

# Stem respiration and carbon dioxide efflux of young *Populus deltoides* trees in relation to temperature and xylem carbon dioxide concentration

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**Abstract** Oxidative respiration is strongly temperature driven. However, in woody stems, efflux of CO<sub>2</sub> to the atmosphere ( $E_A$ ), commonly used to estimate the rate of respiration ( $R_S$ ), and stem temperature ( $T_{st}$ ) have often been poorly correlated, which we hypothesized was due to transport of respired CO<sub>2</sub> in xylem sap, especially under high rates of sap flow ( $f_s$ ). To test this, we measured  $E_A$ ,  $T_{st}$ ,  $f_s$  and xylem sap CO<sub>2</sub> concentrations ([CO<sub>2</sub>\*]) in 3-year-old *Populus deltoides* trees under different weather conditions (sunny and rainy days) in autumn. We also calculated  $R_S$  by mass balance as the sum of both outward and internal CO<sub>2</sub> fluxes and hypothesized that  $R_S$  would correlate better with  $T_{st}$  than  $E_A$ . We found that  $E_A$  sometimes correlated well with  $T_{st}$ , but not on sunny mornings and afternoons or on rainy days. When the temperature effect on  $E_A$  was accounted for, a clear positive relationship between  $E_A$  and xylem [CO<sub>2</sub>\*] was found. [CO<sub>2</sub>\*] varied diurnally and increased substantially at night and during periods of rain. Changes in [CO<sub>2</sub>\*] were related to changes in  $f_s$  but not  $T_{st}$ . We conclude that changes in both respiration and internal CO<sub>2</sub> transport altered  $E_A$ . The dominant component flux of  $R_S$  was  $E_A$ . However, on a 24-h basis, the internal transport flux represented 9–18% and 3–7% of  $R_S$  on sunny and rainy days, respectively, indicating that the contribution of stem respiration to forest

C balance may be larger than previously estimated based on  $E_A$  measurements. Unexpectedly, the relationship between  $R_S$  and  $T_{st}$  was sometimes weak in two of the three trees. We conclude that in addition to temperature, other factors such as water deficits or substrate availability exert control on the rate of stem respiration so that simple temperature functions are not sufficient to predict stem respiration.

**Keywords** Carbon dioxide transport · Mass balance · Sap flow · Xylem sap pH · Woody tissue respiration

## Introduction

CO<sub>2</sub> efflux from a stem segment ( $E_A$ ) is expected to be closely related to the rate of physiologically active oxidative respiration of the living tissues (phloem, cambium and xylem parenchyma) in that segment. Since respiration consists of enzymatic reactions, it is known to be highly temperature dependent (Amthor 1989). Strong relationships between  $E_A$  and temperature ( $T$ ) have been reported (e.g. Levy and Jarvis 1998; Damesin et al. 2002; Gansert et al. 2002). However, the relationship between  $E_A$  and  $T$  has also been reported to be unclear (Edwards and McLaughlin 1978; Kakubari 1988; Kaipainen et al. 1998). These observations gave rise to the idea that  $E_A$  is not just a reflection of the rate of actual respiration ( $R_S$ ) of the living cells in the woody tissues. It has been suggested that sap flow ( $f_s$ ) might influence  $E_A$ . Given the high solubility of CO<sub>2</sub> in water, respired CO<sub>2</sub> at stem level can dissolve in the xylem sap and may be carried upward with the transpiration stream instead of diffusing radially through the bark (Negisi 1979; Martin et al. 1994). Additionally, CO<sub>2</sub> originating from root tissue or soil microbial respiration

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can dissolve in xylem sap, be carried upward, and be released from the stem remote from the site of respiration (Levy et al. 1999; Teskey and McGuire 2007). Hence,  $E_A$  may under- or overestimate the rate of respiration of the stem tissue due to transport of  $\text{CO}_2$  in the transpiration stream. Several studies have established the relationship between  $f_s$  and  $E_A$  (Negisi 1979; Martin et al. 1994; Levy et al. 1999; Edwards and Wullschlegel 2000; Bowman et al. 2005; Gansert and Burgdorf 2005; Maier and Clinton 2006). However,  $f_s$  measurements do not provide direct information about  $\text{CO}_2$  transport inside the stem.

Recently, McGuire and Teskey (2004) proposed a mass balance approach for estimating  $R_S$  of a stem segment that accounts for both outward (i.e.  $E_A$ ) and internal fluxes of  $\text{CO}_2$ . Internal fluxes comprise the  $\text{CO}_2$  transport by flowing sap ( $F_T$ ) and the transient changes in the storage of  $\text{CO}_2$  within the stem ( $\Delta S$ ). To calculate the rate of  $\text{CO}_2$  transport,  $f_s$  and the xylem  $\text{CO}_2$  concentration ( $[\text{CO}_2]$ ) above and below the stem segment are needed. Recently, methods have been developed to directly measure the dynamics of xylem  $[\text{CO}_2]$  in situ (McGuire and Teskey 2002; McGuire et al. 2007; Teskey and McGuire 2007). A few reports have established the relationship between xylem  $[\text{CO}_2]$  and  $E_A$  (Teskey and McGuire 2002, 2005, 2007; Maier and Clinton 2006; McGuire et al. 2007; Saveyn et al. 2006, 2007a; Steppe et al. 2007). However,  $R_S$  has actually been calculated in very few studies (McGuire and Teskey 2004; Bowman et al. 2005; Teskey and McGuire 2007). Because internal fluxes are accounted for, it is expected that calculated  $R_S$  would correlate better with stem  $T$  ( $T_{st}$ ) than ( $E_A$ ). However, as far as we know, the relationship between calculated  $R_S$  based on mass balance and  $T_{st}$  has never been investigated.

The first objective of this work was to elucidate the driving variables of  $E_A$  in a clone of *Populus deltoides* during autumn. The effects of both  $T_{st}$  and xylem  $[\text{CO}_2]$  were analysed under different weather conditions (sunny and rainy days). The second objective was to calculate  $R_S$  by mass balance and to examine the relationship between  $T_{st}$  and  $R_S$ . Because calculated  $R_S$  accounts for both outward and internal fluxes of  $\text{CO}_2$ , and represents the actual respiration rate of the stem tissue, it was hypothesized that the correlation between  $R_S$  and  $T_{st}$  would be better than the correlation between  $E_A$  and  $T_{st}$ .

## Materials and methods

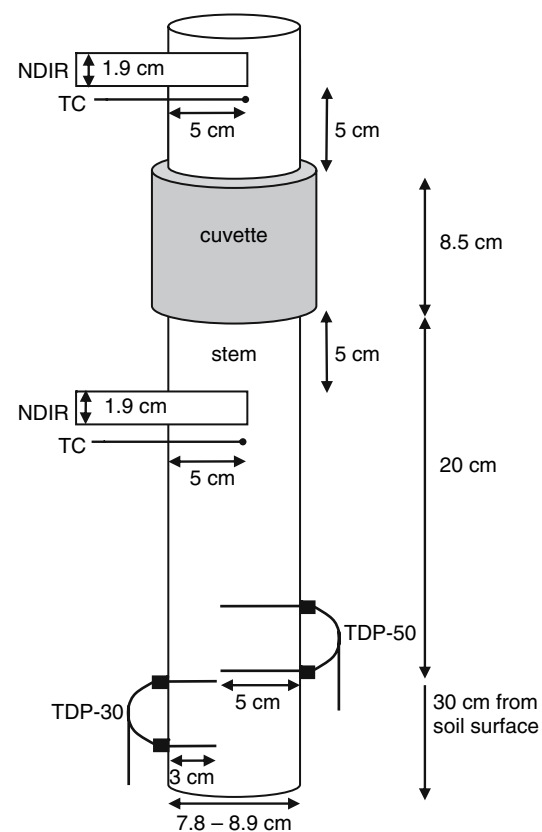
### Plant material and experimental conditions

Field experiments were conducted during autumn in a nursery at Whitehall Forest, an experimental forest of the University of Georgia near Athens, Georgia, from 13

October to 1 November 2006 (days 286–305). Measurements were made on three 3-year-old trees of an eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) clone (clone 98; Meadwestvaco, Paducah, Ky.). Stem diameters were 8.9, 7.7 and 7.8 cm at 0.5 m above ground for trees 1, 2 and 3, respectively. Due to limited available equipment, only one tree at a time was measured. Measurement periods were days 286–291 for tree 1, days 292–298 for tree 2 and days 299–305 for tree 3.

