Fate and importance of respired CO$_2$ transport in trees

ir. Jasper Bloemen
What could possibly go wrong?

- Robert O. Teskey
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Fate and importance of respired CO$_2$ transport in trees

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for the degree of Doctor (PhD) in Applied Biological Sciences
Dankwoord

Beste lezer, waarom bestaan dankwoorden?

Ondertussen zou het namelijk al zeer duidelijk moeten zijn hoe dankbaar ik je ben voor de hulp en steun bij dit doctoraat de afgelopen vier jaar. Ik denk dat ik vrij duidelijk ben geweest in het 'bedanken' gedurende de voorbije vier jaar. Echter mensen vergeten snel (of worden gewoon wat ouder) of luisteren soms niet goed. Daarom een korte recapitulatie van mijn bedankingen zodat iedereen een neergeschreven bewijs heeft van mijn dankbaarheid.

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the statistics and your help on the isotope experiments. Once I’ll be living in the Alps you
can pass by, so you can take a break from the Georgia flatlands.

Now I could try to write the next paragraph in Tyrolean, but I still need to decipher their
vocabulary and work on my Schuhplattler skills. Nonetheless, I want to thank prof. dr.
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discussions, while sitting in the office or walking up- and downhill in the mountains. I’m
looking forward to working together in the future, eating some more Strudel and tour
skiing in the winter and hiking in the summer.

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for his help and his knack in creating labeling systems from bits and pieces during our
small $^{11}$C project at INFINITY lab.

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gemiddeld Belgisch bedrijf er echt aan toe gaat… . Echter, in het labo voor Plantecologie,
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naar de Lekkerbek moeten gaan met mijn busje voor mijn vertrek naar het buitenland.
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trouwkostuum. En Margot, spijtig dat je weg bent, maar toch bedankt voor de administratieve hulp. Ik hoop dat we elkaar nog eens tegen het lijf lopen.

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Gent, december 2013
Jasper
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<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Acoustic emission</td>
</tr>
<tr>
<td>AIC&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Akaike information criterion corrected for small sample sizes</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine-triphosphate</td>
</tr>
<tr>
<td>C</td>
<td>Carbon content</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DBH</td>
<td>Diameter at breast height</td>
</tr>
<tr>
<td>DIC</td>
<td>Dissolved inorganic carbon</td>
</tr>
<tr>
<td>DM</td>
<td>Dry mass</td>
</tr>
<tr>
<td>DOY</td>
<td>Day of the year</td>
</tr>
<tr>
<td>DG</td>
<td>Stem daily growth rate</td>
</tr>
<tr>
<td>EA</td>
<td>Elemental Analyzer</td>
</tr>
<tr>
<td>EC</td>
<td>Electron capture</td>
</tr>
<tr>
<td>FACE</td>
<td>Free-air CO&lt;sub&gt;2&lt;/sub&gt; enrichment</td>
</tr>
<tr>
<td>HB</td>
<td>Heat balance</td>
</tr>
<tr>
<td>HL</td>
<td>High label</td>
</tr>
<tr>
<td>IPCC</td>
<td>Intergovernmental panel climate change</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>IRGA</td>
<td>Infrared gas analyzer</td>
</tr>
<tr>
<td>IRMS</td>
<td>Isotope ratio mass spectrometer</td>
</tr>
<tr>
<td>LA</td>
<td>Leaf area</td>
</tr>
<tr>
<td>LL</td>
<td>Low label</td>
</tr>
<tr>
<td>LVDT</td>
<td>Linear variable displacement transducer</td>
</tr>
<tr>
<td>NDIR</td>
<td>Non dispersive infrared</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetic active radiation</td>
</tr>
<tr>
<td>PEPc</td>
<td>Phosphoenolpyruvate carboxylase</td>
</tr>
</tbody>
</table>
Abbreviations and symbols

PET  Positron emission tomography
RH   Relative humidity
Rubisco Ribulose-1,5-biphosphate carboxylase oxygenase
ROI  Region of interest
SFD  Sap flux density
TDP  Thermal dissipation probe
VPD  Vapor pressure deficit
VPDB Vienna pee dee belemnite

Latin symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_s$</td>
<td>Surface area of the stem enclosed in a stem cuvette</td>
<td>$m^2$</td>
</tr>
<tr>
<td>$A$</td>
<td>Atom%</td>
<td>%</td>
</tr>
<tr>
<td>$A_t$</td>
<td>Atom% of the labeled tissue</td>
<td>%</td>
</tr>
<tr>
<td>$A(C)$</td>
<td>Atomic weight of carbon</td>
<td>g mol$^{-1}$</td>
</tr>
<tr>
<td>$A_{atm}$</td>
<td>total amount of atmospheric carbon assimilated</td>
<td>g</td>
</tr>
<tr>
<td>$A_{net}$</td>
<td>Net photosynthesis</td>
<td>$\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$A_{max}$</td>
<td>Maximum photosynthesis</td>
<td>$\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$A_{root}$</td>
<td>Soil area occupied by the root system</td>
<td>$m^2$</td>
</tr>
<tr>
<td>$[CO_2]$</td>
<td>Gaseous CO$_2$ concentration</td>
<td>% or $\mu$mol mol$^{-1}$</td>
</tr>
<tr>
<td>$[CO_2^*]$</td>
<td>Dissolved CO$_2$ concentration</td>
<td>mM</td>
</tr>
<tr>
<td>$^{13}C_t$</td>
<td>$^{13}$C tissue content</td>
<td>mg</td>
</tr>
<tr>
<td>$^{13}C_{assim}$</td>
<td>Amount of $^{13}$C assimilated</td>
<td>mg</td>
</tr>
<tr>
<td>$^{13}C_{uptake}$</td>
<td>Amount of $^{13}$C label taken up</td>
<td>mg</td>
</tr>
<tr>
<td>$^{13}C_{efflux}$</td>
<td>Amount of $^{13}$C label lost to the atmosphere by efflux</td>
<td>mg</td>
</tr>
<tr>
<td>$\Delta T$</td>
<td>Temperature difference between heated and reference needle</td>
<td>°C</td>
</tr>
<tr>
<td>$\Delta T_0$</td>
<td>Temperature difference between heated and reference needle when there is no sap flow</td>
<td>°C</td>
</tr>
<tr>
<td>$\Delta CO_2$</td>
<td>Gasous CO$_2$ concentration difference between measurement and reference air</td>
<td>$\mu$mol mol$^{-1}$</td>
</tr>
<tr>
<td>$D$</td>
<td>Stem diameter</td>
<td>mm</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>E</td>
<td>Transpiration</td>
<td>mmol H₂O m⁻² s⁻¹</td>
</tr>
<tr>
<td>Eₘₕₕₜₛ</td>
<td>Soil CO₂ efflux</td>
<td>mg C m⁻² h⁻¹</td>
</tr>
<tr>
<td>Eₘₕₜₚₚ</td>
<td>Stem CO₂ efflux</td>
<td>μmol CO₂ m⁻² s⁻¹</td>
</tr>
<tr>
<td>Eₘₕₜₚₚ(ref)</td>
<td>Stem CO₂ efflux at reference stem temperature</td>
<td>μmol CO₂ m⁻² s⁻¹</td>
</tr>
<tr>
<td>fₐ</td>
<td>Flow rate through stem cuvette</td>
<td>l s⁻¹</td>
</tr>
<tr>
<td>Fₗ</td>
<td>Sap flow rate</td>
<td>l h⁻¹</td>
</tr>
<tr>
<td>Fₕ</td>
<td>Xylem transport of CO₂ derived from belowground respiration</td>
<td>mmol h⁻¹</td>
</tr>
<tr>
<td>Fₕₘₕₜₚₚ</td>
<td>Xylem transport of CO₂ derived from</td>
<td>mg C m⁻² h⁻¹</td>
</tr>
<tr>
<td>Fₕₘₕₜₚₚ-g</td>
<td>Xylem transport of CO₂ derived from</td>
<td>mg C m⁻² h⁻¹</td>
</tr>
<tr>
<td>Fₕₘₕₜₚₚ-c</td>
<td>Xylem transport of CO₂ derived from</td>
<td>mg C m⁻² h⁻¹</td>
</tr>
<tr>
<td>gₛ</td>
<td>Stomatal conductance</td>
<td>mol H₂O m⁻² s⁻¹</td>
</tr>
<tr>
<td>k</td>
<td>Site and species specific correction factor for sap flow rate</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>Acidity constant</td>
<td>-</td>
</tr>
<tr>
<td>Kₜₜ</td>
<td>Henry’s constant</td>
<td>-</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
<td>kg/C</td>
</tr>
<tr>
<td>n</td>
<td>Amount of gas</td>
<td>mol</td>
</tr>
<tr>
<td>[O₂]</td>
<td>Gaseous O₂ concentration</td>
<td>%</td>
</tr>
<tr>
<td>P</td>
<td>Atmospheric pressure</td>
<td>Pa</td>
</tr>
<tr>
<td>pₜₘₜₚₚ</td>
<td>Partial pressure of CO₂ over the xylem sap</td>
<td>Pa</td>
</tr>
<tr>
<td>Qₜ₀</td>
<td>Change in rate for a 10°C change in temperature</td>
<td>-</td>
</tr>
<tr>
<td>r</td>
<td>Radius of the soil area occupied by the root system</td>
<td>cm</td>
</tr>
<tr>
<td>R</td>
<td>Universal gas constant</td>
<td>J mol⁻¹ K⁻¹</td>
</tr>
<tr>
<td>Rₗ</td>
<td>Autotrophic component of respiration</td>
<td>mg C m⁻² h⁻¹</td>
</tr>
<tr>
<td>Rₗₘₕₜₚₚ</td>
<td>Autotrophic component of belowground respiration</td>
<td>mg C m⁻² h⁻¹</td>
</tr>
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Abbreviations and symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{ab\text{-}conv}$</td>
<td>Conventional estimate of the autotrophic component of belowground respiration</td>
<td>mg C m$^{-2}$ h$^{-1}$</td>
</tr>
<tr>
<td>$R_{ab\text{-}new}$</td>
<td>New estimate of the autotrophic component of belowground respiration</td>
<td>mg C m$^{-2}$ h$^{-1}$</td>
</tr>
<tr>
<td>$R_h$</td>
<td>Heterotrophic component of respiration</td>
<td>mg C m$^{-2}$ h$^{-1}$</td>
</tr>
<tr>
<td>$R_{h,b}$</td>
<td>Heterotrophic component of belowground respiration</td>
<td>mg C m$^{-2}$ h$^{-1}$</td>
</tr>
<tr>
<td>$R_{sample}$</td>
<td>Abundance ratio of the heavier to lighter isotope in sample</td>
<td>-</td>
</tr>
<tr>
<td>$R_{standard}$</td>
<td>Abundance ratio of the heavier to lighter isotope in standard</td>
<td>-</td>
</tr>
<tr>
<td>$S_i$</td>
<td>Illuminated stem section number $i$</td>
<td>-</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
<td>s</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
<td>°C or K</td>
</tr>
<tr>
<td>$T_{air}$</td>
<td>Air temperature</td>
<td>°C</td>
</tr>
<tr>
<td>$T_{stem}$</td>
<td>Stem temperature</td>
<td>°C</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>Half life</td>
<td>yr or min</td>
</tr>
<tr>
<td>$V$</td>
<td>Gas volume</td>
<td>m$^3$</td>
</tr>
<tr>
<td>$X_{13C}$</td>
<td>Number of $^{13}$C atoms present in the sample</td>
<td>-</td>
</tr>
<tr>
<td>$X_{12C}$</td>
<td>Number of $^{12}$C atoms present in the sample</td>
<td>-</td>
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Greek symbols

