

RESEARCH PAPER

# Assimilation of xylem-transported CO<sub>2</sub> is dependent on transpiration rate but is small relative to atmospheric fixation

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Received 2 November 2012; Revised 5 February 2013; Accepted 22 February 2013

## Abstract

The effect of transpiration rate on internal assimilation of CO<sub>2</sub> released from respiring cells has not previously been quantified. In this study, detached branches of *Populus deltoides* were allowed to take up <sup>13</sup>CO<sub>2</sub>-labelled solution at either high (high label, HL) or low (low label, LL) <sup>13</sup>CO<sub>2</sub> concentrations. The uptake of the <sup>13</sup>CO<sub>2</sub> label served as a proxy for the internal transport of respired CO<sub>2</sub>, whilst the transpiration rate was manipulated at the leaf level by altering the vapour pressure deficit (VPD) of the air. Simultaneously, leaf gas exchange was measured, allowing comparison of internal CO<sub>2</sub> assimilation with that assimilated from the atmosphere. Subsequent <sup>13</sup>C analysis of branch and leaf tissues revealed that woody tissues assimilated more label under high VPD, corresponding to higher transpiration, than under low VPD. More <sup>13</sup>C was assimilated in leaf tissue than in woody tissue under the HL treatment, whereas more <sup>13</sup>C was assimilated in woody tissue than in leaf tissue under the LL treatment. The ratio of <sup>13</sup>CO<sub>2</sub> assimilated from the internal source to CO<sub>2</sub> assimilated from the atmosphere was highest for the branches under the HL and high VPD treatment, but was relatively small regardless of VPD×label treatment combination (up to 1.9%). These results showed that assimilation of internal CO<sub>2</sub> is highly dependent on the rate of transpiration and xylem sap [CO<sub>2</sub>]. Therefore, it can be expected that the relative contribution of internal CO<sub>2</sub> recycling to tree carbon gain is strongly dependent on factors controlling transpiration, respiration, and photosynthesis.

**Key words:** carbon budget, carbon isotope, internal CO<sub>2</sub> transport, leaf mesophyll, transpiration, woody tissue photosynthesis, xylem.

## Introduction

A portion of CO<sub>2</sub> released by mitochondrial respiration of living cells in tree stems and branches diffuses to the atmosphere through woody tissues (Saveyn *et al.*, 2010). However, branch and stem tissues impede gaseous diffusion (Kramer and Kozłowski, 1979; Lendzian, 2006; Sorz and Hietz, 2006; Steppe *et al.*, 2007), resulting in internal CO<sub>2</sub> concentrations ([CO<sub>2</sub>]) (range <1 to 26%) that substantially exceed atmospheric [CO<sub>2</sub>] of ~0.04% (Teskey *et al.*, 2008; McGuire *et al.*, 2009).

The importance of accounting for internal transport of CO<sub>2</sub> to accurately assess estimates of respiration based on

measurements of CO<sub>2</sub> efflux has been described previously (e.g. Levy *et al.*, 1999; Teskey and McGuire, 2007; Saveyn *et al.*, 2008b). CO<sub>2</sub> originating from respiration both below (Aubrey and Teskey, 2009; Grossiord *et al.*, 2012; Bloemen *et al.*, 2013) and above ground (Teskey and McGuire, 2007) can dissolve in xylem sap and be transported upwards via the transpiration stream from the stem base to the top of the canopy (Bloemen *et al.*, 2013), and can diffuse to the atmosphere via above-ground tissues remote from the site of respiration (Teskey and McGuire, 2007).

Along the transport pathway, this internal CO<sub>2</sub> can be assimilated by active chloroplasts within the stem, branches and leaves (McGuire *et al.*, 2009; Saveyn *et al.*, 2010), thereby contributing to whole-plant carbon gain. In woody tissues, chloroplasts occur in both the xylem and inner bark, which justifies the use of the term woody tissue photosynthesis instead of bark or corticular photosynthesis when referring to stem CO<sub>2</sub> assimilation (Saveyn *et al.*, 2010). In the leaves, it is believed that internally transported carbon is mainly fixed in the cells surrounding the xylem vasculature in the petioles and near the veins (Stringer and Kimmerer, 1993; McGuire *et al.*, 2009). McGuire *et al.* (2009) found that assimilation of xylem-transported <sup>13</sup>CO<sub>2</sub>, supplied at high concentrations as a tracer, averaged 6% of the assimilation of atmospheric CO<sub>2</sub> by the leaves in detached sycamore branches. Given that respired CO<sub>2</sub> is redistributed by the transpiration stream, it might be expected that, at a high transpiration rate and/or at high xylem sap [CO<sub>2</sub>], a larger amount of CO<sub>2</sub> could be transported in the xylem towards photosynthetically active tissues in woody organs and leaves. Therefore, transpiration rate and xylem sap [CO<sub>2</sub>] might be critical factors to consider when assessing assimilation of internally transported CO<sub>2</sub> in the context of whole-plant carbon cycling.

However, to our knowledge, the combined effect of transpiration and xylem sap [CO<sub>2</sub>] on the assimilation of xylem-transported CO<sub>2</sub> has not been investigated. In this study, detached branches of *Populus deltoides* Bartr. Ex. Marsh trees were allowed to take up an aqueous solution enriched with either a low or a high concentration of <sup>13</sup>CO<sub>2</sub>, whilst the transpiration rate was manipulated by varying the atmospheric vapour pressure deficit (VPD). Simultaneous leaf gas-exchange measurements allowed us to compare the contributions of assimilation of internally transported CO<sub>2</sub> and atmospheric CO<sub>2</sub>. We hypothesized that more <sup>13</sup>C label would be assimilated in chlorophyll-containing woody and leaf tissues under the high [<sup>13</sup>CO<sub>2</sub>] solution treatment and at high transpiration rates because a greater quantity of <sup>13</sup>C-labelled substrate would be transported under these conditions.

## Materials and methods

### Plant material

Branches were cut from *P. deltoides* trees that were part of a 7-year-old plantation in Whitehall Forest, a research facility of the University of Georgia near Athens, GA, USA. Branches (length >2 m) were collected around 06.30 h on 16 and 17 July 2010 and their cut ends were placed immediately in water to prevent wilting. In the laboratory, lateral segments of these branches that were between 20 and 50 cm long and <1 cm in diameter were cut under water to prevent embolism formation in the xylem. The experiment was conducted on these lateral segments, hereafter referred to as branches.

