Research paper

Precipitation is not limiting for xylem formation dynamics and vessel development in European beech from two temperate forest sites

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We investigated the dynamics of xylem differentiation processes and vessel characteristics in *Fagus sylvatica* L. to evaluate the plasticity of xylem structures under different environmental conditions. In 2008–10, analyses were performed on microcores collected weekly from two temperate sites: Menina planina (1200 m above sea level (a.s.l.)) and Panska reka (400 m a.s.l.). The duration between the onset and end of major cell differentiation steps and vessel characteristics (i.e., density, VD; mean diameter, MVD; mean area, MVA; and theoretic conductivity area, TCA) were analysed in the first and last quarters of the xylem rings, also in respect of local weather conditions (precipitation, temperature). Although the onset, duration and end of xylem formation phases differed between the two sites, the time spans between the successive wood formation phases were similar. Significant differences in MVD, MVA and TCA values were found between the first and last quarters of xylem increment, regardless of the site and year. Vessel density, on the other hand, depended on xylem-ring width and differed significantly between the sites, being about 30% higher at the high elevation site, in beech trees with 54% narrower xylem rings. Vessel density in the first quarter of the xylem ring showed a positive correlation with the onset of cell expansion, whereas a negative correlation of VD with the cessation of cell production was found in the last quarter of xylem increment. This may be explained by year-to-year differences in the timing of cambial reactivation and leaf development, which effect hormonal regulation of radial growth. No significant linkage between intra-annual weather conditions and conduit characteristics was found. It can thus be presumed that precipitation is not a limiting factor for xylem growth and cell differentiation in beech at the two temperate study sites and sites across Europe with similar weather conditions.

Keywords: cambium, conductivity, *Fagus sylvatica*, vessel density, weather conditions, wood formation, xylem anatomy.

Introduction

European beech (*Fagus sylvatica* L.) is a widespread European temperate tree species (von Wühlisch 2008) and is among the most economically important species for Central Europe due to its availability and the diverse usage of the wood (Meier and Leuschner 2008). In past centuries, the total area of beech forests decreased considerably due to human activity (Diekmann et al. 1999). However, in recent decades, as nature-based forestry has become widely accepted, an expansion of beech has been reported for most Central European countries, including Slovenia (Ficko et al. 2008). Changes in forest composition due to anthropogenic and/ or climatic impacts will undoubtedly affect future silviculture practice and forest management, as well as forest-based industry (Poljanec et al. 2010). Beech is considered to be a drought sensitive species (Zimmermann et al. 2015, Dulamsuren et al. 2017). On more extreme sites, such as those in the area between the Mediterranean and Alps, summer drought generally negatively affects its growth in the lowlands, whereas on high elevation sites, summer temperatures have a positive effect on tree ring width (Di Filippo et al. 2007). Despite its ecological tolerance, therefore, the

competitive capacity (advantage) of beech may change due to the increased frequency and severity of hot, dry summers (Geßler et al. 2007, Zimmermann et al. 2015) and late frosts (Menzel et al. 2015), not only at the edge of its distribution range but also in other areas that have been considered optimal for its growth (Hacket-Pain et al. 2016, Dulamsuren et al. 2017). Knowledge of intra-annual growth patterns of beech may therefore help to predict its future distribution in the context of anticipated climate change scenarios (Martinez del Castillo et al. 2016).

Beech responds plastically to environmental conditions (e.g., Rose et al. 2009, Cocozza et al. 2016), indicating that, in addition to genetic predisposition (e.g., Buiteveld et al. 2007, Vitasse et al. 2010), climatic conditions are important for its growth (Peuke et al. 2002, Di Filippo et al. 2007, Eilmann et al. 2014). Its wood formation dynamics have been monitored in various locations across Europe: for one growing season in north and south Germany (Schmitt et al. 2000, Kraus et al. 2016), in the Netherlands (van der Werf et al. 2007) and in France (Michelot et al. 2012). Observations extending over several growing seasons have been reported for Slovenia (Prislan et al. 2013), Romania (Semeniuc et al. 2014), the Czech Republic (Giagli et al. 2016) and Spain (Martinez del Castillo et al. 2016). However, the relationship between weather conditions and intra-annual variation of xylogenesis in beech is still not fully explained, due to its high intra-specific plasticity (e.g., Vitasse et al. 2010). However, studies performed along an altitudinal gradient (e.g., Kraus et al. 2016, Martinez del Castillo et al. 2016) can provide additional insight into the influence of temperature on the phenology of xylem formation in different regions of the beech distribution area.

Secondary xylem (wood), and the process of its formation (= xylogenesis), can be divided into five steps: cambial cell division, xylem cell expansion, secondary wall formation and lignification, and, in the case of sclerenchyma cells (vessels and fibres), also programmed death. After xylem differentiation is completed, the cells become functional; vessels start to conduct water and fibres mainly provide mechanical support (Plomion et al. 2001). In addition to information on wood formation patterns, detailed anatomical analyses of wood features of trees are important for understanding their short-term (e.g., Eilmann et al. 2014, Giagli et al. 2016) and long-term (Carrer et al. 2015) growth responses and functioning under different climatic conditions (Fonti and Jansen 2012, Sass-Klaassen et al. 2016). For example, water transport capacity is an important xylem functional trait because it is closely connected with a tree's water use strategy (Fonti and Jansen 2012). Although general vessel features are species-specific characteristics and hence genetically determined, their actual characteristics in different parts of xylem rings vary within these internally set limits, due to several external factors (Sass and Eckstein 1995). The plastic response of beech in terms of adjusting the wood-anatomical structure in unfavourable years (e.g., drought) has already been previously proven (e.g., Stojnic et al. 2013, Eilmann et al. 2014, Oladi et al. 2014).