<table>
<thead>
<tr>
<th>Symbol</th>
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</tr>
</thead>
<tbody>
<tr>
<td>$\beta^-$</td>
<td>Electron</td>
</tr>
<tr>
<td>$\beta^+$</td>
<td>Positron, i.e. an anti-electron</td>
</tr>
<tr>
<td>$\delta^{13}C$</td>
<td>Carbon isotopic composition ($^{13}$C/$^{12}$C)</td>
</tr>
<tr>
<td>$\delta^{13}C_{a}$</td>
<td>Carbon isotopic composition ($^{13}$C/$^{12}$C) related to enrichment of the air inside the cuvette</td>
</tr>
<tr>
<td>$\delta^{13}C_{b}$</td>
<td>Carbon isotopic composition ($^{13}$C/$^{12}$C) in baseline samples</td>
</tr>
<tr>
<td>$\delta^{13}C_{s}$</td>
<td>Carbon isotopic composition ($^{13}$C/$^{12}$C) in $^{13}$C enriched samples</td>
</tr>
<tr>
<td>$\delta^{13}C_{t}$</td>
<td>Difference in carbon isotopic composition ($^{13}$C/$^{12}$C) related to tissue enrichment</td>
</tr>
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Introduction and outline of the thesis

Since the foundation of the intergovernmental panel on climate change (IPCC) in 1988, substantial progress has been made in understanding greenhouse gas induced changes in the earth’s climate. The fourth assessment report of working group three of the IPCC states that global greenhouse gas emissions due to human activities have increased with 70% between 1970 and 2004, with carbon dioxide (CO₂) considered as the primary source, having grown by approximately 80% (Rogner et al., 2007). However, we still lack detailed information on what the future holds for climate-mediated changes in the CO₂ emission by plant respiration and its role in the biosphere’s response to climate change. While providing the energy and respiratory products necessary for biosynthesis and cellular maintenance (e.g. adenosine-triphosphate (ATP), reducing equivalents, and carbon skeletons (Atkin et al., 2010)), plant respiration releases eighteen times as much CO₂ into the atmosphere than does the burning of fossil fuels in the 1990s (Prentice et al., 2001; Trumbore, 2006). For instance in forests, ecosystem respiration is of particular importance for the local carbon balance with an estimated >50% release of the annual amount of carbon fixed by photosynthesis (Janssens et al., 2001; Luyssaert et al., 2007; Baldocchi, 2008; Schulze et al., 2010). Forests in general act as a sink for CO₂, but there is a delicate balance between the amount of carbon imported into ecosystems and the fraction released back into the atmosphere. Only a detailed understanding of how both processes respond to climate change, from the cellular to the ecosystem level, will enable us to predict whether forests will act as sources or sinks in the future.

Compared with the relatively comprehensive understanding of photosynthesis, we lack basic information on the key determinants of respiration rates in plant organs (Atkin et
Moreover, our ability to match predictions of process-based models with upscaled bulk CO₂ flux respiration estimates remains limited (Trumbore, 2006). The main bottleneck to accurately predict and measure ecosystem respiration is its complex nature. Where photosynthesis is a single well-known process, respiration at ecosystem level integrates the variety of plant and microbial processes both above- and belowground by which CO₂ is released back into the atmosphere (Fig. 1, Trumbore, 2006).

Fig. 1 Release of carbon by respiration into the atmosphere after carbon is imported into the forest ecosystem by photosynthesis (adapted from Trumbore et al., 2006).

In the literature, these processes are functionally divided in an autotrophic (Rₐ) and heterotrophic (Rₕ) component according to the substrate that living organisms use to sustain their metabolism. Rₕ involves the decomposition of organic material in the soil by micro-organisms and is generally considered as being part of a slow cycling process (in the order of months to centuries; Epron, 2009). On the other hand, Rₐ occurs both above- (in foliar and woody tissues) and belowground (in roots, their mycorrhizal fungal symbionts, and rhizosphere micro-organisms) and relies on carbohydrates that are allocated from the canopy to the other plant tissues, referred to as sinks (Litton et al., 2007).

Adding to the complex nature of ecosystem respiration, a fraction of CO₂ derived from Rₐ remains within trees and diffuses remote from the site of respiration (Teskey &
McGuire, 2002) or is assimilated by chlorophyll containing tissues (Zelawski et al., 1970; Stringer & Kimmerer, 1993; McGuire et al., 2009), which reminds us to be careful about invoking respiration when we are actually measuring the CO$_2$ flux from a tissue surface (Trumbore et al., 2013). Since the first observation by Bushong in 1907 on the occurrence of high CO$_2$ concentrations ([CO$_2$]) in tree stems, scientists have been wondering about the different physiological processes that produce the CO$_2$ present inside trees. Local respiration of living cells has been described as an important source of CO$_2$ in tree stems, because of the high resistance to radial diffusion exerted by stem tissues (Boysen-Jensen, 1933; Teskey & McGuire, 2002; McGuire & Teskey, 2004; Teskey et al., 2008). A fraction of respired CO$_2$ has been found to originate lower down the stem (McGuire & Teskey, 2004; Teskey & McGuire, 2007), or even in the root system from belowground $R_a$ ($R_{ab}$), because a fraction of the respired CO$_2$ dissolves in the transpiration stream and is transported upwards throughout the tree (Teskey et al., 2008). A substantial amount might move with the transpiration stream into foliage (Teskey & McGuire, 2002), but the relative proportions of respired CO$_2$ that diffuse into the atmosphere or that are re-assimilated in chlorophyll containing tissues during this transport, is yet unknown (Hanson & Gunderson, 2009). Therefore the main objectives of this PhD study were to elucidate sources and fate of internal CO$_2$ and to assess the mechanistic significance of internal CO$_2$ for understanding tree functioning under varying conditions. To this end, a series of experimental studies on different tree species under controlled or field conditions was conducted using carbon isotopes and detailed plant measurements.

Since the first continuous in situ measurements of stem CO$_2$ by McGuire & Teskey (2002), an increasing number of studies have been reporting on the significance of internal CO$_2$ in trees (e.g. Teskey & McGuire, 2002; McGuire & Teskey, 2004; Maier & Clinton, 2006; Teskey & McGuire, 2007; Aubrey & Teskey, 2009; Grossiord et al., 2012). Therefore, research on internal CO$_2$ is of topical interest in plant sciences and further pushes the boundaries of our understanding of plant functioning. In Chapter 1 of this thesis, current knowledge about the sources and fate of internal CO$_2$ is summarized. In particular the contribution of root-respired CO$_2$ to internal CO$_2$ and transport of internal CO$_2$ with the transpiration stream is assessed in detail. In addition, the second part of
Chapter 1 describes the different techniques used in this thesis to quantify xylem CO₂ transport and to trace the fate of xylem-transported CO₂.

In the past years stable isotopes have been recognized as a powerful tool to trace carbon fluxes and to disentangle soil and plant processes at an unprecedented resolution and scale (Bahn et al., 2012). In particular ¹³C pulse-labeling has been previously used to trace the fast transfer of recently assimilated carbon from leaves to sink tissues via the phloem (e.g. Epron et al., 2011; 2012 and references therein). Based on the utility of ¹³C to understand carbon cycling in trees, results from pulse labeling studies are described in Chapters 2, 3, and 4, which used ¹³C to trace xylem CO₂ transport. As described in Chapter 2 and Chapter 3, dissolved ¹³C-labeled CO₂ (referred to in the text as ¹³CO₂) was introduced in detached leaves and branches under controlled conditions, respectively and analyzed for ¹³C tissue enrichment, as outlined in Chapter 1. Moreover, in Chapter 2 ¹¹C labeling was used to obtain a more accurate tracer distribution at leaf level based on positron autoradiography. In Chapter 3, measurements of xylem CO₂ assimilation were compared relative to atmospheric CO₂ assimilation in the leaves. In Chapter 4, an experiment on 7-year old field-grown Populus deltoides Bartr. ex Marsh. trees was performed, where the ¹³C label was infused at the stem base to simulate belowground respired CO₂ entering the stem. It was hypothesized that as the label was transported throughout the tree, a portion would be assimilated by chlorophyll containing woody and leaf tissues and a portion would diffuse into the atmosphere. To this end, the isotopic composition of branch and stem CO₂ efflux (E_{stem}) was measured in order to validate the radial diffusion of the ¹³C label into the atmosphere.

In the past years, several researchers have pondered over the nature and physiological significance of internal CO₂ assimilation by woody tissue photosynthesis (e.g. Bossard & Rejmanek, 1992; Nilsen, 1995; Eyles et al., 2009; Saveyn et al., 2010). Overall, woody tissue photosynthesis contributes to the tree carbon income by recycling respired CO₂ that would otherwise be lost from the plant to the atmosphere (Wittmann et al., 2001; Pfanz et al., 2002; Cernusak & Hutley, 2011). Under specific environmental or physiological conditions woody tissue photosynthesis might gain more significant importance for plant metabolism. In dormant trees, Saveyn et al. (2008a) hypothesized that woody tissue photosynthesis induced non-temperature related depressions in
efflux-based stem respiration measurements. In **Chapter 5**, this hypothesis was tested on dormant *Quercus robur* L. trees during a light-manipulation experiment under temperature controlled conditions. Woody tissue photosynthesis is assumed to be less drought-sensitive compared to leaf photosynthesis (Pfanz *et al.*, 2002), but its physiological significance in tree resilience to drought stress remains unclear. To this end, the impact of woody tissue photosynthesis on tree physiology under drought stress was experimentally tested in **Chapter 6**. Detailed stem diameter and leaf gas exchange measurements were compared for control and light-excluded *Populus deltoides x nigra* ‘Monviso’ trees under well-watered and dry conditions. Moreover, it was tested whether woody tissue photosynthesis plays a role in light-dependent repair of cavitated vessels, as hypothesized by Schmitz *et al.* (2012), which links plant hydraulics to the local plant carbon status.

Observations on the internal transport of belowground respired CO$_2$ with the transpiration stream (F$_t$, Aubrey & Teskey, 2009; Grossiord *et al.*, 2012) have raised important questions about our understanding of the tree carbon cycling and root metabolism (Hanson & Gunderson, 2009). Generally, studies exclusively measure soil CO$_2$ efflux (E$_{soil}$) when estimating belowground respiration and therefore neglect a fraction of R$_{a,b}$ that is transported with the transpiration stream inside the tree. In **Chapter 7**, F$_t$ and E$_{soil}$ were simultaneously measured according to the techniques outlined in **Chapter 1**, and compared for non-girdled and girdled *Quercus robur* L. trees. The girdling technique, which is based on the removal of a circumferential band of bark and phloem from the tree stem to interrupt belowground carbon transfer (De Schepper *et al.*, 2010), is frequently applied to estimate R$_{a,b}$ (Högberg *et al.*, 2001; 2009; Subke *et al.*, 2011). The girdling approach used in this PhD study allowed a reevaluation of R$_{a,b}$ which is important for understanding belowground respiration, from a mechanistic perspective. Moreover, manipulation of R$_{a,b}$ by tree girdling enabled assessment of the root-respired nature of xylem CO$_2$ imported into the stem.

