Trees (2016) 30:63–72
DOI 10.1007/s00468-014-1132-9

ORIGINAL PAPER

How important is woody tissue photosynthesis in poplar during drought stress?

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Received: 5 July 2014 / Revised: 15 October 2014 / Accepted: 7 November 2014 / Published online: 4 December 2014

Abstract

Key message **Woody tissue photosynthesis might play a key role in maintaining plant carbon economy and hydraulic function under unfavourable conditions such as drought stress.**

Abstract Within trees, a portion of respired CO_2 is assimilated by bark and woody tissue photosynthesis, but its physiological role remains unclear, in particular under unfavour able conditions like drought stress. We hypothesised that woody tissue photosynthesis will contribute to overall tree carbon gain both under sufficient water supply and during drought, and plays a role in maintaining the hydraulic function. We subjected half of the trees to a stem and branch light-exclusion treatment to prevent bark and woody tissue photosynthesis. Then, we measured leaf gas exchange and stem growth in *Populus deltoides x nigra* 'Monviso' trees both under well-watered and dry conditions. We additionally measured cavitation using acoustic emission in detached control and light-excluded branches to illustrate the role of woody tissue photosynthesis in xylem embolism repair. Under well-watered conditions, light exclusion resulted in reduced stem growth relative to control trees by 30 %. In response to drought, stem shrinkage of light-excluded trees was more pronounced as compared to control trees. During drought stress also maximum photosynthesis and transpiration rate tended to decrease more rapidly in light-excluded trees compared to control trees. Leaf fall in light-excluded branches together with the larger number of acoustic emissions in control branches indicates that in the latter more xylem vessels were still hydraulically functional under drought. Therefore, our study highlights that photosynthesis at branch and stem level might be a key factor in the resilience of trees to drought stress by maintaining both the plant carbon economy and hydraulic function.

Keywords Carbon · Cavitation · Drought stress · Internal CO_2 transport · Stem diameter variations · Water deficit · Woody tissue photosynthesis

Communicated by A. Braeuning.

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Introduction

Chlorophyll in stem and branch tissues has been found to play a role in carbon acquisition. By assimilating respired CO_2, which is present at high concentrations in trees (ranging from <1 to over 26 vol %, Teskey et al. 2008), these photosynthetically active tissues photo-reduce CO_2 similarly as described for green leaves (Pfanz et al. 2002; Berveiller et al. 2007). This re-assimilated CO_2 would otherwise be lost to the surrounding atmosphere, which explains why photosynthesis in chloroplasts present in the bark (Pfanz 2008), xylem rays (Rentzou and Psaras 2008), and pith tissues (Van Cleve et al. 1993; Berveiller et al. 2007; Rentzou and Psaras 2008) of the stem and branches is often described within the context of a tree carbon

recycling mechanism (Aschan and Pfanz 2003; Cernusak and Hutley 2011). In the text, we refer to photosynthesis in these bark, xylem rays and pith tissues as woody tissue photosynthesis, as done previously by Saveyn et al. (2010).

Woody tissue photosynthetic rates are relatively low compared to leaf photosynthetic rates and it rarely results in a positive net CO_2 assimilation rate (Wittmann et al. 2001). Wittmann et al. (2006) found that woody tissue photosynthesis was able to assimilate up to 97 % of the dark respired CO_2 in young *Betula pendula* Roth. and Coe and McLaughlin (1980) reported a maximum re-assimilation of respired CO_2 of 31 % for *Acer rubrum* branches, measured under dormancy. Other studies reported similar maximum re-assimilation values for other trees species (see Table 4 in Pfanz et al. 2002), but overall the impact of woody tissue photosynthesis on plant carbon economy under normal conditions is expected to be limited.

Nevertheless, woody tissue photosynthesis might play an important role during particular events. Different authors consider woody tissue photosynthesis as a potentially important means of bridging the carbon balance between defoliation and re-foliation (Bossard and Rejmanek 1992; Wittmann et al. 2001; Pfanz 2008; Eyles et al. 2009) or during bud development (Saveyn et al. 2010). Other studies suggest that woody tissue photosynthesis might improve the stem carbon balance under limited water availability (Wittmann and Pfanz 2008). In desert and semi-desert habitats, many drought-adapted plants have photosynthetic stems (Gibson 1983; Nilsen and Sharifi 1994), which potentially provide a major fraction of the carbon used to sustain plant metabolism (Comstock and Ehleringer 1990; Nilsen and Bao 1990; Aschan and Pfanz 2003). For temperate plants under moderate drought stress, decreased stomatal conductance can limit the assimilation of atmospheric CO_2 in the leaves (Flexas and Medrano 2002; Chaves et al. 2003), whereas woody tissue photosynthesis is supplied by endogenously respired CO_2 (Pfanz et al. 2002; Aschan and Pfanz 2003; Wittmann and Pfanz 2008). Therefore, the fraction of tree carbon derived from woody tissue photosynthesis might become more important under drought conditions.