In this respect, combined studies, including wood formation and quantitative wood anatomy data, are particularly valuable for clarifying site- and species-specific plasticity of xylogenesis resulting in variable wood structure (Oladi et al. 2014, Diaconu et al. 2016, Giagli et al. 2016). Certain wood-anatomical features, such as the dimensions of water-conducting cells, have been proved to be reliable ecological indicators, containing different environmental information than, for example, xylem ring widths or earlywood/latewood proportions (Fonti et al. 2010). A multiproxy approach using various growth parameters would thus permit a more holistic understanding of the influence of climate on tree growth and function (McCarroll et al. 2003).

The high intra-specific plasticity of secondary tissues in *F. sylvatica* has already been presented (Prislan et al. 2013), while the impact of the seasonal dynamics of individual phases of wood formation on wood anatomy, including vessel features, remains to be elucidated. To this purpose, we studied the seasonal dynamics of xylem radial increments in *F. sylvatica* at two forest sites in Slovenia, differing in altitude and temperature regime, during 2008–10, in order to: (i) compare the temporal dynamics of wood formation phases; (ii) compare vessel features (density, VD; mean diameter, MVD; mean vessel area, MVA; and theoretical conductivity area, TCA) between vessels formed at the beginning and at the end of the growing season, as well as between sites and among years; and (iii) explore the influence of local weather conditions on relationships between wood formation patterns and vessel features. We hypothesized that differences in weather conditions between the selected sites affect the dynamics of xylem formation (time differences in observed xylem phenological phases), which results in differences in wood anatomy (i.e., vessel features).

Materials and methods

Study site characteristics

The study was conducted at two uneven-aged mixed beech (*F. sylvatica* L.) forest sites differing in altitude. The low elevation

Table 1. Site description and average meteorological data for the period 2008–10 at ME and PA.

Site	Elevation m a.s.l.	Location	Average max. temp. (°C)	Average min. temp. (°C)	Average mean temp. (°C)	Sum of precipitations (mm)
Menina planina (ME)	1200	46°16′N, 14°48′E	10.1 ± 0.2	4.1 ± 0.6	6.7 ± 0.6	1855.7 ± 57.6
Panska reka (PA)	400	46°00′N, 14°40′E	15.9 ± 0.9	7.2 ± 0.3	11.3 ± 0.6	1564.7 ± 206.4

site, Panska reka (PA), is located near Ljubljana and the high elevation site, Menina planina (ME), is located in the Kamnik–Savinja Alps (Table 1). A detailed description of the sites was presented by Prislan et al. (2013). At both sites, there are mixed uneven-aged forests managed by sustainable forest practices.

A weather station (Davis Instruments, Hayward, CA, USA) was installed in 2008 at the high elevation site ME, in a forest clearing ~2 m above the ground, to measure air temperature and precipitation hourly. Weather data for the low elevation site PA were obtained from the meteorological station Ljubljana–Bežigrad (46°03′N, 14°30′E, 299 m above sea level (a.s.l.)) of the Environmental Agency of the Republic of Slovenia, located 10 km away. Both sites are considered favourable for beech growth; however, the annual temperatures at ME were on average 5 °C lower and precipitation 200 mm higher than at PA.

Sample collection and preparation

At each site, six dominant or co-dominant, healthy *F. sylvatica* trees were selected, with an average diameter at breast height around 50 cm and average height around 20 m. The estimated age of the trees at PA and ME was 100 and 130 years, respectively. Microcores of stem tissues were collected at weekly intervals between March and October, using a Trephor tool (Rossi et al. 2006). The samples were collected at breast height (1.3 m) following a spiral up the stem. To avoid wound effects, sampling locations were at 10 cm distance. Each micro-core contained phloem (non-collapsed and collapsed), vascular cambium and at least two of the last-formed xylem growth rings. The collected samples were embedded in paraffin, and 8–12 μm thick slides were cut with a rotary microtome and stained with a water mixture of safranin and astra blue. The sample preparation procedure is described in detail in Prislan et al. (2013). The prepared cross-sections were observed under a Nikon Eclipse 800 microscope (Tokyo, Japan) using transmission and polarized light. Histometrical analyses were performed with a DS-Fi1 digital camera and a NIS Elements BR3 image analysis system (Tokyo, Japan), with an accuracy of 1.14 μm pixel^{-1} and 0.46 μm pixel^{-1} at 40× and 100× magnification, respectively. While both magnifications were used for wood formation observations, 40× magnification was used to measure vessel features.

Analysis of xylem phenology and vessel features

The widths of the developing xylem increments were measured for three radial files in each histological section to evaluate the dynamic of xylem growth ring formation. The day of the year was recorded for the phenological phases of xylem formation, including: (i) onset of cell enlargement (bE), (ii) onset of secondary wall formation (bW), (iii) first mature cells (bM), (iv) cessation of cambial cell production, which corresponds to the end of cell expansion (cE) and (v) cessation of xylem differentiation in terminal xylem cells (cW). The phenological phases of xylem formation were identified according to the criteria described in detail in Prislan et al. (2013).

We calculated the duration of the different wood formation phases at the beginning and end of xylem growth ring formation. In the first part of the growing season, we assessed the time difference between (i) the onset of secondary wall formation and the onset of cell expansion (bW – bE), which represents the duration of cell expansion of initial xylem cells; (ii) the occurrence of the first mature cells and the onset of secondary wall formation (bM – bW), which corresponds to the duration of secondary cell wall formation of initial xylem cells; and (iii) the first mature cell and the onset of cell expansion (bM – bE), which provides the duration of cell differentiation of initial xylem cells. In the last part of the growing season, we assessed the time difference between the cessation of secondary wall formation and cell expansion (cW – cE), which corresponds to the duration of cell differentiation of terminal xylem cells. Finally, the duration of xylogenesis was evaluated as the time difference between the onset of cell production of initial xylem cells and the cessation of secondary wall formation of terminal xylem cells (cW – bC).