In conclusion, the main findings and main contributions of this study to the fields of internal CO$_2$ and plant respiration research are summarized in **Chapter 8**. Based on this summary, implications and unanswered research questions for ongoing and current research are formulated. Finally, promising future avenues of research which will
improve our current knowledge on the role of internal CO₂ in trees are identified and discussed.
1.1 Introduction

Understanding and characterizing variation in plant respiration is of paramount importance for global change science, as well as fundamental to plant ecology and physiology, because the efflux of CO$_2$ from plant respiratory processes is a critical and uncertain component of plant, ecosystem, and global carbon budgets (King et al., 2006; Houghton, 2007; Reich et al., 2008). To this end, above- and belowground respiration measurements are performed according to the long standing approach that CO$_2$ efflux reflects underlying local respiration rates because all respired CO$_2$ diffuses locally into the atmosphere (Teskey et al., 2008; Atkin, 2011; Trumbore et al., 2013).

However, the observation that respired CO$_2$ can accumulate in tree stems challenges this generally accepted measurement practice to estimate respiration. Transport of internal CO$_2$ in plants can lead to respiration rates being underestimated at source level, while overestimated in remote organs (Teskey et al., 2008; Atkin, 2011). The discovery that in plants, and in particular trees, high gaseous CO$_2$ concentrations ([CO$_2$]) are present relative to the atmospheric one (c. 0.04% at 2013) is not new. Based on extraction of gases from stems, Bushong (1907), MacDougal et al. (1927), MacDougal & Working (1933), and Chase (1934) discovered early 20$^{th}$ century that CO$_2$ can build up in stems because the different woody tissues (xylem, vascular cambium and bark layers) exert a
resistance to radial diffusion of CO$_2$ into the atmosphere (Eklund & Lavigne, 1995; Steppe et al., 2007; Teskey et al., 2008). [CO$_2$] in tree stems is reported within the range of <1 to over 26% for various tree species (see Table 1 in Teskey et al., 2008). Due to the high solubility of CO$_2$ in water, a substantial amount of this respired CO$_2$ can dissolve in the xylem sap and is transported upwards with the transpiration stream. In the liquid phase, dissolved CO$_2$ is present in different forms ([CO$_2$]$_{aq}$, [HCO$_3^-$], and [CO$_3^{2-}$]) which are collectively referred to as dissolved inorganic carbon (DIC) and indicated in the text as [CO$_2^*$].

Recognition of this internal CO$_2$ flux has lead to novel insights on the role of respired CO$_2$ recycling in tree physiology and a new proposed methodology for estimating aboveground woody tissue respiration (see review by Teskey et al., 2008). Recent studies have indicated that even belowground respired CO$_2$ might remain inside the root system and dissolve in the transpiration stream. Aubrey & Teskey (2009) observed for Populus deltoides Bartr. ex Marsh trees that the amount of xylem-transported root-respired CO$_2$ rivaled the amount of belowground respired CO$_2$ that diffused into the soil environment and contributed to soil CO$_2$ efflux (E$_{soil}$). Once transported aboveground, this respired CO$_2$ can diffuse into the atmosphere or is assimilated in chlorophyll containing tissues in stems and branches, as previously observed for locally respired CO$_2$ (Teskey et al., 2008). However, the actual impact of the internal transport of root-respired CO$_2$ on aboveground tree physiology remains unclear.

In this chapter the different sources, fate, and transport of internal CO$_2$ are reviewed. In particular the fate of root-respired CO$_2$ and the characteristic of high internal [CO$_2$] in roots will be highlighted in this review, because to date detailed knowledge on the belowground contribution to internal CO$_2$ is lacking. Finally in the second part of this chapter an overview of different non-isotopic and isotopic methods used to quantify and trace xylem transport of internal CO$_2$ in trees is given. The use of isotopes to trace internal CO$_2$ is new and therefore methods based on carbon isotope labelling of the transpiration stream are highlighted.
1.2 Sources of internal CO$_2$ in stems

The different sources of internal CO$_2$ can be functionally and spatially divided in different groups. Aboveground the different sources (i.e. stem and branch respiration) that contribute to internal CO$_2$ are part of the autotrophic component of respiration (R$_a$), while belowground both R$_a$ (referred to in the text as R$_{a,b}$ for belowground R$_a$) and to a lesser extent the heterotrophic component of respiration (referred to in the text as R$_{h,b}$ for belowground R$_h$) will contribute to respired CO$_2$ imported into the stem. Although information on the contribution of both above- and belowground sources to stem [CO$_2$] is scanty, most recent reports indicate that a substantial fraction of belowground respired CO$_2$ might contribute to stem [CO$_2$] (Aubrey & Teskey, 2009; Grossiord et al., 2012). These belowground sources of internal CO$_2$ have not been described in detail previously and will therefore be highlighted.

1.2.1 Belowground respiration

1.2.1.1 Uptake of soil dissolved inorganic carbon

In forests soils, the [CO$_2$] ranges through the rooting zone from less than 0.1% to more than 1% and generally increases with depth (Pumpanen et al., 2003). A part of this respired CO$_2$ is dissolved in the soil solution as DIC. It is hypothesized that this soil DIC might influence plant growth, either by affecting the [CO$_2$] gradient from the roots to the soil, or by supplying substrate for aboveground plant fixation after uptake by the roots (Enoch & Olesen, 1993; Ford et al., 2007). Vuorinen et al. (1989) supplied $^{14}$C-labeled NaHCO$_3$ as a proxy for soil DIC to roots of hydroponically grown willow plants and detected the radiocarbon tracer within 24 h in the shoot. In a similar hydroponics experiment with *Nicotiana tabacum*, Hibberd & Quick (2002) proposed that assimilation of $^{14}$C supplied to the roots in cells around the vascular tissue supports the existence of a C$_4$-like mechanism in C$_3$ plants. However, under natural conditions the [CO$_2$] within tree tissues is generally reported higher than observed for the soil (Teskey et al., 2008), indicating that a decreasing [CO$_2$] gradient exist from the root tissues towards the soil. Teskey & McGuire (2007) measured simultaneously soil [CO$_2$] at 15 cm depth and stem [CO$_2$] at 0.1 m height and observed that mean [CO$_2$] at stem level (7.6%) was
significantly higher than reported at soil level (mean 1.2%). Similarly, Ubierna et al. (2009) observed higher [CO\(_2\)] at the base of the stem than in the soil. Moreover, based on [CO\(_2\)] measurements in the stem and in the soil, Aubrey & Teskey (2009) estimated that for *Populus deltoides* only up to 8% of belowground respired CO\(_2\) transported with the transpiration stream in the stem is derived from the uptake of soil DIC.

Moreover, there is little evidence that the uptake of soil DIC by roots has a substantial impact on plant physiology. By irrigating crops with a CO\(_2\) enriched solution, it was believed that yield could be increased. While theoretically, aboveground assimilation of CO\(_2\) dissolved in the soil solution by chlorophyll containing tissues could contribute up to 5% of plant carbon assimilation, results indicated that it usually contributed less than 1% (see review by Enoch & Olesen, 1993). More recently, Ford et al. (2007) and Ubierna et al. (2009) applied a \(^{13}\)C labeled solution isotope to soil around the stem of conifer seedlings in pot and field-grown conifer trees, respectively, which served as a proxy of the uptake of soil DIC via the roots. Ford et al. (2007) concluded that assimilation of soil DIC will only contribute to a very limited extent to the tree carbon income. Ubierna et al. (2009) observed no \(^{13}\)C enrichment of stem CO\(_2\) efflux (E\(_{stem}\)) after application of the label to the soil. Therefore it is unlikely that uptake of soil DIC will contribute substantially to internal CO\(_2\) in stems.

### 1.2.1.2 Root respiration

Similar as for aboveground organs, CO\(_2\) in roots is derived from respiration in living cells. These are found in the vascular cambium and the parenchyma cells of the phloem and the xylem rays (Fig. 1.1A,B). However substantial parts of the xylem are composed of non living cells. The overall structure of the root is similar as described for tree stems (compare Fig 1.1 with Fig 1.4). In roots, xylem and phloem strands originate which extend throughout the plant body. The periderm, which is composed of cork cambium, cork, and the phelloderm forms a protective exodermis in tree roots after secondary growth of woody plants and is mainly constructed of suberin (Taiz & Zeiger, 2006). Lenticels in the periderm allow gas exchange of O\(_2\) and CO\(_2\) between the root and soil environment. The suberized periderm makes more mature regions of the root system relatively impermeable to water. Therefore, water uptake in the root system mainly occurs by the less suberized root tips (Kramer & Boyer, 1995; Zwieniecki et al., 2002).
Many plant and environmental factors determine short- and long-term dynamics in root respiration rate. These factors might contribute to variations in internal \([\text{CO}_2]\) observed at stem level.

**Temperature**

Among the environmental factors, temperature is most commonly correlated with respiration. From a theoretical point of view, respiration is partitioned in growth and maintenance respiration and the latter is considered to be highly temperature-sensitive (Amthor, 1989). Variations in soil temperature are often dampened relative to air temperature \((T_{\text{air}})\) dynamics, because the soil takes much longer to warm and cool down. Nonetheless, substantial short- and long-term variation in soil temperature might occur, inducing varying root respiration rates at daily to seasonal scale (Atkin et al., 2000).

Respiration is assumed to increase exponentially with an increase in temperature and a \(Q_{10}\) factor (the proportional change in respiration rate with a 10°C increase in temperature) is commonly used to describe this relationship. For roots, \(Q_{10}\) values in the range from 1.1 to 2.9 are reported (see Table 1 in Atkin et al., 2000). Boone et al. (1998) reported substantially higher \(Q_{10}\) values for root respiration and rhizosphere decomposition (4.6) than for respiration in soils lacking roots (2.5) or in bulk soil (3.5).
Therefore, they hypothesized that within the context of climate change, roots will exert a strong influence on the sensitivity of belowground respiration to increasing temperatures. However, $Q_{10}$ values that are significantly above 2.5 may indicate that substrate supply confounds an observed temperature effect, because diffusion of substrates used for respiration might covary with temperature (Davidson et al., 2006a). Moreover, recent studies consider soil temperature to only directly affect the decomposition rate of substances in soil, i.e. $R_{h,b}$, while $R_{a,b}$ is directly related to belowground transport of photosynthates (see review by Kuzyanov & Gavrichkova, 2010). Finally, $Q_{10}$ values of respiration are found to vary among a temperature range and thermal acclimation of respiration might occur (Atkin et al., 2005) and therefore results from temperature-based models of root respiration are often incorrect.