### Measurements

$E_A$  was measured in the field, in a fan-stirred 8.5-cm-long cylindrical Lexan cuvette that completely surrounded the stem at 0.5 m above ground (Fig. 1). The cuvette was secured to the stem at both ends with closed cell foam gaskets and hose clamps. To provide a gas-tight seal, glazing putty was used to cover the gaskets. The cuvette was covered with aluminium foil to prevent cortical photosynthesis. Compressed air at near-ambient  $[\text{CO}_2]$  was supplied to the cuvette at  $0.5 \text{ l min}^{-1}$  using a mass



**Fig. 1** Schematic drawing of the experimental set-up, indicating the dimensions of stem diameter, holes, distances and positions of equipment installed on the stem. *NDIR* Non-dispersive infrared  $\text{CO}_2$  sensor, *TC* thermocouple, *TDP* thermal dissipation probe

flow controller (model FMA 5514; Omega Engineering, Stamford, Conn.). Air leaving the cuvette was dried over a column of magnesium perchlorate, and its CO<sub>2</sub> concentration was measured with an infrared gas analyser (IRGA) (model 7000; Li-Cor, Lincoln, Neb.). The IRGA was operated in open configuration and  $E_A$  ( $\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$ ) was calculated as:

$$E_A = (f_a/v) \times \Delta[\text{CO}_2] \quad (1)$$

where  $f_a$  is the flow rate of air through the cuvette ( $3.7 \times 10^{-4} \text{ mol s}^{-1}$ ),  $v$  is the sapwood volume of the stem segment ( $\text{m}^3$ ) and  $\Delta[\text{CO}_2]$  is the difference between  $[\text{CO}_2]$  of air flowing into and out of the cuvette ( $\mu\text{mol mol}^{-1}$ ). To determine sapwood volume, stem diameter was measured with a diameter tape at the top and bottom of the cuvette, and bark thickness was measured from the increment cores that were taken for pH measurements (described below). The trees contained no heartwood.

$[\text{CO}_2]$  (%) was measured in situ with two solid state non-dispersive infrared (NDIR) CO<sub>2</sub> sensors (model GMM221; Vaisala, Helsinki) inserted in the stems in holes of 50-mm length and 19-mm diameter. The holes were drilled 50 mm above and below the cuvette (Fig. 1). Flexible putty adhesive (Qubitac; Qubit Systems, Kingston, Ontario) was placed at the junction of the sensor and stem to provide a gas-tight seal. The CO<sub>2</sub> sensors measured  $[\text{CO}_2]$  of the gas in the headspace of the holes, which is in equilibrium with the xylem sap (Hari et al. 1991; Levy et al. 1999). Gas concentration (%) was converted to total dissolved C ( $[\text{CO}_2^*]$ ;  $\mu\text{mol l}^{-1}$ ) using temperature-dependent Henry's solubility and dissociation constants for CO<sub>2</sub> in water (Butler 1991; Stumm and Morgan 1996; McGuire and Teskey 2002). For this conversion,  $T_{\text{st}}$  and pH of the xylem sap must be known.  $T_{\text{st}}$  was measured with two copper-constantan thermocouples inserted 50-mm deep in the stem near the CO<sub>2</sub> sensors (Fig. 1). Two methods were applied to collect the xylem sap for pH measurements. First, sap was expressed with a pressure chamber (PMS Instruments, Corvallis, Oreg.) from excised twigs. Second, sap was expressed with a vise from 5-mm-diameter stem increment cores. The pH of the expressed sap was measured with a solid state pH microsensor connected to a pH meter (Red-Line standard sensor, Argus meter; Sentron, Roden, The Netherlands). Because sampling was destructive, pH measurements were performed after the experiments and we assumed that the pH was constant during the experiments.

Sap velocity ( $\text{cm h}^{-1}$ ) was measured at two xylem depths (15 and 25 mm) with thermal dissipation sensors (model TDP-30 and TDP-50; DynaMax, Houston, Tex.) (Granier 1987) installed on opposite sides of the stem 0.3 m above ground (Fig. 1). The sensors were thermally

insulated with open cell foam and several layers of aluminium foil. Average sap velocity was converted to total  $f_s$  ( $\text{g h}^{-1}$ ) by multiplying by the sapwood area. To determine sapwood area, stem diameter was measured with diameter tape at sensor level, and bark thickness was measured from the increment cores that were taken for pH measurements.

All sensors and the cuvette were protected from solar radiation by wrapping the stem with reflective bubble insulation (Reflectix; Reflectix, Markelville, Ind.). Sensor data were collected simultaneously every 10 s and recorded every 300 s using a datalogger (model CR23X; Campbell Scientific, Logan, Utah). In addition, a weather station measured ambient air temperature and relative humidity (CS500; Campbell Scientific) and rainfall (TE525; Campbell Scientific) every 300 s and recorded 900-s means (CR10X; Campbell Scientific). Vapour pressure deficit (VPD) (kPa) was calculated from air temperature and relative humidity data.

#### Calculation of CO<sub>2</sub> flux components and stem respiration rate

The mass balance approach developed by McGuire and Teskey (2004) was applied to estimate the  $R_S$  ( $\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$ ) of the stem segment enclosed by the cuvette. This balance consists of three terms: the radial efflux of CO<sub>2</sub> from the stem to the atmosphere (i.e.  $E_A$ ), the axial flux of dissolved CO<sub>2</sub> entering and leaving the segment in the flowing sap (i.e. transport flux,  $F_T$ ) and the increase or decrease in sap  $[\text{CO}_2^*]$  of the segment over time (i.e. storage flux,  $\Delta S$ ).

$$R_S = E_A + F_T + \Delta S \quad (2)$$

Transport flux ( $\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$ ) was calculated as:

$$F_T = (f_s/v) \times \Delta[\text{CO}_2^*] \quad (3)$$

where  $f_s$  is sap flow through the segment ( $\text{l s}^{-1}$ ),  $v$  is the sapwood volume of the stem segment ( $\text{m}^3$ ) and  $\Delta[\text{CO}_2^*]$  is xylem  $[\text{CO}_2^*]$  measured above the cuvette minus xylem  $[\text{CO}_2^*]$  measured below the cuvette ( $\mu\text{mol l}^{-1}$ ).

Storage flux ( $\mu\text{mol CO}_2 \text{ m}^{-3} \text{ s}^{-1}$ ) was calculated as:

$$\Delta S = \left( \overline{[\text{CO}_2^*]}_{t1} - \overline{[\text{CO}_2^*]}_{t0} \right) \times w / \Delta t \quad (4)$$

where  $\overline{[\text{CO}_2^*]}_{t0}$  is the mean of  $[\text{CO}_2^*]$  above and below the cuvette at time  $t0$  and  $\overline{[\text{CO}_2^*]}_{t1}$  is the mean of  $[\text{CO}_2^*]$  above and below the cuvette at time  $t1$ ,  $w$  is the volumetric water content of the segment ( $\text{l m}^{-3}$ ) and  $\Delta t$  is the time interval ( $t1-t0$ ) (300 s). We assumed a volumetric sapwood water content of  $500 \text{ l m}^{-3}$ , based on the average specific gravity for hardwoods (Panshin and de Zeeuw 1980).

Temperature response of stem  $\text{CO}_2$  efflux and the rate of respiration

To investigate the temperature response of  $E_A$ , the data for days without rainfall were used from each tree.  $E_A$  was regressed against  $T_{\text{st}}$  ( $^{\circ}\text{C}$ ) with the following equation:

$$E_A = E_A(20) \times Q_{10}^{\frac{T_{\text{st}}-20}{10}} \quad (5)$$

where  $E_A(20)$  is  $\text{CO}_2$  efflux at  $20^{\circ}\text{C}$  and  $Q_{10}$  is the relative increase in  $\text{CO}_2$  efflux with a  $10^{\circ}\text{C}$  rise in temperature. Parameters  $E_A(20)$  and  $Q_{10}$  were estimated by ordinary least squares. A second regression was performed, with the dataset further limited to periods where  $f_s$  was zero (night-time data).