### Baseline sampling

Prior to the start of the labelling experiment, baseline samples were taken from the same trees to determine the natural abundance carbon isotopic composition (δ<sup>13</sup>C) to which δ<sup>13</sup>C values of treated tissues would be compared. For baseline sampling, branches of similar dimensions and at approximately the same canopy position were cut from the trees and separated into woody and leaf tissues. Baseline

samples were frozen immediately in liquid nitrogen and then moved to a freezer at -9 °C for storage before processing.

### Experimental setup

Treatment solutions were prepared as described by McGuire *et al.* (2009). Solutions were enriched with 99 atomic percentage (at%) <sup>13</sup>CO<sub>2</sub> either at a high (high label, hereafter referred to as HL) or a low (low label, hereafter referred to as LL) concentration or with CO<sub>2</sub> at atmospheric isotope composition and concentration (control). To prepare the labelled solutions, 10 l bottles were completely filled with deionized water, which was amended with KCl to 40 mM concentration to facilitate solution uptake (Zwieniecki *et al.*, 2001). Depending on the desired concentration, 1 or 3 l of the solution was displaced with 99 at% <sup>13</sup>CO<sub>2</sub> gas from a compressed gas cylinder (ICON Services, Summit, NJ, USA). The gas was then circulated with a pump through the water in a closed loop for at least 3 h. Ambient air was circulated through the control solution for the same amount of time. Solutions were amended with sodium bicarbonate to control pH and achieve dissolved inorganic carbon ([<sup>13</sup>CO<sub>2</sub>\*], mol l<sup>-1</sup>) target according to modelled [CO<sub>2</sub>\*] at a set pH (Erda *et al.*, 2013). Gaseous CO<sub>2</sub> concentration ([CO<sub>2</sub>], %) and [<sup>13</sup>CO<sub>2</sub>\*] concentration were 8.9 % and 0.003 mol l<sup>-1</sup> for the LL solution, and 18.5% and 0.013 mol l<sup>-1</sup> for the HL solution. Based on preliminary measurements, we assumed that the [CO<sub>2</sub>] of the HL and LL solutions represented high and low xylem sap [CO<sub>2</sub>], respectively. The pH values of the HL and LL solutions were 6.4 and 4.8, respectively, which is within the range measured previously in *P. deltoides* (Aubrey and Teskey, 2009; Aubrey *et al.*, 2011). On the first day (16 July), the experiment was performed with ten branches using the LL solution, whilst on the following day (17 July), the experiment was repeated with the HL solution on another set of ten branches collected that morning.

The cut end of each branch was placed in a 500 ml glass bottle containing 300 ml of solution enriched with CO<sub>2</sub>. Immediately after placing a branch into a solution, the top of the bottle was sealed with a closed-cell foam gasket to minimize diffusion of CO<sub>2</sub> to the atmosphere. Bottles were placed in two growth chambers (model GC36; Environmental Growth Chambers, Chagrin Falls, OH, USA) where different VPD treatments were imposed to manipulate transpiration rate and thus uptake of the treatment solutions. Five treatment branches were placed in each chamber. In addition, two control branches with non-labelled solution were placed in each chamber to determine whether any of the <sup>13</sup>CO<sub>2</sub> label diffused from the treatment bottles to the atmosphere and was subsequently assimilated by leaf photosynthesis during the course of the experiment. Control and treatment branches were randomly placed 20 cm apart on the floor of the growth chambers. Air temperature (25 °C) and photosynthetic active radiation (500 μmol m<sup>-2</sup> s<sup>-1</sup>) were similar in both chambers. In the first chamber, low atmospheric relative humidity was imposed (30%) producing the high VPD treatment (2.23 kPa). In the second chamber, higher atmospheric relative humidity was imposed (60%), producing the low VPD treatment (1.27 kPa).

Branches were allowed to transpire for 1 h prior to the start of measurements to ensure that the labelled solution had reached the leaves. This time period was determined through a preliminary experiment in which branches were placed in a dye solution. The dye was clearly visible in the leaves within 30 min at low VPD, indicating that 1 h was more than sufficient for internally transported CO<sub>2</sub> to reach the leaves under both VPD treatments.

### Leaf gas-exchange measurements

Leaf net photosynthesis ( $A_{\text{net}}$ , μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance ( $g_s$ , mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), and transpiration ( $E$ , mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were measured on a fully expanded lower leaf of all treatment and control branches with a portable photosynthesis system (model Li-6400; Li-Cor, Lincoln, NE, USA).

Measurements were repeated four times over an 8 h period at prevailing growth chamber temperature, light, and humidity conditions and a set atmospheric [CO<sub>2</sub>] of 400 ppm. At each measurement time, the average of five measurements recorded at 10 s intervals was used for analysis. Following the last gas-exchange measurements, the branches were allowed to transpire for another hour before the lights were switched off and woody and leaf tissues were sampled.

#### Tissue sampling for stable isotope analysis

At the end of the 9 h uptake period, branches were removed from the bottles and the remaining amount of solution was subtracted from the original amount to determine the quantity of solution taken up by each branch. Uptake of <sup>13</sup>C (g) was calculated as described by McGuire *et al.* (2009):

$$^{13}\text{C uptake} = [\text{CO}_2^*] * a * w \quad (\text{Eq. 1})$$

where [CO<sub>2</sub>\*] (mol l<sup>-1</sup>) is calculated using Henry's coefficients (Butler, 1991; McGuire and Teskey, 2002), *a* is at% <sup>13</sup>C and *w* is water uptake of the branch (l).

For every branch, the portion above the foam gasket at the top of each bottle was divided into three equal-length sections (lower, mid, and upper), and all woody and leaf tissues were collected from each section separately. Samples were immediately frozen in liquid nitrogen and transferred to an ultralow freezer at -25 °C to stop all metabolic activity. Tissues were later transferred to a walk-in freezer and stored at -9 °C.

Each sample was thawed individually before further processing. The leaf area of each branch section was measured with a leaf area meter (model Li-3100; Li-Cor). Mean total leaf area per branch ± standard error of the mean (SEM) was 1109.1 ± 56.7 cm<sup>2</sup>. Non-living bark was removed from woody tissue samples and discarded. The remaining tissue was separated into inner bark and xylem tissue component subsamples. A sample of three mature leaves was removed from each of the three branch sections and divided into subsamples of petiole, primary vein, secondary veins, and mesophyll. The remaining mature leaves of each branch were processed as whole leaves. Baseline samples were processed in the same manner. All xylem, inner bark and leaf samples were dried to a constant weight in an oven at 65 °C for at least 72 h. The dry mass of all tissues was recorded for scaling the data from the stable isotope analysis to branch level. Finally, subsamples were ground to a powder in a ball mill (8000-D Mixer Mill; SPEX SamplePrep, Metuchen, NJ, USA) for carbon isotope analysis.

