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Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation

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ABSTRACT

Measurements of diurnal diameter variations of the xylem and phloem are a promising tool for studying plant hydraulics and xylem–phloem interactions in field conditions. However, both the theoretical framework and the experimental verification needed to interpret phloem diameter data are incomplete. In this study, we analytically evaluate the effects of changing the radial conductance between the xylem and the phloem on phloem diameter variations and test the theory using simple manipulation experiments. Our results show that phloem diameter variations are mainly caused by changes in the radial flow rate of water between the xylem and the phloem. Reducing the hydraulic conductance between these tissues decreases the amplitude of phloem diameter variation and increases the time lag between xylem and phloem diameter variation in a predictable manner. Variation in the amplitude and timing of diameter variations that cannot be explained by changes in the hydraulic conductance, could be related to changes in the osmotic concentration in the phloem.

Key-words: hydraulic conductance; sap flow; stem diameter variation; xylem diameter variation.

INTRODUCTION

Phloem transport is one of the keys to understanding plant function from resource allocation and long-distance signalling to survival (Thompson 2006; Hölttä, Mencuccini & Nikinmaa 2009; McDowell & Sevanto 2010; Mencuccini & Hölttä 2010; Sala, Piper & Hoch 2010). Yet, our knowledge of phloem transport remains rudimentary because measuring phloem function is challenged by the complexity of the tissue and the difficulty of measuring flow and pressure gradients non-intrusively.

From a hydraulic perspective, phloem, defined in this study as the tissue between the xylem and outer bark, serves two important roles: (1) it is the pathway of sugar transport from sources or storage to sinks; and (2) it can act as a water reserve for the transpiration stream (see, e.g. Zweifel, Item & Häsler 2000 and references therein). Both of these functions rely on water exchange between the xylem and the phloem. In the first case, sugar transport rate depends on the pressure gradient generated by osmotic changes in the phloem and is affected by the hydraulic conductance between the xylem and the phloem (Hölttä *et al*. 2006, 2009). In the second, the reserve availability depends directly on the hydraulic coupling between the two tissues.

One outcome of the hydraulic coupling between xylem and phloem is that high xylem water tension reduces phloem turgor pressure and flow rate (Hölttä *et al*. 2006). Therefore, one could argue that to stabilize phloem function against fast changes in xylem water tension, it would be beneficial for a plant to have a hydraulic barrier between the xylem and the phloem. However, a modelling study shows that tight coupling enables higher sugar flux rates in the phloem as less osmotic 'pull' in the phloem is required for phloem water acquisition (Hölttä *et al*. 2006). Tight coupling also allows faster information transmission in the phloem (Thompson & Holbrook 2004; Thompson 2006). The hydraulic coupling between the xylem and the phloem is, therefore, important for long distance transport in plants, but it may also have importance for local processes such as embolism refilling (Salleo *et al*. 2004), circulation of nutrients (Biddulph and Cory 1957, Zwieniecki, Melcher & Holbrook 2001a) and growth (Hölttä *et al*. 2010).

Hydraulic conductance between the xylem and the phloem is difficult to measure; therefore, little is known about its variability between and within species, especially for woody plants. Recently, advances have been made using nuclear magnetic resonance (NMR) (Köckenberger *et al*. 1997; Rokitta *et al*. 1999) or magnetic resonance imaging (MRI) (Windt *et al*. 2006; Sibgatullin *et al*. 2010). At the moment, these are the only non-destructive methods for measuring phloem transport and water exchange between the xylem and the phloem. These methods, however, are currently not available for field use or for measurements on mature trees.

Diurnal tree stem diameter variations, when measured simultaneously on the xylem and on the bark, have shown promise in differentiating xylem and phloem action (Sevanto *et al*. 2002, 2003a, De Schepper *et al*. 2010). These measurements are easy to maintain and readily available

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for field use, but the extraction of information of phloem function from the data lacks both theoretical and experimental verification. The timing of diameter variations measured on bark has been shown to depend on its sugar content by a girdling study (De Schepper *et al*. 2010), and model studies support the idea that diurnal changes in osmotic concentration in the phloem could cause detectable changes in the dynamics of xylem and phloem diameter variations (Genard *et al*. 2001, Hölttä *et al*. 2006; De Schepper & Steppe 2010), as suggested by Sevanto *et al*. (2003a). One of the least well-known parameters in the models, however, is the hydraulic coupling between the xylem and the phloem, which could affect the timing of the diameter variations and, therefore, modelling studies are only suggestive in this aspect (see Steppe *et al*. 2006).

Diurnal phloem diameter variation measurements that are obtained by subtracting simultaneously measured xylem diameter variations from whole stem diameter variations (measured on outer bark) record the aggregate response of all tissue between the xylem and the outer bark. In many woody plants, the majority of this tissue (referred to as phloem in this study) consists of cells other than phloem conduits (e.g. parenchyma cells), with the sieve element-companion cell complexes that are responsible for the sugar transport imbedded between other cells (Bowes 1997; see also Zweifel *et al*. 2000; van Bel 2003). At the moment, we do not know the relative contribution of changes in water and solute content of these tissues to phloem diameter variations. If the major part of diameter variations was due to changes in sieve elements, phloem diameter variations could be a direct means for measuring changes in turgor pressure that drive phloem flow in axial direction. On the other hand, if sieve elements are hydraulically relatively isolated from the surrounding tissue or if they are very rigid, their contribution would be minor and phloem diameter variations would mostly reflect changes in the contents of the surrounding cells. In this case, diameter variations could reflect changes in phloem transport or osmotic pressure in the sieve elements, provided that the surrounding cells actively interact with the sieve elements to regulate phloem flow. Therefore, to correctly interpret the relationship between phloem diameter variations and phloem transport, it is important to understand not only the effects of changes in sugar content on diameter variations but also the effects of hydraulic coupling of the xylem and the phloem and hydraulic coupling of sieve elements and other phloem cells on phloem diameter variations.

In this study, we develop theory and present measurements on how changing the hydraulic conductance between the xylem and the phloem affects the diurnal diameter variation of the phloem. Specifically, our objectives were to quantify: (1) what causes the daily, tissue-level changes in phloem diameter; and (2) how changing the hydraulic conductance between the xylem and the phloem is reflected in the amplitude and mutual timing of xylem and phloem diameter variations. In our experiments, we consider phloem as a three-dimensional tissue, where flow contributing to diameter variations can occur in any direction, but we do not address the possible effects of the interaction between sieve elements and the surrounding tissue on phloem diameter variations, which, with current knowledge of diameter variations, are undetectable with this method and, therefore, out of the scope of this study. We first show that the relative amplitudes and timing of xylem and phloem diameter variations are determined by the radial hydraulic conductance between the phloem and the xylem, assuming constant xylem and phloem elasticity and osmotic concentration. Then, we combine theory with manipulation experiments conducted to test model predictions and to estimate the hydraulic conductance between the xylem and the phloem.

THEORY

Tree stem diameter variations occur as a result of variation in the water tension inside the stem (Irvine & Grace 1997). Xylem diameter variations are tightly linked with transpiration (Perämäki *et al*. 2001) and diameter variations measured on the bark (whole stem measurements) generally follow the xylem with a time lag (Sevanto *et al*. 2002, 2003a). Because phloem tissue is much more elastic than the xylem, the diurnal amplitude of phloem diameter variations, relative to the tissue thickness, is much larger than the amplitude of xylem diameter variations (Sevanto *et al*. 2002, 2003b).

In this study, we define phloem as the tissue between the xylem and the outer (non-living) bark, and treat it as a homogenous elastic tissue, consciously omitting the fine structure and division to sieve elements and other cells. This does not limit our argumentation regarding the responses of phloem diameter variation to changes in hydraulic conductance because diameter variation measurements essentially treat phloem as a bulk tissue.