The same procedure was used to investigate the temperature response of  $R_S$  of each tree, using the datasets without rainfall:

$$R_S = R_S(20) \times Q_{10}^{\frac{T_{\text{st}}-20}{10}} \quad (6)$$

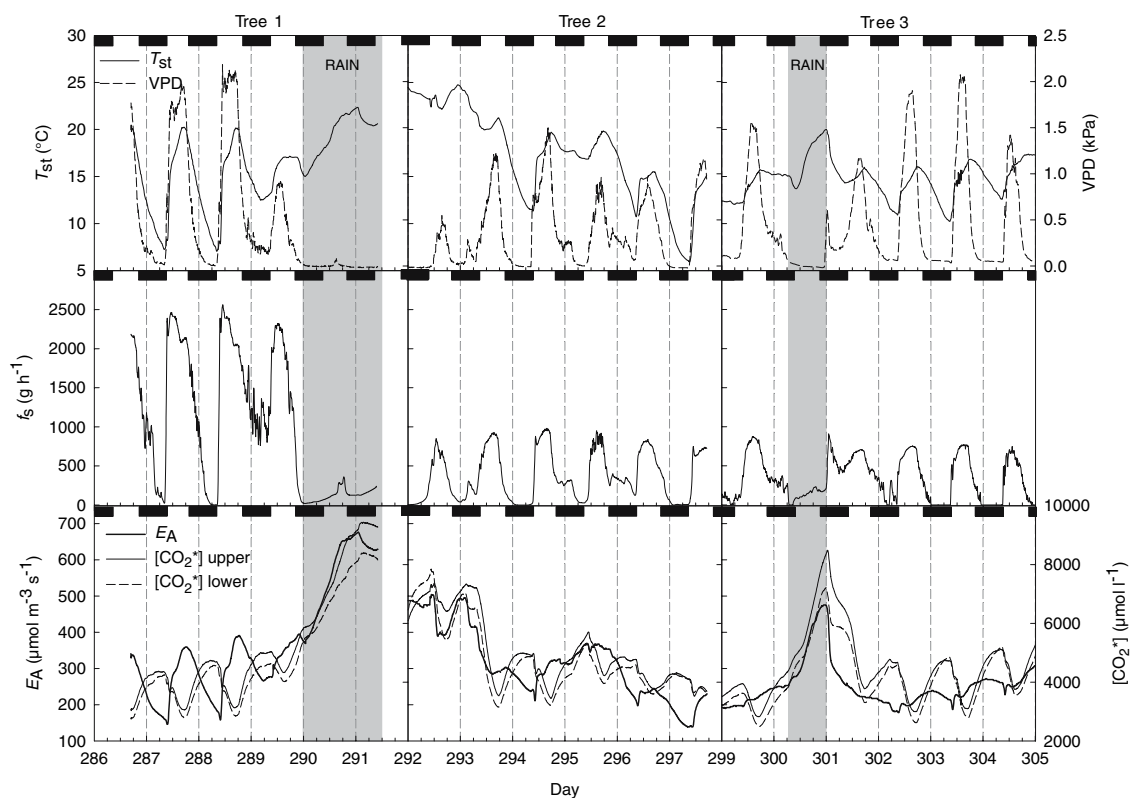
where  $R_S(20)$  is the rate of respiration at  $20^{\circ}\text{C}$  and  $Q_{10}$  is the relative increase in respiration with a  $10^{\circ}\text{C}$  rise in temperature.

Values of  $T_{\text{st}}$  and the parameters obtained from the regression between  $R_S$  and  $T_{\text{st}}$  were used to predict the rate of respiration of the stem segment with Eq. 6. Residual  $E_A$  and  $R_S$  were calculated as measured  $E_A$  or calculated  $R_S$  minus predicted  $R_S$  based on Eq. 6.

## Results

### Microclimatological and physiological variables

Most days during the experiment were sunny, except for day 290 (tree 1) and day 300 (tree 3), when it rained, and day 289 (tree 1) and day 292 (tree 2), when it was heavily clouded (Fig. 2). Stem temperatures ranged from  $6$ – $25^{\circ}\text{C}$  over the measurement period. Maximum  $f_s$  was  $2,537$ ,  $971$  and  $910 \text{ g h}^{-1}$  for trees 1, 2 and 3, respectively (Fig. 2). It is noteworthy that during some nights,  $f_s$  never reached zero. When night-time VPD remained high ( $>0.2 \text{ kPa}$ ) on days 288–289, 294–295, 295–296, 300–301 and 301–302, substantial night-time  $f_s$  occurred. The two different methods for measuring sap pH gave similar mean results:  $6.80 \pm 0.55$  with the pressure bomb and  $6.83 \pm 0.17$  with the vise. Sap pH did not significantly differ between the



**Fig. 2** Six-day periods of stem temperature ( $T_{\text{st}}$ ), vapour pressure deficit (VPD), sap flow ( $f_s$ ), stem  $\text{CO}_2$  efflux ( $E_A$ ) and xylem sap  $\text{CO}_2$  concentration ( $[\text{CO}_2^*]$ ) above and below the stem cuvette as

measured in the three 3-year-old field-grown trees of a *Populus* clone. Black boxes indicate night-time periods and vertical dashed lines indicate midnight

three trees, so an average value (6.80) was used to calculate xylem  $[CO_2^*]$ . Xylem  $[CO_2^*]$  ranged from 2.78 to 9.45, 3.16 to 7.85 and 2.51 to 8.49  $mmol\ l^{-1}$  for trees 1, 2 and 3, respectively (Fig. 2). These values correspond with a  $[CO_2]$  range of 3.9–17.6%. Xylem  $[CO_2^*]$  above the cuvette was generally slightly higher than  $[CO_2^*]$  below the cuvette. The mean difference was 0.32, 0.31 and 0.32  $mmol\ l^{-1}$  for trees 1, 2 and 3, respectively. Diurnal patterns of both upper and lower  $[CO_2^*]$  measurements were very similar. On sunny days, xylem  $[CO_2^*]$  exhibited clear daytime depressions, despite the higher  $T_{st}$  during the day compared with the night. During rain (days 290 and 300),  $[CO_2^*]$  increased dramatically. In tree 3  $[CO_2^*]$  more than doubled during the rain, and from the moment  $f_s$  restarted (night-time  $f_s$  due to a sudden increase in VPD when rainfall stopped),  $[CO_2^*]$  showed a large decline, reaching its original value in the late afternoon the next day.  $E_A$  ranged from 154–673, 146–499 and 183–475  $\mu mol\ m^{-3}\ s^{-1}$  for trees 1, 2 and 3, respectively (Fig. 2). Expressed per unit stem surface area, these values correspond with 3.4–15.0, 2.8–9.6 and 3.5–9.2  $\mu mol\ m^{-2}\ s^{-1}$ , respectively. Similar to  $[CO_2^*]$ ,  $E_A$  showed a large increase on the rainy days.