**Soil moisture**

Soil moisture is considered as another important abiotic factor determining soil and root respiration rates. Overall, soil moisture will reduce the contribution of root respiration to $E_{soil}$ under drought conditions (Bryla et al., 1997; Burton et al., 1998; Moyano et al., 2009). During drought events, a reduction in root cell turgor will lead to a reduced growth and consequently to reduced growth respiration rates. Therefore, observed summer drought induced reductions in $E_{soil}$ (e.g. Davidson et al., 1998; Epron et al., 1999; Curiel Yuste et al., 2004) are partially explained by the reduction in root growth respiration. On the other hand, excess soil water will reduce root metabolism, mainly because oxygen availability in the soil becomes sub-optimal (Moyano et al., 2009).

**Carbohydrate supply**

Within the soil, root respiration is strongly tied to the consumption of carbon allocated belowground (Hanson et al., 2000). On average nearly half of the carbon assimilated at leaf level is allocated to the root system via the phloem where between one-quarter and two-thirds of this carbon is used for respiration (Lambers, 1987; Farrar & Williams, 1990; Lambers et al., 2002; Moyano et al., 2009). The remainder of the carbon allocated belowground is used to sustain root growth, is stored or is released from roots as root exudates into the rhizosphere.
The strong coupling of tree canopy photosynthesis with $R_{a,b}$ has been overlooked in the past because temperature variations are highly correlated with solar radiation and therefore mask the effect of carbohydrate supply on belowground metabolism (see review by Kuzyakov & Gavrichkova, 2010). Results from recent studies that measured the reduction in $E_{\text{soil}}$ after girdling (e.g. Högberg et al., 2001; Högberg et al., 2009; Subke et al., 2011) or traced $^{14}$C and $^{13}$C in $E_{\text{soil}}$ after pulse labeling the canopy with $^{14}$CO$_2$ (Carbone et al., 2007) or $^{13}$CO$_2$ (e.g. Högberg et al., 2008; Epron et al., 2011; Epron et al., 2012) (Fig. 1.2), respectively, found that current photosynthesis drives belowground respiration in the order of days or less. Observed lag times in assimilate allocation depended on tree height (Fig. 1.2), and are found to correlate well with results predicted on estimated phloem rates, which typical range from 0.5 to 1 m h$^{-1}$ (Kuzyakov & Gavrichkova, 2010 and references therein). Moreover, the higher lag times observed for coniferous species relative to deciduous species most likely reflect a difference in phloem anatomy (Epron et al., 2012; Jensen et al., 2012) (Fig. 1.2). A rapid coupling of above- and belowground processes by pressure-concentration waves through the phloem is theoretically possible (Thompson & Holbrook, 2004) but its role in trees remains yet unclear.

![Fig. 1.2](image)

**Fig. 1.2** Time lag of peak $^{14}$CO$_2$ in soil CO$_2$ efflux or coarse roots after pulse labeling the canopy with $^{14}$CO$_2$ for varying species with a varying height (after Epron et al., 2012).

The impact of belowground carbohydrate supply on root respiration is assumed to vary on a seasonal scale, in particular for deciduous trees. While during the dormant season root reserves are used to fulfill maintenance root respiration, growing season root respiration and root growth will mainly be fueled by the belowground supply of fresh
assimilates (Moyano et al., 2008 and references therein). However, presence of large carbohydrate reserves in roots in the growing season might explain observed decoupling of belowground carbon allocation and root respiration in some species (Farrar, 1999). Regardless whether belowground respired CO$_2$ is derived from stored carbohydrates or recent photosynthates, a fraction is transported within the tree with the transpiration stream (Aubrey & Teskey, 2009). However, the coupling of xylem CO$_2$ transport with belowground metabolism remains unclear.

**Soil nitrogen**

Supplying plants with inorganic nutrients is one of the major functions of the roots (Jungk, 2002) and therefore a change in soil nutrient concentration will importantly affect root metabolism. In this section we highlight the effect of soil nitrogen on root respiration. Overall, nitrogen content is believed to be a key determinant of (root) maintenance respiration (Comas & Eissenstat, 2004; Atkinson et al., 2007; Wang et al., 2010), because nitrogen plays an important role in metabolic processes in the form of enzymes or co-factors (Reich et al., 2008) of which the replacement and repair are tightly linked with cellular activity (Ryan et al., 1996). Therefore, root respiration is expected to increase with greater soil nitrogen availability (Wang et al., 2010). Ryan et al. (1996) found a strong correlation of fine root nitrogen content with root respiration for Pinus radiata trees either subjected to a control or fertilization treatment. Moreover, Reich et al. (2008) reported based on an extensive dataset that root respiration rates per unit of nitrogen are generally higher than what is measured for leaves, presumably because nitrogen in roots are involved in high demanding metabolic functions, while in leaves a large fraction of the nitrogen is used in photosynthetic machinery. Hence, an important fraction of the nutrients absorbed at root level like nitrogen might be used to sustain aboveground plant functioning for instance at leaf level. In addition, this transport of nutrients provides a means for achieving root-shoot signalling of whole-plant nutrients status, leading to a proper integration of root nutrient uptake in response to whole plant demand (Glass, 2002).

**Morphology and age**

The root system is composed of roots with different diameters (Fig 1.3A,B), which possess different morphological traits and have a wide range of different physiological
functions (Eissenstat et al., 2000; Chen et al., 2010). As a result, metabolic root tissue demands are highly variable and therefore a large range in respiration rates is observed among the different roots of the root system. Fine roots (diameter <2 mm or <5mm, depending on the definition by the author) are assumed to be more metabolically active, with higher reported respiration rates (Fig. 1.3A), and sustain physiological functions such as water and nutrient uptake of trees (Gill & Jackson, 2000; George et al., 2003). The larger roots, which are often suberized and whose primary function is support and transport of nutrients and water from the very fine roots to other portions of the tree, are not as active in nutrient uptake and are expected to have lower respiration rates than the very fine roots (Fig. 1.3a, Pregitzer et al., 1998). This decrease in specific root respiration (i.e. respiration per unit dry mass) with increasing diameter is found to correlate with decreasing nitrogen content (Ryan et al., 1996; Pregitzer et al., 1998; Bahn et al., 2006), an increase in tissue and plant age (George et al., 2003; Volder et al., 2005; Moyano et al., 2009), and with differences in growth respiration (Lambers et al., 2002).

![Fig. 1.3](image)

**Fig. 1.3** (A) Specific respiration rate (measured as O₂ uptake) of Acer saccharum Marsh roots of different diameters measured for two sites and at different depths (after Pregitzer et al., 1998). (B) View of the excavated root system of a 9-y old Quercus robur tree.

**CO₂ and O₂ concentration**

Overall, roots thrive in substantially higher [CO₂] as compared to aboveground tissues (see Section 1.2.1.1). Soil [CO₂] is found to vary strongly across the growing season (Sands et al., 2000) and even during the day (Bouma & Bryla, 2000) and there are conflicting views regarding the sensitivity of root CO₂ efflux to varying soil [CO₂] (Subke
et al., 2006). Ryan et al. (1996) observed an exponential increase in root CO$_2$ efflux of *Pinus radiata* roots with an artificial decrease in surrounding [CO$_2$] from 1500 to 400 μmol mol$^{-1}$. Similarly, Qi et al. (1994) and McDowell et al. (1999) observed a decrease in root CO$_2$ efflux with increasing [CO$_2$] for *Pseudotsuga menziesii* and *Tsuga heterophylla*, respectively, while other authors reported little or no sensitivity of root CO$_2$ efflux to soil [CO$_2$] for temperate (Burton & Pregitzer, 2002) and Mediterranean (Bouma et al., 1997) species, tested in a range of 100-2000 μmol mol$^{-1}$ and 400-25000 μmol mol$^{-1}$, respectively. The exact mechanism responsible for the [CO$_2$] sensitivity of root CO$_2$ efflux remains unclear and to date the respiratory inhibition of root respiration by increase [CO$_2$] is assumed to be taxon-specific (Subke et al., 2006). In spite of the conflicting views regarding the sensitivity of root respiration to soil [CO$_2$], efflux-based measurements of root respiration should preferably be made at concentrations closest to those present in the soil (Hanson et al., 2000; Subke et al., 2006).

Less controversial seems the inhibitory effect of the low soil O$_2$ concentrations ([O$_2$]) on root respiration. Limited soil [O$_2$] in soils prone to flooding is an important constraint for plant metabolism because gas diffusivity is $10^4$ fold slower in water than in air (Nobel, 1999; Abiko et al., 2012). Therefore, internal transport of O$_2$ from the aerial shoot to the root is crucial for these plants, which is enhanced by tissues high in porosity and the formation of aerenchyma in cortical root and stem tissues (Armstrong, 1979; Colmer, 2003). On the other hand, root-respired CO$_2$ might be transported via the aerenchyma towards the shoot, where it is used as substrate in photosynthesis (Wetzel & Grace, 1983). Moreover, many of these wetland species possess roots with strongly suberized cells walls and/or a thickened exodermis (De Simone et al., 2003), which form an important barrier to radial oxygen loss (Colmer, 2003). At the same time, these barriers will also limited radial CO$_2$ diffusion of root-respired CO$_2$ and sediment-derived CO$_2$, leading to an accumulation of CO$_2$ in the roots of these wetland and aquatic species (Brix, 1990; Li & Jones, 1995; Colmer, 2003; Aubrey & Teskey, 2009).

1.2.2 Stem and branch respiration

Within forest ecosystems stem and branch respiration is considered as an important component of the ecosystem respiration, with an estimated 30-70% of Gross primary
productivity released back to the atmosphere via aboveground woody tissue respiration (Ryan et al., 1996; Damesin, 2003). While results from these studies were based on the generally accepted assumption that stem and branch respired CO₂ diffused immediately into the atmosphere (Hölttä & Kolari, 2009), it is now generally accepted that a substantial quantity of locally respired CO₂ is retained in the xylem and contributes to internal CO₂ (Steppe et al., 2007; Teskey et al., 2008) beside belowground respired CO₂. Within trees, gaseous CO₂ can accumulate to concentrations (range <1 to over 26%) substantially higher than the atmospheric one (C. 0.04% in 2013), implying that woody tissues like the xylem (Sorz & Hietz, 2006), cambium (Hook et al., 1972) and bark (Lendzian, 2006) exert an important resistance for radial diffusion of locally respired CO₂. Moreover, variation in these resistances to radial CO₂ diffusion might contribute to intra-species variation in CO₂ efflux (Steppe et al., 2007) (see Section 1.3.3).