#### Isotopic analysis of samples

Ground tissue samples were weighed to µg precision in tin capsules that were flash combusted and analysed by isotope-ratio mass spectrometry at the Stable Isotope and Soil Biology Laboratory (SISBL), Odum School of Ecology, University of Georgia, Athens, GA, USA.

Enrichment of the labelled tissues (δ<sup>13</sup>C<sub>t</sub>, ‰) was calculated as the difference between the δ<sup>13</sup>C value of the labelled sample (δ<sup>13</sup>C<sub>s</sub>, ‰) and the δ<sup>13</sup>C value of the baseline sample (δ<sup>13</sup>C<sub>b</sub>, ‰) of similar tissue:

$$\delta^{13}\text{C}_t = \delta^{13}\text{C}_s - \delta^{13}\text{C}_b \quad (\text{Eq. 2})$$

The ratio of <sup>13</sup>C to <sup>12</sup>C relative to tissue enrichment (*R<sub>t</sub>*) in each sample was calculated as:

$$R_t = \left( \frac{\delta^{13}\text{C}_t}{1000} \right) 0.0112372 \quad (\text{Eq. 3})$$

where 0.0112372 is the ratio of <sup>13</sup>C/<sup>12</sup>C of the PeeDee Belemnite standard.

#### Scaling isotope measurements of tissue component samples to branch level

The dry mass of the tissue component subsamples was used to determine the mass proportions of living bark and xylem of the woody tissue and of the different leaf components for each individual branch. These mass proportions were used to separate the total biomass of woody and leaf tissue into component parts by branch section.

Based on the ratio of <sup>13</sup>C to <sup>12</sup>C relative to tissue enrichment determined using Eq. 3, the amount of <sup>13</sup>C assimilated in each tissue component (<sup>13</sup>C<sub>t</sub>, g) was calculated for each branch as:

$$^{13}\text{C}_t = \left( \frac{R_t}{(1 + R_t)} * \text{DM} * C \right) \quad (\text{Eq. 4})$$

where DM is the dry mass of the tissue per section (g) and *C* is the carbon content of the tissue component (%). Mean <sup>13</sup>C<sub>t</sub> was calculated for each label×VPD treatment combination.

#### Ratio of <sup>13</sup>C assimilation to atmospheric CO<sub>2</sub> assimilation

To obtain the total amount of atmospheric carbon assimilated via photosynthesis (*A<sub>atm</sub>*, g) during the period of label uptake, *A<sub>net</sub>* averaged for the entire measurement period was scaled to the branch level:

$$A_{atm} = A_{net} * \text{LA} * 10^{-6} * M(C) * t \quad (\text{Eq. 5})$$

where LA is the total leaf area of all branch sections (m<sup>2</sup>), *M(C)* is the atomic weight of C and *t* is the length of the period of CO<sub>2</sub>-enriched solution uptake (s).

To determine the ratio of total <sup>13</sup>C<sub>t</sub> (Σ<sup>13</sup>C<sub>t</sub>, g) to *A<sub>atm</sub>* (Σ<sup>13</sup>C<sub>t</sub>/*A<sub>atm</sub>*, ‰), <sup>13</sup>C<sub>t</sub> was summed for all the sections and tissues and divided by *A<sub>atm</sub>*:

$$\text{Ratio} = \frac{(\Sigma^{13}\text{C}_t)}{A_{atm}} \quad (\text{Eq. 6})$$

#### Data processing and statistical analysis

We analysed enrichment of the woody and leaf tissue components (δ<sup>13</sup>C<sub>t</sub>) of the treatment branches, as well as the total quantity of <sup>13</sup>C assimilated in each tissue component (<sup>13</sup>C<sub>t</sub>), using multifactorial analysis of variance (ANOVA). For the woody tissues, <sup>13</sup>C label concentration (*n*=2), branch section (*n*=3), VPD treatment (*n*=2), and tissue component (*n*=2, inner bark and xylem) were treated as fixed factors and individual branch (*n*=5 per label×VPD treatment combination) was treated as a random factor. A similar ANOVA model was used for leaf components (*n*=4; petiole, primary vein, secondary veins, and mesophyll). We also used a similar ANOVA model to compare δ<sup>13</sup>C<sub>t</sub> in the treatment versus control branches with branch type (*n*=3; control, HL, and LL), <sup>13</sup>C label concentration (*n*=2), branch section (*n*=3), VPD treatment (*n*=2), and tissue component (*n*=6) treated as fixed factors and individual branch (*n*=5) treated as a random factor. Ratios of Σ<sup>13</sup>C<sub>t</sub> to *A<sub>atm</sub>* were analysed using an ANOVA model similar to that used for δ<sup>13</sup>C<sub>t</sub>, but with <sup>13</sup>C label concentration (*n*=2) and VPD treatment (*n*=2) used as fixed factors and individual branch (*n*=5) treated as a random factor. Solution uptake was analysed using a similar ANOVA model with only branch type (*n*=3) and VPD treatment (*n*=2) considered as fixed factors. Average *A<sub>net</sub>*, *g<sub>s</sub>* and *E* were analysed using a repeated-measures ANOVA model with <sup>13</sup>C label concentration (*n*=2), VPD (*n*=2), and time

( $n=4$ ) treated as fixed factors and individual branch ( $n=5$ ) treated as a random factor. Akaike Information Criterion corrected for small sample sizes ( $AIC_C$ ) was used to determine the covariance structure that best estimated the correlation among individual branches over time. Treatment means were compared using Fisher's least significance difference test. ANOVA analyses were performed using the mixed model procedure (PROC MIXED) of SAS (version 9.1.3, SAS, Cary, NC, USA) with  $\alpha=0.05$ .

## Results

### *Uptake of CO<sub>2</sub>-enriched solution*

Solution uptake was influenced by VPD ( $P < 0.0001$ ), but not by label treatment ( $P = 0.179$ ). Mean uptake of the <sup>13</sup>CO<sub>2</sub>-enriched solution for all treatment branches at high VPD was  $144.0 \pm 11.0$  ml, which was significantly higher than the uptake at low VPD ( $61.5 \pm 5.2$  ml). Similar results were observed for the amount of solution taken up by the control branches ( $148.8 \pm 11.8$  ml and  $82.5 \pm 6.3$  ml under high and low VPD treatment, respectively).