However, the exact role of woody tissue photosynthesis during drought stress is not yet well understood. Woody tissue photosynthesis has been shown to be less sensitive to drought stress than leaf photosynthesis (Nilsen 1992). Moreover, Schmitz et al. (2012) hypothesised that photosynthesis in stem chloroplasts might be important for maintaining the hydraulic function of the vasculature, which is crucial during drought stress. Photosynthetic activity in these chloroplasts potentially fulfils local energetic and carbohydrate demands for repair of cavitated vessels (Zwieniecki and Holbrook 2009; Secchi and Zwieniecki 2011; Schmitz et al. 2012). Therefore, by maintaining the carbon balance as well as the plant water status, woody tissue photosynthesis might play a crucial dual role in tree drought stress resilience.

The purpose of this study was to investigate the importance of woody tissue photosynthesis in one-year-old poplar (*Populus deltoides x nigra* 'Monviso') trees under both well-watered and drought stress conditions. To illustrate its role in tree physiology, we measured photosynthesis and transpiration at leaf level and diameter growth at stem level (both manually and automatically) while manipulating the light availability to stem and branches (control and 100 % light-excluded trees). This was complemented with light response curves, to show that the light-exclusion treatment impeded woody tissue photosynthesis in covered stem and branch sections. We hypothesised that woody tissue photosynthesis will contribute to overall tree carbon gain both under sufficient water supply and during drought stress. We also hypothesised that light-excluded trees will suffer faster and more dramatically from drought stress than (non-light excluded) control trees. Finally, we aimed at illustrating the significance of stem chloroplasts in maintaining hydraulic functioning of drought-stressed trees, as suggested Schmitz et al. (2012), by measuring for first time xylem cavitation with acoustic emission sensors in light-excluded branches. We hypothesised that the hydraulic functioning of xylem tissue in light-excluded trees will be less than in control trees.

Materials and methods

Plant material and experimental design

One-year-old cutting-derived trees of *Populus deltoides x nigra* 'Monviso' were used for this study. Twelve branch cuttings were planted mid-June 2012 in 50 L containers filled with a potting mixture (LP502D, Peltracom nv, Gent, Belgium) and slow-releasing fertiliser (Basacot Plus 6 M, Compo Benelux nv, Deinze, Belgium) and grown in the greenhouse facility at the Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium. Eight trees were selected based on uniform height (approximately 1.6 m) and stem diameter (approximately 2.4 cm at 30 cm) and were randomly assigned to two treatments. The remaining four trees were used for destructive chlorophyll concentration measurements. Four trees served as control, while other four trees were used for the light-exclusion treatment, which started on 2 August 2012 (day of year, DOY 214). The stem and woody branches of these light-excluded trees were loosely wrapped with aluminium foil following Saveyn et al. (2010), so gaseous diffusion from

woody tissues to the atmosphere was not restricted. Results from a previous shading experiment on trees of similar dimensions as the ones considered in this study showed that the average daytime and nighttime stem temperature measured in the xylem with a thermocouple in light-excluded stems was 2.2 °C lower and 1.3 °C higher than in control trees. At this time, all trees considered in our study were irrigated at least twice a week, ensuring adequate water supply. Subsequently, trees were irrigated for the last time on 30 September 2012 (DOY 273) to impose drought stress. No leaf fall occurred until 1 week after the start of the drought stress treatment. The fully developed leaf area before drought stress was determined by randomly sampling ten leaves per tree and multiplying this average leaf area with the number of leaves per tree. Leaf area ranged from 3.2 to 4.7 m^2 and from 2.8 to 3.9 m^2 for control and light-excluded trees, respectively, and average leaf area was not different between control and light-excluded trees ($P = 0.20$).

Relative humidity (RH) in the greenhouse was measured with a capacitive RH sensor (Type hih-3610, Honeywell, Morristown, NJ, USA), air temperature (T_{air}) with a copper constantan thermocouple (Type T, Omega, Amstelveen, USA), and photosynthetic active radiation (PAR) with a quantum sensor (LI-190S, Li-COR, Lincoln, TE, USA). Sensors were installed at a height of approximately 2 m. Measurements were conducted from July 2012 until mid-October 2012.

Stem diameter measurements

Stem diameter variations were continuously measured with linear variable displacement transducers (LVDT; model DF5.0, Solartron Metrology, Bognor Regis, UK), installed with custom-made stainless steel holders on one control tree and one light-excluded tree randomly chosen from the eight selected trees. On 21 September 2012 (DOY 264), before the start of the drought stress, two additional trees (one control and one light-excluded tree) were instrumented with LVDT sensors. Stem radial daily growth rate (DG, mm day^{-1}) was calculated as the difference between two successive daily maximum values of the stem diameter. Continuous stem diameter measurements were complemented with weekly calliper-based manual measurements for all trees, conducted between 10 and 14 h. Two measurements were made at a height of 30 cm along perpendicular axes. The average of these two measurements was used for analysis. Relative reductions in stem diameter growth were calculated as the difference in stem diameter increment between control and light-excluded trees during light exclusion, normalised with the control value.