To compare differences between vessels formed at the beginning and at the end of the growing season and to estimate their relations with early (bE, bW, bM) and late (cE, cW) phenological phases of xylem formation, the widths of fully formed xylem increments of all analysed years (2008, 2009 and 2010) were divided into four equal parts. All vessels were then counted, and their diameters and areas were measured in the first (1/4) and last (4/4) quarters of the xylem increment, as shown in Figure 1. The analyses of vessel features were limited to the first and last quarters of xylem ring because only these two parts are possibly linked with the above-mentioned xylem phenological phases, as demonstrated in Prislan et al. (2009), in which the dynamics of vessel and fibre differentiation were studied in detail. In addition, this decision was also based on the findings of Giagli et al. (2016), who found a significant correlation between weather data and vessel features mainly in the first and last parts of growth rings in beech. The mean vessel diameter (MVD), mean vessel area (MVA), vessel density (VD, number of vessels mm^{-2}) and percentage of lumen, i.e., water conductive area (TCA, theoretical vessel area/measured xylem area) were then calculated for each sample and finally averaged (Figure 1).

Wood formation dynamics

The dynamics of xylem formation were analysed by Gompertz function (Rossi et al. 2003), using the CAVIAR package (Rathgeber et al. 2011) built for R statistical software (R Development Core Team 2014). Gompertz functions were fitted to mature increments (M) and to total increments (EWM), comprising enlarging (E), wall thickening (W) and mature cells (M), using fixed asymptotes.

$$y_{EWM,t} = A \cdot e^{-e^{\beta - k \cdot t}}$$

where $y_{EWM,t}$ is the total xylem ring width at time t; A is the upper asymptote, representing the final xylem growth ring width at the end of the growing season; β is the time axis placement
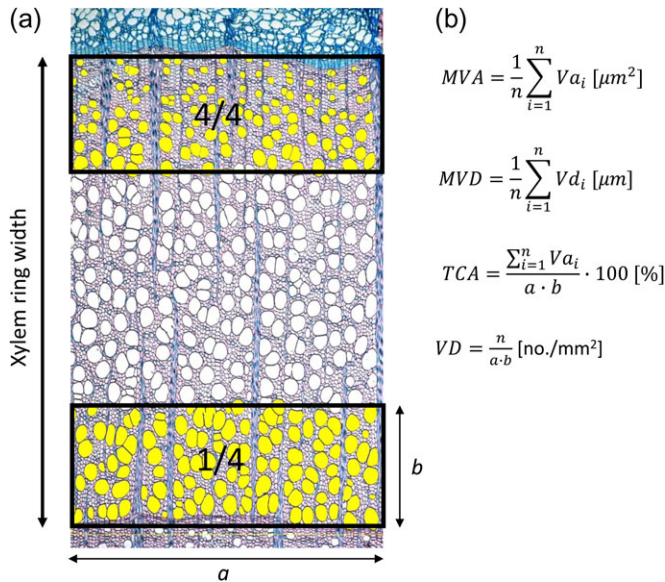

Figure 1. (a) Schematic illustration of the analysed vessel parameters in the first (1/4) and the last (4/4) quarters of the xylem ring in beech. (b) Vessel area (Va) and number of vessels (n) were measured and counted in each quarter. Vessel diameter (Vd) was calculated based on Va assuming circular lumen shape. Mean vessel area (MVA), mean vessel diameter (MVD), vessel density (VD) and theoretic conductivity area (TCA) were calculated for each sample. The tangential width (a in panel (a)) of the measured area was in general $1500 \pm 300\,\mu m$ width and varied based on slide section size and quality, while the radial dimension (b in panel (a)) is related to tree ring width.

parameter; and k is the growth-rate parameter (Rathgeber et al. 2011).

Differences in wood formation phenology and vessel features

The differences among years and between sites in the evaluated xylem formation phases, as well as the duration between these phases, were determined with one-way repeated measurements ANOVA, in which 'site' was the treatment factor and 'year of growth' was the repeated measurement. The differences in vessel characteristics (MVD, MVA, VD and TCA) measured in the first and last quarters of the tree-ring were evaluated using mixed linear models, whereby both the 'year of growth' (2008, 2009 and 2010) and the 'percentage' (first or last quarter) were included as repeated factors and the site as a fixed factor. Normality of distribution and homogeneity of variance were verified using the Shapiro–Wilk W test and Levene's test, respectively (Quinn and Keough 2002).

Relationships between wood formation phenology, weather data and vessel features

Vessel characteristics and weather relationships were evaluated by calculating average mean (T_{avr}), maximum (T_{max}) and minimum (T_{min}) temperatures and the sum of precipitation (PP_{sum}) for the periods between bE and the time when 25% of fully mature xylem increment was formed ($t_{M25\%}$), representing the conditions in which the first quarter of 'functional' growth ring increment was formed. Similarly, weather data were calculated for the period between the date on which 75% of the total annual increment was formed ($t_{EWM75\%}$) and the cW date, representing the weather conditions in which the last quarter of the annual xylem growth ring was formed. Gompertz functions
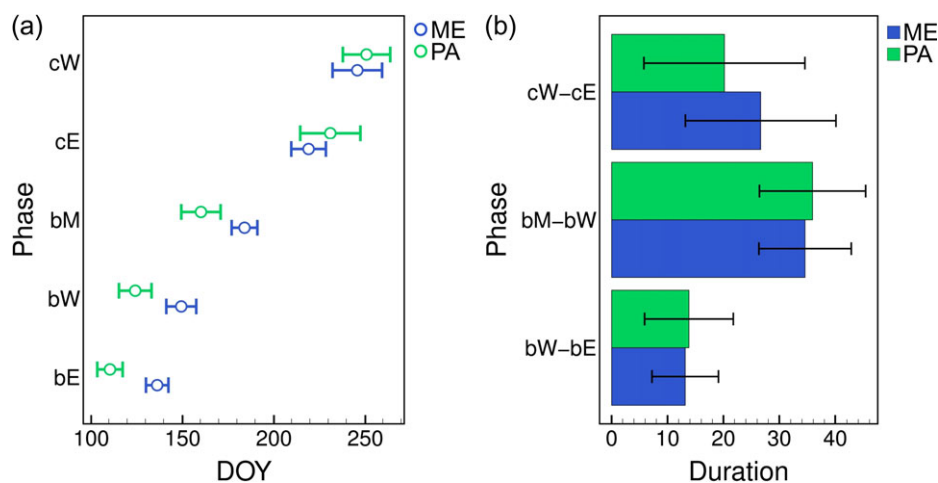

