

Xylem traits in European beech (*Fagus sylvatica* L.) display a large plasticity in response to canopy release

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Abstract

• **Key message** The position of trees in the canopy impacts xylem structure and its inter-annual variation. After canopy release, the increase in the hydraulic conductivity of growth rings was driven by an increase in radial growth in large trees, and by both an increase in radial growth and changes in xylem structure in saplings.
• **Context** Forest canopies are frequently subjected to disturbances that allow understory trees to access the upper canopy. The effect of canopy release on xylem anatomy has been assessed in juvenile trees and saplings, while the potential acclimation of larger trees remains poorly documented.

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Contribution of the co-authors

Estelle Noyer collected the data, ran the data analysis and was the main writer.

Barbara Lachenbruch, Jana Dlouha, Catherine Collet and Meriem Fournier contributed to the discussions and to the writing.

Julien Ruelle developed image analysis tools and contributed to the discussions.

François Ningre designed the Grand Poiremont forest experiment.

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• **Aims** We estimated the potential hydraulic conductivity of growth rings in large understory trees compared to overstory trees, and evaluated the responses to canopy release in large trees and in saplings.

• **Methods** We recorded radial growth, wood density, and vessel structure in beech trees according to their position within the canopy and their size. Xylem traits were followed during 6 years after canopy release for large trees, and during 2 years for saplings. Vessel diameter and frequency as well as ring area were used to compute the potential annual ring hydraulic conductivity.

• **Results** Large understory trees displayed lower radial growth increments and lower potential annual ring hydraulic conductivity than overstory trees. After canopy release, potential annual ring hydraulic conductivity increased in large trees, due exclusively to increased radial growth without any change in specific hydraulic conductivity. It increased in saplings due to both increased radial growth and increased specific conductivity.

• **Conclusion** Tree size impacted xylem structure and resulted in plasticity of the potential hydraulic conductivity of the annual tree ring following canopy release.

Keywords Xylem anatomy · Vessel · Potential hydraulic conductivity · Radial growth · Wood density

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1 Introduction

Trees face changing environments throughout their lifetime because their locations may experience environmental change, and also because they sample locations differently as they grow. By examining modifications in stem structure such as microclimate changes, ecophysiological responses may be uncovered. The present work focuses on plasticity in xylem traits of stems in response to tree position within the canopy. European beech (*Fagus sylvatica* L.), a diffuse-porous species with a wide functional sapwood area (Gasson 1985), was chosen as the model species because of its known plasticity to growth conditions (Stojnic et al. 2013; Eilmann et al. 2014; Schuldt et al. 2016). It is a shade-tolerant species that can survive under a high level of inter-tree competition, and can rapidly respond to changes in canopy structure and local irradiance (Lemoine et al. 2002a, 2002b; Caquet et al. 2009a).

The potential specific hydraulic conductivity at tissue level (K_S) is a function of vessel diameter and frequency (Tyree and Zimmermann 2002). Slight modifications in vessel diameter have a large impact on hydraulic properties (Gartner 1991; Badel et al. 2015). Trees can also alter the conductivity in the whole stem by increasing sapwood width or by increasing the width of the new growth rings (Maherali et al. 1997), independently of any change in K_S .

Understory trees grow under lower irradiance levels, lower wind speeds and lower extremes in air temperature and vapor pressure deficit (VPD) (Parker et al. 1995; Aussenac 2000). They are shorter, exhibit smaller stem diameters, narrower annual growth increments, and lower sapwood area (Nicolini et al. 2001; Löf et al. 2005; Petritan et al. 2009; Klopčic and Boncina 2010; Fajardo 2016), and may have access to less water than overstory trees (Bréda et al. 1995). While modifications in xylem traits reflect tree acclimation (Fonti et al. 2010; Anderegg and Meinzer 2015), there is little literature on xylem anatomy and function in overstory vs understory trees from temperate forests. Most studies focus on sun-exposed and shaded branches on the same individual (Lemoine et al. 2002a; Gebauer et al. 2014), which is not representative of acclimation at tree level. Some studies suggest that because of lower access of understory trees to water, it would be to their advantage to display a larger resistance to soil water shortage than overstory trees. And because of lower irradiance (Hacke 2014), shorter path length (Preston et al. 2006), and possibly lower dry growth conditions (Hajek et al. 2016), smaller vessels might occur in understory trees. However, other studies suggest that the lower evaporative demand and shorter stem could lead to lower tension in the xylem column of understory trees (Granier et al. 2000) and, therefore, to wider vessels.

Another case in which xylem plasticity could play an important role is after understory trees are released from inter-tree competition. Natural or anthropogenic disturbances periodically affect most forest ecosystems (Rentch et al. 2010), and provide the opportunity for understory trees to reach the canopy (Webster and

Lorimer 2005). Such released understory trees then have to cope with severe and sudden changes in their environment. To our knowledge, the acclimation process of stem xylem after canopy release, including the timing and nature of changes in xylem traits, has not been fully characterized. Various studies analyzing xylem traits of released angiosperm trees focused on the instant response to canopy release. While some authors observed an increase in vessel diameter and a decrease in vessel frequency (Hoffmann and Schweingruber 2002; Caquet et al. 2009a), others did not observe such changes (Maherali et al. 1997). Moreover, enhanced radial growth following canopy release has been repeatedly observed (Collet et al. 2001; Hartmann and Messier 2011). Large-diameter trees have a greater potential than smaller trees to increase stem conductivity through enhanced radial growth, due to wider annual rings (Caquet et al. 2009b; Klopčic and Boncina 2010) and larger relative basal area increments. Therefore, the relative importance of changes in xylem structure vs changes in ring width for altering water transport capacity may depend on tree size.