Water movement inside a stem can be treated as flow through porous media where the flow rate (volume flux density) J [$m^3\ m^{-2}\ s^{-1}$] is directly proportional to the water potential gradient and the coefficient of proportionality $\bar{k}$ [$m^2\ Pa^{-1}\ s^{-1}$] is the hydraulic specific conductivity tensor that takes into account the conductivity in every flow direction:

$$\bar{J} = \bar{k} \cdot \nabla \Psi \tag{1}$$

Each component of the water potential gradient $\nabla \Psi$ consists of the gradient in hydrostatic pressure determined by the gravitational force and the pressure losses (or gains) resulting from water flow at the location in question and the spatial variation in osmotic pressure (see, e.g. Nobel 1991). Equation 1 can be reduced to three one-dimensional equations if the primary flow directions in a cylindrical plant stem are axial, radial and tangential (note: we chose a cylindrical coordinate system). In the xylem, the osmotic term of the water potential gradient can be excluded and for flow in the radial or tangential direction, there is no gravitational gradient.

The rate of change of the volume (V) of a stem element depends on the accumulation or removal of water (and solutes) from that element:

$$\frac{dV}{dt} = \sum_i (A_{\text{in,i}} J_{\text{in,i}} - A_{\text{out,i}} J_{\text{out,i}}) \quad (2)$$

where A is the area through which the flow occurs at the inner (subscript *in*) and outer (subscript *out*) boundaries of the stem element and index i runs over all relevant flow directions.

As long as changes in volume are small and elastic, they are related to the change in pressure (P) via Hooke's law:

$$\frac{dP}{dt} = \frac{E}{V_0}\frac{dV}{dt} \quad (3)$$

where E is the modulus of elasticity of the tissue and V_0 is the initial volume of the element.

If the flow causing phloem diameter variations is primarily radial, the flow rate at the radial boundaries of a cylindrical stem element determine the majority of the change in the volume of the element and combining Eqns 1 and 2, we can write

$$\frac{dV}{dt} = A_{r+\Delta r} k_{r+\Delta r} \left.\frac{d\Psi}{dr}\right|_{r+\Delta r} - A_r k_r \left.\frac{d\Psi}{dr}\right|_r \quad (4)$$

where A refers to the area of exchange at locations r and $r + \Delta r$ and the gradients of the water potential are taken at the same surfaces. The hydraulic specific conductivity in radial direction at each location is denoted by k.

Substituting the rate of change in volume by the rate of change in pressure using Eqn 3, writing the initial volume V_0 for the cylindrical volume element and reducing Δr to zero leads to a second-order partial differential equation for the pressure field in the radial direction

$$\frac{\partial P}{\partial t} = k_r E_r \left(\frac{1}{r}\frac{\partial P}{\partial r} + \frac{\partial^2 P}{\partial r^2}\right) \quad (5)$$

where k_r is the hydraulic specific conductivity [$m^2\ Pa^{-1}\ s^{-1}$] and E_r the elastic modulus of the tissue in radial direction. Here, k_r and E_r are assumed to be constant. In general, this equation applies for radial water movement both in the xylem and in the phloem separately, as well as between those tissues, as long as the possible changes in k_r and E_r between tissues are taken into account. Note that we also assume here that phloem osmotic concentration does not change in time and is constant inside the phloem in the radial direction at this particular location. Therefore, changes in water potential and pressure become interchangeable.

Diameter variations of the phloem that are a result of changes in radial water transport between the xylem and the phloem can now be calculated using the pressure field obtained as a solution of Eqn 5. This can be inserted into Eqn 3 to obtain the variation in volume and further converted to diameter variation using the relation between changes in volume and changes in diameter (d) for a cylindrical stem:

$$\frac{dd}{dt} = \frac{2}{\pi h d_0}\frac{dV}{dt} \quad (6)$$

where d_0 is the initial diameter and h is the height of the element under consideration.

When solving Eqn 5 for the phloem, xylem pressure at the location of the phloem element under consideration can be treated as a boundary condition, and the propagation of the xylem pressure wave into the phloem is determined by phloem elasticity (E_r) and the radial hydraulic conductivity (k_r) (see also Levin 1996 and references therein). These two parameters then also determine the amplitude and response time of phloem diameter variation relative to xylem diameter variation for a phloem of certain thickness. This applies generally in the absence of osmotic changes and if the primary flow direction that contributes to diameter variations is radial. Any changes in the osmotic concentration or primary flow direction should appear as deviations from this theory as long as phloem elasticity and the hydraulic conductivity remain constant. On the other hand, if osmotic concentration is constant and flow in the radial direction dominates the diameter variations, solving Eqn 5 enables the determination of any one of the other parameters, elasticity, hydraulic conductivity or phloem thickness, provided the other two are known. This can be demonstrated by writing Eqn 5 into dimensionless form. In that case, a dimensionless number with E_r and k_r in the nominator and phloem thickness explicitly in the denominator appears as a coefficient controlling the dynamics of the pressure field (see also Levin 1996).

Empirically, if the hydraulic coupling between the xylem and the phloem is weak (i.e. low radial hydraulic conductance between those tissues), water fluxes in axial and tangential directions inside the phloem should dominate in Eqn 2, and if a region of phloem is then isolated from the rest of the phloem in these directions, we should see a reduction in the amplitude of diameter variation of that piece, and the time lag between xylem and phloem diameter variation should decrease, because flow into the phloem is reduced and phloem starts to act like a dead cover on the xylem (see Sevanto *et al.* 2003a). On the other hand, if the coupling is strong, water exchange in radial direction will dominate and the manipulation would have only a minor effect or no effect at all on the amplitude and timing of phloem diameter variation, the magnitude of the effect depending on the relative hydraulic conductivities in these directions. Note that the conductivity in the axial direction here is not necessarily the conductivity of sieve elements as long as flow through those cells does not result in increase or decrease of water (or solute) content of the phloem element [i.e. either steady-state flow through the phloem element or no flow at all (our manipulation experiments; see further discussion)]. Only if the flow rates in and out of a phloem element are not equal will flow in that direction contribute to phloem diameter variations.

MATERIALS AND METHODS

To test this theory, we built a model for calculating xylem and phloem diameter variations from a pre-described xylem water tension using Eqns 3, 5 and 6, and made manipulation experiments, where we isolated a piece of phloem from the rest of the phloem by cutting a gap into the phloem and changed the radial hydraulic conductance between the xylem and the piece of phloem by inserting thin films of different hydraulic conductance between those tissues, while simultaneously measuring the diameter variation of the xylem and the phloem.

Model

For the model, we solved Eqn 5 numerically using the explicit Euler-finite difference scheme and simulated the dynamics of xylem and phloem diameter variations with various radial hydraulic conductivities. The model calculates the radial pressure distribution from Eqn 5 in the phloem and converts that to diameter using Eqns 3 and 6.

In the model, the phloem (thickness 2 mm) was divided into five equal-size numerical elements in the radial direction (for model parameters see Table 1). Xylem pressure was calculated from sapflow rate and axial hydraulic conductance, along with the radial water exchange between the xylem and the phloem as the radial water potential differences influence the pressures and diameters in each of the numerical elements (Eqns 2–6 in three-dimensional forms). Sapflow rate was made to equal a pre-described transpiration rate with a sinusoidal diurnal pattern reaching a maximum rate of 3.5 gs^{-1} at midday. The xylem was not divided into elements, i.e. water potential was assumed to be homogenous in the xylem. This is justified by our treatment of xylem pressure as a boundary condition for the phloem pressure field. A time step of 0.1 s was used in the simulations. This, together with the small radial dimension of the phloem elements, ensured the stability and robustness of the numerical solution, that is, the changes in pressure and flow rate per time step were small enough for the numerical solution to be robust (see further discussion below).

