla et al. 2001).

We found that the majority of the annual radial increment, especially in branches, was formed in the period of leaf development. When leaves were fully unfolded in mid-May, about 40–80% of xylem and 40–60% of phloem rings were formed in stem and branches. Initial earlywood vessels were already mature in order to provide essential hydraulic conductivity for axial water flow during leaf development, which is in line with the findings of Zweifel et al. (2006) for the same species. We observed the transition from early to late increment parts in a week (phloem) or two (xylem) after full development of new leaves. Ladefoged (1952) attributed this transition to differences in hormonal regulation originating from the developing and fully developed leaves. In ring-porous trees, wood formation is most active when the foliage is young, i.e. during the period of formation of early increment parts (Aloni 2015). Young leaves develop for only a short period of a few weeks in spring and produce auxin, which stimulates sieve tube and vessel formation (Aloni et al. 1997). Mature leaves, on the other hand, induce a gibberellin signal (Dayan et al. 2012), stimulating the development of lignified fibres (Aloni 2015). In addition, the second part of the growing season is also important for storage of carbohydrate resources (in parenchyma) (Barbaroux and Bréda 2002).

## Conclusions

Our study provides fundamental information on differences in seasonal dynamics of xylem and phloem formation in stem and branches, corresponding to their different anatomies. The different anatomical structures of the studied

tissues in adult stem and branches are in line with different roles of these two tree parts in terms of tree functioning. The observations further suggest that anatomical data from stem/branches cannot be extrapolated and interpreted to the whole tree level. In addition, the age, species and site dependence of these processes needs to be taken into consideration when comparing the findings of different studies.

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**Author contributions** JG, ML and KE conceived and designed the work; ML, JG and KE collected the data; all authors analysed and interpreted the data; JG and ML drafted the manuscript; all authors critically revised the manuscript; all authors approved the final version of the manuscript to be published.

## Compliance with ethical standards

**Conflict of interest** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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