The study focuses on the response of stem xylem traits to tree position within the canopy and to canopy release, assessed in experimental sites with either large or small beech trees. We recorded xylem structure and growth increment to reconstruct hydraulic conductivity of tree-rings in the past. Such a retrospective approach does not allow either any direct measurement of stem conductivity or the ability to access past sapwood width or total stem conductance. Thus, we concentrated on changes in potential hydraulic conductivity at tree ring level. We tested the following hypotheses:

- (H1) understory trees display a xylem structure with smaller vessel diameter and higher vessel frequency;
- (H2) because of lower year-to-year variation in the micro-environment, understory trees exhibit a more stable xylem structure from year to year than overstory trees;
- (H3) after canopy release, understory trees increase the conducting area and change the number and size of vessels, and attain similar potential annual ring hydraulic conductivity than overstory trees within a few years;
- (H4) after canopy release, small trees display larger adjustments in xylem anatomy, in addition to radial growth as a basis for increased potential ring conductivity, than large trees which have larger tree-ring sections.

2 Material and methods

2.1 Study sites and plant material

2.1.1 Large trees

The site for large trees was a 13-ha stand in a managed forest in north-eastern France (47.9507°N, 6.3857°E, alt: 470 m).

The soil was dystric to hyperdystric cambisol with a luvic layer between the A and S horizons (IUSS Working Group WRB 2014). Meteorological data were recorded from 2001 to 2013 from the Aillevillers-et-Lyaumont weather station (French National Climatic Network, Météo-France) 5 km from the stand. Over that period, the mean annual temperature was 10.3 °C (Online Resource 1). The mean annual precipitation was 1,218 mm. During 2003 and 2011, years with very dry summers in Europe, temperatures were slightly higher and precipitation slightly lower than long-term means.

The broadleaved stand had formerly been managed as a coppice-with-standards. In 1955–1956, however, the stand was thinned and converted to a high forest. Records show it was further thinned between 1956 and 1995, but the years of thinning were not recorded. After 1995, there was no further thinning. In 2006, the stand was dominated by *Fagus sylvatica* L. (basal area: 21 m² ha⁻¹) with another 5.5 m² ha⁻¹ of *Quercus* spp., *Fraxinus excelsior* L., *Acer pseudoplatanus* L., *Carpinus betulus* L., *Betula* spp., *Abies alba* Mill, and *Picea abies* L. (H) Karst. Stand density was 513 stems ha⁻¹ and the mean height of the overstory trees was 31.6 m.

During fall 2007, we selected a sample of 42 understory beech trees distributed throughout the stand and at least 18 m from one another. The trees originated from seeds, and grew under closed canopy or in small gaps. Sample trees met the following criteria: DBH was 7.5 to 17.5 cm, stems were unforked, leaned <11°, had fewer than 25 epicormic branches (sensu Colin et al. 2012) along the lowest 4 m of stem, and had no visible injury, spiral grain, canker, or top dieback. The sample trees were then split into two subsamples with similar mean values for diameter, height, and relative vertical crown length (Table 1). During winter 2007–2008, one subsample was released by a thinning that removed the competing trees in a 12-m radius around each target tree (hereafter referred to as “released” trees) and the other subsample of trees was left unreleased (“suppressed” trees) (Ningre et al. 2011).

Five overstory, healthy, and relatively straight beech trees were also selected. They were later found to be among the oldest canopy trees of the stand (Table 1).

2.1.2 Saplings

The site for the saplings (49.0778°N, 6.0172°E) (Caquet et al. 2009b; Caquet et al. 2009a) is at a distance of about 150 km from the large tree site and at a similar altitude (300 m). The soil is a calcisol (40–60 cm depth). Mean annual temperature is 10.1 °C and annual precipitation is 745 mm (French National Climatic Network, Météo-France).

The stand was formerly a broadleaved coppice-with-standards converted to a high forest during the 1960s. In 1999, a storm created scattered canopy gaps. In 2006, the forest canopy was mainly composed of *F. sylvatica*, *Acer pseudoplatanus* L., *Acer platanoides* L., *Carpinus betula* L. and *Quercus* spp.

During January 2005, four advanced regeneration patches were selected under closed canopy and at least 300 m from one another. Canopy gaps of 500 to 1,900 m² were created by removal of all overstory trees above two patches (leaving the “released” understory saplings) and the two other patches were left unthinned (“suppressed” saplings). Two 0.5- to 1-m-tall beech saplings were selected for measurement in each patch. Additionally, four beech saplings were selected in gaps that had been created by the 1999 storm (“acclimated” saplings) (Table 1). During summer 2005, relative irradiance (with respect to the irradiance above canopy) was estimated above each selected sapling with hemispherical photography. It reached, on average, 5, 31, and 60% for the suppressed, released, and acclimated saplings respectively (Caquet et al. 2009b).