Leaf level measurements

At leaf level, maximum net photosynthesis (A_{max}, μmol CO_2 m^{-2} s^{-1}) and transpiration rate (T, mmol H_2O m^{-2} s^{-1}) were measured on all control and light-excluded trees on two fully expanded leaves per tree with a portable photosynthesis system (model Li-6400, Li-Cor, Inc., Lincoln, Nebraska, USA). Measurements were conducted at 25 °C, prevailing RH conditions, a set atmospheric CO_2 concentration of 400 ppm and a PAR level of 1,500 μmol m^{-2} s^{-1} and made alternately for leaves of control and light-excluded trees. The PAR level of 1,500 μmol m^{-2} s^{-1} at which A_{max} occurred was determined via a preliminary experiment in which light response curves were obtained for one leaf on six trees. Leaf gas exchange measurements were conducted biweekly during the period before drought stress between 10 and 16 h, and at a 2-day interval during the drought stress treatment. At each measurement time, the average of five measurements recorded at 10 s intervals was used for the analysis.

Light response curves for woody tissue photosynthesis

We measured woody tissue photosynthesis light response curves on five *Populus* trees additional to the trees that were dried out during the stem diameter, leaf level and chlorophyll concentration measurements to show that the light-exclusion treatment impeded woody tissue photosynthesis in covered stem and branch sections. We used an opaque conifer cuvette (model 6400-22L, Li-Cor Inc., Lincoln, Nebraska, USA) connected to a portable infrared gas analyzer (IRGA; model Li-6400, Li-Cor Inc., Lincoln, Nebraska, USA) to measure stem net CO_2 exchange. The Li-6400 was operated as an open system while gas exchange was in transient state to prevent accumulation of CO_2 and depletion of O_2 within the cuvette (Cernusak and Marshall 2000). The air temperature inside the cuvette was kept constant at 25 °C. We used ambient relative humidity, and CO_2 concentration in the cuvette was set at 400 ppm. Net CO_2 flux was first measured in the dark, and then incrementally at photosynthetically active radiations (PAR) of 25, 50, 100, 250, 500, 1,000, 1,500, 1,800 μmol m^{-2} s^{-1}. Given the limited dimensions of the opaque conifer cuvette, measurements were conducted on smaller branches, ranging in diameter from 0.66 to 0.86 cm. Negative net CO_2 exchange indicates that CO_2 diffuses from the branch into the chamber. Based on net CO_2 efflux measurements in the dark (E_d) and under illumination (E_{il}) the refixation rate of respired CO_2 was calculated as:

$$\% \text{ Refixation} = \left(\frac{E_d - E_{il}}{E_d}\right) \times 100$$

Cavitation measurements

To illustrate the potential role of woody tissue photosynthesis in the light-dependent repair of cavitated vessels, as suggested by Schmitz et al. (2012), we conducted a dehydration experiment in the lab on cut branches of one non-instrumented control and one light-excluded tree from the eight selected trees, while measuring xylem cavitation with acoustic emission sensors as been used previously by Logullo and Salleo (1993) and Rosner et al. (2006). Measurements were repeated three times: once 9 days before the start of the drought stress treatment (21 September 2012, DOY 265) and twice during the drought stress treatment (i.e. 5 and 20 days after the start of the drought stress treatment, 5 and 19 October 2012, DOY 279 and 293, respectively). After cutting the branches, parafilm was used to prevent dehydration and again removed at the start of the cavitation experiment after which branches were left to dry out. In the lab, one acoustic emission (AE) sensor (VS150-M sensor and ASCO-P signal conditioner, Vallen systeme, Icking, Germany) was installed per branch. The AE sensors were clamped to the middle of the branches according to Rosner et al. (2009) but in contrast bark was not removed. Measurements were conducted over a 60-h time span. The maximum peak amplitude per second was recorded using a data acquisition system (Type NI USB 6009, National Instruments, Austin, TX, USA) and software (Labview 8.2, National Instruments, Austin, TX, USA). The AE signals above a certain threshold were assumed to occur due to cavitation of xylem vessels. The threshold was set at the background noise level (35.5 and 31 dB) and was determined by waving the sensors in the air. The cumulated number of AE signals was determined over the 60-h measurement period.