Figure 2. Phenological phases of xylem formation in *Fagus sylvatica* at Panska reka (PA) and Menina planina (ME) in 2008–10. (a) Phases of xylem growth ring formation; beginning of cell enlargement (bE), beginning of wall thickening in xylem cells (bW), first mature xylem cells (bM), cessation of cell enlargement (cE), cessation of cell wall formation and lignification (cW). (b) Duration of successive differentiation phases; duration of cell enlargement (bW − bE), duration of secondary wall formation and lignification (bM − bW) and duration of wall formation and lignification in the terminal part of the growth ring (cW − cE). Bars represent standard deviation.

calculated for each tree in each year were used to calculate the date on which a certain proportion of the growth ring was formed:

$$t_l = \frac{\beta - \ln(-\ln(l))}{k}$$

where t_l is the date on which a certain proportion of growth ring (l) was formed and β and k are the parameters of the Gompertz function.

Pearson's product moment correlation coefficient was used to measure the strength of the association among xylem formation phases, the selected weather variables (T_{avr}, T_{min}, T_{max} and PP_{sum}) and vessel characteristics (MVD, MVA, VD and TCA) calculated as yearly averages at PA and ME.

Results

Timing of phenological phases of xylem formation

Time differences (duration) among the successive phases of xylogenesis were comparable at the two sites, while the timing of their beginning and cessation differed (Figure 2; see Table S1 available as Supplementary Data at *Tree Physiology* Online). At PA, bE (see Figure S1 available as Supplementary Data at *Tree Physiology* Online) was in general observed in the middle of April and at ME almost 4 weeks later (Figure 2a), although significant differences were also found among years (Table 2; Table S1 available as Supplementary Data at *Tree Physiology* Online). bW (see Figure S1d and e available as Supplementary Data at *Tree Physiology* Online) was in general observed around 2 weeks after bE at both locations (Table 2; Table S1 available as Supplementary Data at *Tree Physiology* Online; Figure 2b), although the bW occurrence date differed significantly between the sites in all 3 years (Figure 2a; Table 2; and see Table S1 available as Supplementary Data at *Tree Physiology* Online). bM (see Figure S1f available as Supplementary Data at *Tree Physiology* Online) was observed 7 weeks after bE and 5 weeks after bW and there were no

differences between sites (Figure 2b). cE was first observed at ME and around 2 weeks later at PA (Figure 2; see Table S1 available as Supplementary Data at *Tree Physiology* Online). Xylem growth rings were fully developed (cW) at both sites at approximately the same time (see Figure S1i available as Supplementary Data at *Tree Physiology* Online); around 9 September at PA and about 5 days earlier at ME (Figure 2; Table 2). The time interval between cE and cW differed slightly but not significantly; at PA, the period lasted around 3 weeks and at ME, 4 weeks (Table 2; Figure 2b).

The duration of xylem growth ring development was considerably longer at PA than at ME. The final width of xylem increments in beech substantially differed between the sites, being twice as wide at PA (Figure 3a and b; see Table S2 available as Supplementary Data at *Tree Physiology* Online). In general, the first quarter (25%) of the total xylem increment (with cells in different differentiation stages) was formed at the end of May at PA and 2 weeks later at ME. The initial 25% of annual xylem increment was fully developed (with functional vessel elements) at the beginning of July at PA and at the end of July at ME (Figure 3c and d; see Table S2 available as Supplementary Data at *Tree Physiology* Online). The development of three-quarters of the annual xylem increment was completed by the end of July at PA and mid-August at ME (Table S2 available as Supplementary Data at *Tree Physiology* Online). The maturation of cells in the last 25% (last quarter) of the growth ring lasted ~1 month at both sites.

Vessel properties

Mean vessel area in the first 25% of the annual xylem increment was smaller at ME than at PA (Figure 4a; Table 3; see Table S3 available as Supplementary Data at *Tree Physiology* Online). However, in the last 25% of the annual xylem increment, MVA was around 55% smaller than in the first 25% of the annual xylem increment at both sites in all three years (Table 3; see Table S3 available as Supplementary Data at *Tree Physiology* Online). Mean vessel diameter was on average 34% smaller in the last than in the

Table 2. Differences between successive phenological phases of xylem formation and time differences between mayor phenological phases as evaluated with one-way repeated measurements ANOVA in which 'site' was the treatment factor and 'year of growth' was the repeated measure. Bold values are significant at a 0.05 level.

|		Site		Year		Site × Year	
		F	p	F	p	F	p
Phenological phases							
First enlarging cells	bE	**167.12**	**<0.001**	**13.77**	**<0.001**	0.43	0.656
First wall thickening cells	bW	**371.62**	**<0.001**	**12.91**	**<0.001**	1.091	0.355
First mature cells	bM	**73.31**	**<0.001**	0.988	0.344	0.001	0.001
End of cell enlargement	cE	**7.76**	**0.019**	5.205	0.046	0.511	0.491
End of lignification	cW	1.09	0.321	0.47	0.507	0.134	0.721
Time differences between phenological phases							
bW–bE		0.147	0.710	0.33	0.581	0.37	0.554
bM–bW		0.163	0.695	0.03	0.861	1.58	0.237
cW–cE		1.39	0.267	0.49	0.502	0.19	0.677
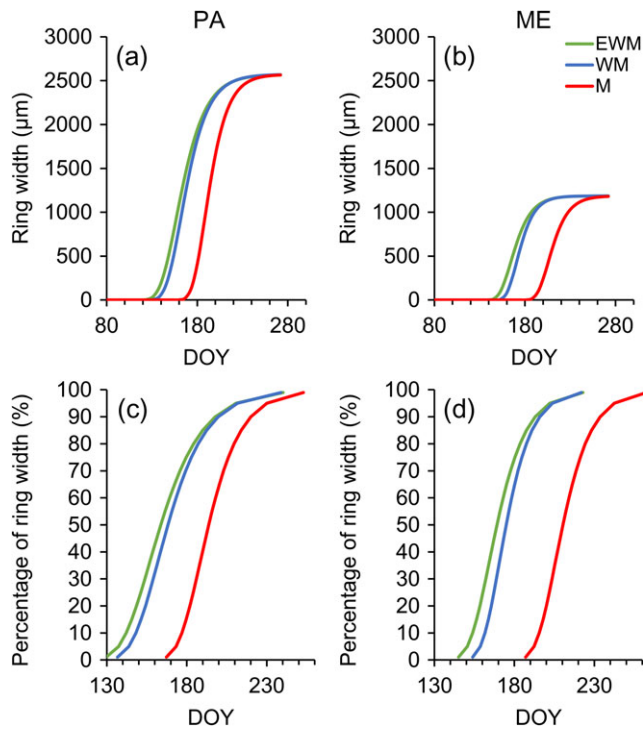
Figure 3. Intra-annual dynamics of xylem formation at Panska reka (PA) (a and c) and Menina planina (ME) (b and d) between 2008 and 2010. (a and b) Gompertz functions fitted to weekly xylem ring increments; i.e., total increment (EWM) comprised of enlarging (E) wall thickening (W) and mature cells (M), increment of mature and secondary wall forming cells (WM) and increment with only mature cells (M). (c and d) Percentage of formed annual xylem increment in different stages of development EWM, WM and M in relation to day of the year (DOY).