2.2 Measurements

2.2.1 Large trees

The released and suppressed understory trees were felled during March 2014, 6 years after canopy release. Two 5-cm-thick disks were collected at a height of 1.30 m from each tree and wrapped in plastic film. The first disk, used for anatomical measurements, was stored at 4 °C immediately after

Table 1 Sample tree characteristics (number of trees, age, and tree height range, mean ± standard error of stem diameter and cross-section area) in 2013 for large trees and in 2006 for saplings

	Large			Sapling		
	Overstory	Understory suppressed	Understory released	Acclimated	Understory suppressed	Understory released
Number of trees	5	21	21	4	4	4
Age	83–117*	54–108		11–18		
Tree height (m)	27.7–31.6	12.4–25.1		0.5–1.0		
Stem diameter (cm)	52.60 ± 0.91	12.13 ± 0.52	14.98 ± 0.57	0.92 ± 0.48	0.50 ± 0.04	0.74 ± 0.07
Stem cross-section area (cm ²)	2150.81 ± 150.49	132.93 ± 16.01	181.29 ± 13.68	0.68 ± 0.11	0.20 ± 0.03	0.44 ± 0.08

* at breast height (1.30 m)

harvesting. Using growth stress indicator measurements (Fournier et al. 1994), we identified the radial position of expected tension-wood (Purba et al. 2015). Radii perpendicular to the tension-wood zone and without knots were considered to be normal wood. A radial strip (0.5 cm × 1.8 cm, T × L) was then cut in the normal wood zone and divided into successive 1.5-cm-long segments. Each segment was embedded in successive polyethylene glycol baths (30%, 50%, and 100% w/v). Twelve-μm-thick cross-sections were cut from each segment with a sliding microtome. They were double-stained with a specific staining procedure as follows. They were first stained for 1 min in aqueous Safranin (1% w/v), treated with hydrochloric alcohol for 1 min, rinsed with distilled water, then stained with Astra blue (1% w/v) for 5 min, rinsed with distilled water, and dehydrated sequentially with 96% and absolute ethanol. Finally, the sections were mounted on glass slides in Histolaque LMR©. Each section was then imaged at ×10 magnification with a digital camera (Sony XCD-U100CR) through a light microscope (Zeiss AxioImagerM2) with image acquisition software (Archimed software, Microvision Instruments, France). For each image, three to six sectors bounded by rays were defined per ring to collect data related to vessel number and geometry. Sectors typically contained 25 to 200 vessels.

The second disk was used for ring width and wood density measurements. It was stored at −20 °C immediately after harvest. After disks were sanded, four perpendicular radii were imaged by digital camera. On each radius, the width of each ring (RW, mm) from pith to bark was measured to a precision of 0.01 mm, by image analysis using TSAP-Win (Rinntech, Germany).

One of the normal wood radii was used for wood density measurements. A radial strip (2.5 cm × 5 cm, T × L) was cut and conditioned for 1 month at 12% relative humidity at ambient temperature. A radial slice (1.0 × 0.2 cm², T × L) was then cut for X-ray micro-densitometry measurements with a microfocus X-ray source (Hamamatsu L9181–02130 kV) and a digital X-ray detector (Varian PaxScan 4030R). Pixel size varied between 20 to 30 μm. The Crad and Cerd software suite was used to compute radial wood density (WD, kg m⁻³) profiles (Mothe et al. 1998).

The selected overstory trees were sampled with an increment borer. In June 2015, two 5-mm-diameter cores were collected at 1.30 m in height. Cores were stored at 4 °C. These cores were used for anatomical and X-ray micro-densitometry measurements, using a protocol similar to the one used for understory trees. Ring widths for the overstory trees were estimated from X-ray data.

2.2.2 Saplings

Understory saplings were harvested from late August to mid-September 2006, two growing seasons after treatments had

been applied. A 2- to 3-cm-long stem portion was cut from each sapling from just above ground level. It was stored at −20 °C until it was analyzed.

A 12-μm-thick cross-section was then cut from each stem portion using a sliding microtome (SM2000, Leica Jung, Nussloch, Germany). Sections were double-stained as for the large trees, and mounted on slides with Canada balsam. Images were taken and analyzed as above, to measure ring width. Vessel number and geometry were measured in three to six sectors bounded by rays in each of the three most recent (youngest) growth rings.

2.3 Data analysis

For each tree and sapling, annual ring width and annual basal area increment (BAI, cm²) was estimated from the position data for ring boundaries, averaged over the four radii. BAI was estimated as follows:

$$BAI = \pi (r_1^2 - r_2^2) \quad (1)$$

where r_1 and r_2 (cm) are the average of the radii from 2 successive years.

The Hagen–Poiseuille diameter is the vessel diameter which, if multiplied by the observed vessel frequency, gives the same potential specific conductivity as the actual vessel diameter (VD) distribution. Following Steppe and Lemeur (2007), the Hagen–Poiseuille vessel diameter D_{HP} (μm) equation was adjusted for elliptical vessel (Fig. 1) and computed as:

$$D_{HP} = \sqrt[4]{\frac{1}{n} \sum_{i=1}^n \frac{2a_i^3 b_i^3}{a_i^2 + b_i^2}} \quad (2)$$

where n is the number of measured vessels, and a and b (μm) are the two axes of the ellipse (maximum and minimum lumen diameter, respectively).