Measurements of bark chlorophyll concentration

We determined the effect of the light-exclusion treatment on bark chlorophyll concentration by sampling the bark of stems of the four trees which were not assigned to the control or light-exclusion treatment. Two out of these four trees were also light excluded as described above and at the same time as the other treated trees. We sampled bark because we assumed that light-exclusion would have a larger impact on chlorophyll concentrations in the bark than in the deeper xylem tissue. Moreover, for chlorophyll concentration measurements of the xylem tissue a larger amount of sample is needed as for the bark chlorophyll determination, potentially impacting tree physiology when sampling repeatedly over time. Bark samples of the additional trees were collected on three dates: at the day of light exclusion (2 August 2012, DOY 214), 53 days after the start of light exclusion (24 September 2012, DOY 267) and at the end of the measurement period, 99 days after the start of light exclusion (9 November 2012, DOY 313). At the last date, bark was additionally sampled from all control and light-excluded trees. Per stem, three bark samples of control and light-excluded trees were collected at approximately the same height, to account for potential internodal variation in bark chlorophyll concentration, immediately frozen in liquid nitrogen and stored at −80 °C. Samples were ground (A11 basic analytic mill, IKA-Werke GmbH & Co. KG, Staufen, Germany) and chlorophyll was extracted by adding 7.5 ml aceton (80 %) to 150 mg of sample. After 24 h extraction in the dark, samples were centrifuged and the supernatant was transferred to a glass cuvette and analysed for chlorophyll concentration with a spectrophotometer (UVIKON XL, Bio-Tek Instruments, Winooski, VT, USA) at wavelengths of 663.6 and 646.6 nm. Chlorophyll concentrations were calculated according to Porra et al. (1989) and expressed per unit of bark fresh weight (mg chl g^{-1} FW).

Data and statistical analysis

Data from automated measurements were recorded at 1 min intervals with a datalogger (CR1000, Campbell Scientific, Logan, Utah, USA) and averaged over 30 min intervals. Data were analysed using Excel 2007 (Microsoft Inc., Redmond, WA, USA).

Radial stem growth and DG derived from manual and automated diameter measurements, respectively, leaf gas exchange data (A_{max} and T), and bark chlorophyll concentrations were analysed using a repeated measures multifactorial analysis of variance (ANOVA). Radial stem growth data from manual measurements were analysed with treatment ($n = 2$) and date ($n = 23$) treated as fixed factors and individual tree ($n = 8$) as random factor. A similar model was used to analyse DG data from the automated measurements. In this case, our data were derived from a lower number of individual trees ($n = 4$) and confined to the measurements taken 1 week before and during the drought stress treatment (date, $n = 16$). Similarly, A_{max} and T measurements were confined to those taken during the drought stress treatment (date, $n = 12$) with a higher number of tree replicates (individual tree, $n = 8$). Bark chlorophyll concentration was analysed with date ($n = 3$), treatment ($n = 2$) and repetition ($n = 3$ per tree) as fixed factors, and tree ($n = 2$ per treatment for the first two dates and $n = 4$ for the last date) as random factor. Treatment means were compared using Fisher's least significant difference (LSD) test. Akaike Information Criterion (AIC) was used to determine the covariance structure that best estimated the correlation among

individual trees over time. All analyses were conducted using the mixed model procedure (PROC MIXED) of SAS (Version 9.1.3, SAS Inc., Cary, NC, USA) with $\alpha = 0.05$.

Results

Light response curves for woody tissue photosynthesis

The net CO_2 flux from branches into the cuvette was less negative with increasing light intensity up to 1,800 µmol m^{-2} s^{-1}, which indicates that a fraction of the respired CO_2 diffusing out of the branches was re-fixed by woody tissue photosynthesis in the stem chloroplasts (Fig. 1a). This refixation of respired CO_2 expressed as percentage of dark respiration within the light intensity range 1,000–1,800 µmol m^{-2} s^{-1} ranged on average from 61.7 ± 7.4 to 69.6 ± 3.9 % (Fig. 1b). High refixation rates of respired CO_2 occurred even under low light conditions, with an average (±SE) refixation of 56.6 ± 5.7 % at 250 µmol m^{-2} s^{-1}.

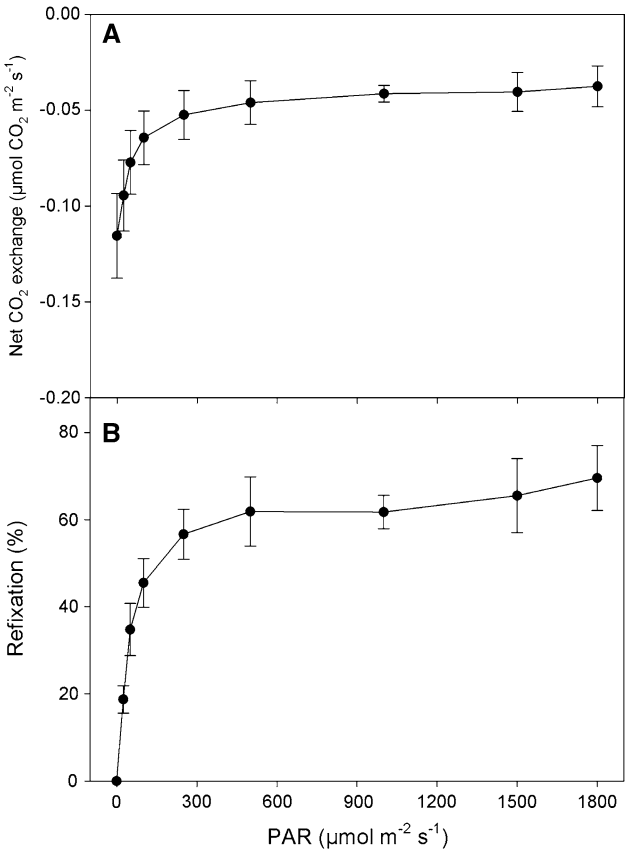

Fig. 1 The light response of branches of young poplar trees expressed as **a** mean net CO_2 flux and **b** mean refixation as a percentage of dark respiration. Measurements were made on branches of five additional trees at ambient RH and a set CO_2 concentration and air temperature of 400 ppm and 25 °C, respectively. Refixation was calculated as the difference in net CO_2 flux between illuminated and dark conditions, normalised for the net CO_2 flux under dark conditions. *Bars* indicate the standard error of the mean