CO<sub>2</sub> efflux in relation to temperature

For the entire dataset (except for days with rain),  $Q_{10}$  of  $E_A$  ranged between 1.59 and 1.89,  $E_A(20)$  between 299.8 and 374.7  $\mu mol\ m^{-3}\ s^{-1}$  and the coefficient of determination ( $R^2$ ) for the exponential regression ranged between 0.42 and 0.88 (Table 1). When only data at zero  $f_s$  were taken into account, Eq. 5 was a better fit (higher  $R^2$ ). Parameter values obtained from the regression based on zero  $f_s$  data were higher for all trees (Table 1). In tree 1, measured  $E_A$  was much lower than the temperature prediction (with parameters obtained for  $R_S$  from Table 1) during the daytime on sunny days 287 and 288 (Fig. 3). On average, on these days, measured  $E_A$  was only 71% of the temperature-predicted flux during daytime (0900–1900 hours). Residual

**Table 1** Characteristics of the response of measured CO<sub>2</sub> efflux ( $E_A$ ;  $\mu mol\ m^{-3}\ s^{-1}$ ) and calculated rate of respiration ( $R_S$ ;  $\mu mol\ m^{-3}\ s^{-1}$ ) to stem temperature ( $T_{st}$ ) during the entire measurement period, except for days with rainfall, in three trees of a *Populus* clone. SEs are given

	Tree 1			Tree 2			Tree 3		
	$Q_{10}$	$E_A(20)^a$ or $R_S(20)^b$	$R^2$	$Q_{10}$	$E_A(20)$ or $R_S(20)$	$R^2$	$Q_{10}$	$E_A(20)$ or $R_S(20)$	$R^2$
$E_A$	1.89 (0.012)	357.5 (1.94)	0.88	1.73 (0.016)	374.7 (1.95)	0.83	1.59 (0.023)	299.8 (2.43)	0.42
$E_A$ at $f_s = 0$	2.16 (0.025)	500.8 (6.73)	0.99	1.82 (0.011)	384.1 (1.22)	0.96	1.84 (0.035)	350.9 (4.36)	0.67
$R_S^c$	1.90 (0.035)	497.5 (4.87)	0.64	1.91 (0.015)	394.40 (0.96)	0.89	2.20 (0.039)	399.2 (3.88)	0.59

<sup>a</sup> See Eq. 5 for calculation of  $E_A(20)$

<sup>b</sup> See Eq. 6 for calculation of  $R_S(20)$

<sup>c</sup> See Eq. 2 for calculation of  $R_S$

$E_A$  (i.e. measured  $E_A$  minus temperature-predicted  $R_S$ ) exhibited a clear diurnal variation, with the lowest values occurring during daytime (Fig. 4). During the rain (day 290), residual  $E_A$  was positive, so  $E_A$  was larger than expected based on temperature. In tree 2, residual  $E_A$  exhibited a consistent diurnal pattern on all days: it fluctuated closely around zero during the night and was negative during the daytime. In tree 3, residual  $E_A$  also exhibited a clear diurnal pattern, fluctuating around zero or slightly positive during the night and negative during the daytime. Residual  $E_A$  deviated from this pattern only during the rain (day 300), and was highly positive, indicating that measured  $E_A$  was larger than what would be expected based on temperature.

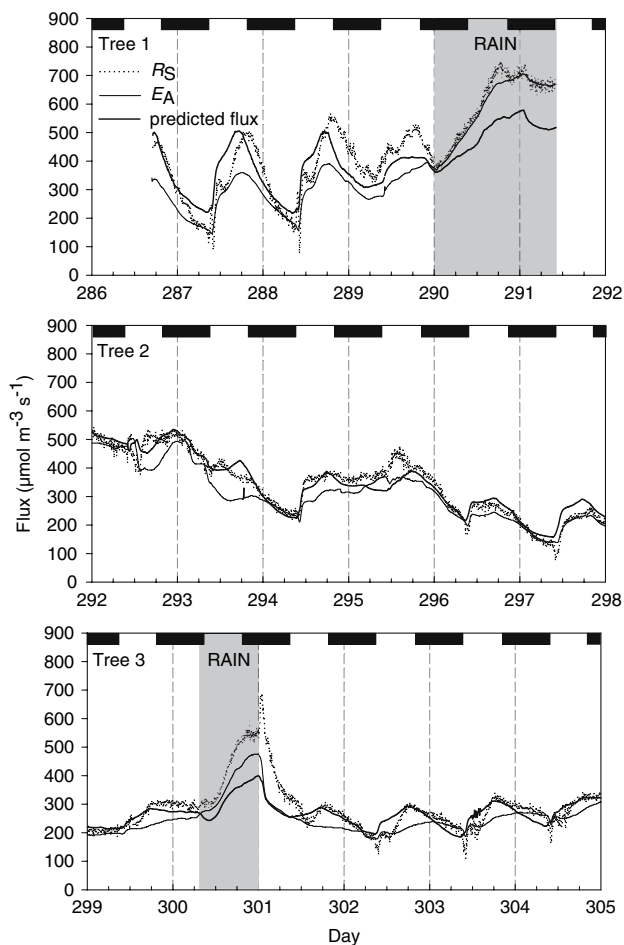
CO<sub>2</sub> efflux in relation to xylem CO<sub>2</sub> concentration

The correlation between  $E_A$  and xylem  $[CO_2^*]$  was unclear. In tree 1 on sunny days,  $E_A$  and xylem  $[CO_2^*]$  appeared to be in opposite phases (Fig. 2). In tree 2, there was no apparent correspondence between diurnal patterns of  $E_A$  and xylem  $[CO_2^*]$ . In tree 3, it also appears that  $[CO_2^*]$  and  $E_A$  were decoupled.  $E_A$  and xylem  $[CO_2^*]$  were positively correlated only during rainy days (days 290 and 300). In contrast, residual  $E_A$  correlated fairly well with  $[CO_2^*]$  (Fig. 4). On the sunny days, the daytime depressions in residual  $E_A$  corresponded with the reductions in xylem  $[CO_2^*]$  and on the rainy days (days 290 and 300), the large increase in  $[CO_2^*]$  coincided with an increase in residual  $E_A$ .  $R^2$ -values of the linear regression between  $[CO_2^*]$  and  $E_A$  ranged between 0.63 and 0.94 (Table 2).

Calculation of CO<sub>2</sub> flux components and the rate of stem respiration

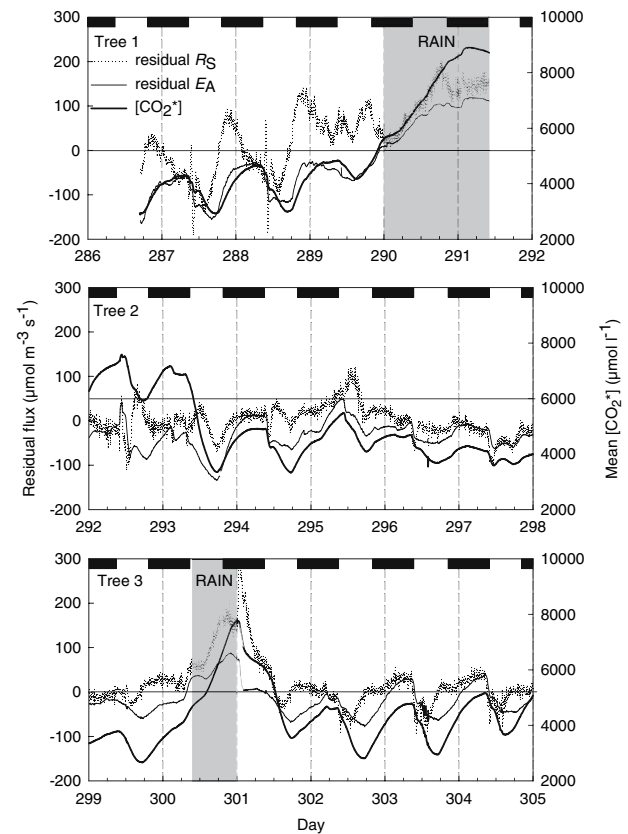
Table 3 summarizes 6-h mean fluxes of CO<sub>2</sub> and their relative contribution to total  $R_S$  for a 24-h period on

*in parentheses.*  $Q_{10}$  Increase in  $E_A$  or  $R_S$  for a 10°C rise in temperature,  $E_A(20)$  CO<sub>2</sub> efflux at 20°C, and  $R_S(20)$  rate of respiration at 20°C,  $R^2$  coefficient of determination for the exponential regression between  $E_A$  or  $R_S$  and  $T_{st}$