The potential hydraulic conductivity is the total estimated conductivity of a sample of vessels assuming that their flow is governed by the Hagen–Poiseuille equation. Potential specific hydraulic conductivity K_s (kg m⁻¹ s⁻¹ MPa⁻¹) was calculated as follows:

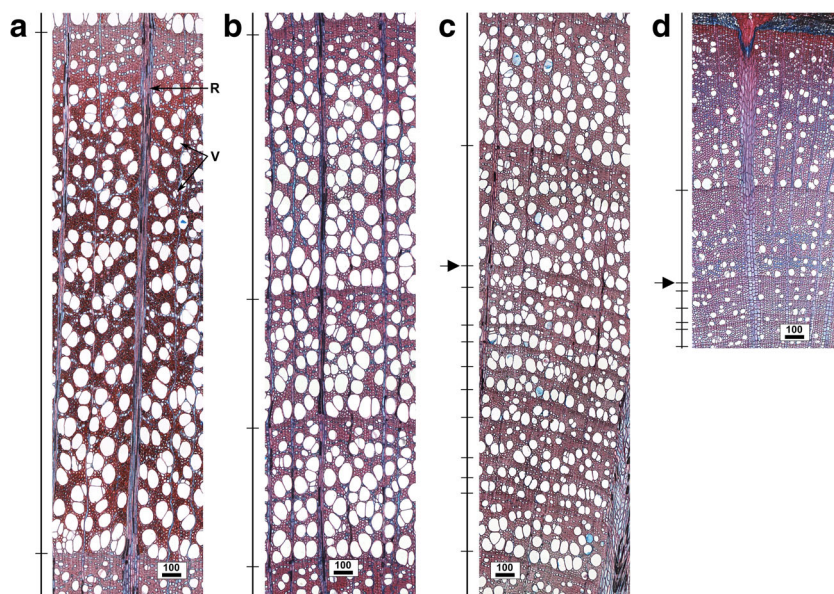
$$K_s = \left(\frac{\pi \rho}{128 \eta} \right) \cdot D_{HP}^4 \cdot VF \quad (3)$$

where η is the viscosity of water (1.002 × 10⁻⁹ MPa s⁻¹), ρ the density of water (998.21 kg m³), D_{HP} is the Hagen–Poiseuille vessel diameter (m) and VF (n m⁻²) is the mean vessel number per square meter.

The potential hydraulic conductivity for a ring K_{ring} (kg m s⁻¹ MPa⁻¹) is estimated by:

$$K_{ring} = K_s \cdot BAI \quad (4)$$

Fig. 1 Images of large overstory (a and b) and understory (c) trees and saplings (d). At the left of each image, ticks represent tree-ring boundaries and the arrow the ring the first year after a release event. V: vessels; R: ray parenchyma. Bars: 100 μm



For each measured or computed trait and for each year, trees or saplings in different treatments were compared using a Kruskal–Wallis test. On large trees, if the associated p -value was below 0.05, a post-hoc Wilcoxon–Mann–Whitney test was made. Large trees and saplings were tested independently. All statistical analyses were performed using R software, version 3.2.3 (R Core Team 2015).

To identify the variance contribution of each factor (D_{HP} , VF and BAI) to the K_{ring} variation, we computed variance partitioning of log transformed Eq. (5) as follows:

$$\begin{aligned} \text{Var}(\log_{10}K_{ring}) &= \text{Var}(4\log_{10}D_{HP}) + \text{Var}(\log_{10}VF) \\ &+ \text{Var}(\log_{10}BAI) \\ &+ 2 \text{Cov}(4\log_{10}D_{HP}, \log_{10}BAI) \\ &+ 2 \text{Cov}(4\log_{10}D_{HP}, \log_{10}VF) \\ &+ 2 \text{Cov}(\log_{10}VF, \log_{10}BAI) \end{aligned} \quad (5)$$

The covariance factor expresses the interaction between individual factors that can be synergistic (positive value) or compensative (negative value). A covariance value close to zero means that factors are independent.

Results are presented as a relative contribution to total variance and are computed for the second year after release for understory saplings and large trees in comparison to suppressed individuals.

3 Results

3.1 Large trees

BAI of overstory trees varied widely among years (Fig. 2a) and BAI was at least 15-fold higher in overstory than in