Radial stem growth

Automated and manual stem diameter measurements showed that woody tissue photosynthesis contributes for 28.1 and 31.1 % to stem growth under well-watered conditions, respectively. Before light exclusion, radial stem growth was similar for trees of both treatments under well-watered conditions (Fig. 2). However, during light exclusion, radial stem growth of light-excluded trees (2.2 mm) was systematically lower than observed for the control trees diameter of control and light-excluded trees were observed from 19th September onwards, 48 days after the start of light exclusion. The average radial stem increment during the light-exclusion well-watered period was smaller for the light-excluded trees (3.1 ± 0.2 mm) than for the control trees (4.5 ± 0.5 mm).

Daily growth rate during drought stress

During the initial drought stress period, DG of both control and light-excluded trees was positive, indicating that both control and light-excluded trees were growing. However, under prolonged drought (from 1 week onwards, 7 October, Fig. 3) stems of light-excluded and control trees shrank with DG of light-excluded trees being systematically lower compared to DG of control trees. Hence, under prolonged drought light-excluded trees were shrinking faster than control trees (Fig. 3).

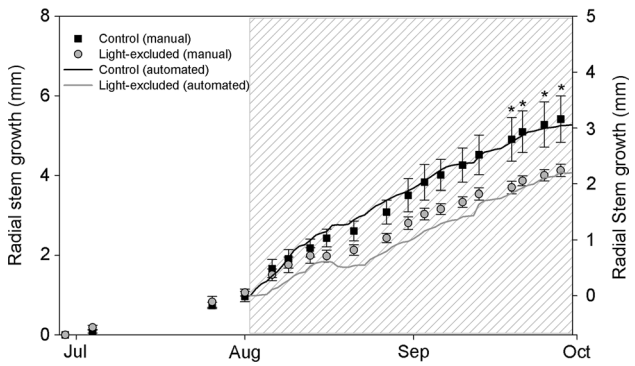

Fig. 2 Radial stem growth under well-watered and drought conditions derived from manual stem diameter measurements averaged for control (*black square*) and light-excluded (*grey dot*) trees ($n = 4$ per treatment, left *y*-axis) and automated diameter measurements on one control (*black*) and light-excluded (*grey*) tree ($n = 1$ per treatment, right *y*-axis). Radial stem growth was calculated as the cumulative increase in stem diameter over the experimental period. The *dashed area* indicates the period of stem and branch light exclusion. *Asterisks* indicate significant ($P < 0.05$) differences in average stem diameter between both treatments at each observation derived from manual measurements. *Bars* indicate the standard error of the mean

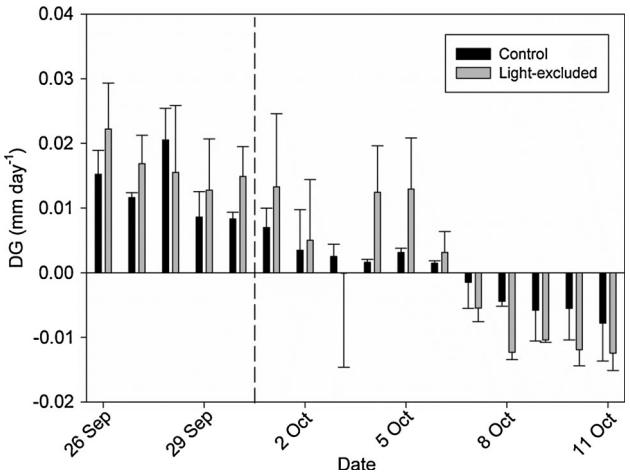

Fig. 3 Radial daily stem growth rate (DG) before and during drought stress derived from automated measurements of the stem diameter and averaged for control and light-excluded trees ($n = 2$ per treatment). DG was calculated as the difference between two successive daily maximum values of the stem diameter. The *dashed line* indicates the timing of last irrigation before the onset of drought stress. No significant differences ($P < 0.05$) in DG between both treatments were observed. *Bars* indicate the standard error of the mean

Maximum net photosynthesis and transpiration rate

Light exclusion of the stem and woody branches additionally affected leaf gas exchange during drought stress (Fig. 4). Before drought stress, similar rates of maximum net photosynthesis (A_{max}) were observed in leaves of control and light-excluded trees. During the drought treatment, A_{max} in light-excluded trees started to decrease about 3 days earlier than in control trees (4 vs. 7 October, Fig. 4a) and this was approximately simultaneous to the onset of negative DG during drought stress (Fig. 3). A similar difference between control and light-excluded trees was observed in leaf transpiration (T, Fig. 4b).