### *Carbon isotope composition of woody and leaf tissue components*

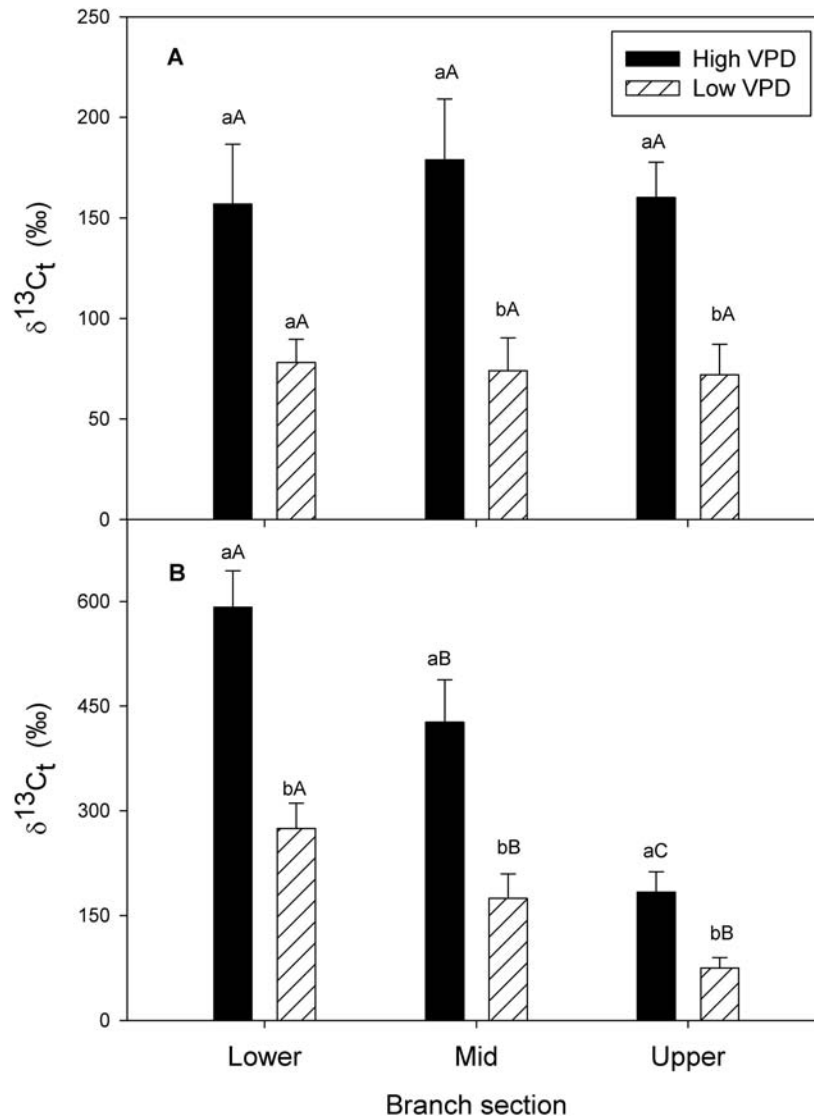
The <sup>13</sup>C enrichment of woody tissues ( $\delta^{13}C_t$ ) was influenced by label concentration, VPD treatment, branch section, and tissue component; however, these individual effects were not independent of each other (i.e. VPD treatment  $\times$  branch section  $\times$  tissue component interaction,  $P < 0.0001$ , and a label concentration  $\times$  VPD treatment  $\times$  branch section interaction,  $P < 0.0001$ ). Averaged across both label treatments, the  $\delta^{13}C_t$  of xylem and inner bark was higher at high VPD than at low VPD with the exception of xylem in the lower branch (Fig. 1). The  $\delta^{13}C_t$  of the inner bark was higher in the lower branch section than in the mid- and upper-branch section, irrespective of VPD treatment, whereas no such variation among branch sections was observed for the xylem (Fig. 1). Averaged across xylem and inner bark tissues, the mean  $\delta^{13}C_t$  of the woody tissue was higher under the HL treatment than under the LL treatment at the mid-branch section ( $305.22 \pm 24.23$  vs  $122.18 \pm 22.93$  ‰) and upper-branch section ( $223.88 \pm 14.89$  vs  $21.50 \pm 10.15$  ‰), but there was no difference between label treatments at the lower branch. The  $\delta^{13}C_t$  of the woody tissue components of the control branches varied between  $-0.92 \pm 0.07$  ‰ and  $2.72 \pm 0.84$  ‰ and was substantially lower than the  $\delta^{13}C_t$  observed for the woody tissue components of the treatment branches ( $P < 0.0001$ ).

For leaf tissue, enrichment of all components was observed under the HL treatment, whereas under the LL treatment, only  $\delta^{13}C_t$  of the petioles was elevated. Similar to the  $\delta^{13}C_t$  of the woody tissue, the  $\delta^{13}C_t$  of the leaf tissue was influenced by the same individual effects, which were not independent of each other (i.e. there was a significant VPD treatment  $\times$  tissue component  $\times$  branch section  $\times$  label concentration interaction,  $P = 0.0166$ ). For example, under the HL treatment, the average  $\delta^{13}C_t$  of petiole (Fig. 2A), primary vein (Fig. 2B), secondary veins (Fig. 2C), and leaf

mesophyll (Fig. 2D) was higher under the high VPD than under the low VPD treatment, regardless of branch section, whereas under the LL treatment, higher  $\delta^{13}C_t$  at high VPD was observed only for the petioles (Fig. 2A). Under the high VPD treatment, the  $\delta^{13}C_t$  of all leaf tissue components was higher under the HL treatment than under the LL treatment in all branch sections (Fig. 2). Under the low VPD treatment, the  $\delta^{13}C_t$  of the petiole (Fig. 2A) was higher under the HL treatment than under the LL treatment regardless of branch section, but the other leaf tissue components were not influenced by the label treatment (Fig. 2B–D). The  $\delta^{13}C_t$  of the petioles decreased with increasing branch section (lower to upper), regardless of VPD treatment or label concentration, whereas the  $\delta^{13}C_t$  of the other leaf tissue components decreased with increasing section only under the HL treatment at high VPD. For the samples processed as whole leaves, the effect of VPD and label treatment on  $\delta^{13}C_t$  was similar to that observed for the leaf blade tissue components (primary and secondary veins and leaf mesophyll). The  $\delta^{13}C_t$  of the leaf tissue components of the control branches varied between  $-0.99 \pm 0.07$  and  $3.39 \pm 0.32$  ‰. This was lower than the  $\delta^{13}C_t$  of the leaf tissue components under the HL treatment ( $P < 0.0001$ ), regardless of branch section, and the  $\delta^{13}C_t$  of the petioles of the branches under the LL treatment ( $P < 0.0001$ ).