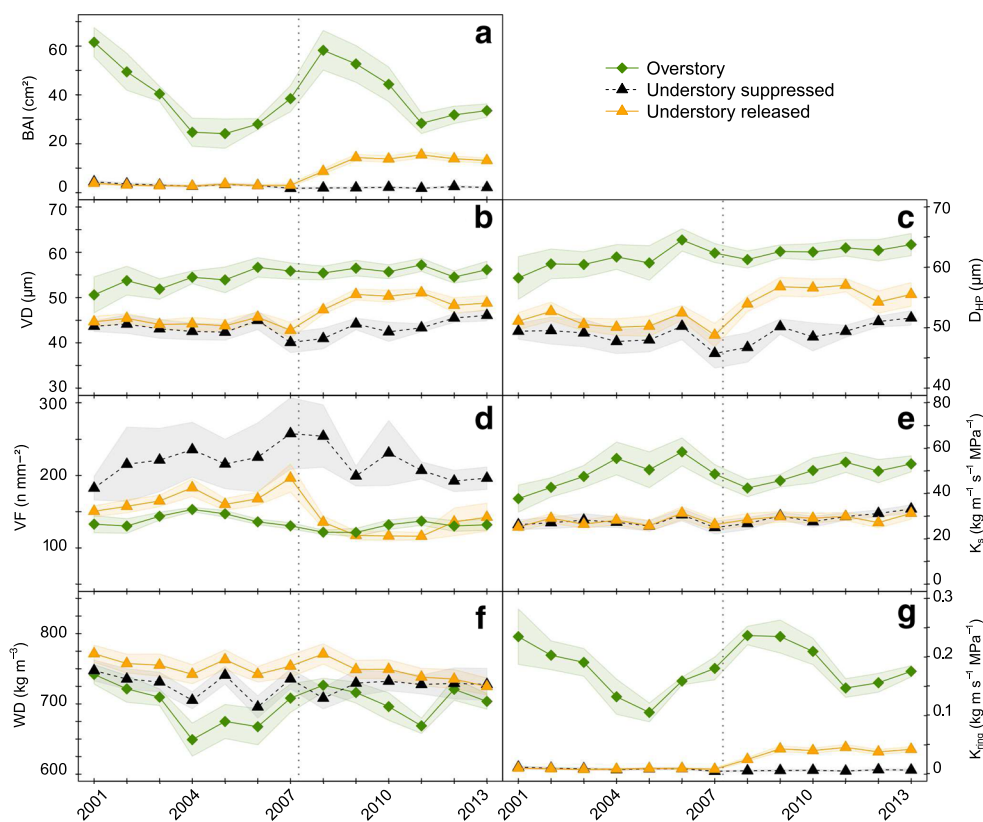
understory trees in all years. Before the release in 2007, understory trees showed stable BAI values. After 2007, BAI of understory suppressed trees remained at 2.1 cm^2 whereas, for released trees, it significantly increased until 2009 and leveled off at 14.0 cm^2 . However even after stabilization, BAI remained significantly lower for released than for overstory trees (Fig. 2a; Table 2).

During most years before the release, overstory trees exhibited significantly higher vessel diameter (51.9 to 56.7 μm) than understory trees (40.1 to 45.7 μm). After release, vessel diameter significantly increased in released trees. In 2009 and afterwards, it was 43.7 and 49.4 μm in suppressed and released understory trees respectively (Fig. 2b; Table 2).

Vessel frequency was significantly higher in understory trees than in overstory trees and significantly decreased in released trees (Fig. 2d; Table 2). Wood density was not significantly lower in overstory than understory trees except during in 2004 and 2011 (Fig. 2f; Table 2). In overstory trees, wood density and BAI displayed similar inter-annual variations.

From 2002 to 2007, D_{HP} and K_S were higher in overstory than understory trees (61.7 to 49.6 μm , 50.6 to 27.6 $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ respectively) (Fig. 2c, e; Table 2). After release, D_{HP} showed the same trend as VD: an increase until 2009 followed by a stabilized range of values around 55.7 μm . Annual variations in D_{HP} in suppressed trees were significantly lower than in released trees and overstory trees. K_S fluctuated between 42.5 and 53.9 $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ in overstory trees whereas it was very stable in understory trees and did not significantly increase after release (Fig. 2e; Table 2). In all trees, K_{ring} showed inter-annual fluctuations similar to BAI. K_{ring} was significantly higher in overstory ($10.5 * 10^{-2}$ to $23.7 * 10^{-2} \text{kg m s}^{-1} \text{MPa}^{-1}$) than in understory trees ($0.9 * 10^{-2} \text{kg m s}^{-1} \text{MPa}^{-1}$) (Fig. 2g; Table 2). K_{ring} of released trees increased after release and stabilized at around $3.9 * 10^{-2} \text{kg m}$

Fig. 2 Mean (\pm SE) of xylem traits (**a, b, d, f**): basal area increment (BAI), vessel diameter (VD), vessel frequency (VF) and wood density (WD); and potential hydraulic performances (**c, e, g**): vessel conductivity-weight (D_{HP}), potential specific hydraulic conductivity (K_S) and ring conductivity (K_{ring}) of overstory (green) and understory suppressed (black, dotted lines) and released (yellow) large trees. The vertical dotted line shows the canopy release of understory released trees in winter 2007–2008



$s^{-1} MPa^{-1}$, whereas it remained at $0.65 * 10^{-3} kg m s^{-1} MPa^{-1}$ in suppressed trees (Fig. 2g; Table 2).

The relative contribution of D_{HP} , VF and BAI, to K_{ring} variation was calculated for 2009, 2 years after release. Variation of BAI after release contributed up to 101% to the variation in K_{ring} whereas D_{HP} and VF contributions were low or negative. BAI exhibited a positive covariance with D_{HP} and a negative one with VF (Table 3).

3.2 Saplings

In all saplings, BAI increased between 2004 and 2006, from an average of 0.121 to 0.273 cm^2 , from 0.014 to 0.046 cm^2 and from 0.014 to 0.205 cm^2 , in acclimated, suppressed, and released saplings respectively (Fig. 3a). Differences among treatments were statistically significant in all years (Table 4).