To use our model to predict how the ratio of the daily amplitude of phloem and xylem diameter variations and the time lag between them would change when the hydraulic conductance between these tissues varied, we first created a model base case by setting the phloem elasticity and radial hydraulic conductivity to give an amplitude ratio of 1.8 (average of maple, see Figs 2 and 3), as well as a time lag of 75 min (average of maple, see Fig. 4) with the pre-described transpiration rate. The values obtained were 3×10^{-14} m^3 m^{-2} Pa^{-1} s^{-1} for the hydraulic conductance (or, alternatively, 3×10^{-17} m^4 m^{-2} Pa^{-1} s^{-1} for hydraulic conductivity) and 16 MPa for the phloem elastic modulus. We then simulated the diameter variations under changing radial conductance and calculated the amplitude ratio and the time lag as a function of radial conductance for stems with different elasticities. In all simulations, phloem thickness was set to 2 mm. We chose to vary phloem elasticity instead of phloem thickness, but it can be seen from Eqn 5 that increasing the elastic modulus of phloem tissue has a similar effect on the amplitudes and time lags of the diameter variation as decreasing the phloem thickness and, therefore, this choice does not limit us to discuss only variation in phloem elasticity.

In the model, we used a scenario where the majority of hydraulic resistance between the xylem and the phloem was in the cambium; therefore, we refer to the values as radial hydraulic conductance. We chose this approach because it was closer to our experimental set up, where the conductance limiting transport was created by the inserted film. This approach resulted in the water potential in the phloem being homogenous in the radial direction. We also tested a version of the model where the hydraulic resistance was evenly distributed inside the phloem. In this case, there was a radial gradient in the water potential in the phloem, but the effects of the gradient on the diameter variations were minuscule because of the small thickness of the phloem. Therefore, the values reported here as hydraulic conductance can be converted to an average hydraulic conductivity by dividing by a transport distance of 1 mm. The base case values for transpiration rate and xylem conductance led to a diurnal variation of 0.4 MPa in the xylem water potential and a 0.06 MPa maximum difference in the water potential between the xylem and the phloem.

Measurements

Diameter variations were measured with linear displacement transducers (LVDT; Solartron AX/5.0/S, Solartron Inc., West Sussex, UK) attached to a rectangular metal

Table 1. Model parameterization for the base case

Parameter	Value	Reference
Xylem thickness	9 cm (of which 3 cm heartwood)	Measured
Phloem thickness	2 mm	Measured
Maximum transpiration rate	3.5 g/s	Estimated
Elastic modulus of the xylem	0.75×10^9 Pa	Irvine & Grace (1997)
Axial hydraulic conductance the of xylem up to the measuring point	8×10^{-12} m^3 $s^{-1}Pa^{-1}$	Iterated to obtain a diurnal variation of 0.4 MPa in xylem water potential (see also Perämäki *et al*. 2001)
Elastic modulus of the phloem	16×10^6 Pa	Iterated (a result)
Radial hydraulic conductance	3×10^{-14} m^3 m^{-2} Pa^{-1} s^{-1}	Iterated (a result)

frame mounted around the stem. The sensors were attached via two holes drilled through one of the metal bars of the frame and tightened using small bolts. For the xylem measurement, two screws were screwed on opposite sides of the stem through the bark and cambium. One of the sensor tips was set to rest on one screw (xylem measurement) and the opposite-side frame bar on the other. The other sensor tip was set on a small metal plate glued on smoothed bark where most of the dead bark was removed, leaving only a thin layer to protect the phloem from evaporative water loss. With this system, we detected the variation in the distance between the back-side screw and the sensor tip (see, e.g. Sevanto *et al*. 2005a).

Measurements were carried out at Harvard Forest, central Massachusetts, USA (42°32′N 72°10′W, elevation 340 a.s.l.) from July to September 2006 on five maple (*Acer rubrum* L.), two birch (*Betula papyrifera* L.) and two oak (*Quercus rubra* L.) trees. On each tree, diurnal diameter variations of the xylem and the whole stem (on bark) were measured at two locations some 30 cm apart at approximately breast height (1.3 m). One measurement from each tree was used as a control. To avoid the effect of possible embolization resulting from the frame installation, the two sets of sensors on each tree measured diameter variation perpendicular to each other. The instruments were protected from the warming of direct sunlight using a conical shade made of a polyethylene sheet (thickness 0.8 mm) and a reflective 'rescue' blanket. The temperature of the metal frames and the stem (1 cm depth) were measured using copper-constantan thermocouples and the data were corrected for the effects of thermal expansion, as described in Sevanto *et al*. (2005a).

Manipulation experiments

Two sets of manipulations were used: one where the flow in the radial direction was kept intact and one where it was severed (Fig. 1). In the first set, we isolated a piece of phloem from the rest of the phloem by cutting a 1-cm-wide gap either around the whole stem (blocking the axial phloem flow) or around the sensors (blocking axial and tangential flow in the phloem) (Fig. 1, group 1). The gap was filled with Vaseline grease to prevent drying of the xylem and the cuts reopened every week to remove possible re-growth of the phloem. In the second set, we used parafilm or aluminium foil to change the hydraulic conductance in radial direction between the xylem and the phloem by inserting the film between those two tissues (Fig. 1, group 2). Here, cambium was included in the piece of phloem. To insert the film we had to sever the hydraulic connection of the piece of the phloem in at least two directions. To test the possible effects of severing tangential and axial flow in addition to changing the hydraulic conductance in the radial direction we used three different approaches: we either (1) cut a whole piece (size ~1–2 cm^2) of phloem and wrapped it in a film; (2) cut three sides open so that the piece was still attached to the surrounding phloem from the top and wrapped it in the film; or (3) made two vertical cuts, forced the phloem between the cuts to separate from the xylem by carefully pulling the bark tissue and slid the film between the xylem and the phloem. In the first case, in addition to radial flow, both axial and tangential flow directions were severed. In the second case, tangential flow was severed totally but the axial was intact from the top, and in the third, the axial connection of the phloem under the sensor

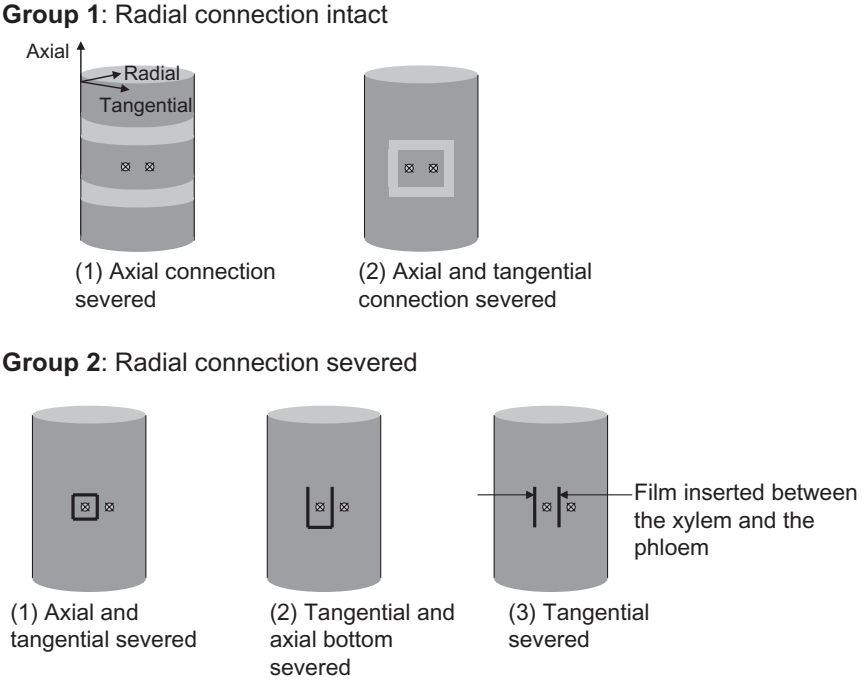

Figure 1. Summary of manipulation protocols. In group 1, the radial connection was intact. The light thick lines in the figures indicate the gaps made to the bark and phloem in the manipulations. The flow directions are indicated by arrows on the top left figure. In group 2 the radial connection was severed by inserting either parafilm or aluminium foil between the xylem and the phloem. The black lines indicate the cuts made for the insertion. The circles mark the positions of the sensors. In every case, the left side sensor was measuring on the whole stem. The illustrations are not to scale.