Local respiration in stem and branches is performed by mitochondria in living phloem parenchyma, cambium, and parenchyma cells in the xylem rays of heart and sapwood (Fig 1.4). The dead xylem vessels in the sapwood are used for water transport. In particular the cells in the inner bark (part of the stem from vascular cambium till the last formed periderm, which includes the phloem, Pfanz & Aschan, 2001) are considered to have a higher respiratory activity as compared to xylem ray cells (Teskey et al., 2008; Araki et al., 2010), in particular during periods of high metabolic activity like during springtime (Maier & Clinton, 2006). Goodwin & Goddard (1940) measured oxygen consumption rate as a proxy for respiration rate in black ash stems and observed that uptake rates were several magnitudes higher near the inner bark compared to the xylem. Pruyn et al. (2002a; 2002b; 2003) measured CO₂ efflux and observed that respiration of the inner bark of different conifer species was between 2-15 times greater than that of the sapwood. Therefore in smaller woody organs (≤~0.10 m diameter) inner bark respiration will be the largest respiratory component. However, when the number of living cells is scaled with tissue volume for stems and larger branches, generally more living cells are found in the xylem than in the inner bark. For instance, Ryan (1990) determined for Pinus contorta and Picea engelmanni trees that the xylem contained more than 80% of the total live-cell volume in their stems. Similarly, Ceschia et al. (2002) found that sapwood in Fagus sylvatica stems contained an important
fraction of total number of living cells in the stem. Therefore, in stem and larger branches, CO₂ derived from living xylem parenchyma cells will be the paramount local respiratory source that contributes to internal CO₂ at stem level.

**Fig. 1.4** Anatomical structure of a tree stem (adapted from Raven et al., 1999)

Besides distribution and the amount of living cells, other factors that affect woody tissue respiratory activity will influence internal CO₂ at stem level. Overall, these are similar as the factors that influence respiratory activity at root level, discussed in Section 1.2.1.2.

### 1.3 Fate of internal CO₂

Depending on the site of respiration discussed in the previous sections and on plant physiological factors like sap flow rate (Fₛ) and xylem sap pH, internal CO₂ is transported within the tree towards the canopy, assimilated by chlorophyll containing tissues or diffuses through stems and branches towards the atmosphere (Fig. 1.5). In particular, the aboveground transport of (belowground) respired CO₂ either by axial diffusion or by bulk flow with the transpiration stream is an important new aspect of internal CO₂ research.
1.3.1 Transport of respired CO₂ in trees

1.3.1.1 Transport with the transpiration stream

While most studies report on gaseous CO₂ present in trees, a large quantity of respired CO₂ in stems and branches is dissolved in the xylem sap (Teskey et al., 2008) and is transported away from the site of respiration (number 1 in Fig. 1.5B). The gaseous phase in intercellular spaces, pore spaces within the cell walls, and the lumen of cells is in contact with this liquid phase (Hari et al., 1991), and an equilibrium exists between both gaseous and the different forms of dissolved CO₂. [CO₂*] can be calculated according to the law of Henry based on measurements of gaseous [CO₂]. Therefore measurements of
gaseous [CO₂] in holes drilled in the stem are commonly used to estimate the amount of CO₂ dissolved in the xylem sap and the total quantity of CO₂ transported internally (see Section 1.4.1.1).

High Fₛ can substantially dilute xylem [CO₂*] and therefore diurnal variation in [CO₂*] are inversely correlated to transpiration and Fₛ dynamics. Based on laboratory and field experiments Teskey & McGuire (2002) reported xylem sap [CO₂*] in the range of 0.9 to 8.3 mM at seasonal scale for Quercus alba L. and Liriodendron tulipifera L., which differ in xylem anatomy, and observed that diurnal dynamics were negatively correlated with Fₛ. Similarly, McGuire et al. (2007) artificially manipulated Fₛ in branch segments of Platanus occidentalis L. and found that xylem [CO₂*] strongly decreased from around 1.5 to 0.2 mM when increasing sap flux density (SFD) from around 0.008 cm³ cm⁻² h⁻¹ to 0.2 cm³ cm⁻² h⁻¹. Maier & Clinton (2006) progressively removed the canopy of Pinus taeda L. and observed that xylem [CO₂] and therefore [CO₂*] gradually increased over time. However, when expressed as the total quantity of CO₂ transported with the transpiration stream (as calculated in Section 1.4.1.4), the largest amount of xylem-dissolved CO₂ will be transported at high Fₛ (McGuire et al., 2007), because the increase in Fₛ compensates for the decrease in xylem sap [CO₂*] due to dilution.

While previous studies focused on internal transport of respired CO₂ to account for observed midday depressions in E.stem (see Section 1.3.3 and references therein) or to provide additional substrate for photosynthesis in leaves (see Section 1.3.2 and references therein), results from recent studies highlight the transport of belowground respired CO₂ within trees. In the root system, respired CO₂ may dissolve in water taken up by the roots, instead of contributing to E.soil, thereby representing an internal flux that rivals the autotrophic contribution to E.soil (Fig. 1.6). Teskey & McGuire related high internal [CO₂] measured at the stem base to the transport of root-respired CO₂ with the transpiration stream. Aubrey & Teskey (2009) were the first that quantified the internal flux of root-respired CO₂ versus the contribution of Rₐ,b to E.soil. With simultaneous measurements of Fₛ and [CO₂] in stems and soil [CO₂] profiles, they estimated that for eastern cottonwood (Populus deltoides Bartr. ex Marsh), twice the amount of CO₂ derived from Rₐ,b was transported with the transpiration stream compared with that which diffused into the soil environment and contributed to E.soil (Aubrey & Teskey,
Similarly, transport of root-respired CO$_2$ with the transpiration stream was observed for *Eucalyptus* (Grossiord *et al.*, 2012). However, in their study only 24% of root-respired CO$_2$ was found to be diverted from E$_{soil}$ to transport with the transpiration stream.

![Fig. 1.6](image)

**Fig. 1.6** Schematic of the fate of CO$_2$ respired within living cells of the roots. Number 1 indicate transport of root-respired CO$_2$ with the transpiration stream, number 2 diffusion of respired CO$_2$ into the soil environment which contributes to soil CO$_2$ efflux (E$_{soil}$) (adapted from Cruiziat & Tyree, 1990).

Nevertheless, the transport of root-respired CO$_2$ raises important questions about our understanding of belowground respiration because efflux-based measurements of belowground respiration routinely underestimate the carbon needed to sustain belowground tissues (Aubrey & Teskey, 2009; Hanson & Gunderson, 2009). Moreover, increased [CO$_2^*$] in the xylem at night suggests that roots might possess substantial barriers to radial diffusion of respired CO$_2$ into the soil environment (Aubrey & Teskey, 2009), resulting in high [CO$_2$] in the roots relative to the soil (Box 1.1). Nevertheless, most studies on belowground respiration neglect the transport of root-respired CO$_2$ with the transpiration stream or the possible occurrence of high internal [CO$_2$] in roots.
Chapter 1

1.3.1.2 Axial diffusion of respired CO_2

During the dormant season, when trees are leafless, no xylem transport of respired CO_2 occurs due to the lack of sap flow. As a result, dormant season E_stem is assumed to be a good estimator of stem respiration (McGuire et al., 2007) which equals maintenance respiration as radial stem growth does not occur during dormancy (Maier, 2001).

However, when sap flow is absent, respired CO_2 in gaseous form may be transported by axial diffusion along a [CO_2] gradient in the stem (number 2 in Fig. 1.5B), according to the law of Fick (Jones, 1992; Sorz & Hietz, 2006). While the formation of aerenchyma tissue is mainly constrained to plants growing on waterlogged soils (see Section 1.2.1.2),

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Box 1.1 Occurrence of high internal [CO_2] in roots?

Occurrence of high [CO_2] in tree roots might be related to an oxygen concentrating trait in roots of wetland species (Aubrey & Teskey, 2009). As a result a substantial fraction of respired CO_2 might accumulate in roots, in particular in the older sections of the root system where root tissue is more suberized.

Different observations underpin the possible existence of high internal [CO_2] in roots. Root respiration is frequently estimated in the field based on excised root measurements (see review by Kuzyakov, 2006). By excising the roots, barriers to radial diffusion are removed, and a large amount of root-respired CO_2 concentrated in the roots might flush into the incubation chamber, accounting for the high increase and subsequent rapid decline in respiration rate estimates observed within one hour after root excision (Rakonczay et al., 1997; Kuzyakov, 2006; Marsden et al., 2008) and the significantly higher respiration rates measured for excised roots compared to those for living ones (Makita et al., 2012). In addition, variation in the resistance to radial diffusion among roots of different species might account for the taxon-specific sensitivity of root CO2 efflux to increased [CO_2] levels in the soil (see Section 1.2.1.2). Upward transport of root-respired CO_2 which partially accumulates in the roots may interfere with efflux-based estimates of root and belowground respiration. Subke et al. (2009) observed unexpected decreases in daytime E_soil related to daytime depressions in the autotrophic component of E_soil. At root level, similar depressions in CO_2 efflux related to varying transpiration rates were observed (Bekku et al., 2009; 2011), presumably because a fraction of respired CO_2 will be retained in the root and transported away with the transpiration stream instead of diffusing into the soil environment.
trees contain large gas volumes (range 18-50%, Gartner et al. (2004)) allowing considerable gas movements in trunks along axial and radial axis (MacDougal & Working, 1933; Hook & Brown, 1972; Armstrong & Armstrong, 2005). Saveyn et al. (2008a) reported non-temperature related depressions in $E_{stem}$ and $[CO_2]$ for dormant *Quercus robur* and *Fagus sylvatica* trees concomitant with a 12h light period under a controlled light-regime. They hypothesized that during the light period, vertical internal CO$_2$ gradients arose due to local decreases in internal CO$_2$ by woody tissue photosynthesis in uncovered stem sections relative to internal [CO$_2$] in the stem section covered with an opaque cuvette for efflux-based stem respiration measurements. As a consequence, CO$_2$ molecules were transported away from the site of respiration along this CO$_2$ gradient, detected as a reduction in $E_{stem}$ and local internal [CO$_2$]. Similarly, illumination triggered stem photosynthesis in young *Alnus glutinosa* trees which increased local [O$_2$] in the cortex and enabled O$_2$ transport from the shoot to the roots (Armstrong & Armstrong, 2005). Therefore, axial diffusion of respired CO$_2$ might account for dormant season $E_{stem}$ and [CO$_2$] dynamics, even in large field-grown trees (Etzold et al., 2013), however we currently lack detailed information on the significance of axial CO$_2$ diffusion for efflux-based estimates of stem respiration.

1.3.2 Re-fixation of respired CO$_2$

Within trees, chlorophyll containing stem and branch tissues are capable of assimilating respired CO$_2$ in addition to atmospheric CO$_2$ assimilation by green leaves (Pfanz et al., 2002; Berveiller et al., 2007a). Despite the fact that the photosynthetic biochemistry is similar, both processes show distinctly different characteristics (Table 1.1). Within stems and branches, most chlorophyll containing woody tissue can be found in the inner bark (Fig. 1.7) where woody tissue photosynthesis occurs (number 3 in Fig. 1.5B). However a fraction of the chlorophyll is present in the ray cells of the wood (Rentzou & Psaras, 2008) and even in the pith (Van Cleve et al., 1993), justifying the use of woody tissue photosynthesis instead of cortical or bark photosynthesis when referring to stem CO$_2$ assimilation (Saveyn et al., 2010).
Fig. 1.7  (A) Chlorophyll present in segments of young *Platanus occidentalis* stems. In the current year stem segment (far right) chlorophyll is found throughout the stem. With increasing size, age, and bark thickness, chlorophyll is mostly present in the inner bark (after Teskey et al., 2008). (B) Bark chlorophyll in a branch of a young *Populus canadensis* tree.