In 2004, average VD was 22.5 μm in all saplings (Fig. 3c). Acclimated and released saplings showed an increase in VD of up to 29.3 μm and 29.7 μm respectively, in 2006, whereas VD remained stable in suppressed saplings and was significantly lower than in acclimated and released saplings (Table 4). In 2004, VF was higher in acclimated than in suppressed and released saplings. In 2005 and 2006, values were stable in suppressed saplings, and significantly decreased in released and acclimated saplings (Fig. 3e; Table 4). In 2006, released and acclimated saplings had 203 and 121 vessels mm^{-2} respectively.

D_{HP} increased each year in acclimated (from 24.7 to 32.6 μm) and released (from 26.3 to 33.9 μm) saplings. Suppressed saplings showed stable D_{HP} of around 24.2 μm in all years (Fig. 3b; Table 4). K_S of acclimated saplings increased in 2005 (from 1.9 to 3.4 $kg m^{-1} s^{-1} MPa^{-1}$), whereas K_S of released saplings increased in 2006, the second growing season after release (6.5 $kg m^{-1} s^{-1} MPa^{-1}$) (Fig. 3d; Table 4). Similar to the other measured variables, suppressed saplings had very stable values of K_S (Fig. 3d). K_{ring} increased in all years in both acclimated and released saplings, up to $0.95 * 10^{-4}$ and $1.28 * 10^{-4} kg m s^{-1} MPa^{-1}$ respectively (Fig. 3f; Table 4), the result of increases in K_S and BAI.

In saplings, BAI contributed up to 45% to variation in K_{ring} , whereas D_{HP} contributed up to 38% and VF 8%. The covariance between BAI and D_{HP} was 83%, $-36%$ between D_{HP} and VF, and $-38%$ between BAI and VF (Table 3).

4 Discussion

4.1 Effect of tree position within the canopy on xylem traits in large trees

In large trees, xylem structure strongly differed between understory and overstory trees, except for wood density (Fig. 2). As expected, understory trees presented a xylem structure with smaller vessel diameter and higher vessel frequency than

Table 2 Overall treatment effects on anatomic and hydraulic traits for large trees and for each year. *P*-values (*P*) are from the Kruskal–Wallis test. Treatments showing significant differences for a year were then tested with a Wilcoxon test. A plus sign (+) indicates significant differences between overstory and understory released (O.R); overstory and understory suppressed (O.S); and understory released-suppressed (R.S) trees

Year	BAI			VD			VF			D _{HP}			K _s			K _{ring}			WD					
	P	O.R	O.S	R.S	P	O.R	O.S	R.S	P	O.R	O.S	R.S	P	O.R	O.S	R.S	P	O.R	O.S	R.S	P	O.R	O.S	R.S
2001	0.0014	+	+	+	n.s				n.s				n.s				0.0014	+	+	+	n.s			
2002	0.0014	+	+	+	n.s				0.0269				0.03	+	+	+	0.0014	+	+	+	n.s			
2003	0.0015	+	+	+	0.0166	+			0.011				0.0101	+	+	+	0.0015	+	+	+	n.s			
2004	0.0012	+	+	+	0.0013	+			0.0013				0.0015	+	+	+	0.001	+	+	+	0.0225	+		
2005	0.0011	+	+	+	0.0097	+			0.0158				0.0124	+	+	+	0.001	+	+	+	0.0297			
2006	0.0008	+	+	+	0.0023	+			0.0023				0.003	+	+	+	0.001	+	+	+	0.0337			
2007	0.0006	+	+	+	0.0027	+			0.005				0.0098	+	+	+	0.001	+	+	+	n.s			
2008	0	+	+	+	0	+			0				0.0036	+	+	+	0	+	+	+	n.s			
2009	0	+	+	+	0.0001	+			0.0004				0.0039	+	+	+	0	+	+	+	n.s			
2010	0	+	+	+	0	+			0.0001				0.0032	+	+	+	0	+	+	+	n.s			
2011	0	+	+	+	0	+			0				0.0024	+	+	+	0	+	+	+	0.0130	+		
2012	0	+	+	+	0.0011	+			0.0005				0.0019	+	+	+	0	+	+	+	n.s			
2013	0	+	+	+	0.0002	+			0.0001				0.0018	+	+	+	0	+	+	+	n.s			

Table 3 Relative contribution (%) of D_{HP}, VF and BAI to K_{ring} variance in understory released trees and saplings, 2 years after canopy release