tip remained completely intact (Fig. 1). In every case, we measured xylem and phloem diameter variations simultaneously next to each other so that the phloem sensor was on the manipulated piece of phloem. The manipulation was conducted after 6–15 d of intact measurements and the measurements were continued at least for 2 weeks after the manipulation or until the response to the damage had stabilized and the detected pattern did not change significantly from day to day.

We measured the hydraulic conductance of both aluminium foil and parafilm in the laboratory by filling a Petri dish with water and letting it evaporate at room temperature through the film under investigation. We measured the change in the mass of the Petri dish with a balance simultaneously with relative humidity and air temperature (Rotronic Hygrolog, Rotronic, Huntington, NY, USA) and calculated the hydraulic specific conductance of parafilm and aluminium foil by assuming saturation vapour pressure above the covered water surface in the Petri dish. Special attention was paid to the sealing of the film to the Petri dish using Vaseline grease so that no water loss could occur through the seam. The hydraulic conductance (±standard error) of parafilm and aluminium foil determined as an average of 5 and 4 measurements was $1.8 \pm 0.4 \times 10^{-14}$ m^3 m^{-2} Pa^{-1} s^{-1} and $1.1 \pm 0.3 \times 10^{-14}$ m^3 m^{-2} Pa^{-1} s^{-1}, respectively. We also checked the effect of stretching on the parafilm conductance and did not find a significant difference between un-stretched and stretched film. Because in the manipulations we used aluminium foil in one- and twofold layers, we also measured the conductance of one- and twofold layers in the laboratory and found that the conductance reduced to half as expected.

Calculations and statistical analysis

Phloem diameter variation was calculated as the difference between xylem diameter variation and the measurement on the bark (whole stem measurement). In some cases, the treatment led to an overall swelling of the phloem during the first 2 to 3 d after the treatment, after which the diurnal diameter variation resumed. The days of swelling right after the treatment were omitted form the analysis.

The amplitude of diurnal xylem and phloem diameter variation was determined by fitting a four-component discrete Fourier series presentation to daily data. The coefficients for each term were determined from Fourier transformation using FFT (Fast Fourier Transformation; MATLAB 6.0) algorithm and adjusted by minimizing the deviation of the fit from the data. The fifth and higher terms of the series presentation contributed less than 5% to the power spectrum and were omitted. This procedure reduced the scatter in amplitudes by 15–20% compared to using means of time windows. The amplitude was defined as a difference of the highest value of the fitted curve before noon and the lowest value after 0900 h.

The daily time lags between xylem and stem diameter variation were determined by correlation analysis (see Sevanto *et al*. 2002, 2003a) where we moved one data set in time with respect to the other until the highest correlation coefficient was found. For this analysis, days with no diurnal variation in diameter (rainy or very cloudy days see Sevanto *et al*. 2005b) were omitted. The statistical significance of the difference of average amplitudes and time lags before and after treatment was determined using Student's *t*-test.

RESULTS

Our manipulation experiments confirmed that flow in the radial direction between the xylem and the phloem dominates phloem diameter variations. After blocking the axial and tangential flow directions (Group 1 experiments, Fig. 1), the ratio of the amplitudes of xylem and phloem diameter variations remained the same as before the treatment, and there was no statistically significant change in the relative timing ($P > 0.1$ for the differences in the average amplitude and average time lag before and after treatment).

Changing radial conductance by inserting parafilm or aluminium foil between the xylem and the phloem, on the other hand, resulted in a reduction in the ratio of the daily amplitudes of phloem and xylem diameter variation (Figs 2 and 3), as well as an increase in the time lag between them (Fig. 4, see also Fig. 2).

Although the changes in the amplitude ratio were clear, they were not large enough to be statistically significant when parafilm was used, but became significant with aluminium foil. For maple, reducing radial conductance with one layer of aluminium foil (second T3 from the left in Fig. 3) did not result in a statistically significant reduction in the amplitude ratio, while the same treatment with two layers of aluminium foil did (first T3 from the left in Fig. 3). Interestingly for oak, the treatment with a double layer of aluminium foil (oak T2, Fig. 3) led to a larger variation in amplitude ratios than the treatment with one layer of aluminium foil (oak T1, Fig. 3), although in this case, the response could also be partially due to the difference in the treatment (see Fig. 1). In general, the lower the radial conductance was, the larger the effect on the amplitude. For oak and birch, the treatments resulted even in the phloem swelling simultaneously with the xylem shrinking, which is shown as a negative amplitude ratio in Fig. 3 (see also Fig. 2). The amplitude of xylem diameter variation did not change in the treatments. Therefore, the change in the amplitude ratio was solely due to a decrease in the amplitude of phloem diameter variation. There was also no statistically significant change in the amplitude ratios of the control measurements.

Similar to the amplitude ratios, the effects of the treatments on the time lag between xylem and phloem diameter variation was clear, but not statistically significant in every case (Fig. 4). There were, however, two cases when the change in time lag was statistically significant while the change in amplitude ratio was not; namely parafilm treatment 1 for maple and one layer aluminium foil treatment 3 for maple (Figs 3 and 4). Contrary to other cases, for birch