first quarter of the annual increment, while no significant differences in MVD in the first/last quarter of ring were observed between the two sites or among years (Figure 4b; Table 3; see Table S3 available as Supplementary Data at *Tree Physiology* Online). Theoretic conductivity area significantly differed between the first and last quarters of annual xylem ring at both sites in all three years (Figure 4c; Table 3; see Table S3 available as Supplementary Data at *Tree Physiology* Online). Vessel density, on the other hand, differed significantly between the sites in all three years, being higher at the high elevation site ME (Figure 4d; Table 3; see Table S3 available as Supplementary Data at *Tree Physiology* Online); around 20% in the first quarter and 28% higher in the last quarter. Differences between the first and last quarters of the xylem ring increment were also significant (Table 3). In general, VD was smaller in the last quarter of xylem increment; by about 25% and 8% at PA and ME, respectively (Figure 4d). Significant differences in VD were also found among years (Table 3); the lowest VD values in the first quarter of xylem increment were found in 2008 and highest in 2011 at both sites. Similar year-to-year variations in VD were also found in the last quarter of xylem increment, except for high elevation site ME, where the lowest VD values were observed in 2009 (see Table S3 available as Supplementary Data at *Tree Physiology* Online).

Relationship between vessel features, the phenology of xylem formation and weather data

In terms of vessel features, only VD showed statistically significant correlations with most of the xylem formation milestones (Table 4). In the first quarter of the annual xylem increment, VD showed a positive correlation with bE, indicating that VD is higher if cell production and cell enlargement start later (Figure 5). The duration of cell enlargement (bW − bE) and duration of wall thickening (bM − bE) in the first quarter of the growth ring showed no significant correlations with any observed vessel features (Table 4). In the last quarter of xylem increment, significant negative correlations were found between cE and VD, indicating lower VD in the case of later cessation of cell production (Table 4). In addition, the relation between VD and the duration of wall thickening (cW − cE) was found to be significant (Table 4). Furthermore, negative correlations were observed between VD in the first and last quarters of xylem ring and final xylem ring width; VD values were smaller if xylem rings were wider (Table 5). However, other vessel features (MVA, MVD and TCA) showed no correlation with xylem ring width (Table 5).

Weather conditions (T_{avr}, T_{max}, T_{min} and PP_{sum}) during the period in which the first and last quarters of the xylem ring increment were formed showed no correlation with the observed vessel features (see Table S4 available as Supplementary Data at *Tree Physiology* Online).