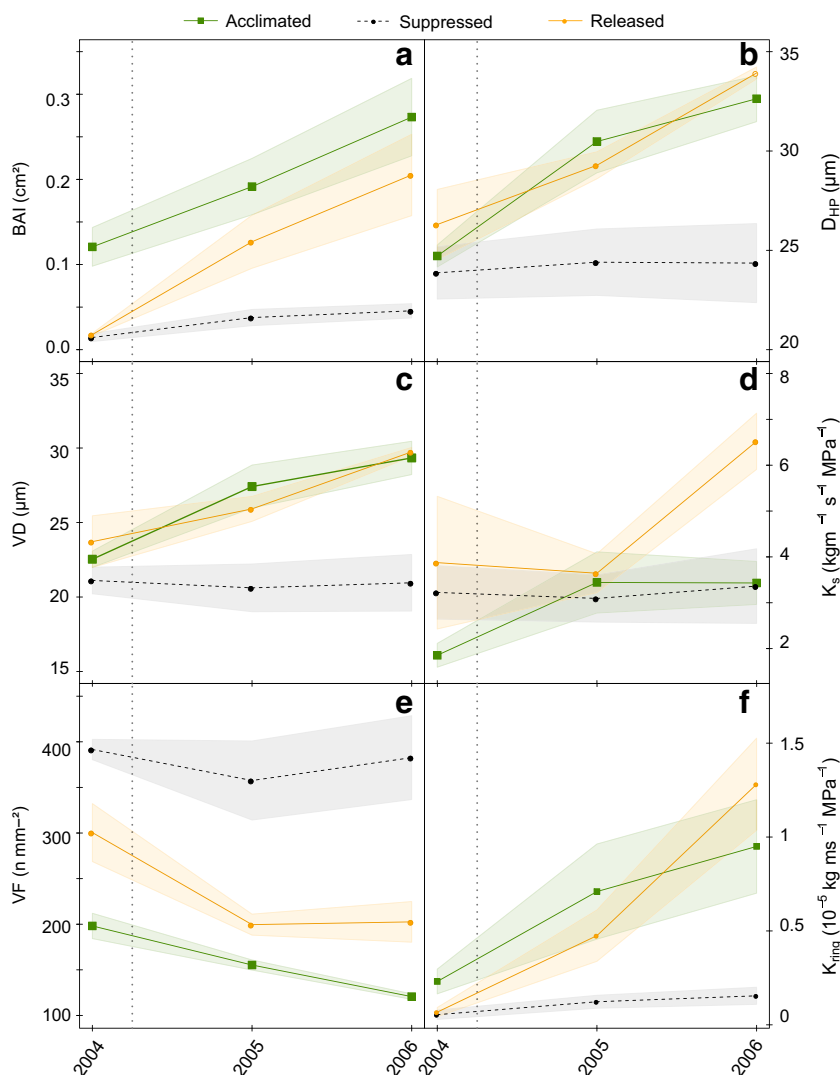
	D _{HP}	VF	BAI	D _{HP} , BAI	DHP, VF	BAI, VF
Large trees	4.32	4.56	101.10	41.82	-8.88	-42.92
Saplings	38.40	8.25	44.55	82.72	-35.59	-38.34

overstory trees (Hypothesis 1). Understory trees are simultaneously subjected to lower soil water supply (related to higher inter-tree competition and lower root biomass, Delagrange et al. 2004; Matjaž and Primož 2010) and to lower potential evapotranspiration (related to lower VPD, lower irradiance, and lower leaf area, Barigah et al. 2006; Klopčič and Boncina 2010; Hajek et al. 2016) than overstory trees. Schuldt et al. (2016) reported that the reduction of water availability generated narrower vessels. In our case, tree water availability was limited by soil conditions and could have been one of the drivers of reduced xylem vessel size in understory trees.

The consequences of these anatomical differences coupled with stem diameter differences would be a lower potential for water transport through the annual ring in understory trees compared to overstory trees. As stated in previous studies (McCulloh et al. 2010; McCulloh et al. 2015), a small stem diameter induces reduced potential specific conductivity and potential ring conductivity, although xylem structure adjustments that resulted in smaller D_{HP} (through vessel diameter and vessel frequency) also contributed. Furthermore, the differences in xylem anatomy of understory vs overstory trees may be also related to the longer water path length from roots to canopies of overstory trees (Preston et al. 2006; Fan et al. 2012).

As expected, the growth and wood traits displayed smaller inter-annual variations in understory than overstory trees (Hypothesis 2, Fig. 2). The largest differences between understory and overstory trees were observed in annual fluctuation in wood density, BAI and in K_{ring} which is strongly related to BAI. Through wood density adjustments, overstory trees may modulate their water transport capacity with regard to annual growth conditions (Stratton et al. 2000; Anderegg and Meinzer 2015). The relationship between BAI and wood density reflects hydraulic adjustments in many situations (Preston et al. 2006), but it can also reflect developmental constraints, such as fast cambial growth involving shorter cell-wall thickening stages (Cuny et al. (2012) on gymnosperms) that produce thinner cell walls, or thinner slowly growing stems that require mechanically stiffer and stronger wood (Lei et al. 1997). Our finding of the relatively constant xylem structure and potential specific conductivity suggests that the understory trees, in their less fluctuating environment, did not receive sufficient signals to adjust xylem structure from year to year (Fonti et al. 2010), and/or the trees' reactions were constrained

Fig. 3 Mean (\pm SE) of xylem traits (**a, c, e**): basal area increment (BAI), vessel diameter (VD) and vessel frequency (VF); and potential hydraulic performances (**b, d, f**): vessel conductivity-weight (D_{HP}), potential specific hydraulic conductivity (K_s) and ring conductivity (K_{ring}) of acclimated (green), understory suppressed (black, dotted lines) and understory released (yellow) saplings. The vertical dotted line shows the canopy release of released saplings in January 2005



by a limited growth potential. In contrast, the larger overstory trees, in their more fluctuating environments, showed adjustments of their BAI and potential water transport ability each year.