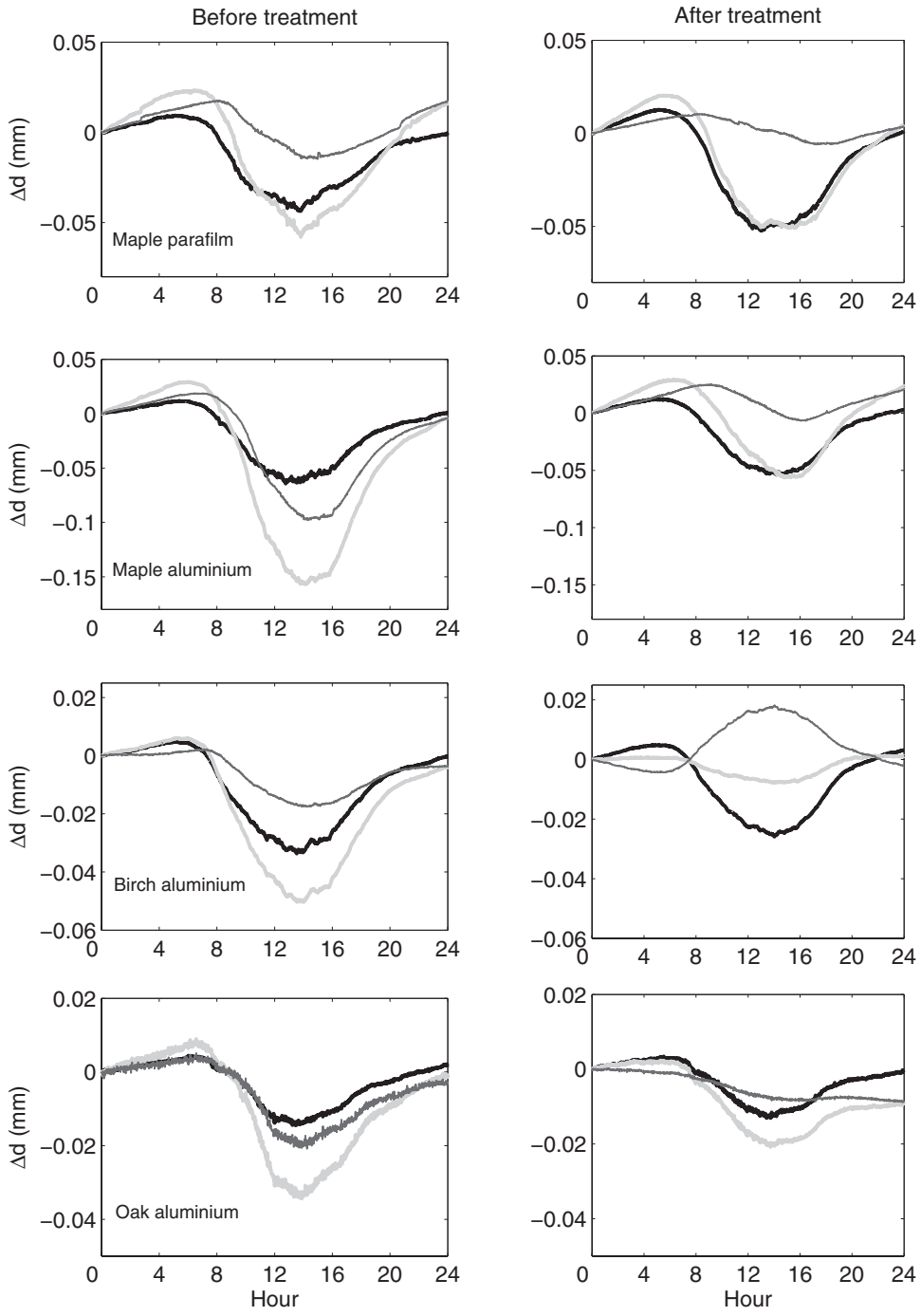

Figure 2. Examples of average diurnal diameter variation of the xylem (black line), whole stem (light gray line) and phloem (dark gray line) before and after treatments. From the top, the panels represent maple treatment 1 (see Fig. 1) with parafilm, maple treatment 1 aluminium foil, birch treatment 1 aluminium foil and oak treatment 2 aluminium foil. All treatments refer to group 2.

and oak, the time lag decreased instead of increasing. This was caused by the phloem starting to swell almost exactly simultaneously with xylem shrinking (see Fig. 2). In one case (oak T2, Fig. 4), the shrinkage of xylem even lagged slightly behind the phloem swelling, which is shown as a positive time lag in Fig. 4. The treatments did not have any statistically significant effect on the time lags of the control measurements.

We did not observe any consistent effect in either the amplitude ratios or the time lags when going from reducing only radial conductance to also blocking the axial and tangential flow directions (Fig. 1 group 2 going from treatment 1 to 3). With parafilm, blocking all directions had a larger effect on both the amplitude ratio and the time lag than leaving the axial direction intact at the top (T2 vs. T1 in Figs 3 and 4). Also, blocking all the directions with

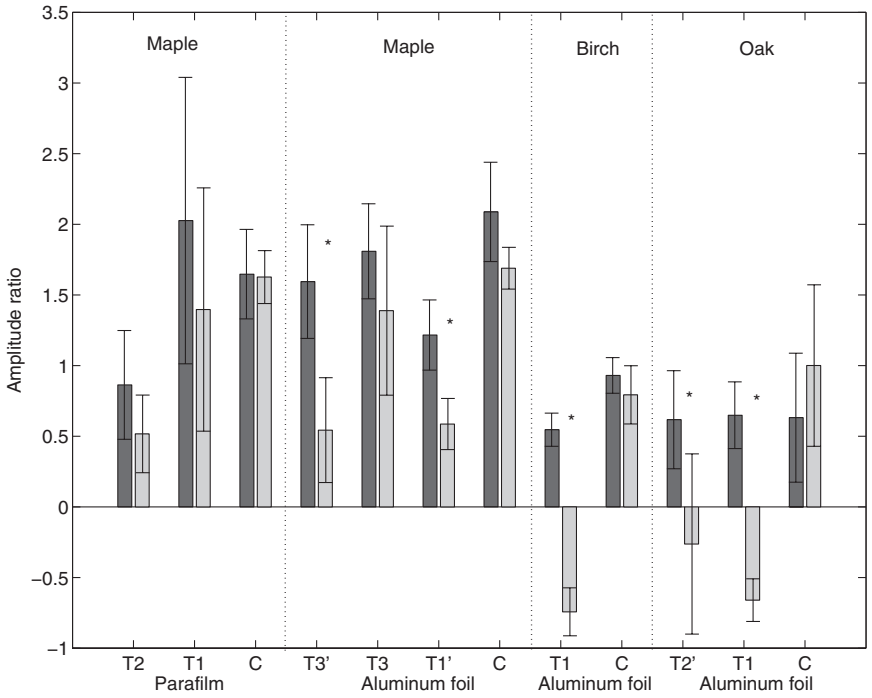

Figure 3. The response of the ratio of the daily amplitude of phloem and xylem diameter variation to the treatments. Each group of two bars, dark and light, represents the average (±STD) ratio of one manipulation experiment before and after the treatment, respectively. The treatments are indicated on the *x*-axis. All treatments refer to group 2 (see Fig. 1). T1 refers to treatment 1, T2 to treatment 2 and T3 to treatment 3. C refers to control measurement. The treatments using a double layer of aluminium foil are marked with an apostrophe. All the other treatments used a single film layer between the xylem and the phloem. The axial lines separate different tree species or the parafilm and aluminium treatments. Statistically significant (Student's *t*-test $P < 0.01$) response to the treatment is indicated by an asterisk. Negative amplitude ratio refers to that phloem started swelling while the xylem shrank after the treatment (see Fig. 2).