**Fig. 3**  $E_A$ , calculated rate of stem respiration ( $R_S$ ) and predicted rate of respiration for the three 3-year-old field-grown trees of a *Populus* clone.  $R_S$  was calculated with Eq. 2 (see text).  $R_S$  was predicted with Eq. 6 (see text), using  $Q_{10}$  and  $R_S$  at 20°C [ $R_S(20)$ ] from the temperature-response curve of  $R_S$  (Table 1). Black boxes indicate night-time periods and vertical dashed lines indicate midnight

selected sunny days and rainy days, together with their 24-h totals. Over a 24-h period,  $E_A$  was by far the most important component on all days, and represented between 82 and 94% of the total flux of respired  $\text{CO}_2$ , resulting in a generally close correspondence between  $R_S$  and  $E_A$  (Fig. 3).  $\Delta S$  and  $F_T$  were rather small, and their relative contribution to  $R_S$  differed between sunny and rainy days. On the sunny days,  $\Delta S$  was zero over a 24-h period, indicating that there was no net change in  $[\text{CO}_2^*]$  over a 24-h period. On these days,  $\Delta S$  was positive during the evening and night, whereas it was negative in the morning and afternoon. Hence,  $\text{CO}_2$  was stored in the sap during evening and night, and released during the daytime. On the rainy days,  $\Delta S$  was always positive, indicating that  $\text{CO}_2$  was being stored the entire day. The 24-h contribution of  $F_T$  to  $R_S$  accounted for 9–18% and 3–7% of  $R_S$  on the sunny and rainy days, respectively. The relative



**Fig. 4**  $E_A$ , residual respiration ( $R_S$ ) and mean xylem sap  $\text{CO}_2$  concentration ( $[\text{CO}_2^*]$ ) for the three 3-year-old field-grown trees of a *Populus* clone. Residuals were calculated as the difference between measured  $E_A$  or calculated  $R_S$  and predicted flux, where the flux was predicted with Eq. 6 (see text) using  $Q_{10}$  and  $R_S(20)$  parameters of the temperature-response curve of  $R_S$  (Table 1). Black boxes indicate night-time periods and vertical dashed lines indicate midnight. For abbreviations, see Figs. 2 and 3

contribution of  $F_T$  to  $R_S$  was higher on the sunny days because of the higher  $f_s$  on those days. On the sunny days, the relative contribution of  $F_T$  showed a diurnal variation, whereas during the rain,  $F_T$  showed no pronounced diurnal trend. Summarized, during rain the discrepancy between  $E_A$  and  $R_S$  was mainly due to the storage of  $\text{CO}_2$  inside the stem, whereas during the daytime of sunny days, the discrepancy was mainly attributed to the transport of  $\text{CO}_2$  in the stem.

#### Respiration rate in relation to temperature and xylem $\text{CO}_2$ concentration

Estimated parameter values of  $R_S(20)$  and  $Q_{10}$  for the fit of Eq. 6 to calculated  $R_S$  are listed in Table 1. Generally, the parameters were higher than for  $E_A$  obtained with the first regression (all data except rain days).  $R^2$ -values were higher than for  $E_A$  in tree 2 and 3 but lower in tree 1.

**Table 2** Coefficients and  $R^2$  for the linear regression: residual  $E_A = a [\text{CO}_2^*] - b$  or residual  $R_S^a = a [\text{CO}_2^*] - b$ .  $E_A$  ( $\mu\text{mol m}^{-3} \text{s}^{-1}$ ) and xylem sap  $\text{CO}_2$  concentration ( $\mu\text{mol l}^{-1}$ ) were measured in three trees of a *Populus* clone.  $R_S$  (respiration rate,  $\mu\text{mol m}^{-3} \text{s}^{-1}$ ) was calculated with Eq. 2 (see text). Residuals were calculated as the difference

between measured  $E_A$  or calculated  $R_S$  and predicted flux, where the flux was predicted with Eq. 6 (see text) using  $Q_{10}$  and  $R_S(20)$  parameters of the temperature-response curve of  $R_S$  (Table 1). SEs are given in parentheses. For abbreviations, see Table 1

	Tree 1			Tree 2			Tree 3		
	<i>a</i>	<i>b</i>	$R^2$	<i>a</i>	<i>b</i>	$R^2$	<i>a</i>	<i>b</i>	$R^2$
Residual $E_A^b$	0.043 (0.0003)	243.49 (1.60)	0.94	0.040 (0.0009)	203.79 (4.12)	0.63	0.027 (0.0004)	135.05 (1.90)	0.71
Residual $R_S^b$	0.032 (0.0008)	124.84 (4.39)	0.54	0.024 (0.0015)	104.55 (6.69)	0.13	0.041 (0.0008)	156.48 (3.51)	0.61

<sup>a</sup> See Eq. 2 for calculation of  $R_S$

<sup>b</sup> Residuals were calculated as the difference between measured  $E_A$  or calculated  $R_S$  and predicted flux, where the flux was predicted with Eq. 6 (see text) using  $Q_{10}$  and  $R_S(20)$  parameters of the temperature-response curve of  $R_S$  (Table 1)

Compared with the second regression of  $E_A$  (only zero  $f_s$  data), parameters were higher for tree 1, but lower for tree 2 and 3, and  $R^2$ -values were lower for all trees. Although the temperature prediction (with parameters obtained for  $R_S$  from Table 1) correlated generally well with  $R_S$ , residual  $R_S$  deviated several times from zero (Fig. 4). In tree 1, on sunny days 287 and 288, residual  $R_S$  was negative in the afternoon, indicating that  $R_S$  was lower than what would be expected based on temperature. During the rain (day 290), residual  $R_S$  was positive and reached  $205 \mu\text{mol m}^{-3} \text{s}^{-1}$ . On the other days, the temperature prediction fitted  $R_S$  better. In tree 2, residual  $R_S$  was small, and showed no consistent diurnal pattern. In tree 3, residual  $R_S$  showed a diurnal pattern on sunny days 302–304: it was zero during the night and negative during the daytime. During the rain (day 300), residual  $R_S$  was positive and reached  $300 \mu\text{mol m}^{-3} \text{s}^{-1}$ . A positive correlation between residual  $R_S$  and xylem  $[\text{CO}_2^*]$  was found, but the relationship was weaker than for residual  $E_A$  (Fig. 4; Table 2).

## Discussion

### Xylem $\text{CO}_2$ concentrations

Consistent with previous reports, xylem  $[\text{CO}_2^*]$  was several orders of magnitude higher than the  $[\text{CO}_2]$  in air (Levy and Jarvis 1998; Teskey and McGuire 2002), indicating that there are large barriers to radial  $\text{CO}_2$  diffusion. In most species, the cambium significantly inhibits radial gas diffusion (Kramer and Kozlowski 1979). The xylem itself also forms a barrier to free gas exchange. Sorz and Hietz (2006) found that diffusion coefficients for  $\text{O}_2$  in water-saturated xylem were always lower than the diffusion coefficients in water alone, illustrating that the xylem cell walls present a major barrier to gas diffusion. Moreover, it has been demonstrated that differences in  $[\text{CO}_2]$  between xylem and bark tissues are large: values for xylem  $[\text{CO}_2]$  have been

reported to be as high as 26% (MacDougal and Working 1933), while  $[\text{CO}_2]$  in bark tissues are reported to be around 0.06–0.17% (Cernusak and Marshall 2000; Wittmann et al. 2006). Due to these barriers, respired  $\text{CO}_2$  builds up in the xylem instead of directly escaping to the atmosphere. This build-up of  $\text{CO}_2$  was clearly visible during the rain events, when  $[\text{CO}_2^*]$  more than doubled (Fig. 2, days 290 and 300).

A diurnal pattern was observed in xylem  $[\text{CO}_2^*]$  on sunny days. Despite higher  $T_{st}$  during the day,  $[\text{CO}_2^*]$  was lower during the day than during the night (Fig. 2). When  $f_s$  was high, the sap in the stem at high  $[\text{CO}_2^*]$  appears to have been diluted with water from the soil at lower  $[\text{CO}_2^*]$ . During sunny days,  $[\text{CO}_2^*]$  always decreased as  $f_s$  increased (Fig. 2), suggesting that although the rate of respiration of the living xylem cells might be greater during the day due to higher  $T_{st}$ , the decrease in xylem  $[\text{CO}_2^*]$  due to dilution by soil water can overwhelm any increase in xylem  $[\text{CO}_2^*]$  due to  $\text{CO}_2$  production. However, because we did not measure water status of the living stem tissues, we cannot exclude a possible decrease in the rate of actual respiration of the living stem tissues due to water stress. When transpiration starts, tension develops in the xylem and turgor pressure of the living cells in the stem decreases. A drop in turgor pressure may reduce rates of growth and maintenance processes and the respiratory processes which support those (Woodruff et al. 2004; Proseus and Boyer 2006; Steppe et al. 2006). Water deficits in living stem tissues have been used to explain midday depressions in stem  $\text{CO}_2$  efflux (Edwards and McLaughlin 1978; Lavigne 1987; Kakubari 1988; Wang et al. 2003; Daudet et al. 2005; Saveyn et al. 2007b). However, it is extremely difficult to distinguish between the effects of  $\text{CO}_2$  transport in the xylem and those of turgor dynamics in the living stem tissues because both processes are determined by sap flow (Saveyn et al. 2007b). Hence, it is possible that water deficit in the living stem tissues may play a role in the diurnal pattern of  $[\text{CO}_2^*]$ .