### *<sup>13</sup>C assimilation*

Generally, more <sup>13</sup>C was assimilated (<sup>13</sup>C<sub>t</sub>) in leaf tissue than in woody tissue under the HL treatment, whereas the opposite pattern was observed under the LL treatment (Table 1). The <sup>13</sup>C<sub>t</sub> of the woody and leaf tissue components was influenced by label concentration, VPD treatment, branch section, and tissue component, and these individual effects were not independent of each other (i.e. VPD treatment  $\times$  tissue component  $\times$  branch section  $\times$  label concentration interaction,  $P = 0.0137$ ). Under the HL treatment, the <sup>13</sup>C<sub>t</sub> of the inner bark, petiole, and mesophyll was higher under the high VPD treatment than under the low VPD treatment, whereas the <sup>13</sup>C<sub>t</sub> of both woody tissue components and the petiole was higher under the high VPD treatment than under the low VPD treatment in the LL treatment (Table 1). Averaged across VPD and label concentration treatments, most assimilation of <sup>13</sup>C in woody tissue occurred in the inner bark ( $65.07 \pm 4.57$  %). Averaged across VPD and label concentration treatments, most <sup>13</sup>C assimilation in leaves occurred in the mesophyll compared with the petiole, primary vein, and secondary vein (Table 1). Under the HL treatment, the <sup>13</sup>C<sub>t</sub> of the petioles and mesophyll was higher at high VPD than at low VPD in all branch sections, whereas a VPD effect on <sup>13</sup>C<sub>t</sub> under the LL treatment was observed only in the petioles of the lower branch section. In total, between 62.71 and 80.36% of the applied label was assimilated, whilst it was assumed that the remainder diffused to the atmosphere. Regardless of VPD and label treatment, the <sup>13</sup>C<sub>t</sub> of the woody and leaf tissue of the control branches was lower than the <sup>13</sup>C<sub>t</sub> of the woody and leaf tissue of the treatment branches ( $P < 0.0001$ ).



**Fig. 1.** Mean tissue enrichment ( $\delta^{13}\text{C}_t$ , ‰) of xylem (A) and inner bark (B) of three equal-length sections of five branches under high (filled bars) or low (hatched bars) atmospheric VPD averaged for high and low  $^{13}\text{CO}_2$  concentration solution.  $\delta^{13}\text{C}_t$  was calculated by subtracting the  $\delta^{13}\text{C}$  value of baseline samples of non-labelled tissue from the  $\delta^{13}\text{C}$  value of the enriched sample of the same tissue. Different lower-case letters indicate significant differences ( $P < 0.05$ ) in  $\delta^{13}\text{C}_t$  in inner bark and xylem between VPD treatments within each branch section. Different upper-case letters indicate significant differences among different branch sections within tissue component and VPD treatment. Results are shown as means  $\pm$  SEM.

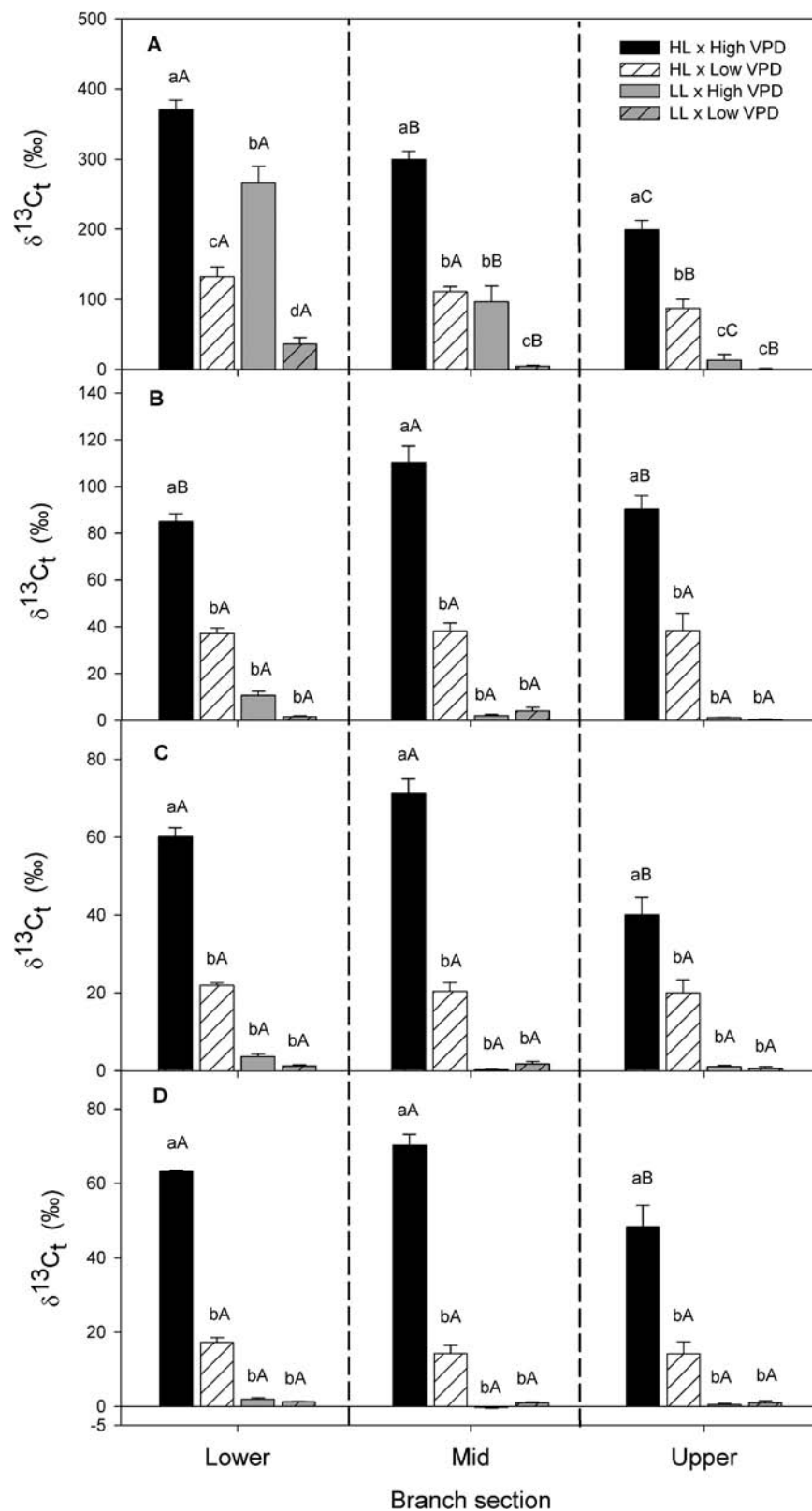
### Leaf gas exchange

On average, transpiration rate ( $E$ ) in the high VPD treatment was approximately twice as high as under the low VPD treatment, whereas photosynthesis ( $A_{\text{net}}$ ) and stomatal conductance ( $g_s$ ) averaged over all measurements were similar under both VPD treatments (Table 2). However, for  $E$ ,  $A_{\text{net}}$ , and  $g_s$ , both treatment responses changed through time (i.e. VPD treatment  $\times$  time interaction for  $E$ ,  $P < 0.0001$ ; VPD treatment  $\times$  time interaction for  $A_{\text{net}}$ ,  $P = 0.0363$ ; and VPD treatment  $\times$  time interaction for  $g_s$ ,  $P < 0.0001$ ). Under both the high and low VPD treatment,  $E$  and  $A_{\text{net}}$  tended to decrease slightly over time. At the last measurement time,  $A_{\text{net}}$  under the low VPD treatment

tended to be higher than under the high VPD treatment, which coincided with a difference in  $g_s$  observed between the VPD treatments.