Table 1.1  Features of woody tissue photosynthesis and leaf photosynthesis. Modified according to Aschan & Pfanz (2003). WUE: Water Use Efficiency; PAR: Photosynthetic Active Radiation; Diff. conduct.: Diffusive conductance

<table>
<thead>
<tr>
<th>Type of photosynthesis</th>
<th>Carbon source</th>
<th>Longevity</th>
<th>WUE</th>
<th>Limiting factor</th>
<th>Diff. conduct.</th>
<th>Significance?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woody tissue photosynthesis</td>
<td>Respired CO₂</td>
<td>&lt; than sufficient to pay for construction and maintenance</td>
<td>High</td>
<td>PAR</td>
<td>Low</td>
<td>In leafless trees or when leaf photosynthesis is limited (e.g. stress)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; than required for construction and maintenance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf photosynthesis</td>
<td>Atmospheric CO₂</td>
<td>Low</td>
<td>CO₂</td>
<td>High</td>
<td></td>
<td>Main carbon income of plants</td>
</tr>
</tbody>
</table>

Woody tissue photosynthesis is generally widespread among plants and trees (see Table 3 in Teskey et al., 2008) and it is believed to positively contribute to the overall plant carbon economy (Pfanz et al., 2002), by recapturing respired CO₂ that otherwise would have been lost from the plant to the atmosphere (Cernusak & Hutley, 2011). Within illuminated branches of *Betula pendula* Roth. woody tissue photosynthesis was able to refix up to 97% of respired CO₂ (Wittmann et al., 2006). According to Aschan et al. (2001) chlorophyll in current year *Populus tremula* L. twigs refixed around 80% of internal CO₂. However, the efficiency of refixation decreased with increasing plant age.
Sources, fate, and transport of internal CO₂

(Pfanz et al., 2002) and it is well accepted that rates of woody tissue photosynthesis are relatively low compared to atmospheric assimilation rates and stem CO₂ assimilation very rarely leads to a net carbon gain, but merely entails a recycling of respired CO₂ (Pfanz et al., 2002).

Overall, woody tissue photosynthesis is important for the tree carbon income but it might gain even more importance when trees are leafless (Eyles et al., 2009; Saveyn et al., 2010) or when atmospheric CO₂ uptake by leaves is reduced under limited water conditions (Wittmann & Pfanz, 2008). During a light exclusion experiment, Saveyn et al. (2010) observed that young Prunus ilicifoila, Umbellularia californica, and Arctostaphylos Manzanita plants rely on sugars from woody tissue photosynthesis for bud development at the end of the dormant season. Moreover, Eyles et al. (2009) performed a defoliation experiment on young Eucalyptus globulus seedlings and observed that defoliated seedlings had an increased capacity to refix respired CO₂ (up to 96%). One of the fundamental advantage of woody tissue photosynthesis over leaf photosynthesis is that in order to take up CO₂, leaves have to open their stomata, hence exposing the drought-sensitive mesophyll tissue to the drying power of the air, whereas woody tissue chlorophyll is supplied with endogenously respired CO₂ (Pfanz et al., 2002). Moreover, there is very limited water loss via the stem (Cernusak & Marshall, 2000; Wittmann & Pfanz, 2008). Therefore, woody tissue photosynthesis is expected to increase the plants resilience to drought, since partial closure of stomata has only a limited effect on the xylem [CO₂] (Teskey et al., 2008; Angert et al., 2012). Moreover, xylary chloroplasts potentially play an important role in providing sugars needed for the refilling and sensing of cavitated xylem vessels (Box 1.2).

Finally, recent studies indicate that xylem-transported CO₂ can be assimilated in photosynthetic cells in woody tissues and leaves. McGuire et al. (2009) supplied a dissolved ¹³C isotope to detached Platanus occidentalis L. branches and observed that on average 35% of the label was assimilated. The majority of the label was assimilated by the woody tissue, where at leaf level the petiole assimilated most of the label. These results corroborate with previous findings on the role of xylem-transported CO₂ as substrate for photosynthesis in detached leaves (Stringer & Kimmerer, 1993) and herbaceous plants (Hibberd & Quick, 2002). Moreover, assimilation of xylem-
transported CO₂ fits seamlessly with the possible existence of a C₄-like mechanism in C₃ plants (Hibberd & Quick, 2002). However, to date we lack knowledge on the extent of assimilation of xylem-transported CO₂ in field-grown trees and its significance in maintaining the carbon and water status of plants under varying environmental conditions.

### Box 1.2 Role of woody tissue photosynthesis in xylem cavitation repair

Embolism results in a dramatic loss of the xylem hydraulic function (Zwieniecki & Holbrook, 2009). Therefore cavitation repair, by refilling embolized vessels, is crucial for plant survival. Schmitz et al. (2012) observed that for a diverse range of mangrove species stem hydraulic conductivity was lower in branches where woody tissue photosynthesis was excluded relative to control branches. They suggested that xylary chloroplasts in particular could play an important role in light-dependent repair of embolized xylem vessels, because of their proximity to the xylem vasculature. Sugars synthesized by these chloroplasts potentially supply the necessary energy for vessel refilling (Zwieniecki & Holbrook, 2009) or are used for xylem embolism sensing which is crucial within the context of a refilling mechanism (Secchi & Zwieniecki, 2011).

### 1.3.3 Diffusion of respired CO₂ into the atmosphere

Internal CO₂ derived from local respiration or imported by the transpiration stream with the xylem sap can diffuse radially to the atmosphere (number 4 in Fig. 1.5), thereby confounding efflux-based estimates of local stem respiration rates (Teskey et al., 2008). Moreover, when the transpiration stream acts as a sink, a large fraction of respired CO₂ can be transported upwards to the canopy and diffuse remote from the site of respiration, leading to an underestimation of local stem respiration.

Based on measurements of $F_s$, internal [CO₂], and $E_{stem}$ (see Section 1.4.1), McGuire & Teskey (2004) and Teskey & McGuire (2007) have quantified the contribution of different sources to $E_{stem}$. When $F_s$ are low, $E_{stem}$ is primarily derived from local tissue respiration by living cells and CO₂ stored temporarily in the stem. The amount of respired CO₂ diffusing into the atmosphere is dependent on the [CO₂] gradient and on the diffusion resistance (Steppe et al., 2007; Hölttä & Kolari, 2009), as characterized by a
modified version of Fick’s law of diffusion (Pfanz & Aschan, 2001; Teskey et al., 2008), where resistance in trees is highly variable among individual trees (Steppe et al., 2007) and diffusion changes at least according to tissue type (Teskey et al., 2008) and stem gas and water content (Sorz & Hietz, 2006). Within the bark, resistance is determined by the cortex, periderm (consisting of phelloderm, cork cambium and cork) and the rhytidome (dead outer tissue of the bark) (Ziegler, 1957; Teskey et al., 2008) (Fig. 1.4). Additionally, the vascular cambium limits radial diffusion of respired CO2 from the xylem towards the atmosphere (Hook et al., 1972; Kramer & Kozlowski, 1979). Therefore the stem of some species could contain two disconnected CO2 pools (Ubierna et al., 2009), one inside the part of the stem enclosed by the cambium, with high xylem [CO2] (see Section 1.1) and one in the stem outside of the cambium, containing lower [CO2] (Cernusak & Marshall, 2000; Wittmann et al., 2006; Teskey et al., 2008).

At high Fs, a large fraction of locally respired CO2 might be transported away from the site of respiration leading to an underestimation when using efflux-based measurements to quantify stem respiration (McGuire et al., 2007). First studies reported daytime Estem rates lower than those modeled from bark or sapwood temperature, due to transport of respired CO2 with the transpiration stream (e.g. Edwards & Mclaughlin, 1978; Negisi, 1979; Kaipiainen et al., 1998; Gansert & Burgdorf, 2005). McGuire & Teskey (2004) proposed a new mass balance approach for quantifying stem respiration and estimated that up to 55% of locally respired CO2 could be transported away from the site of respiration in the transpiration stream of Platanus occidentalis L. stems. Angert et al. (2012) measured the ratio of Estem to O2 influx for different Amazonian species and observed that on average around 35% of locally respired CO2 was transported away with the transpiration stream.

At the same time that respired CO2 is transported away from the site of respiration, at high xylem sap [CO2*] or high Fs a large amount of xylem-transported CO2 is imported into the stem section, from lower stem segments or the root system, which partially contributes to Estem. By manipulating sap [CO2*], Teskey & McGuire (2002) and Steppe et al. (2007) showed that CO2 efflux was strongly dependent on xylem sap [CO2*]. However, a decoupling between Estem and xylem [CO2*] has been observed in other studies, due to the large barriers for radial diffusion (Saveyn et al., 2008b) or due to the
high activity of cambial and phloem cells during spring (Maier & Clinton, 2006; Gruber et al., 2009). All previous studies mainly focused on the radial diffusion of xylem-transported CO$_2$ derived from aboveground sources. The recent discovery of an internal xylem-transported flux of belowground respired CO$_2$ of large magnitude by Aubrey & Teskey (2009) might imply that a fraction of $E_{stem}$ is derived from belowground sources. Therefore, the interpretation of efflux-based estimates of respiration are potentially even more complex than previously assumed (Box 1.3), and the fraction of belowground respired CO$_2$ released via the stem is as yet unknown (Hanson & Gunderson, 2009).

**Box 1.3 Is belowground respired CO$_2$ released into the atmosphere from stems?**

An important consequence of the internal transport of CO$_2$ within the soil plant continuum is that a substantial fraction of belowground respired CO$_2$ might be released through stems (Aubrey & Teskey, 2009; Hanson & Gunderson, 2009). Levy et al. (1999) was the first to hypothesize that a fraction of $E_{stem}$ was derived from belowground sources. They observed for Betula pendula Roth. and varying tropical species a positive correlation between sap flow and $E_{stem}$, which they attributed to the import of xylem sap at high [CO$_2$] from the roots. During a free-air CO$_2$ enrichment (FACE) experiment on Pinus taeda L., Moore et al. (2008) estimated that 19% of the average $E_{stem}$ was contributed by CO$_2$ taken up from the soil instead of local respiration. Recently, Yang et al. (2012) observed a correlation between root-respired CO$_2$ efflux with $E_{stem}$ in a temperate forest in China. However, during a $^{13}$C soil labeling experiment near the stem of various conifer species, used as a proxy of DIC uptake, Ubierna et al. (2009) found no $^{13}$C enrichment in $E_{stem}$ after label application. Moreover Ubierna et al. (2009) did not observe a reduction in $E_{stem}$ after artificially reducing sap flow by crown removal. They hypothesized that their conifer species had lower $F_s$ and a more impermeable cambium, thereby reducing the potential diffusion of belowground respired CO$_2$ through the stem.