**Table 3** Six-hour mean (minimum–maximum) flux components [ $E_A$ , transport flux ( $F_T$ ) and storage flux ( $\Delta S$ )] and  $R_S$ , calculated from 5-min measurements; and relative contribution of the flux components to  $R_S$ . Twenty-four hour totals ( $\text{mol m}^{-3}$ ), calculated from 5-min measurements are also given (*in bold*). For other abbreviations, see Table 1

Tree	Time (hours)	Flux components and total respiration ( $\mu\text{mol m}^{-3} \text{ s}^{-1}$ )				Relative contributions to $R_S$			
		$E_A$	$F_T$	$\Delta S$	$R_S$	$E_A$	$F_T$	$E_A$	$\Delta S$
Tree 1 day 287 (sunny)	0000–0600	192.1 (171.5–225.5)	30.5 (2.9–67.6)	6.8 (–20.4–34.5)	229.4 (158.4–309.0)	0.84	0.13	0.84	0.03
	0600–1200	188.7 (146.0–281.4)	24.3 (0.4–98.3)	–12.4 (–89.8–42.1)	200.6 (89.5–339.7)	0.94	0.12	0.94	–0.06
	1200–1800	324.6 (283.1–359.6)	68.6 (43.3–104.3)	–18.0 (–46.5–15.6)	375.2 (292.0–479.1)	0.87	0.18	0.87	–0.05
	1800–0000	328.9 (281.4–359.6)	98.3 (64.8–118.7)	27.9 (–5.6–52.4)	455.2 (359.2–505.7)	0.72	0.22	0.72	0.06
	24-h total	<b>22.4</b>	<b>4.8</b>	<b>0.1</b>	<b>27.3</b>	<b>0.82</b>	<b>0.18</b>	<b>0.82</b>	<b>0.00</b>
Tree 1 day 290 (rain all day)	0000–0600	401.1 (368.9–445.8)	1.9 (1.2–2.9)	9.2 (–16–37.2)	412.2 (360.7–480.4)	0.97	0.00	0.97	0.02
	0600–1200	493.7 (446.2–540.9)	5.2 (2.8–7.6)	21.4 (–2.9–45.8)	520.3 (460.5–583.8)	0.95	0.01	0.95	0.04
	1200–1800	606.5 (542.1–652.9)	22.6 (8.3–51.2)	23.1 (–7.2–64.1)	652.2 (554.2–740.4)	0.93	0.03	0.93	0.04
	1800–0000	659.9 (651.5–671.5)	32.4 (22.6–68.0)	15.0 (–21.9–48.3)	707.3 (667.7–753.4)	0.93	0.05	0.93	0.02
	24-h total	<b>46.7</b>	<b>1.3</b>	<b>1.5</b>	<b>49.5</b>	<b>0.94</b>	<b>0.03</b>	<b>0.94</b>	<b>0.03</b>
Tree 2 day 296 (sunny)	0000–0600	271.0 (233.7–317.5)	23.8 (15.7–31.0)	–0.6 (–18.5–22.6)	294.3 (234.7–357.4)	0.92	0.08	0.92	0.00
	0600–1200	223.1 (197.3–238.4)	21.0 (5.2–53.8)	–11.5 (–57.8–18)	232.6 (164.0–281.2)	0.96	0.09	0.96	–0.05
	1200–1800	237.7 (230.9–244.8)	38.6 (18.0–54.4)	–8.8 (–54.4–18.6)	267.4 (230.1–285.7)	0.89	0.14	0.89	–0.03
	1800–0000	219.4 (200.2–235.9)	8.8 (1.5–18.1)	10.7 (–11.5–34.2)	239.0 (190.3–267.7)	0.92	0.04	0.92	0.04
	24-h total	<b>20.5</b>	<b>2.0</b>	<b>–0.2</b>	<b>22.0</b>	<b>0.93</b>	<b>0.09</b>	<b>0.93</b>	<b>0.00</b>
Tree 3 day 299 (sunny)	0000–0600	193.3 (189.6–197.9)	5.2 (0–13.4)	7.6 (–11.4–24.7)	206.1 (186.4–224.3)	0.94	0.03	0.94	0.04
	0600–1200	200.6 (194.9–214.2)	23.5 (4.5–51.3)	–4.2 (–51.9–26.4)	219.9 (187.0–255.9)	0.91	0.11	0.91	–0.02
	1200–1800	217.6 (214.1–228.0)	54.7 (46.5–86.2)	–15.6 (–41.9–17.7)	256.7 (222.6–304.9)	0.85	0.21	0.85	–0.06
	1800–0000	238.3 (227.9–246.8)	46.2 (35.7–62.4)	19.1 (–0.8–32.4)	303.6 (282.3–316.6)	0.78	0.15	0.78	0.06
	24-h total	<b>18.4</b>	<b>2.8</b>	<b>0.1</b>	<b>21.3</b>	<b>0.86</b>	<b>0.13</b>	<b>0.86</b>	<b>0.00</b>
Tree 3 day 300 (rain 0620–2250 hours)	0000–0600	249.7 (247.2–252.9)	36.3 (20.0–49.7)	12.3 (–11.5–28.9)	298.3 (273.1–315.0)	0.84	0.12	0.84	0.04
	0600–1200	273.9 (252.8–294.3)	6.4 (0.0–37.3)	20.2 (–0.1–45.2)	300.5 (270.7–328.6)	0.91	0.02	0.91	0.07
	1200–1800	360.1 (295.5–423.6)	22 (8.8–43.3)	30.4 (1–61.3)	412.6 (310.5–522.5)	0.87	0.05	0.87	0.07
	1800–0000	457.6 (423.6–475.4)	45.3 (38.5–73.1)	35.1 (–13.1–82.8)	537.9 (503.0–591.1)	0.92	0.04	0.92	0.04
	24-h total	<b>29.0</b>	<b>2.4</b>	<b>2.3</b>	<b>33.5</b>	<b>0.87</b>	<b>0.07</b>	<b>0.87</b>	<b>0.07</b>



During rainy days (days 290 and 300), xylem  $[\text{CO}_2^*]$  increased sharply (Fig. 2). Due to the lack of  $f_s$  during the rain, xylem  $[\text{CO}_2^*]$  was not diluted and transport of  $\text{CO}_2$  did not occur, resulting in a build-up of xylem  $[\text{CO}_2^*]$ . However, the large increase in  $[\text{CO}_2^*]$  in the absence of  $f_s$  might also be partly due to a better stem water status and, hence, higher rates of cell metabolism and respiration.