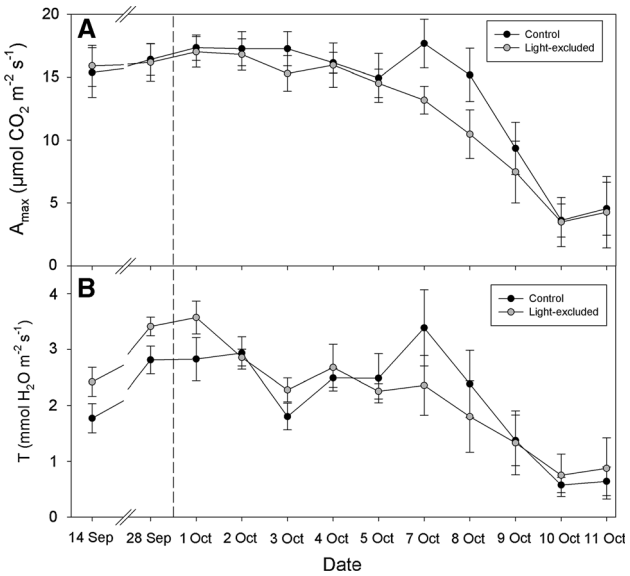

Fig. 4 Maximum leaf net photosynthesis (A_{max}) (**a**) and transpiration rate (T) (**b**) before and during drought stress, averaged for leaves ($n = 2$ per tree) of control and light-excluded trees ($n = 4$ per treatment). The *dashed line* indicates the timing of the last irrigation before the onset of drought stress. No significant differences ($P < 0.05$) in A_{max} neither T between both treatments were observed. *Bars* indicate the standard error of the mean

Cavitation

The cumulative number of acoustic emissions was similar in branches of control and light-excluded trees before being subjected to drought stress (Fig. 5). Five days after the start of the drought treatment, we found a larger cumulative number of acoustic emissions in the control branches 5 days after the start of the drought stress treatment (Fig. 5). Finally, 20 days after the start of the drought stress treatment, a low cumulative number of AE signals were recorded (Fig. 5), regardless of the treatment, implying that the majority of the xylem vessels in the branches were already cavitated before the last branch dehydration experiment started.

Bark chlorophyll concentration

At the start of the light-exclusion treatment, similar bark chlorophyll concentrations were detected in the bark of control and light-excluded trees (Table 1). However, light

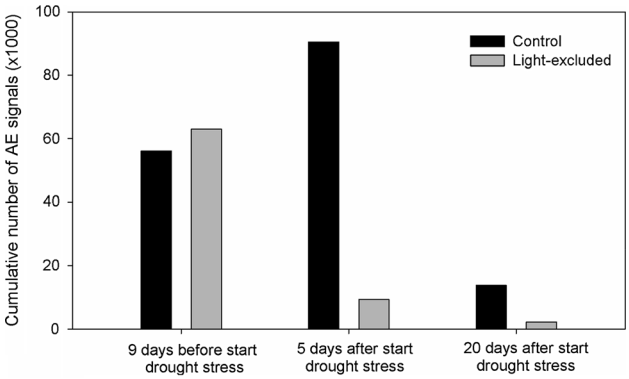

Fig. 5 Cavitation in branches of control and light-excluded trees measured as the cumulative number of acoustic emissions over a 60-h dehydration period. Measurements were repeated three times: at 9 days before the start of the drought stress treatment (22–24 September 2012), and 5 and 20 days after the start of the drought stress treatment (5–7 and 19–21 October 2012), respectively. The larger the cumulative number of acoustic emission (AE) signals, the more xylem vessels were hydraulically functional just before being excised and the more vessels could thus still cavitate during dehydration

Table 1 Chlorophyll concentration of bark, sampled from *Populus deltoides x nigra* 'Monviso' trees, on different dates (2 August 2012, 24 September 2012, and 9 November 2012) during the light-exclusion experiment

Chlorophyll concentration (mg Chl g FW^{-1})			
Treatment	Day of light exclusion	53 days after start light exclusion	99 days after start light exclusion
Control	0.33 ± 0.03^{a}	0.41 ± 0.01^{a}	0.73 ± 0.02^{a}
Light excluded	0.38 ± 0.02^{a}	0.24 ± 0.01^{b}	0.28 ± 0.01^{b}

On the first two dates, bark was sampled ($n = 3$ per tree) from additional trees ($n = 2$ per treatment) treated similarly as the measured control and light-excluded trees, while on the last date bark was sampled from all control and light-excluded trees ($n = 6$ per treatment). Data are averaged (±SE) per treatment for all samples and all trees. Different letters indicate significant differences in chlorophyll concentration between treatments for a specific date ($P < 0.001$)

exclusion resulted in a significant decrease in bark chlorophyll concentration, whereas bark chlorophyll concentration in control trees (no light-exclusion) increased over time. In particular, 99 days after the start of the light-exclusion treatment, when bark of all control and light-excluded trees was sampled, a large difference was observed in bark chlorophyll concentration between both treatments (Table 1).