To date, information on the extent of aboveground diffusion of belowground respired CO$_2$ among trees is scanty. This characteristic is potentially species specific. First, stem anatomy will affect radial diffusion and xylem transport rates of belowground respired CO$_2$. Second, trees with larger root systems potentially transport more belowground respired CO$_2$ upwards into the stem, as hypothesized by Teskey & McGuire (2007).
1.4 Measuring and tracing internal CO₂ in trees

Different methods are used to study the fate of internal CO₂ in trees or to measure related ecophysiological variables. These methods can be divided in two groups: non-isotopic measurements and isotopic measurements. The first non-isotopic measurements of internal CO₂ date from early 20th century (Bushong, 1907; MacDougal, 1927; MacDougal & Working, 1933). Since then studies on internal [CO₂] have been using different techniques, ranging from non-continuous gas extraction combined with gas chromatography to continuous solid state non dispersive infrared (NDIR) sensor-based measurements (see Table 1 in Teskey et al., 2008). Moreover, with the discovery of xylem CO₂ transport in trees, assimilation of internal CO₂, and the diffusion of xylem-transported CO₂ to the atmosphere in the past years, the entire gamut of different non-isotopic techniques applied in internal CO₂ research have been extended tremendously. In particular, combined F₅ and xylem sap [CO₂⁺] measurements are now being used to estimate the magnitude of xylem CO₂ transport in trees.

The use of carbon isotopes, either stable or unstable, have only recently received attention as a tool for gaining insight into the fate of internal CO₂ in trees. Isotopes are introduced in the xylem as a tracer of xylem CO₂ transport, and either diffuse into the atmosphere or are assimilated in plant tissue (McGuire et al., 2009). To our knowledge, the first who introduced isotopes directly in the xylem to trace respired CO₂ transport in trees were Stringer & Kimmerer (1993). In their experiment, excised Populus deltoides leaves were allowed to transpire a 1 mM [¹⁴C]NaHCO₃ solution and analysis revealed that 99.6% of the label was assimilated by the leaf and this mainly in the xylem vasculature. Recently, McGuire et al. (2009) introduced ¹³C in the xylem of excised Platanus occidentalis L. branches and observed that the label was transported with the transpiration stream from the branch base to the leaves.

In the next sections, the most common (non)-isotopic techniques used in internal CO₂ research will be reviewed. In particular, we will highlight the methods used in this thesis and discuss their benefits and shortcomings and their applicability within the context of internal CO₂ and plant respiration research.
1.4.1 Non-isotopic techniques

1.4.1.1 Measuring xylem CO$_2$ concentration

In this thesis, xylem [CO$_2$] was measured using a solid state NDIR sensor (Model GMM 221, Vaisala inc., Helsinki, Finland). After drilling a hole of 20 mm wide and approximately 5 cm deep, sensors are installed in the stem base and soft rubber foam or a flexible putty adhesive is used to seal off the space between sensor housing and stem, allowing CO$_2$ to build up at the site of sensor insertion (Fig. 1.8) (e.g. Teskey & McGuire, 2007; Saveyn et al., 2008b; Etzold et al., 2013). In this thesis, sensors were installed at stem base, because xylem CO$_2$ measurements were used to estimate the flux of xylem-transported CO$_2$ derived from belowground respiration ($F_t$) entering the stem (see Section 1.4.1.4).

![Fig. 1.8](image)

**Fig. 1.8** (A) NDIR sensor installed in the stem base of a 9-y old *Quercus robur* L. tree. An additional thermocouple is installed to measure stem temperature ($T_{stem}$). (B) Structure of the sensor head of the Vaisala NDIR sensor (adapted from Vaisala application note B211228EN) IR: infrared.

The NDIR measurement of [CO$_2$] is based on Vaisala’s Carbocap technology, which uses infrared sensing to measure the volumetric concentration of CO$_2$ within a range of 0-20%. The sensor consists of an infrared (IR) source, measurement chamber, interference filter and IR detector (Fig. 1.8B). When gas samples pass through the measurement chamber, CO$_2$ molecules absorb a fraction of the IR light at a specific wavelength. The filter in front of the detector prevents wavelengths other than the specific one to pass to the detector. This filter is an electrically tunable interferometer
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filter, allowing an additional compensation for any changes in the light source intensity by dirt accumulation and contamination. Finally, the IR light reaches the detector, where the light intensity is converted into a gas concentration value.

Because most gas produce a signal proportional to molecular density of gases according to the ideal gas law (Equation 1.1), a twofold correction of the sensor signal is needed when measuring internal \([\text{CO}_2]\) (%) (Equation 1.2) at different conditions than the standard one (25°C, 1013 hPa).

\[
P V = n R T \quad (1.1)
\]

\[
[\text{CO}_2](T, P) = [\text{CO}_2](25^\circ C, 1013 \text{ hPa}) \frac{P}{1013} \left(\frac{298.15}{T}\right) \quad (1.2)
\]

where \(P\) is the atmospheric pressure (Pa), \(V\) is the gas volume (m³), \(n\) the amount of gas (mol), \(R\) is the universal gas constant (8.3145 J mol\(^{-1}\) K\(^{-1}\)), and \(T\) the temperature (K).

First of all a gas pressure correction is needed because the IR-detector will detect less CO₂ molecules for the same \([\text{CO}_2]\) at lower atmospheric pressures. Secondly, continuous stem temperature \((T_{\text{stem}})\) data is needed to correct for a temperature effect, because under increasing temperature less CO₂ molecules will be detected at a constant \([\text{CO}_2]\). Correcting for the sensitivity of the NDIR sensor for temperature can also be performed while calibrating the NDIR sensors with different reference gases with a known \([\text{CO}_2]\) in range of 0 to 20%. The experimental setup for this calibration and additional temperature correction is described in Saveyn (2007).

During their long-term internal \([\text{CO}_2]\) measurements, Etzold et al. (2013) have raised concerns about the formation of wound tissue around the sensor head, thereby limiting diffusion of respired CO₂ to the sensor head. Therefore, re-installation of sensors in neighboring bottom stem sections is potentially needed in long-term studies. Overall, Vaisala sensors are very stable over time, in particular compared to previously used micro-electrodes (McGuire & Teskey, 2002). However an important shortcoming of the NDIR sensors is that the IR-based technology is very sensitive to water present in the stem tissue, which might eventually lead to sensor breakdown at high stem water content. Therefore, sensors can be wrapped in porous tubing, allowing diffusion of CO₂ to the sensor head, while protecting the sensor to moisture.
1.4.1.2 Conversion of xylem [CO$_2$] to [CO$_2^*$]

Although measurements of xylem-dissolved CO$_2$ in woody tissue cannot currently be made in situ (Aubrey et al., 2011), the amount of all products of CO$_2$ dissolved in the xylem sap can be calculated based on measurements of xylem [CO$_2$], T$_{stem}$, and pH. Because both the gaseous and aqueous phase are in contact, an equilibrium exists between the [CO$_2$] present in both phases. According to Henry’s law, the partial pressure of a gas over a solution is proportional to the concentration of that gas in the solution (mM) (Stumm & Morgan, 1996; McGuire & Teskey, 2002):

$$[CO_2^*] = \left(1 + \frac{K_1}{10^{-pH}} + \frac{K_2K_1}{(10^{-pH})^2}\right) K_H pCO_2 \quad (1.3)$$

with $K_1$ and $K_2$, the first and second acidity constant, respectively, $K_H$, Henry’s constant, and pCO$_2$ the partial pressure of CO$_2$ over the xylem sap (= gaseous [CO$_2$] expressed in atm). Gaseous [CO$_2$] and T$_{stem}$ can be measured continuously with a NDIR sensor and thermocouple, respectively (Fig. 1.8A). The constants $K_1$, $K_2$, and $K_H$ are temperature-dependent (see Butler, 1991) and the solubility of CO$_2$ decreases with increasing temperature (Stumm & Morgan, 1996; Teskey et al., 2008). Xylem sap pH has a pronounced effect on the solubility of [CO$_2$] within the xylem sap (Fig 1.9) and on the distribution of dissolved CO$_2$ among its different forms (Erda et al., 2013). Within the xylem sap pH range reported for trees, 4.5 to 7.4 among different species (see Table 2 in Teskey et al., 2008), most dissolved CO$_2$ is present in the carbonic acid (H$_2$CO$_3$) or bicarbonate (HCO$_3^-$) form. In spite of recent developments in online measuring of pH in liquids (e.g. Presens microsensor®), xylem sap is still being measured non-continuously and destructively (Aubrey et al., 2011). The major problem with sampling xylem sap is the negative pressure in the xylem of transpiring plants (Schneider et al., 1997). Therefore xylem sampling in the field is based on taking detached parts of a plant, putting them under pressure and collecting the sap that is squeezed out of the cut end. Xylem sap can be extracted from stems, branches, and twigs and this sap is then applied on a pH electrode. In this PhD thesis we expelled xylem sap from twigs with a Scholander pressure bomb and used xylem sap twig pH to convert xylem [CO$_2$] to [CO$_2^*$] measured at stem base. Previously, Aubrey et al. (2011) observed that for *Populus*
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deltoides Bartr. ex. Marsh xylem sap pH derived from twigs is a good proxy for stem xylem sap pH.

![Figure 1.9 Effect of xylem sap pH on the concentration of all products of CO₂ dissolved in water ([CO₂*], after Erda et al., 2013).](image)

A point of discussion regarding xylem sap pH measurements is the sampling frequency required to account for potential variation. Recent studies indicate that at diurnal scale variations in xylem sap pH are limited (Aubrey et al., 2011; Erda et al., 2013). At seasonal scale, variation in xylem sap pH might occur and might cause substantial errors in [CO₂*] estimates, in particular when xylem sap pH is more in the upper range reported within trees (pH > 6.5) (Erda et al., 2013). Additionally, pH can be influenced by environmental factors that affect transpiration rates (Stoll et al., 2000; Beis et al., 2009; Aubrey et al., 2011), such as vapor pressure deficit (VPD) or solar radiation. Increases in xylem sap pH have been observed under reduced transpiration rates (Campbell & Strother, 1996) or when the soil dries out (Wilkinson, 1999). In this PhD thesis we sampled xylem sap biweekly to account for potential variation in xylem sap pH at the seasonal scale.

1.4.1.3 Measuring sap flow

Accurate determination of Fₛ within tree stems is another key issue when estimating xylem CO₂ transport in trees or for understanding diurnal dynamics in stem [CO₂] and Eₘₚ. Commonly, sap flow measurements for woody species and some herbaceous plants are based on heat transport within the stem (Smith & Allen, 1996; Vandegehuchte
& Steppe, 2013) but utilized methods are fundamentally different in their operating principles (Smith & Allen, 1996).

In this thesis, two types of sap flow sensors were used to measure sap flow, the heat balance (HB) and the thermal dissipation probes (TDP) (Fig. 1.10). The heat balance sensor is used to determine sap flow rate in the stem or in a specific stem segment. The sensor itself consists of a heater element, a few centimeters in width, and thermocouple pairs both embedded in a cork shielding. The shielding is wrapped around the stem to ensure good contact of both the heater and thermocouples with the stem surface. Heat is applied to the entire circumference of the stem and the balance of the heat fluxes into and out of the stem section registered with the thermocouple pairs is used to quantify sap flow (Sakuratani & Abe, 1985; Baker & Van Bavel, 1987). These sensors are used to estimate whole plant water use, however limited to small herbaceous species and small trees (Smith & Allen, 1996).