#### $\text{CO}_2$ efflux in relation to temperature

$E_A$  correlated generally well with  $T_{st}$ . Temperature has an effect on three different processes, which all affect  $E_A$ : increase in  $T_{st}$  causes: (1) an increase in cellular respiration; (2) an increase in the diffusion coefficient of  $\text{CO}_2$  (Fick's law); and (3) a decrease in the solubility of  $\text{CO}_2$  in water (Henry's law) and, hence, increase of gaseous  $\text{CO}_2$  (McGuire et al. 2007). The interplay of all of these processes determines the positive relationship between  $T_{st}$  and  $E_A$ . However, we observed that the correlation between  $E_A$  and  $T_{st}$  was sometimes less clear. During the daytime on sunny days,  $E_A$  was generally lower than expected based on the exponential regression of  $R_S$  and temperature, using parameters from Table 1 (Fig. 4). Similar discrepancies have been observed previously in other species, and several explanations for this phenomenon have been proposed:

1. Measurements of  $T_{st}$  at one location might not be representative of the temperature of the whole stem, so that temperature predictions of  $E_A$  are not representative for the whole stem (Stockfors 2000).
2. Bark photosynthesis during daytime may cause refixation of  $\text{CO}_2$  respired by the living stem tissues (Sprugel and Benecke 1991; Saveyn et al. 2006).
3. The rate of oxidative respiration might be higher at night than during the day because a better stem water status in the absence of  $f_s$  at night promotes higher turgor pressure in the living stem tissues and, hence, higher growth and maintenance metabolism (Edwards and McLaughlin 1978; Lavigne 1987; Kakubari 1988; Wang et al. 2003; Daudet et al. 2005; Saveyn et al. 2007b).
4. High  $f_s$  during the daytime causes a dilution of xylem  $[\text{CO}_2^*]$  with water at lower  $[\text{CO}_2^*]$  coming from the soil, resulting in a decrease in stem xylem  $[\text{CO}_2^*]$  and, consequently, a decrease in  $E_A$  due to a reduction in the  $\text{CO}_2$  concentration gradient from stem to atmosphere (Boysen-Jensen 1933; Negisi 1979; Hari et al. 1991; Martin et al. 1994; Kaipiainen et al. 1998; McGuire et al. 2007).

Two of these explanations do not apply in the context of this study. Explanation (1) does not apply because the stem was covered with reflective bubble insulation, which blocked direct sunlight and prevented the occurrence of

large temperature gradients. We measured  $T_{st}$  at two places in the stem and found very similar diurnal patterns. Bark photosynthesis (explanation (2)) was also prevented by covering the cuvette with aluminium foil and by covering the entire stem with reflective bubble insulation. Hence, only explanations (3) and (4) may account for why  $E_A$  was lower than predicted based on temperature during the daytime on sunny days. However, with our data we could not distinguish between the effects of  $\text{CO}_2$  transport in the xylem and those of turgor dynamics in the living stem tissues on  $E_A$ .

#### $\text{CO}_2$ efflux in relation to xylem $\text{CO}_2$ concentration

On sunny days, a (partial) decoupling between  $E_A$  and xylem  $[\text{CO}_2^*]$  was observed (Fig. 2). This decoupling was probably due to a combination of large barriers for radial  $\text{CO}_2$  diffusion presented by the cambium and xylem and a predominant contribution of cambial and phloem cell respiration to  $E_A$ .  $\text{CO}_2$  produced by xylem cells is faced with several diffusion barriers (xylem itself, cambium and bark), while  $\text{CO}_2$  produced by cambium and phloem tissues must overcome only the diffusion resistance of the bark. The phloem itself is aerated by numerous gas spaces, which have continuity with the external environment (Hook et al. 1972). Therefore, measured  $E_A$  is most likely primarily related to the rate of respiration of the phloem and cambium.

Nevertheless, a part of  $E_A$  was definitely related to xylem  $[\text{CO}_2^*]$ . When the temperature effect was removed from the data, a diurnal variation in residual  $E_A$  could still be observed on sunny days (Fig. 4). This variation was well correlated with the diurnal variation in xylem  $[\text{CO}_2^*]$  (Fig. 4; Table 2). The daytime decrease in xylem  $[\text{CO}_2^*]$  on sunny days caused a decrease in the radial  $\text{CO}_2$  diffusion gradient, resulting in a daytime depression in residual  $E_A$ . On rainy days (day 290 and 300), residual  $E_A$  was also well correlated with xylem  $[\text{CO}_2^*]$  (Fig. 4). The build-up of  $\text{CO}_2$  on these days caused a large radial  $\text{CO}_2$  diffusion gradient, resulting in a large positive residual  $E_A$ .

In contrast with our findings, other relationships between  $E_A$  and xylem  $[\text{CO}_2^*]$  have been reported. Maier and Clinton (2006) found that  $E_A$  was completely decoupled from xylem  $[\text{CO}_2^*]$ . On the other hand, Teskey and McGuire (2005, 2007) and Steppe et al. (2007) found a consistent linear relationship between  $E_A$  and xylem  $[\text{CO}_2^*]$ . Our results fall between these extremes. The relationship between  $E_A$  and xylem  $[\text{CO}_2^*]$  may vary with season. Maier and Clinton (2006) performed measurements during spring when cambium and phloem likely respire at a very high rate due to growth processes and highly active phloem transport. Our measurements were in autumn, when

the growth rate of the trees was much slower. It is possible that due to lower rates of respiration of cambium and phloem in autumn, xylem  $[\text{CO}_2^*]$  has a relatively larger influence on  $E_A$  than in spring. The degree of decoupling between  $E_A$  and xylem  $[\text{CO}_2^*]$  may also be related to age. Decoupling may be more pronounced in young trees because of the high secondary growth activity (i.e. high rate of oxidative respiration of cambium and phloem meristems) and smaller ratio of xylem tissue compared to cambium and phloem tissue. In mature trees, with reduced secondary growth and a higher ratio of xylem tissue,  $E_A$  may be more strongly related to xylem  $[\text{CO}_2^*]$ . Further investigations are needed on seasonal and age-related variation in the relationship between xylem  $[\text{CO}_2^*]$  and  $E_A$ .

#### Calculation of $\text{CO}_2$ flux components and rates of stem respiration

We found that the relative contribution of  $E_A$  to  $R_S$  in *Populus deltoides* stems ranged between 82 and 94% over a 24-h period. Likewise, Bowman et al. (2005) found that  $E_A$  in *Dacrydium cupressinum* Lamb stems accounted for 86–91% of  $R_S$  over a 24-h period. On sunny days, the discrepancy between  $E_A$  and  $R_S$  during the daytime was mainly caused by transport of  $\text{CO}_2$  in the transpiration stream.  $F_T$  accounted for up to 18% of  $R_S$  over a 24-h period, which is similar to the findings of McGuire and Teskey (2004) that  $F_T$  was 15 and 14% of  $R_S$  in *Fagus grandifolia* Ehrh. and *Liquidambar styraciflua* L. stems, respectively, and with the findings of Bowman et al. (2005) that  $F_T$  accounted for 11% of  $R_S$  in *D. cupressinum* stems. There was no net change in xylem  $[\text{CO}_2^*]$  on sunny days because the  $\text{CO}_2$  that built up in the stem during the night was released from storage during the daytime. On rainy days,  $\Delta S$  was positive during the entire day, indicating that  $\text{CO}_2$  entered into storage. The relative contribution of  $F_T$  to  $R_S$  was very small during rain periods due to negligible  $f_s$ . Hence, during rain, the difference between  $E_A$  and  $R_S$  was mainly attributed to storage of  $\text{CO}_2$  in the stem.

#### Respiration rate in relation to temperature and xylem $\text{CO}_2$ concentration

Because  $R_S$  accounts for both internal and outward fluxes of  $\text{CO}_2$  and theoretically represents actual stem respiration, it was expected that there would be a strong correlation between  $R_S$  and  $T_{st}$ . However, the relationship was rather weak in two of the three trees (Table 1). The lack of a strong relationship may indicate that the rate of stem respiration is dependent not only on temperature, but also on other factors, or alternatively, that the estimate of the rate

of respiration is incomplete. There are a number of possible factors that may affect respiration. One is stem water status. Saveyn et al. (2007b) demonstrated that temperature-independent diurnal dynamics of stem  $E_A$  in *Fagus sylvatica* L. and *Quercus robur* L. were fairly well correlated with the turgor pressure in the living cells, suggesting that in addition to effects of temperature, respiration was also affected by stem water status. Another factor may be diurnal changes in carbohydrate availability. Rates of leaf and root respiration have been linked to the availability of current photosynthate (Azcon-Bieto and Osmond 1983; Högberg et al. 2001; Johnsen et al. 2007). A similar dependence may exist for stem tissues. Temporal and spatial variation in the concentration of oxygen within the stem (Eklund 2000) may also affect the rate of respiration (Sorz and Hietz 2006) and, hence, the variability in the relationship of  $R_S$  to temperature.