Discussion

The role of woody tissue photosynthesis in trees

Since the first observation of chlorophyll containing woody tissues late 19th–early 20th century (see references in Pfanz et al. 2002), a large number of studies have been conducted on the nature and magnitude of woody tissue photosynthesis in trees (Bossard and Rejmanek 1992; Nilsen 1995; Cernusak and Marshall 2000; Wittmann et al. 2001; Pfanz et al. 2002; Berveiller et al. 2007). These photosynthetic tissues use respired CO_2 as a substrate, thereby partially compensating for the loss of carbon by respiration while providing carbon compounds for tissue synthesis or to sustain plant metabolism (Cernusak et al. 2001). The overall supply of carbon by woody tissue photosynthesis as compared to the fraction of carbon derived from leaf level photosynthesis is assumed to be small (Aschan and Pfanz 2003). However, bark and xylem tissues might play an important role as a means to guarantee photosynthate supply when trees are leafless due to environmental (e.g. due to herbivory or fungal disease) or phenological conditions (Pfanz 2008; Eyles et al. 2009; Saveyn et al. 2010).

Woody tissue photosynthesis contributes to stem growth

In our study, we observed that light exclusion of the stem and the woody branches of foliated *Populus deltoides x nigra* 'Monviso' trees resulted in a decreased radial stem growth and bark chlorophyll concentration, implying that even under well-watered growing conditions woody tissue photosynthesis can significantly contribute to the overall plant carbon income. We are aware of only two studies that have quantified the contribution of woody tissue photosynthesis to stem growth. Saveyn et al. (2010) combined a similar light-exclusion treatment with stem diameter measurements and observed a reduction in stem diameter and stem chlorophyll concentration for light-excluded trees of different native Californian species: *Prunus ilicifolia*, *Umbellularia californica* and *Arctostaphylos Manzanita*. They estimated that for the different species between 25 and 56 % of the stem tissue synthesis was derived from woody tissue photosynthesis. Based on isotope analysis of wood samples of covered and uncovered branch sections of *Eucalyptus miniata*, Cernusak and Hutley (2011) calculated that 11 % of newly formed branch tissue was constructed from stem assimilates. Our estimates of the contribution of woody tissue photosynthesis to radial stem growth for *Populus deltoides x nigra* 'Monviso' were 28 and 31 % using manual and automated measurements, respectively. However, lower contributions might be observed for older trees or for species that contain lower concentrations of bark and pith chlorophyll in their stem and branch tissues (Aschan and Pfanz 2003). In foliated trees, xylem-transported CO_2 derived from below- and aboveground respiration is available as substrate for woody tissue photosynthesis, besides locally respired CO_2 (McGuire et al. 2009; Bloemen et al. 2013a, b; 2014). During leafless periods, woody tissue photosynthesis solely relies on locally respired CO_2, which internally concentrates due to the high resistance to radial CO_2 diffusion exerted by bark and xylem tissues (Steppe et al. 2007). Therefore, woody tissue photosynthesis in foliated trees may benefit from this additional substrate source and use CO_2 respired in organs remote from the site of assimilation to maintain the local stem and branch carbon status.

Woody tissue photosynthesis contributes to tree drought stress resilience

More importantly, our study illustrates the potential role of woody tissue photosynthesis in tree drought stress resilience. While leaf photosynthesis converts atmospheric CO_2 into carbohydrates, woody tissue photosynthesis relies on endogenously respired CO_2 for sugar synthesis, which makes this process less vulnerable to potentially lethal

tissue dehydration under drought conditions (Comstock and Ehleringer 1988; Nilsen 1992; Wittmann and Pfanz 2008). Moreover, the low peridermal water vapour conductance as compared to leaf conductance leads to high water use efficiency (Wittmann and Pfanz 2008). In particular in desert and semi-desert environments, where water scarcity is considered as the most important abiotic constraint for plant growth (Aschan and Pfanz 2003), plants are assumed to benefit from photosynthesis in green non-leaf tissues. We observed that the stem of light-excluded *Populus deltoides x nigra* 'Monviso' trees tended to shrink more drastically in response to severe drought stress than observed in stems of control trees. Growth processes are known to be very sensitive to drought stress (Hsiao 1973; Steppe et al. 2006; Saveyn et al. 2007a) and are related to the water status of living stem tissues, of which the cell turgor pressure in the stem tissue is a good measure (Bradford and Hsiao 1982). In living plant tissues, cell turgor and volume can be maintained by osmotic adjustment (i.e. the accumulation of solutes in the symplast, like for instance sugars) (Woodruff et al. 2004; Saveyn et al. 2007a). Sugars synthesised in chlorophyll containing bark and xylem tissues are potentially used to maintain cell turgor, which might explain why stems of light-excluded trees tended to shrink more drastically relative to control trees. Stem growth cessation can additionally be explained by a decrease in carbohydrate supply under drought stress (Saveyn et al. 2007b; Steppe et al. 2008; Lavoir et al. 2009; De Schepper and Steppe 2010; Simpraga et al. 2011). We observed that stem shrinkage under drought stress (i.e. a negative daily growth rate) occurred simultaneously with the pronounced decrease in leaf photosynthesis, regardless of the treatment. Leaf photosynthesis measured under drought conditions tended to decline more rapidly when woody tissue photosynthesis was impeded by light exclusion. In contrast to previous assumptions (Wittmann and Pfanz 2008), we hypothesise that the role of woody tissue photosynthesis in maintaining the carbon status of temperate species under drought stress conditions might extend beyond the stem level.