4.2 Effects of canopy release on xylem traits of large trees

After understory trees are released, one would expect xylem adjustments reflecting the acclimation to the new environmental conditions. Contrary to our hypothesis, xylem structure and

Table 4 Kruskal and Wallis test (*p*-value) for overall treatment effects (acclimated, understory released, understory suppressed) on xylem structural traits and hydraulic performances for saplings and for each year

Year	BAI	VD	VF	D_{HP}	K_s	K_{ring}
2004	0.0238	n.s	0.0016	n.s	n.s	0.0458
2005	0.0231	0.0308	0.0073	0.0435	n.s	0.0373
2006	0.0210	0.0244	0.0073	0.0231	0.0373	0.0154

potential hydraulic conductivity of released understory trees approached but did not reach those of overstory trees, even 6 years after release (Hypothesis 3). Although potential tree-ring conductivity of released trees was far from that of overstory trees and was probably limited by tree size (Poorter et al. 2010; Fan et al. 2012), all xylem traits as well as basal area growth remained stable after 2 years. Changes that occurred during the first 2 years post-release were apparently sufficient to supply the main hydraulic demands for tree survival, although we are unable to conclude whether and to what extent hydraulic properties actually limited tree growth after release. It is difficult to discuss the dynamics and duration of the acclimation of hydraulic properties because existing studies focused in general on the initial few years after canopy release in saplings or branches (Maherali et al. 1997; Hoffmann and Schweingruber 2002; Lemoine et al. 2002b; Gebauer et al. 2014).

Observed increases in D_{HP} after canopy release may be related to a higher irradiance (Barigah et al. 2006; Hacke 2014) and wind movements in the foliage that increase the potential evapotranspiration: the wider vessels would support

an increased demand while limiting the increase of tension in the xylem sap. However, an increase in D_{HP} was compensated for by lower vessel frequency, so that at the tissue scale, potential hydraulic conductivity (K_S) did not change. This observation may seem contradictory, with large changes observed in the xylem anatomy of beech saplings after canopy release and may be related to differences in tree size, as discussed below.

4.3 Saplings vs large trees

Even if the relative change in K_{ring} after release was similar in saplings and in large trees, the contribution of K_S to K_{ring} release-induced changes was significant only in saplings (hypothesis 4, Table 3). This indicates that the gain of sapwood area with same intrinsic property (K_S) may be sufficient to sustain the increased evaporative demand in large trees, whereas saplings equally rely on adjustments in both the tissue quantity (BAI) and quality (especially D_{HP}). This difference in the nature of the transport adjustment in saplings and large trees may be partly explained by the difference in the relative contribution of one new growth ring in a species such as beech with its diffuse-porous wood and deep sapwood in which sap flow occurs throughout many growth rings (Granier et al. 2000; Luttschwager and Remus 2007; Dalsgaard et al. 2011): the effect of one ring could be much greater in the sapling than the large tree.

Other potential factors could contribute to the observed differences between released large trees and saplings that include differences in water storage and release, relative change in water path length, and specific trajectories of xylem adjustments. Due to their size, large trees may have a greater reliance on water storage and conductivity of older rings (Phillips et al. 2003) than saplings. This hypothesis is obviously only valid for species with wide sapwood area, and cannot be generalized for species like oak, known particularly to rely on the last growth ring to ensure sap flow. In fact, Collet et al. (2001, 2011) reported a 2-years-differed recovery of axial growth in saplings. As the radial growth rate increases, height increment relative to the total height could be larger in saplings than in large trees, increasing the water path length as well. Consequently, a relatively larger increase in D_{HP} could help saplings to partially offset this effect.

5 Conclusion

Large understory *Fagus sylvatica* trees display lower specific hydraulic conductivity and ring conductivity than overstory trees, despite their more stable growth environment. We suggest that these differences are driven by the larger hydraulic constraints of overstory trees because of their geometry (longer path lengths and higher canopies) and their growth

environment (higher VPD and more light, leading to higher rates of transpiration). Overstory trees presented inter-annual fluctuations in growth, specific conductivity, and wood density that could reflect continuous adjustments of their water transport capacity in response to their more fluctuating environments.

After canopy release by thinning, the xylem anatomy of the large understory trees was modified, stabilizing and approaching the trait values of the overstory trees without ever reaching them. The opposing effects of increased vessel diameter and decreased vessel frequency on conductivity compensated for each other in that there was no significant change in potential specific conductivity after canopy release. The gain in radial growth was sufficient to significantly increase potential stem overall performance (ring conductivity) without modifying the potential specific conductivity. This result contrasts with the saplings, for which the increase in conductivity was equally due to radial growth and changes in potential specific hydraulic conductivity (which resulted mainly from an increase in hydraulic diameter). We suggest that this result is due to a dimensional effect, the relatively larger contribution to the potential water transport of a structure via a new growth increment added to a small vs a large pre-existing structure. We emphasize that this result may be applicable only to species with considerable sapwood width that are known to use many growth rings for transport (e.g., not for ring-porous species). Further experiments on acclimation after canopy release in species with different wood types, sapwood widths, and radial sap flow patterns could yield interesting information on the role of xylem structure in tree hydraulic performance.

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Compliance with ethical standards

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