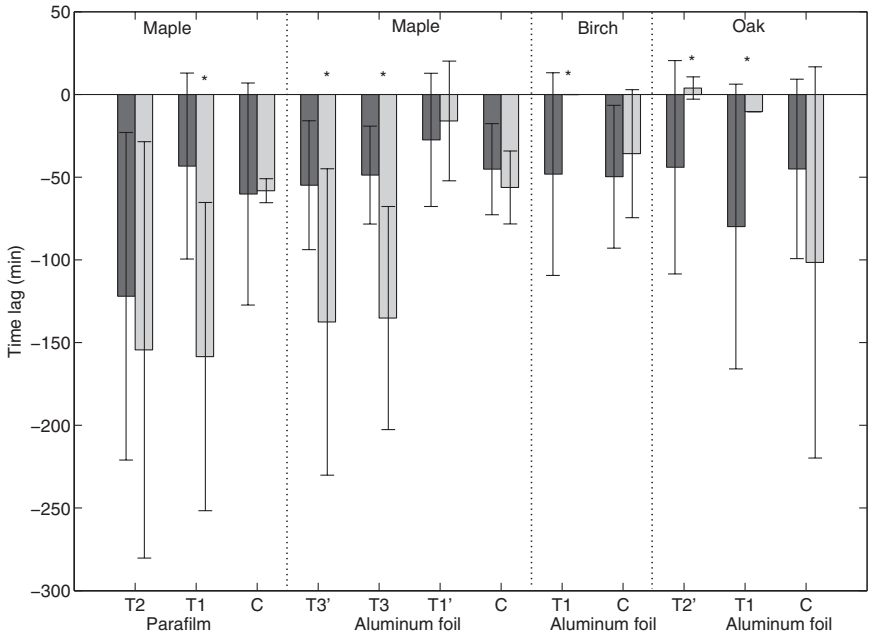

Figure 4. The treatment response of the average daily time lags between the xylem and the phloem diameter variations. Each group of two bars, dark and light, represents the average (±STD) time lag of one manipulation experiment before and after the treatment, respectively. The treatments are indicated on the *x*-axis. All treatments refer to group 2 (see Fig. 1). T1 refers to group 2 treatment 1, T2 to treatment 2 and T3 to treatment 3. C refers to control measurement. The treatments using a double layer of aluminium foil are marked with an apostrophe. All the other treatments used a single film layer between the xylem and the phloem. The vertical lines separate different tree species or the parafilm and aluminium treatments indicated above and below the figure. Statistically significant (Student's *t*-test $P < 0.01$) response to the treatment is indicated by an asterisk. Positive time lag indicates that xylem diameter variation lagged behind phloem diameter variation and negative vice versa. All positive time lags were caused by cases where phloem started swelling while the xylem shrank after the treatment (see Figs 2 and 3).

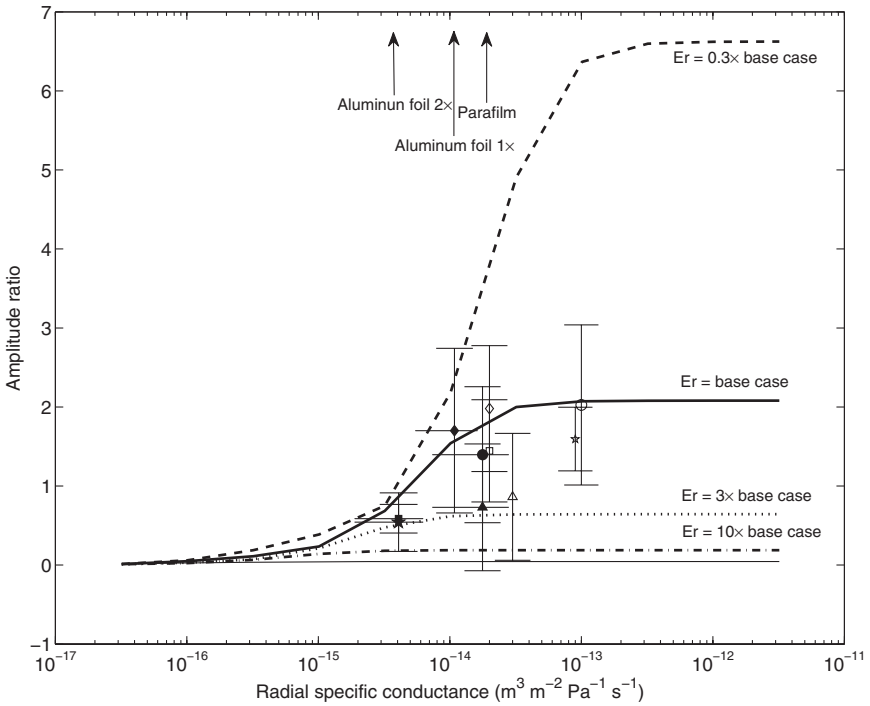

Figure 5. The modelled ratio of daily amplitude of phloem and xylem diameter variation as a function of radial hydraulic conductance for different tissue elasticity and phloem thickness values. The thick line represents the base case, the dash line is the case when $Er/r = 0.3 \times$ base case, dotted line $Er/r = 3 \times$ base case, dash-dot-line $Er/r = 10 \times$ base case and the thin line $Er/r = 30 \times$ base case. The solid symbols present the measured ratios for parafilm, aluminium foil and double-aluminium foil treatments and the open symbols are corresponding values before the treatment extrapolated to correct radial conductivities. Each symbol pair (filled and open) represents one tree. Here we excluded the amplitude ratios for cases when phloem started swelling during the day because assuming constant osmotic concentration in the phloem made our model unable to produce such behaviour (see Materials and Methods). The circles refer to parafilm treatment 2, triangles to parafilm treatment 1, squares to double-aluminium foil treatment 3, diamonds to single aluminium treatment 3 and stars to double-aluminium foil treatment 1 (see Figs 3 and 4). The arrows indicate the hydraulic conductances of the different films used.

aluminium foil caused birch and oak phloem to start to swell during the day, but this did not happen with the same treatment for maple.

The changes in amplitude and time lag could not be caused by changes in the weather. During the experiments with oak and the parafilm experiments with maple there was no statistically significant difference in daily average air temperature, relative humidity, precipitation or photosynthetically active photon flux density before and after treatment. Only during the aluminium foil experiments with maple and birch was the daily average air temperature 3.6 °C higher after the treatments, but even in these cases, there was no statistically significant difference in relative humidity, precipitation or photosynthetically active photon flux density (data obtained from Harvard Forest Fisher meteorological station ~1 km to the east of the measurement site; see Boose 2001). Furthermore, rather than a decrease in the amplitude of phloem diameter variation, increasing air temperature would have caused an increase in the amplitude via increasing stomatal conductance in this well-watered environment (see Sevanto *et al*. 2005b). Also, there were no statistically significant changes in the amplitude ratio (Fig. 3) or time lag (Fig. 4) of the control experiments that were conducted simultaneously with the manipulations. We can thus conclude that all the changes seen in these variables were solely due to our treatments. The average daily air temperature varied between 19–22 °C during all the maple and birch experiments and between 12–16 °C during the oak experiments that were conducted in September.

Our model showed that for fairly elastic phloem ($Er \leq$ base case value, see Materials and Methods), the amplitude ratio decreased with decreasing radial conductance (Fig. 5). The decrease was sharpest when the radial conductance was between 10^{-13} and 10^{-15} m^3 m^{-2} Pa^{-1} s^{-1}, but the exact boundaries of the conductance range of steepest change depended on the phloem elasticity. If the elastic modulus was larger than the base case value, the amplitude ratio remained below one and radial conductance had only a minor effect on the amplitude ratio. Note that the model would never produce negative amplitude ratios (phloem swelling when xylem shrinks) because we assumed constant osmotic concentration in the phloem.

According to the model, the time lag between xylem and phloem diameter variation increased with decreasing radial conductance (Fig. 6a). Similar to the amplitude ratio, there was a threshold conductance, after which the increase became steeper, and that threshold depended on the