### Assimilation of internally transported CO<sub>2</sub> versus atmospheric CO<sub>2</sub> assimilation

The ratio of total  $^{13}\text{C}$  label assimilation to atmospheric CO<sub>2</sub> assimilation ( $\Sigma^{13}\text{C}_t/A_{\text{atm}}$ ) was affected by the VPD and label concentration treatments. The ratio was higher under high VPD than under low VPD (Fig. 3), independent of label concentration ( $P < 0.0001$ ). However, under the high VPD treatment, the ratio was higher under the HL treatment than under the LL treatment, whereas at low VPD, the ratio was



**Fig. 2.** Mean tissue enrichment ( $\delta^{13}C_t$ , ‰) of petiole (A), primary vein (B), secondary vein (C), and mesophyll (D) of leaves of three equal-length sections of five branches allowed to transpire in either high  $^{13}CO_2$  concentration solution (HL) under high (filled bars) or low (hatched bars) atmospheric VPD treatment, or low  $^{13}CO_2$  concentration solution (LL) under high (shaded bars) or low (shaded hatched bars) VPD treatment.  $\delta^{13}C_t$  was calculated by subtracting the  $\delta^{13}C$  value of baseline samples of non-labelled tissue from the  $\delta^{13}C$  value of the enriched sample of the same tissue. Different lower-case letters indicate significant differences ( $P < 0.05$ ) in  $\delta^{13}C_t$  of tissues among the four label $\times$ VPD treatment combinations within each branch section. Different upper-case letters indicate significant differences in  $\delta^{13}C_t$  among different branch sections within tissue component and label and VPD treatment combinations. Results are shown as means  $\pm$  SEM.

**Table 1.** Mean <sup>13</sup>C assimilation (<sup>13</sup>C<sub>t</sub>, mg) (SEM) in woody and leaf tissue components of five branches allowed to transpire solutions with dissolved <sup>13</sup>CO<sub>2</sub> at high (HL) or low (LL) concentrations and at high or low atmospheric VPD. Significant differences between all label×VPD treatment combinations for both woody and leaf tissue components are indicated (*P* < 0.05). <sup>13</sup>C<sub>t</sub> and SEM displayed as 0.00 (0.00), respectively, were not zero but have been truncated due to rounding up.

Treatment combination	Branch		Leaf			Total	
	Xylem	Inner bark	Petiole	Primary vein	Secondary vein		Mesophyll
LL×high VPD	0.39 <sup>a</sup> (0.11)	0.93 <sup>a</sup> (0.16)	0.75 <sup>b</sup> (0.14)	0.01 <sup>a</sup> (0.00)	0.00 <sup>a</sup> (0.00)	0.82 <sup>c</sup> (0.16)	2.9 <sup>b</sup> (0.47)
LL×low VPD	0.11 <sup>b</sup> (0.02)	0.22 <sup>c</sup> (0.05)	0.01 <sup>c</sup> (0.02)	0.00 <sup>a</sup> (0.00)	0.00 <sup>a</sup> (0.00)	0.14 <sup>c</sup> (0.02)	0.48 <sup>c</sup> (0.06)
HL×high VPD	0.60 <sup>a</sup> (0.12)	1.15 <sup>a</sup> (0.14)	1.63 <sup>a</sup> (0.20)	0.18 <sup>a</sup> (0.03)	0.15 <sup>a</sup> (0.01)	5.11 <sup>a</sup> (0.59)	8.11 <sup>a</sup> (0.64)
HL×low VPD	0.41 <sup>a</sup> (0.08)	0.69 <sup>b</sup> (0.13)	0.69 <sup>b</sup> (0.10)	0.09 <sup>a</sup> (0.01)	0.05 <sup>a</sup> (0.00)	1.76 <sup>b</sup> (0.28)	3.69 <sup>b</sup> (0.33)

**Table 2.** Average (SEM) transpiration (*E*, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), net photosynthesis (*A*<sub>net</sub>, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), and stomatal conductance (*g*<sub>s</sub>, mol H<sub>2</sub>O vapour m<sup>-2</sup> s<sup>-1</sup>) measured on leaves of branches allowed to transpire solutions with dissolved <sup>13</sup>CO<sub>2</sub> at high (HL) or low (LL) concentrations and at high or low VPD. Data are averages of four measurements performed at 2h intervals during an 8h measurement period on five leaves (one leaf per treatment branch) per label and VPD treatment combination. Lower-case letters indicate significant (*P* < 0.05) differences between high and low VPD treatments within <sup>13</sup>C label treatments. Values and SEM displayed as 0.00 (0.00), respectively, were not zero but have been truncated due to rounding up.

Label treatment	LL		HL	
	High	Low	High	Low
<i>E</i>	2.57 <sup>a</sup> (0.02)	1.14 <sup>b</sup> (0.03)	2.53 <sup>a</sup> (0.03)	1.07 <sup>b</sup> (0.03)
<i>A</i> <sub>net</sub>	8.98 <sup>a</sup> (0.15)	7.90 <sup>a</sup> (0.22)	8.28 <sup>a</sup> (0.13)	7.47 <sup>a</sup> (0.25)
<i>g</i> <sub>s</sub>	0.15 <sup>a</sup> (0.00)	0.13 <sup>a</sup> (0.00)	0.15 <sup>a</sup> (0.00)	0.11 <sup>b</sup> (0.00)

not significantly different between HL and LL treatments (label concentration×VPD treatment interaction, *P*=0.046; Fig. 3).