Discussion

Site-specific wood formation patterns in beech

We found that time lags among the successive phases of xylogenesis in beech (e.g., duration of cell expansion and duration of secondary cell wall formation of initial/terminal cells) were comparable at the two sites. It is suggested that the process of cell differentiation at the beginning/end of the growing season is not affected by local weather factors. If the investigated physiological processes are not limited by environmental factors, they may be driven mainly endogenously (Samuels et al. 2006). In contrast, the timings of beginning/cessation of xylem formation phases differed between the sites. Although xylogenesis ceased by mid-September at both sites, it started about 4 weeks earlier at the lower elevation, resulting in 121% wider annual increments, indicating that the duration of the growing season is negatively correlated with elevation (Prislan et al. 2013). Oladi et al. (2011) came to similar conclusions for *Fagus orientalis* in the subtropical Hyrcanian forest in Iran. Kraus et al. (2016) found for beech that elevation in the Bavarian Alps has a negative impact on the duration of the growing season because of delayed onset and earlier cessation of xylem formation. Martinez del Castillo et al. (2016) compared xylogenesis data on beech from different locations in Europe and detected a clear north-south trend in xylogenesis duration for beech; it was shorter in the south of Europe. The authors attributed this trend to warmer
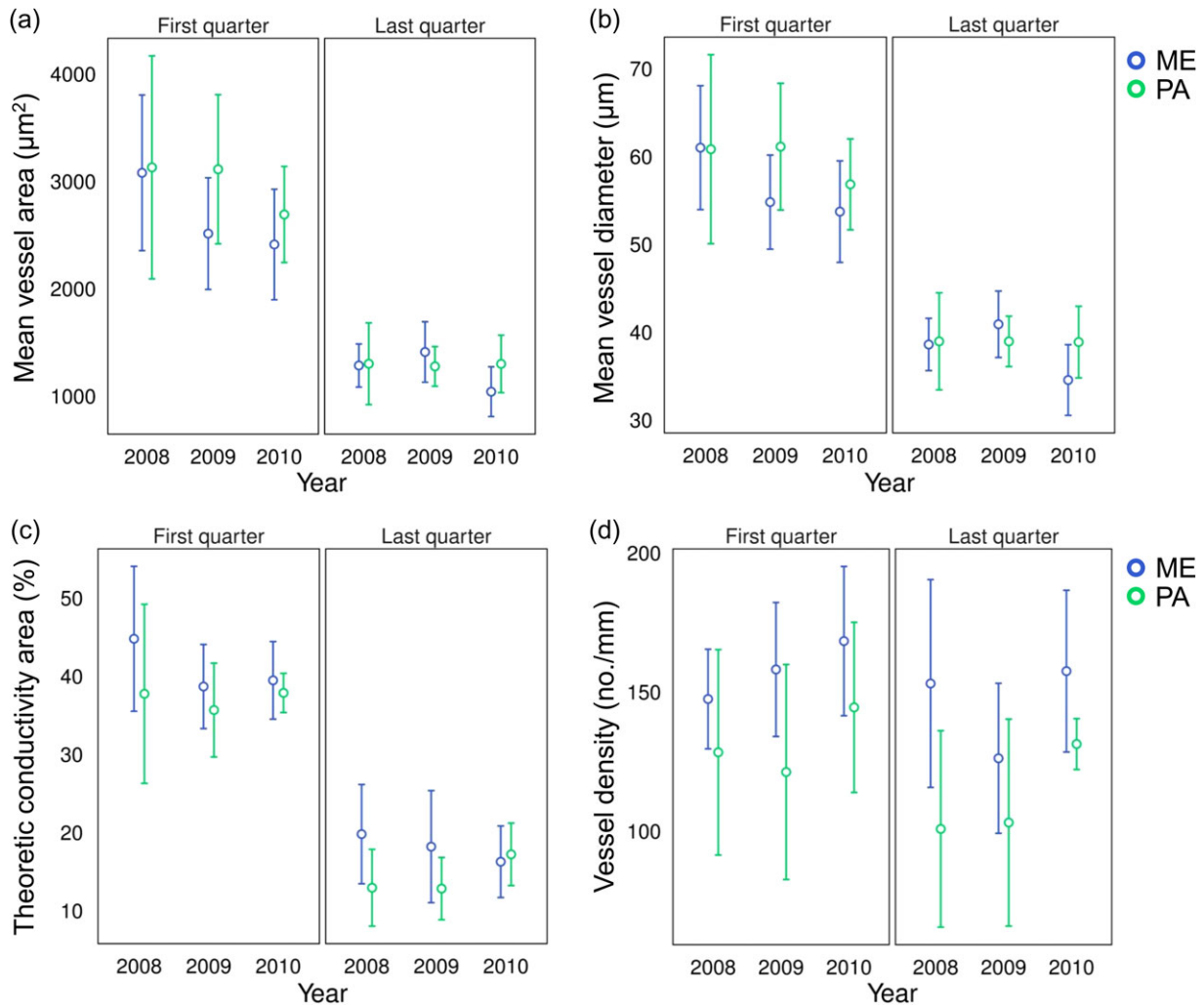
Figure 4. Spatial (Panska reka (PA)–Menina planina (ME)) and temporal (2008–10) comparison of vessel features; (a) mean vessel area (MVA), (b) mean vessel diameter (MVD), (c) theoretic conductivity area (TCA) and (d) vessel density (VD) in the first and last quarter of xylem increment in *Fagus sylvatica*. Bars represent standard deviation.

Table 3. Differences in vessel features between xylem ring part as well as between sites and years as evaluated using mixed linear models in which 'year' and 'percentage' were the repeated factors and 'site' as fixed factor. Bold values are significant at a 0.05 level.

	Mean vessel diameter (MVD)		Mean vessel area (MVA)		Theoretic conductivity area (TCA)		Vessel density (VD)	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Site (S)	2.165	0.149	2.116	0.156	2.116	0.156	**17.372**	**0.000**
Year (Y)	3.271	0.050	2.955	0.068	2.955	0.068	**4.190**	**0.022**
Percentage (P)	**206.770**	**0.000**	**160.742**	**0.000**	**160.742**	**0.000**	**4.491**	**0.039**
S × Y	0.541	0.587	0.285	0.754	0.285	0.754	0.201	0.819
S × P	0.645	0.427	1.149	0.292	1.149	0.292	0.163	0.688
Y × P	0.707	0.500	0.938	0.403	0.938	0.403	0.233	0.793
S × Y × P	1.550	0.226	1.153	0.330	1.153	0.330	0.979	0.384

and drier Mediterranean summer conditions in the south, which negatively affect the duration of xylogenesis in *F. sylvatica* (Martinez del Castillo et al. 2016). However, year-to-year differences in local weather conditions influence the radial growth of beech. For example, unfavourable weather conditions, such as a dry summer or cold spring, may reduce its growth (Dittmar et al. 2003, Čufar et al. 2008). Similarly, Giagli et al. (2016) showed that lack of precipitation caused premature cessation of cambial cell division, which resulted in markedly narrower annual xylem increments in beech.

Table 4. Correlation analysis between phenology of xylem formation and vessel features in first and last quarter of tree ring width; mean vessel area (MVA), mean vessel diameter (MVD), theoretic conductivity area (TCA) and vessel density (VD). Bold values are significant at a 0.05 level.