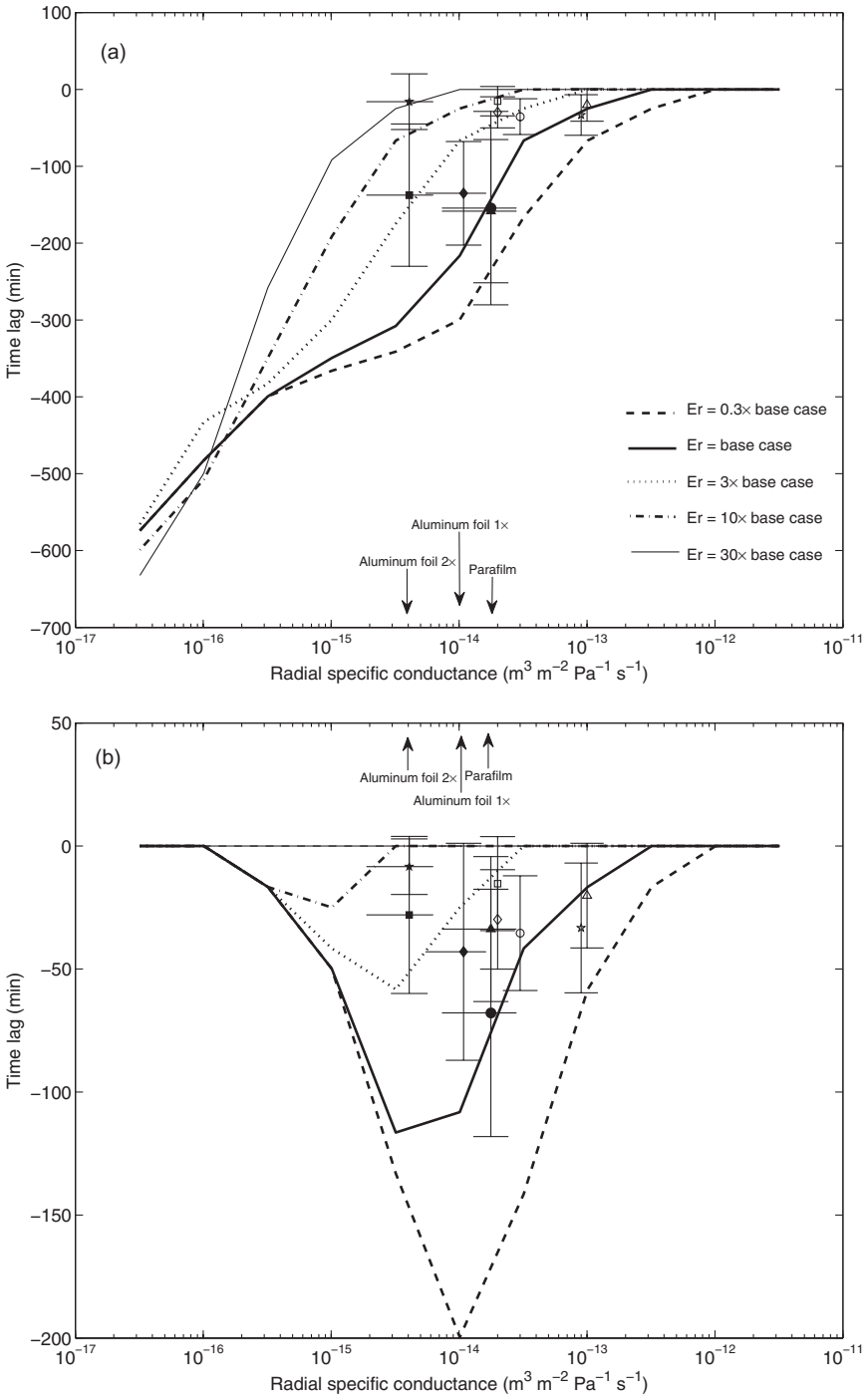

Figure 6. The modelled time lags of xylem and phloem diameter variation a) and xylem and whole stem diameter variation b) as a function of radial hydraulic conductance for different tissue elasticity and thickness values. The thick line represents the base case, the dash line is the case when $Er/r = 0.3\times$ base case, dotted line $Er/r = 3$ x base case, dash-dot-line $Er/r = 10\times$ base case and the thin line $Er/r = 30 \times$ base case. The solid symbols present the measured ratios for parafilm, aluminium foil and double-aluminium foil treatments and the open symbols are corresponding values before the treatment extrapolated to correct radial conductivities. Each symbol pair (open and filled) represents one tree. Here we excluded the amplitude ratios for cases when phloem started swelling during the day because assuming constant osmotic concentration in the phloem made our model unable to produce such behaviour (see Materials and Methods). The circles refer to parafilm treatment 2, triangles to parafilm treatment 1, squares to double-aluminium foil treatment 3, diamonds to single aluminium treatment 3 and stars to double-aluminium foil treatment 1 (see Figs 3 and 4). The arrows indicate the hydraulic conductances of the different films used.

phloem elasticity, so that the more elastic the phloem, the higher the conductance, at which the time lag started to increase.

Because the amplitude of phloem diameter variation decreases with decreasing radial conductance (Fig. 5), there is a maximum time lag for diameter variations between the xylem and the whole stem (xylem + phloem) that can be caused by changing radial conductance (Fig. 6b). That maximum depends on the phloem elasticity, and for our maple, it was 115 min. This means that any time lag larger than that cannot be solely caused by changes in radial conductance and is, therefore, at least partially caused by osmotic changes in the phloem.

The measured amplitude ratios and time lags with known radial conductance (either parafilm or aluminium foil inserted between the xylem and the phloem) were close to those modelled for the base case or between the base case and the curve of the next higher elastic modulus (filled symbols in Figs 5 and 6). This was to be expected because the model base case was calibrated for an average maple

tree of our experiments. Slight deviations in the exact locations of our measurement points compared to the modelled curves in Fig. 6a,b reflects the sensitivity in estimating small time lags between curves of similar amplitude (e.g. the case of xylem and whole stem with low radial conductivity).

Determination of xylem-phloem radial conductance

Using the modelled curves in Figs 5 and 6, we can extrapolate the radial conductance from the observed amplitude ratios and time lags before the treatment (open symbols in Figs 5 and 6). Following the modelled curves, we obtain values $2–10 \times 10^{-14}$ m^3 m^{-2} Pa^{-1} s^{-1} for all the maple trees. Because of the plateauing of the curves at high hydraulic conductance, our estimates are essentially the lower limits. However, because parafilm did not seem to be a large barrier for the flow, it is reasonable to assume that these estimates are fairly close to the real values. Interestingly, the apparent contradiction in the statistical significance of the treatment effects on amplitude ratio and time lag for the maple under parafilm treatment 1 and a single layer aluminium foil treatment 3 (Figs 3 and 4) could be explained by the modelled curve, as the change in conductance was at the range where changes in amplitude were very small, but changes in time lag could already be large (triangle and diamond symbols in Figs 5 and 6).

When making these estimates, we used the measured conductance of parafilm and aluminium foil as values on the *x*-axis of Figs 5 and 6. To be more precise, we should have used the combined value of the original conductance and the film, which would lead to an iterative process when determining the original conductance. Because of the order of magnitude difference between the film conductance and the original conductance, taking into account the original conductance would show only in the second or third figure of the estimated values for total flow resistance, and because of the variability in our data, these figures are insignificant. Therefore, our approach is justified here.

Figures 5 and 6 also provides a tool for estimating the ratio of phloem elastic modulus to phloem thickness. For example, the maple tree undergoing treatment 2 with parafilm (triangle symbol in Fig. 5) had an amplitude ratio of 0.8 ± 0.3 before the treatment. This indicates that the value for Er/r cannot be smaller than $2\times$ the base case value (see Table 1). With a phloem thickness of 2 mm, this leads to an estimate of 32 MPa for maximal phloem elastic modulus. Interestingly, trees of the same species show quite a large range of values. It is worth noting that even a small change in phloem thickness, say 1 mm reduction (here reduced to half), would reduce the estimated elastic modulus to half. Furthermore, we did not measure the daily variation in the tree water potential. An error in the estimation of the actual diurnal variation in the stem water potential by a given factor would lead to an error in the phloem elastic modulus by the same factor (see Eqn 3).