Does woody tissue photosynthesis play a role in xylem cavitation sensing and repair?

While past studies mainly described woody tissue photosynthesis from a plant water use efficiency perspective, recent results indicate that stem and branch CO_2 assimilation might directly affect the xylem hydraulic pathway in drought-stressed plants. In these plants, the decrease in hydraulic function due to xylem cavitation has been considered as the main source of productivity loss (Logullo and Salleo 1993; Hacke et al. 2000; Zwieniecki and Holbrook 2009). It has been shown that cavitation is accompanied by acoustic emissions that can be detected non-destructively at the surface of plant organs (Milburn and Johnson 1966; Tyree and Dixon 1983; Rosner et al. 2006; Vergeynst et al. 2014). Recovery from cavitation may occur under suitable conditions (McCully et al. 1998; Perks et al. 2004; Zufferey et al. 2011) but the actual mechanisms that allow plants to refill embolized xylem conduits and thereby maintain the hydraulic function is still under debate (Clearwater and Goldstein 2005; Zwieniecki and Holbrook 2009). Most recent studies hypothesise that sugars play a crucial role in the creation of an osmotic driving force to refill embolized xylem vessels (Secchi and Zwieniecki 2011) and in the chemical sensing of cavitation (Zwieniecki and Holbrook 2009).

In this context, woody tissue photosynthesis in chlorophyll containing cells might play a role in the local provision of these sugars, in addition to sugars derived from local phloem unloading (Nardini et al. 2011). Our acoustic emission measurements suggest that woody tissue photosynthesis potentially plays an important role in drought-stressed cavitation repair in poplar, as observed previously for mangrove species (Schmitz et al. 2012). Xylem vessels in branches of control drought-stressed trees were hydraulically more functional compared to vessels in light-excluded trees, so that more vessels could still cavitate during the three-day dehydration of the excised branch in the lab as detected as high number of AE signals during the dehydration experiment.

Long-term light-exclusion treatment probably hindered refilling of the embolized xylem vessels due to the lack of leakage of locally synthesised sugars into the xylem vessels. Schmitz et al. (2012) observed that hydraulic conductivity of mangrove branches decreased following light exclusion. They suggested that xylem chloroplasts mainly played a role in xylem refilling, given its location adjacent to the xylem vessels. However, with our setup, we were not able to distinguish the role of chloroplasts in xylem and bark in embolism repair. Stem hydraulics, which are strongly dependent on cavitation of xylem vessels, directly affect leaf water status because plants supply water to their leaves dependent on the degree of cavitation (Hacke et al. 2000). Therefore, the lower cavitation repair after drought stress in light-excluded trees than in control trees might explain their lower transpiration, in particular around 5 days after the start of the drought stress. However, more observations are needed for poplar and other species to confirm the role of woody tissue photosynthesis in xylem embolism repair.

Conclusion

We examined the role of woody tissue photosynthesis in plant functioning under well-watered and drought conditions. Our results show that assimilation of respired CO_2 might play a dual role in tree drought stress resilience. The sugars synthesised in chlorophyll containing woody tissues might either be used to meet the metabolic carbon demand of drought-stressed plants or to maintain the hydraulic function of the xylem. In particular, we believe that our results provide an impetus for future studies aiming at understanding the significance of woody tissue photosynthesis in plant drought stress resilience, as its role in providing local sugars for maintaining cell turgor or repairing xylem embolism merits further study.

Author contribution J. B., L. L. V. and L. O-M performed the measurements. J. B., L. L. V. and K. S. interpreted the results and J. B. wrote the MS. All authors commented on the manuscript during the final stages.

Acknowledgments The authors wish to thank Philip Deman and Geert Favyts of the Laboratory of Plant Ecology, Ghent University, for their enthusiastic technical support. Moreover, we thank Marie-Christine Van Labeke and her colleagues from the Department of Plant Production for the use of the spectrophotometer and help with bark chlorophyll analysis. This project was supported by a starting grant from the Special Research Fund (BOF) of Ghent University to KS and a PhD funding from the Research Foundation—Flanders (FWO) granted to LV.

Conflict of interest The authors declare that they have no conflict of interest.

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