| Xylem formation phases	Vessel features	Pearson's correlation	P-value
First quarter of final xylem increment			
Start of cell production (bE)	MVA	−0.64	0.198
	MVD	−0.62	0.212
	TCA	0.69	0.156
	VD	**0.93**	**0.016**
Duration of cell enlargement (bW − bE)	MVA	0.34	0.525
	MVD	0.31	0.562
	TCA	0.31	0.559
	VD	−0.09	0.872
Duration of wall thickening (bM − bE)	MVA	0.38	0.391
	MVD	0.36	0.477
	TCA	0.20	0.711
	VD	−0.27	0.613
Last quarter of final xylem increment			
Cessation of cell enlargement (cE)	MVA	0.37	0.490
	MVD	0.41	0.436
	TCA	−0.75	0.111
	VD	**−0.88**	**0.033**
Duration of wall thickening (cW − cE)	MVA	−0.61	0.223
	MVD	−0.62	0.208
	TCA	0.61	0.217
	VD	**0.92**	**0.017**
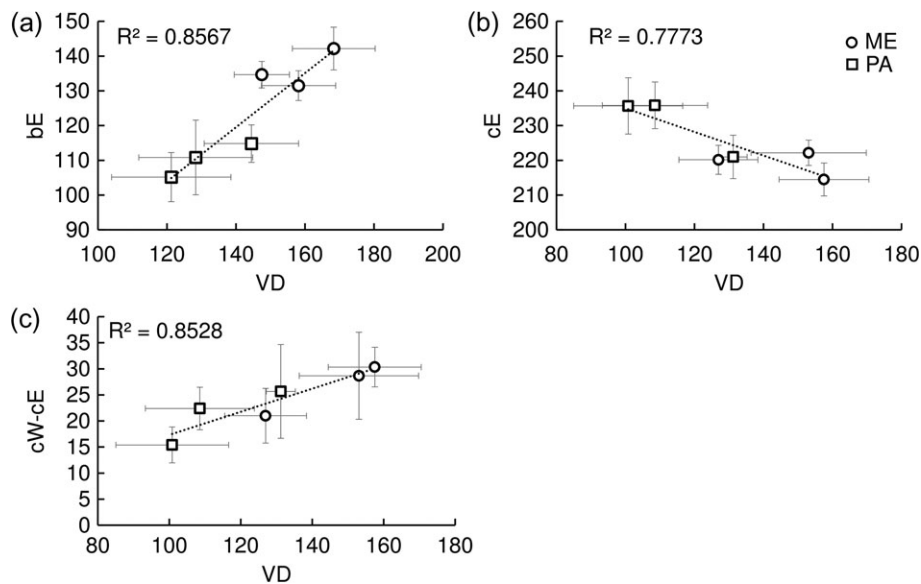
Figure 5. Relationships between bE (onset of cell production), cE (cessation of cell production), cW − cE (duration of cell wall thickening) and vessel density (VD) in first and last quarters of xylem growth ring in *Fagus sylvatica* at Panska reka (PA) and Menina planina (ME) during 2008–10 calculated as yearly averages.

Intra- and inter-annual variation of vessel features between sites

Depending on environmental conditions, the vessel distribution pattern in beech wood may vary from a diffuse-porous pattern formed in favourable growing conditions to a semi-ring-porous pattern in the case of water-limiting conditions (Bosshard 1982). We found significant differences in VD values between the two sites, which also correlated with xylem ring width. The MVA, MVD and TCA values significantly differed between the first and last quarters of xylem increment, irrespective of the site and year, being higher in the first quarter of the xylem ring. Larger vessels at the onset of radial growth can be explained by lower evaporative demand and

Table 5. Relationships between xylem ring width and vessel features in first and last quarter of tree ring width; mean vessel area (MVA), mean vessel diameter (MVD), theoretic conductivity area (TCA) and vessel density (VD). Bold values are significant at a 0.05 level.

	Vessel features	Pearson's correlation	P-value
First quarter	MVA	0.53	0.297
	MVD	0.52	0.307
	TCA	−0.77	0.092
	VD	**−0.87**	**0.038**
Last quarter	MVA	0.34	0.52
	MVD	0.40	0.444
	TCA	−0.70	0.144
	VD	−0.83	0.057

higher water availability in spring (Schume et al. 2004). Sass and Eckstein (1995) found smaller variability in the size of initial vessels and interpreted this by more favourable growing conditions, especially in terms of moisture supply at the beginning of the growing season. Furthermore, Sass and Eckstein (1995) found comparable VD within xylem rings and concluded that this variable does not provide much information on external influences at the study sites. In contrast, we found that VD differed significantly between the first and last quarters of the same xylem ring, as well as between sites, being about 30% higher at the high elevation ME site. The negative relation of VD with xylem ring width in beech found in our study is in line with the previous observations of Pourtahmasi et al. (2011), Stojnic et al. (2013), Eilmann et al. (2014) and Giagli et al. (2016). Furthermore, Schume et al. (2004) detected a negative link between MVD (MVA) and VD. We confirmed this trend only in the first quarter of the xylem ring, while it was not significant in the last quarter. This indicates that relationships among vessel features can vary within a single ring. In addition, it shows that the MVD (MVA) and VD relationship is stable at the beginning of the growing season. Vessel features and their climatic sensitivity are also affected by site properties, including microtopography, soil properties and rock type, which undoubtedly differ among the study sites of the above-mentioned studies, and may considerably influence runoff, distribution and water movement in the soil.

As the conduit diameter is closely related to tree height (Anfodillo et al. 2013), this information is crucial for comparison of conduit diameters among different trees and sites (Carrer et al. 2015). However, the trees selected for our study had comparable heights, so increased VD in the case of reduced increment clearly demonstrated the plasticity of xylem formation processes and anatomy in terms of changing cell type proportions or their characteristics in response to site conditions. Maintaining equilibrium among the main xylem functions (i.e., efficiency, safety, support and storage) may represent an adaptation of beech trees to acclimatize to local environmental conditions (Borghetti et al. 1993, Peuke et al. 2002, Herbette et al. 2010, Pratt and Jacobsen 2016). However, since total TCA in

beech consists of several growth rings, the influence of the last annual increment on the total water conducting capacity is only partial (Gasson 1985, Jupa et al. 2016).