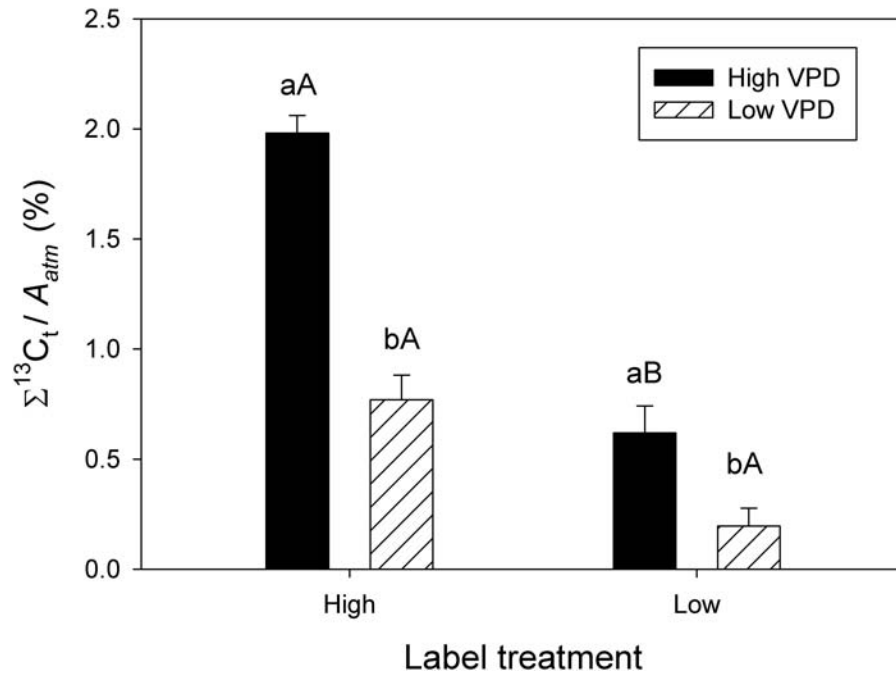
## Discussion

Our results showed that transpiration and xylem sap [CO<sub>2</sub>] both affect the quantity of xylem-transported CO<sub>2</sub> that is assimilated by woody and leaf tissues. Higher <sup>13</sup>C enrichment and a larger quantity of assimilated <sup>13</sup>C were found in branch and leaf tissues of the branches subjected to higher transpiration rate and higher xylem sap [CO<sub>2</sub>]. These results were not surprising, as the amount of label taken up by the branches was greater under high transpiration rate and high label (25.4 ± 2.4 mg <sup>13</sup>C) relative to the other VPD×label treatment combinations (up to 10.7 ± 1.7 mg <sup>13</sup>C). A fraction of the label taken up by the branches under high label and high VPD treatment also diffused to the atmosphere (37.3 ± 4.9 %), potentially affecting the actual amount of label supplied to the tissues. The low δ<sup>13</sup>C<sub>t</sub> found in the control branches relative to the treatment branches confirmed that stomatal uptake of <sup>13</sup>C label that may have diffused from the bottles or

from the treatment branches was negligible, confirming that tissue enrichment in the treatment branches was due solely to the assimilation of xylem-transported <sup>13</sup>C label.

Besides its role in plant water supply, the transpiration stream is also considered an important sink for internally transported CO<sub>2</sub> derived from plant respiration below ground (Grossiord *et al.*, 2012; Aubrey and Teskey, 2009; Bloemen *et al.*, 2013) and above ground (Teskey and McGuire, 2007). It has been suggested that a substantial fraction of root-respired CO<sub>2</sub> is transported upwards in the xylem where it can either be assimilated by stem, branch, and leaf tissues containing chlorophyll (Saveyn *et al.*, 2008a; McGuire *et al.*, 2009; Saveyn *et al.*, 2010) or diffuse to the atmosphere from stem and branch surfaces (McGuire *et al.*, 2007; Teskey and McGuire, 2007; Saveyn *et al.*, 2008b).

We observed that, at higher transpiration rates, more <sup>13</sup>CO<sub>2</sub> was transported in the branches, thereby increasing the amount of <sup>13</sup>C-labelled substrate available for assimilation in woody and leaf tissue. Assimilation occurs both in the xylem and the inner bark of woody tissues (Pfanzen *et al.*, 2002; Aschan and Pfanz, 2003). While McGuire *et al.* (2009) estimated that CO<sub>2</sub> assimilation in the xylem accounted for 42% of woody tissue photosynthesis, we found an average contribution of 35% in small branches. The highest δ<sup>13</sup>C<sub>t</sub> was observed in the inner bark of the lower branch sections, probably due to its proximity to the <sup>13</sup>C source. However, in contrast to previous observations (McGuire *et al.*, 2009), the δ<sup>13</sup>C<sub>t</sub> of the xylem showed only a small variation over the length of the branch. It is possible that our longer period of label application (10 h) compared with the 4.75 h of McGuire *et al.* (2009) induced higher enrichment at mid- and upper-branch sections. McGuire *et al.* (2009) found that most of a <sup>13</sup>C label applied to branches was assimilated in the bottom section of the branch xylem, whilst in our experiment we observed only a small variation in assimilation in the xylem along the branch. The concentration of the <sup>13</sup>C label provided at the lowest branch section was very high in our experiment, which suggests that the enzymes that fix the <sup>13</sup>C label might have been saturated by the substrate, according to Michaelis–Menten kinetics. Therefore, not all the label could be assimilated in the lower branch, and the remaining portion would be transported upwards and assimilated higher in the branch. Thus, the longer exposure time in our experiment would result in transfer of more label to the



**Fig. 3.** Mean ratio of total assimilation of <sup>13</sup>C label in branches (both woody and leaf tissues) to total atmospheric CO<sub>2</sub> assimilation by leaves ( $\Sigma^{13}\text{C}_t/A_{\text{atm}}$ , %) of five branches allowed to transpire solutions with dissolved <sup>13</sup>CO<sub>2</sub> label at high or low concentrations and at high (filled bars) or low (hatched bars) VPD. Different lower-case letters indicate significant differences ( $P < 0.05$ ) in the ratio of <sup>13</sup>C assimilation to atmospheric CO<sub>2</sub> assimilation between VPD treatments within <sup>13</sup>CO<sub>2</sub> concentration treatments. Different upper-case letters indicate significant differences between <sup>13</sup>CO<sub>2</sub> concentration treatments within VPD treatments. Results are shown as means  $\pm$ SEM.

higher branch sections, and would explain our observation of similar enrichment of the xylem in all branch sections. Similar high enrichment of woody tissues more distal from the point of <sup>13</sup>C infusion was observed for the same species in a whole-tree experiment in the field (Bloemen *et al.*, 2013). However, in whole trees, a larger proportion of <sup>13</sup>C tracer diffused to the atmosphere (up to 94%; Bloemen *et al.*, 2013) than in this study (up to 55%), probably due to the longer transport pathway from the point of infusion to these more distal tissues.