We observed that during the rain,  $R_S$  was substantially higher than on sunny days (Table 3), and also substantially higher than the temperature prediction as indicated by the positive  $R_S$  residuals (Fig. 4). The most likely explanation for these discrepancies is water stress during sunny days that reduced cell metabolism. On rainy days stem  $[\text{CO}_2^*]$  increased throughout the day, i.e.  $\Delta S$  was positive, as were  $E_A$  and  $F_T$ . We assumed that  $E_A$  was measured the most accurately, and  $\Delta S$  and  $F_T$  were both small relative to  $E_A$  on rainy days. Therefore it appears that the high  $R_S$  on rainy days was an actual biological response and not an error in measurement. On sunny days it is likely that water stress reduced  $R_S$ , but it is also possible that the measurements of  $F_T$  or  $\Delta S$  were inaccurate. With the current data, it is not possible to determine if either or both fluxes were underestimated on sunny days. These results suggest the need for manipulative studies under carefully controlled conditions to examine the component fluxes of  $R_S$  as well as the effects of stem water stress on  $R_S$ .

It should be noted that there are some potential sources of error in our calculations and measurements. One is the assumption of constant xylem sap pH. Diurnal changes in xylem sap pH have been reported (Schurr and Schulze 1995). Irradiance and temperature change diurnally and both factors affect proton pumping activity, which in turn affects pH (Wilkinson 1999). pH changes have a substantial effect on calculation of xylem  $[\text{CO}_2^*]$  (Henry's law) and, hence, on calculation of  $F_T$  and  $\Delta S$ . Another source of error may originate from drilling the holes for inserting the NDIR probes. The NDIR  $\text{CO}_2$  sensor was used because of its good reliability, but it is relatively large compared to a  $\text{CO}_2$  microelectrode that can also measure stem  $[\text{CO}_2]$  but is much less reliable (McGuire and Teskey 2002). We recognize that the 19 mm diameter hole required to insert the probe may have had the potential to cause wound respiration, but we think it had a minimal

effect on the measurements because (1) the pattern of response was very similar to previous measurements made with CO<sub>2</sub> microelectrodes inserted in much smaller (4 mm) diameter holes (McGuire and Teskey 2004); (2) we observed the same increase in [CO<sub>2</sub>] in the stems of large trees when the probes are inserted on the same or different sides of the tree stem (Teskey and McGuire 2007); and (3) the initial apparent wound response in tree stems appears to be due to an increase in CO<sub>2</sub> diffusion from the stem rather than a substantial change in the actual rate of respiration (Teskey and McGuire 2005). Additionally, the drilled holes may have introduced artificial reductions in sap flow and, hence, errors in the calculation of  $F_T$ . The overall reduction in sap flow was probably small because it has been demonstrated in *Acer pseudoplatanus* (which like *Populus deltoides* has diffuse-porous xylem anatomy) that substantial reductions in sapwood area (>50%) induced by cutting did not reduce whole plant transpiration (Mackay and Weatherley 1973).

#### Ecophysiological implications

##### *Stem CO<sub>2</sub> efflux measurements are not sufficient to quantify stem respiration*

Our results showed that patterns of  $E_A$  and xylem [CO<sub>2</sub>\*] were partially decoupled, suggesting that  $E_A$  reflected processes in the external tissues rather than processes in the xylem. Large barriers for radial CO<sub>2</sub> diffusion presented by cambium and xylem and a major contribution to  $E_A$  from active processes in phloem and cambium may explain this decoupling. This finding implies that measurements of  $E_A$  are not representative of the respiration of all living stem tissues because processes in the xylem are not entirely accounted for. Moreover, our results suggest that not all CO<sub>2</sub> respired by xylem parenchyma diffuses radially or remains in the stem, but that a part is transported internally in the transpiration stream. Surveillance of the dynamic pool of CO<sub>2</sub> in the xylem is necessary for deepening our understanding of the production and fluxes of CO<sub>2</sub> in the xylem. Recent advances in measurement techniques have facilitated precise and continuous measurement of xylem CO<sub>2</sub> concentrations. These measurements need to be performed simultaneously with  $E_A$  measurements, in order to quantify the respiration of all living stem tissues.

##### *CO<sub>2</sub> exchange measurements need to be re-interpreted*

In the majority of the reports on CO<sub>2</sub> efflux measurements of woody tissues, CO<sub>2</sub> efflux is used as a synonym for respiration. It has now emerged that the outward flux of

CO<sub>2</sub> only partially reflects the rate of respiration of the stem. Respiration is the active process that produces CO<sub>2</sub>, whereas efflux is a passive process determined by physical factors such as the diffusion coefficient of CO<sub>2</sub> and the CO<sub>2</sub> concentration gradient (Fick's law). The latter is affected not only by the rate of CO<sub>2</sub> production but also by the solubility of CO<sub>2</sub> in xylem sap (which changes with temperature and sap pH) and the rate of CO<sub>2</sub> removal in flowing sap. We hope that our study may convince scientists to clearly distinguish between the terms “woody tissue CO<sub>2</sub> efflux” and “woody tissue respiration”. Furthermore, our results imply that measurements of CO<sub>2</sub> exchange of leaves may need to be re-interpreted. Because CO<sub>2</sub> originating from stem respiration can be transported toward the canopy, it may function there as an internal C source for leaf photosynthesis. In this case, CO<sub>2</sub> exchange measurements may underestimate the actual leaf photosynthesis.

##### *Contribution of stem respiration to forest C balance: larger than previously estimated?*

The study revealed that on 24-h basis,  $E_A$  measurements always underestimated the “actual” stem respiration. Attempts to quantify the contribution of woody tissue respiration to the total C balance of forest ecosystems are generally based on a number of  $E_A$  measurements of branch and stem segments (e.g. Lindroth et al. 1998; Granier et al. 2000; Damesin et al. 2002; Hamilton et al. 2002). Our findings indicate that these values probably underestimate the actual contribution of woody tissue respiration and ask for other methods to quantify respiration. The mass balance approach is a first promising step towards more accurate estimations of stem respiration, but further studies are required to verify the accuracy of this method.

##### *Stem respiration cannot be predicted by temperature alone*

Although  $R_S$  was generally well related to  $T_{st}$ , the correlation was sometimes less clear. Tree physiologists frequently predict the rate of tissue respiration by means of a simple exponential temperature function (Eq. 6). Our results suggest that respiration may be regulated by factors in addition to temperature, such as water status of the living cells, substrate availability or oxygen concentration. Particularly on rainy days  $R_S$  increased dramatically and was much higher than expected from temperature predictions, suggesting that respiration may have increased due to factors other than temperature. The most likely reason for the increase was improved cell water status in the stems. Further study is needed on the effects of water status,

carbohydrate availability and oxygen availability on  $R_S$ . However, it cannot be excluded that measurements of the transport and storage flux and, hence, calculations of  $R_S$  were inaccurate. With the current data, it was not possible to determine if either or both fluxes were erroneous. These results demand more in-depth studies on the transport and storage flux components of  $R_S$ .

## Conclusion

This study demonstrated that  $E_A$  was affected by changes in both  $T_{st}$  and xylem  $[CO_2^*]$ . Changes in xylem  $[CO_2^*]$  were not related to changes in  $T_{st}$ , but were inversely related to changes in  $f_s$ , which probably affected both the rate of  $CO_2$  transport and the water status of the living stem tissues and, hence, the rate of respiration. We conclude that  $E_A$  does not equal stem respiration. It is a process affected by respiration, but also by removal of internal  $CO_2$  in flowing xylem sap.  $E_A$  was the dominant component flux of  $R_S$  calculated by mass balance. However, the internal transport flux also accounted for a substantial portion of stem-respired C. Unexpectedly, the relationship between  $R_S$  and  $T_{st}$  was sometimes weak. Other factors including the water status of the living stem tissues or oxygen and carbohydrate availability may have contributed to variability in  $R_S$ . The experiments demonstrate the need for more refined and detailed measurements of individual components of stem respiration like transport and storage fluxes of  $CO_2$ .

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