Impact of wood formation dynamics on variability in vessel features

Differences in the timing of xylogenesis dynamics between the sites were only partly reflected in different wood structures, which, as already mentioned above, can be ascribed to site-specific strategies of beech to ensure a sustainable water-conducting system. Our findings show a significant relation between VD and the onset/end of cell enlargement. A negative link between cessation of cell enlargement and VD can be linked to xylem-ring width; a later end of cell production is generally related to wider increments, which is inversely related to VD. Processes of vascular differentiation may be regulated by environmental cues through hormonal mechanisms (Aloni 2015). There is a positive correlation between auxin concentration and VD, but a negative correlation between auxin concentration and vessel diameter (Aloni 2015). According to Aloni and Zimmermann (1983), high-auxin concentrations accelerate cell differentiation processes, so smaller vessels are formed, whereas at low auxin concentrations, cell differentiation is slowed down, causing the formation of vessels with wider diameters. However, in addition to auxin, the differentiation process is largely controlled by other hormones, such as gibberellin, which originates in mature leaves, and cytokinin, which is produced in roots (Aloni 2013). Interestingly, no correlation was found between vessel size (MVA, MVD) and the onset of cell enlargement, suggesting that the rate of vessel expansion at the beginning of the growing season may be more important for the final vessel size than the duration of its expansion.

Impact of local weather conditions on vessel features and their hydraulic functioning

Vessel features reveal the environmental conditions that prevailed before and during their formation (Fonti et al. 2010). In particular, vessel size is controlled by water supply, because turgor pressure, as the driving force of cell expansion, is largely affected by water availability (Tyree and Sperry 1989). Drought thus negatively affects vessel growth, which is reflected in reduced vessel size (Sass and Eckstein 1995, Arend and Fromm 2007, Eilmann et al. 2014). In view of the strong vessel size–conductivity relationship, and since the hydraulic efficiency of a vessel is subject to the Hagen–Poiseuille law, even small differences in vessel size would drastically change water transport efficiency and security (Tyree and Zimmermann 2010). Vessel size is a trade-off between water transportation efficiency and vulnerability to cavitation, because wider vessels increase water-transport efficiency (Sperry et al. 2006) but also increase the risk of drought-induced cavitation (Stojnic et al. 2013). In our study, however, we could not confirm any significant link between precipitation (or other weather conditions) and conduit

characteristics in beech at the selected sites. The lower sensitivity of vessel diameter of diffuse porous tree species to hydrological alterations was also demonstrated by Schume et al. (2004) and Oladi et al. (2014). Pourtahmasi et al. (2011) noted that vessel variables as such do not necessarily provide added value in dendroclimatological/dendroecological studies; rather this depends on tree species, tree age, tree condition and site factors. Giagli et al. (2016), on the other hand, demonstrated that wood formation patterns and vessel features in *F. sylvatica* showed different responses to climatic factors in two contrasting years, characterized by different amounts of precipitation. In a normal year (with 590 mm annual precipitation), vessel size was mainly affected by air temperature, particularly at the beginning of xylogenesis, whereas soil moisture content was critical for vessel diameter at the beginning of the growing season in a dry year (344 mm of annual precipitation). As already mentioned above, water availability also impacts the vessel distribution pattern in beech wood, being diffuse-porous in favourable years and semi-ring porous in dry years (Bosshard 1982, Schweingruber 2007). It can thus be presumed that water availability is not a limiting factor for growth of beech at the two temperate study sites, which is evident from the annual amount of precipitation exceeding 1000 mm at both sites in the period 2008–10 (Table 1). Different but still fairly optimal local environmental conditions may allow the relative independence of vessel formation and vessel feature adjustments in *F. sylvatica* from the weather (precipitation) regime. Long-term variations in xylem-ring widths at the lower elevation, however, revealed that summer precipitation favours xylem growth of beech. This indicates that, in the case of dry, hot summers, precipitation may become a limiting factor for the growth of beech (Čufar et al. 2008). In addition, beech wood is vulnerable to freezing-induced embolism, which increases gradually during the winter (Cochard et al. 2001), especially in trees growing at high elevation sites. The vulnerability of xylem to embolism by freezing increases with the size of water-conducting cells (Améglio et al. 2001). Smaller vessel size in the last quarter of the growth ring (formed at the end of the growing season) may also improve resistance to early and late frost events in the next spring (Cochard et al. 2001). However, Lemoine et al. (1999) also pointed out that embolism formation in beech may be more related to the dynamics of sap freezing than to vessel features. Beech is reported to be a very sensitive European tree species to photoperiod in its spring leaf phenology (Vitasse et al. 2014). Since leaf and xylem phenologies are not linearly related and are under different environmental control, the intra-annual temporal sequences of their development may not be constant (Lavrič et al. 2017). This means that these sequences (i.e., the timing of cambial reactivation, leaf-development and their inter-relationship) may also affect the anatomical structure of xylem rings. For example, low temperature in spring may negatively affect leaf phenology, i.e., bud break, and thus polar auxin transport and/or

cambial sensitivity to auxin, which would then influence vessel features (Aloni 2015). Long-term analyses of time-series of the xylem anatomy of trees from contrasting locations in combination with leaf phenology data are therefore required to argue the potential of different vessel features in beech as bioindicators for environmental changes.

Conclusion

The results of our study show differences in the dynamics and phenology of wood formation processes between the selected sites, showing that local weather conditions (predominantly temperature) affect the onset and end of cambial cell production (Prislan et al. 2013). However, time intervals between successive phenological phases (e.g., between onset/end of cell expansion and onset/end of secondary wall formation of initial cells) were similar at the two sites, suggesting that these processes are controlled predominantly endogenously or are subjected to different environmental limits.

Vessel features significantly differed within the xylem growth rings; in the first quarter, values were found to be in general higher than in the last quarter of xylem ring width. However, no differences were found among years and between sites in MVD, MVA and TCA. The only significant differences between sites were found in VD, which showed a strong positive relationship with onset of cell enlargement and a negative relationship with cessation of cell enlargement and, consequently, with xylem ring width. We were not able to confirm any significant relationship between conduit characteristic and intra-annual weather data; our hypothesis was therefore rejected. On the selected sites, environmental factors may affect the timing of xylem formation (Prislan et al. 2013) but not the time differences between successive xylem cell differentiation processes and vessel characteristics. The results suggest that these processes are not directly related and may exhibit different 'critical conditions/environmental thresholds'. In addition, it can be presumed that precipitation is not a limiting factor for xylem growth and vessel development in beech at the two temperate study sites and sites across Europe with similar weather conditions.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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