DISCUSSION

The existence of radial water flow between the xylem and the phloem has been demonstrated before (see, e.g. Bull, Gayler & Glasziou 1972; van Bel 1978; Minchin & Lacointe 2005; Ohya *et al*. 2008; see also Zweifel *et al*. 2000; Zweifel & Häsler 2001), but how that – and more specifically, how changes in the hydraulic coupling between the xylem and the phloem – affect the timing and amplitude of diurnal phloem diameter variations, has not been shown previously. Our results show that phloem diameter variations are mainly due to water flow between the xylem and the phloem in the radial direction, and that the ratio of the amplitudes of phloem and xylem diameter variation is, therefore, mainly determined by the hydraulic conductance between those tissues and the elasticity and thickness of the phloem and the xylem. Our results also suggest that the time lag between xylem and phloem diameter variations can be controlled by the same tissue properties of the phloem as the amplitude ratio. This applies as long as it is justified to assume that the daily variations are elastic (i.e. there is no rupture or large-scale cavitation in the xylem and no excessive changes in the osmotic concentration in the phloem or cambial growth). Because both amplitude ratios and time lags change in a predictable manner when the hydraulic conductance changes, any deviation from the predicted changes could be caused by osmotic changes in the phloem. Therefore, knowing the tissue properties should enable us to calculate the changes in the osmotic concentration in the phloem as a deviation from this theory. On the other hand, if the osmotic concentration in the phloem could be assumed constant, changes in the amplitude ratio and time lag would indicate variation in these tissue properties.

Diameter variation measurements are insensitive to the flow direction. They detect only changes in the thickness of the tissue at the location of measurement. These changes can be caused by inward and outward fluxes of water (and solutes) that are linked with the changes in water potential of the tissue (see, e.g. Irvine & Grace 1997), but the insensitivity to the flow direction means that a change in flow rate could give a similar response in diameter as a change in flow direction along one coordinate axis, provided that fluxes in other directions compensate for the change in direction instantaneously and accordingly. Previous studies have shown that outside of areas that function as sinks, the radial direction of flow is actually from the xylem to the phloem and not the reverse (Hölttä *et al*. 2006; Windt *et al*. 2006). Therefore, phloem diameter variations are mostly caused by diurnal changes in the flow rate between the xylem and the phloem rather than by changes in flow direction. This view is somewhat contradictory to the view of phloem acting as a water reserve for the transpiration stream (see Zweifel *et al*. 2000), but it does not indicate that a change in flow direction could not occur under some conditions (e.g. severe drought).

One question that rises from this and other studies on the dynamics of diameter variation measurements is where the

large day-to-day variation in both amplitude ratios and time lags stem from (Figs 3 and 4; see also Sevanto *et al*. 2002, 2003a). The daily amplitude of tree stem diameter variation depends on both the transpiration rate and soil water availability, which vary from day to day (Perämäki *et al*. 2001; Sevanto *et al*. 2005b). But, based on this study, unless there were day-to-day changes in the radial conductance between the xylem and the phloem, in the elasticity of the tissues or in the osmotic concentration in the phloem, the ratio of the amplitudes should remain fairly constant. Diameter variations measured on the bark could be affected by absorption and evaporation of water from the bark tissue (see Lövdahl & Odin 1992), and it is possible that some of the day-to-day variation could be explained by varying weather conditions even if we removed most of the dead bark under the sensors and excluded rainy days from the analysis to minimize this effect. On the other hand, the weather could also affect the diameter variations via changes in osmotic concentration or the tissue properties via plant physiological processes that could depend on, e.g. temperature. It is also worth noting that when applying the films between the xylem and the phloem we did not use any special means to ensure good hydraulic contact between the film and the stem. We only pushed the piece of phloem firmly back to its place. Therefore, it is possible that there were air pockets between the film and the tissues. Despite this both the time lag and amplitude ratios agree surprisingly well with the theory (Figs 5 and 6), which suggests that the hydraulic connection remained quite good.

All in all, this study thus suggests that the day-to-day variation both in the amplitude ratio and the time lags is linked with either changes in tissue properties of the xylem and phloem or changes in the osmotic concentration in the phloem. This view is supported by that the seasonal variation in the time lags is larger than the day-to-day variation (Sevanto *et al*. 2003a), but further studies specifically concentrating on the origin of variability are needed to fully resolve this question. As to the tissue properties, it is reasonable to assume that xylem and phloem elasticity and thickness remain fairly constant outside the active growing period, again provided that environmental conditions do not change dramatically. Therefore, variations outside the growing period are most likely to results from either changes in osmotic concentration in the phloem or changes in the radial conductance.

Changes in sugar content in the phloem have been shown to cause detectable changes in the timing of stem diameter variations (De Schepper *et al*. 2010). The three cases of our study where the phloem started to swell during the day when the hydraulic conductivity in the radial direction was reduced by aluminium foil could also be viewed as supporting that finding. In the clearest of these cases (treatment 1 for birch and oak) the piece of phloem was entirely wrapped in the foil so that water exchange was hindered in every direction. Because there was no source of external water to the piece of phloem, the swelling had to be caused by internal changes of the molar volume of material, for example, conversions of starch to sucrose or glucose and back. Taking birch as an example, the 0.02 mm swelling during the day (Fig. 4) would have required 265 μmol cm^{-3} of amylose to be transformed to glucose (molar volume of a glucose unit in starch was taken as 97.5 cm^3 mol^{-1} (Kalistratova *et al*. 1999) and the molar volume of glucose 110 cm^3 mol^{-1} (Zhuo *et al*. 2007), phloem area under the sensor was 1 cm^2 and phloem thickness 2 mm), which could easily be achieved. This value was calculated assuming that the change in the osmotic concentration did not pull any water from the xylem to the phloem. If the water flow was included, the amount of starch transformed to smaller sugar molecules needed to achieve the observed swelling in the phloem would be even smaller. The cyclic swelling could further indicate an inbuilt diurnal cycle in these transformations (for more on circadian rhythms see, e.g. Webb 2003).

Our estimates for the radial conductance (10^{-14} to 10^{-13} m^{3}m^{-2}Pa^{-1} s^{-1}) agree with previous estimates (Genard *et al*. 2001 for *Prunus persica;* 2.9×10^{-14} m^{3}m^{-2}Pa^{-1} s^{-1}) and are of the same order of magnitude as reported for the radial conductance from vessel-to-vessel in the xylem (Zwieniecki, Melcher & Holbrook 2001b). This suggests that the hydraulic coupling between the xylem and the phloem is fairly tight and there is no real hydraulic barrier between the xylem and the phloem. This is in accordance with the view of several modelling studies, which show that to function efficiently the radial conductivity has to be relatively large (Thompson & Holbrook 2004; Hölttä *et al*. 2009), supporting the hypothesis that phloem sieve-tubes are close to water potential equilibrium with the surrounding apoplast as well as with the xylem (Daudet *et al*. 2002, Hölttä *et al*. 2006, see also Hölttä *et al*. 2009). Interestingly, several studies report hydraulic conductances of the same order of magnitude for aquaporins in the plasma membrane (see, e.g. Johansson *et al*. 1998) as well as for cell membranes in the cortical tissue of roots (see, e.g. Steudle & Peterson 1998). The conductance of the apoplastic pathway has been reported to be significantly higher (Steudle & Boyer 1985) and therefore our results suggest that water moving between the xylem and the phloem may cross cell walls at least somewhere along the route.

CONCLUSIONS

Diurnal diameter variations of the phloem seem to be dominated by changes in water flow rate in the radial direction between the xylem and the phloem. Therefore, if osmotic concentration in the phloem can be assumed constant at the location of measurement, the relative amplitudes and timing of xylem and phloem diameter variations depend on the hydraulic conductance between these tissues as well as the phloem and xylem elasticity and thickness. Changes in the ratio of the daily amplitudes or relative timing of xylem and phloem diameter variations therefore indicate changes in these properties. If phloem thickness and elasticity can be assumed to be constant, changes in hydraulic conductance would be the source of this variation. Alternatively, if the hydraulic conductance was also

constant, the variation should be caused by changes in osmotic concentration in the phloem. In that case, knowledge of hydraulic conductance would enable osmotic changes in the phloem to be estimated from measurements of diurnal diameter variations of the xylem and the phloem.

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