In the leaves, the effect of transpiration rate on tissue component enrichment was strongly determined by tissue type. In the petioles, a higher  $\delta^{13}\text{C}_t$  was observed compared with that in other tissues at both high and low transpiration rates and independent of label concentration. Stringer and Kimmerer (1993) found that 53.7% of a <sup>14</sup>C label applied to *P. deltoides* leaves via the xylem was assimilated in the petiole. Hibberd and Quick (2002) noted that photosynthetic cells in the petioles increased plant carbon gain, and related petiole photosynthesis to the possible existence of a C<sub>4</sub>-like mechanism in C<sub>3</sub> plants. In our study, transpiration rate determined enrichment in the veins and leaf mesophyll only with the HL treatment. At higher transpiration, a larger fraction of the <sup>13</sup>C label was fixed in leaf veins and mesophyll tissue. High levels of <sup>13</sup>C<sub>t</sub> were found in branches subjected to the HL treatment under high VPD and, in contrast to previous studies (Stringer and Kimmerer, 1993; McGuire *et al.*, 2009), more <sup>13</sup>C was assimilated in the leaf mesophyll ( $5.1 \pm 0.6$  mg) than in the petioles ( $1.6 \pm 0.2$  mg).

Xylem-transported CO<sub>2</sub> has generally been considered as an important potential carbon source only for cells that surround the xylem and phloem in petioles and veins (Stringer and Kimmerer, 1993; McGuire *et al.*, 2009). Our results showed that, at higher xylem sap [CO<sub>2</sub>], internally transported CO<sub>2</sub> can improve carbon substrate availability for the leaf mesophyll as well. Because of their dense vein network and the occurrence of bundle sheath extensions and paraveinal mesophyll tissue, *P. deltoides* leaves are especially well adapted for efficient water transport (Russin and Evert, 1984). Thus, internally transported CO<sub>2</sub> can be delivered very effectively to every part of the leaf, which may compensate for the lack of lateral CO<sub>2</sub> diffusion due to compartmentalization of the mesophyll in leaves with heterobaric anatomy (Pieruschka *et al.*, 2005; Leegood, 2008), which is the case for poplar leaves (McClendon, 1992).

Only a few isotope studies on detached leaves (Stringer and Kimmerer, 1993) and branches (McGuire *et al.*, 2009) have reported on the assimilation of internally transported CO<sub>2</sub> relative to the assimilation of atmospheric CO<sub>2</sub>. McGuire *et al.* (2009) estimated that internal CO<sub>2</sub> assimilation averaged 6% of atmospheric uptake in branches, whilst Stringer and Kimmerer (1993) estimated that, in leaves, the input of CO<sub>2</sub> via the transpiration stream could account for 2.2% of atmospheric uptake. We observed that both a higher transpiration rate and higher xylem sap [CO<sub>2</sub>] increased the contribution of xylem-transported CO<sub>2</sub> to branch carbon gain. There are other factors, not considered in this study, that might increase the ratio of internal compared with atmospheric CO<sub>2</sub>



assimilation (McGuire *et al.*, 2009). For example, the branches used in this experiment had a non-leaf mass to leaf mass ratio of less than 1, whilst a substantially greater non-leaf mass to leaf mass ratio can be expected for branches of other species or for full-grown trees. In addition, there are species with a higher stem chlorophyll content than *P. deltoides* (e.g. *Fraxinus excelsior* and *Quercus robur*; Pfanz *et al.*, 2002), which potentially assimilate more xylem-transported CO<sub>2</sub> than observed in this study. Finally, our <sup>13</sup>C-based estimates of internal CO<sub>2</sub> assimilation relative to atmospheric uptake do not account for the re-assimilation of locally respired CO<sub>2</sub>, which is composed almost entirely of <sup>12</sup>CO<sub>2</sub>. These factors could potentially increase the importance of assimilation of internal CO<sub>2</sub> in woody tissue; however, our results indicated that the overall amount of xylem-transported CO<sub>2</sub> that was assimilated was small compared with the uptake of atmospheric CO<sub>2</sub>.

Previous studies have described the potential benefits of internal CO<sub>2</sub> assimilation from a water-use perspective. Whilst leaves open their stomata to assimilate CO<sub>2</sub>, thus subjecting plants to the ambient drying power of the atmosphere, CO<sub>2</sub> for internal assimilation is supplied endogenously by respiration, thereby avoiding excessive water loss (Pfanz *et al.*, 2002; Aschan and Pfanz, 2003). Our results showed that, at a high transpiration rate, the assimilation of xylem-transported CO<sub>2</sub> increased in importance compared with a low transpiration rate. Therefore, we suggest that the assimilation of xylem-transported CO<sub>2</sub> in woody and leaf tissues is strongly dependent on transpiration, which is directly related to stomatal conductance. Beyond a certain threshold, stomatal conductance will decrease exponentially with increasing VPD (Massman and Kaufmann, 1991; McCaughey and Iacobelli, 1994; Monteith, 1995; Oren *et al.*, 1999), reducing the assimilation of atmospheric CO<sub>2</sub> (Oren *et al.*, 1999). At the same time, transpiration will continue to increase up to some plateau level as VPD increases to a certain level (Jarvis, 1980; Pataki *et al.*, 2000). Therefore, as VPD increases, assimilation of atmospheric CO<sub>2</sub> will reduce, but xylem transport of respired CO<sub>2</sub> will maximize, suggesting that, under these conditions, the contribution of internal fixation to the overall carbon budget might become more important. Moreover, during periods of high VPD or low soil water availability, xylem CO<sub>2</sub> assimilation might also have a role in maintaining stem and branch hydraulics due to light-induced repair of embolized xylem vessels (Schmitz *et al.*, 2012), thereby reducing moisture stress and stomatal closure.

Our results showed that assimilation of xylem-transported CO<sub>2</sub> is affected by both the rate of transpiration and xylem [CO<sub>2</sub>], which is dependent on respiration. Therefore, the contribution and importance of internal CO<sub>2</sub> assimilation to overall plant carbon gain is likely to change with changes in the factors that control rates of transpiration, respiration, and photosynthesis.

## Acknowledgements

We thank T. M. Wertin for assistance with leaf gas exchange measurements and A. Alfred, S. Arnold, J. Audley, M. Cent, L. Coleman, A. Gojman, D. Layfield, E. Oxford, and

P. Tupper for processing the samples. T. Maddox of The Stable Isotope/Soil Biology Laboratory of the Institute of Ecology, University of Georgia, provided advice and conducted isotope analysis. Funding was provided by the Special Research Fund (B.O.F.) of Ghent University and US National Science Foundation Award No. 1021150.

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