Sap flow measurements with TDP sensors are based on a temperature differences between a heated and reference needle and is the most widely applied SFD method, because of its simplicity and low cost (Vandegehuchte & Steppe, 2013). Constant power is applied to the upper heater needle and as sap flow increases, heat is more rapidly dissipated with the transpiration stream, which decreases the temperature difference between heated and reference needle. The empirical basis for measuring sap flow with TDP sensors was developed by Granier (1985). He found experimentally for *Pseudotsuga menziessii* (Mirb.) Franco, *Pinus nigra* Arnold, and *Quercus pedunculata* Ehrh. that SFD (cm³ cm⁻² h⁻¹) was related to measured temperature difference by the following equation

\[ SFD = 0.000119 \frac{(\Delta T_0 - \Delta T)}{\Delta T}^{1.231} \]

(1.4)

where \( \Delta T \) is the temperature difference between heated and reference needle, and \( \Delta T_0 \) is the value of \( \Delta T \) when there is no sap flow. SFD multiplied with the cross sectional area of the sapwood is used to obtain \( F_s \) (l h⁻¹).
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While Granier (1985) assumed that this experimentally determined regression TDP-based measurements is species independent, results from recent studies indicate that SFD is underestimated between 6 and 90% for a wide variety of species compared to other SFD methods or when compared to results from mass-based calibration experiments on excised stem and branch segments or cut trees (Vandegehuchte & Steppe, 2013 and references therein). Therefore species specific correction factors should be used to obtain accurate Fₛ estimates (Steppe et al., 2010). In this PhD thesis, we obtained site and species specific correction factors for SFD measurements on Quercus robur L. by comparing TDP measurements on excised Quercus robur L. stem segments with mass-based estimates of sap flow, using the Mariotte based verification system of Steppe et al. (2010). We observed that on average TDP sensors underestimated actual Fₛ by 66.4% in these Quercus robur trees.

1.4.1.4 Estimating xylem transport of root-respired CO₂

As described by Aubrey & Teskey (2009), measurements of [CO₂*] and Fₛ are used to estimate Fₜ (mmol h⁻¹) according to the following formula:

\[ Fₜ = Fₛ [CO₂^*] k \]  

(1.5)

where k is a site and species specific correction factor for Fₛ based on sap flow calibration experiments. In this PhD thesis, NDIR and TDP sensors (model TDP-10, Dynamax inc., Houston, Texas, USA) were installed at stem base on 5 and 35 cm above the soil, respectively. The diel pattern in Fₜ will mimick that of Fₛ but its magnitude will additionally depend on [CO₂*] dynamics. Aubrey & Teskey (2009) observed large day-
night variations in $F_t$ for *Populus deltoides* with maxima during the day and zero flux at night, when no sap flow occurred (Fig. 1.11).

**Fig. 1.11**  (A) Sap flow rate ($F_s$, blue) and xylem-dissolved CO$_2$ ([CO$_2^+$], red) measured in 9-year old *Populus deltoides* trees.  (B) Comparison of the scaled flux of root-respired CO$_2$ transported via the xylem ($F_t$, green) calculated from data in (A) and soil CO$_2$ efflux ($E_{soil}$, black) (adapted from Aubrey & Teskey, 2009).

### 1.4.1.5 Comparing xylem transport of root-respired CO$_2$ with soil CO$_2$ efflux

$F_t$ and $E_{soil}$ measurements were compared, to quantify the total belowground $R_a$. One of the main issues when comparing $F_t$ with the flux of root-respired CO$_2$ contributing to $E_{soil}$ is that both fluxes are expressed on a different unit scale. Therefore, in this PhD thesis $F_t$ was scaled ($F_{t,scaled}$, mg C m$^{-2}$ h$^{-1}$) based on the soil area occupied by the root system:

$$F_{t,scaled} = rac{F_t \cdot A(C)}{A_{root}}$$

(1.6)

with $A_{root}$ the soil area occupied by the root system (m$^2$), $A(C)$ the atomic mass of carbon (12 g mol$^{-1}$). Data from root excavation measurements on additional trees with similar dimensions was used to estimate $A_{root}$. For older trees, root excavation is an elaborate task and therefore general rule of thumbs regarding the extension of roots relative to an imaginary downward projection of the branch tips can be applied (Lyford & Wilson,
1964; Perry, 1982). Nonetheless, this $A_{\text{root}}$ factor is potentially a large source of uncertainty for $F_{t, \text{scaled}}$ estimates. Therefore other, more precise approaches are needed to estimate this parameter.

Within $A_{\text{root}}$, roots will contribute both to $F_t$ and $E_{\text{soil}}$ (Fig. 1.12A) and therefore it is important that measurements of the latter are representative for efflux rates within this zone, given the potential large spatial variability for $E_{\text{soil}}$ observed within forests (e.g. Soe & Buchmann, 2005; Katayama et al., 2009). Aubrey & Teskey (2009) performed $E_{\text{soil}}$ measurements at a distance of 0.75 m of the stem of 9-y old Populus deltoides trees. In this PhD thesis we measured $E_{\text{soil}}$ at 0.40 m and 0.70 m from the stem of 9-y old smaller Quercus robur trees, within a circular shaped $A_{\text{root}}$ of 3.14 m² (Fig. 1.12B). Measurements of $E_{\text{soil}}$ should be performed at the same high temporal resolution as for $F_t$ measurements, either using the CO₂ gradient method along a vertical soil profile (Aubrey & Teskey, 2009), described in detail in Tang et al. (2003), or by using automatic efflux chambers, as performed in this thesis, based either on an open or closed dynamic system (Pumpanen et al., 2009).

**Fig 1.12** (A) Schematic of the fluxes of root-respired CO₂, both transported internally in trees with the transpiration stream ($F_t$) and contributing to soil CO₂ efflux ($E_{\text{soil}}$) within the soil area occupied by the root system ($A_{\text{root}}$) with radius $r$. (B) Set up used in this PhD study to estimate $F_t$ and the contribution of root respiration to $E_{\text{soil}}$. $F_t$ estimates are derived from stem [CO₂] and sap flow measurements with a non dispersive infrared (NDIR) and a thermal dissipation probe (TDP) sensor, respectively. $E_{\text{soil}}$ is measured in $A_{\text{root}}$ with automatic (black chamber) (see Fig 1.13A,B) and manual chambers (grey chambers) (see Fig. 1.13C). Manual chamber measurements performed at 70 cm are not presented. Measurements of $F_t$ are scaled with $A_{\text{root}}$ ($F_{t, \text{scaled}}$) for comparison with the fraction of $E_{\text{soil}}$ derived from root respiration (see Equation 1.6).
In this PhD study automated dynamic PVC chambers (20 cm diameter, 15 cm height, design adapted from Suleau et al., 2009) were used to measure \( E_{\text{soil}} \) (Fig 1.13A,B). The chambers were connected to an infrared gas analyzer (IRGA) (model GMP 343, Vaisala inc., Helsinki, Finland) in a closed loop, to estimate the slope of the increase in \([\text{CO}_2]\) within the chamber headspace. In addition, manual \( E_{\text{soil}} \) measurements were performed at varying positions and distances from the stem to complement these automated measurements (Fig. 1.13C) and to account for spatial heterogeneity within the soil area occupied by the roots. These measurements were performed using a CO\(_2\) analyzer (EGM-4, PP systems, Amesbury, MA, USA) connected to a soil chamber (SRC-1, PP systems, Amesbury, Massachusetts, USA) which was installed on PVC chambers (20 cm diameter, 12 cm height).

The benefit of estimating \( E_{\text{soil}} \) with chambers is that measurements and calculations are more straightforward compared to the CO\(_2\) gradient method, for which site-specific estimates of soil diffusion and tortuosity are needed, as described by Tang et al. (2003). On the other hand with the CO\(_2\) gradient method, the fraction of xylem-transported CO\(_2\) derived from the uptake of soil solution can be estimated, based on soil \([\text{CO}_2]\) and \(F_s\) measurements (Aubrey & Teskey, 2009). Regardless of the technique, additional manual measurements of \( E_{\text{soil}} \) - at lower temporal frequency - should be performed to account for spatial variation of \( E_{\text{soil}} \) in \( A_{\text{root}} \) and to validate the automatic measurements.

Finally, to quantify the total \( R_{ab} \) accounting both for xylem CO\(_2\) transport and the autotrophic contribution to \( E_{\text{soil}} \), the latter needs to be estimated. In their study, Aubrey
& Teskey (2009) assumed that 50% of $E_{\text{soil}}$ was derived from autotrophic sources, based on the average of the reported range of 10-90% among different studies and different ecosystems (Hanson et al., 2000). However different isotopic as well as non-isotopic methods can be applied to estimate this autotrophic fraction, of which the discussion on their advantages and limitations lies out of the scope of this thesis but is available in reviews by Hanson et al. (2000) and Kuzyakov (2006). Grossiord et al. (2012) estimated the fraction of $E_{\text{soil}}$ derived from $R_{a,b}$ based on the isotopic signature of $E_{\text{soil}}$ after conversion of $C_4$-type vegetation into $C_3$-type vegetation. In this thesis, we used the girdling method to quantify $R_{a,b}$. By removing a circumferential band of bark and phloem from a tree stem during girdling (Högberg et al., 2001; De Schepper et al., 2010), the belowground transport of recent photosynthates is interrupted, decreasing the amount of substrate available for $R_{a,b}$.

### 1.4.1.6 Measuring stem CO₂ efflux

$E_{\text{stem}}$ measurements used to estimate stem respiration during the growing season are considered to be biased by xylem CO₂ transport, because a substantial fraction of respired CO₂ is potentially transported away from the site of respiration with the transpiration stream or a fraction of xylem-transported CO₂ diffuses radially to the atmosphere (see Section 1.3.3). Therefore, $E_{\text{stem}}$ measurements have been used in combination with $[\text{CO}_2^*]$ and $F_s$ measurements to quantify stem respiration, according to the mass balance approach proposed by McGuire et al. (2004) and Teskey & McGuire (2007). In this PhD thesis, we measured $E_{\text{stem}}$ on intact stems of dormant Quercus robur trees and non-dormant Populus deltoides trees. In the study on dormant Quercus robur trees, sap flow and xylem CO₂ transport were absent and $E_{\text{stem}}$ can be assumed to represent local stem respiration rates (McGuire et al., 2007). However, we performed $E_{\text{stem}}$ measurements in combination with artificial illumination of remote stem sections at varying distances from the stem cuvette, to elucidate the impact of axial diffusion of respired CO₂ on dormant season $E_{\text{stem}}$ measurements (Fig 1.14).
We enclosed the stem segments with a flexible fan-stirred stem cuvette constructed of Polycarbonate film and sealed to the stem with adhesive closed-cell foam gasket material and silicone sealer. Previous studies mainly used solid acrylic or Lexan tubes cut in two and clamped around the stem to form a cylindrical cuvette and made leak tight with flexible putty adhesive. However, based on the experience during previous studies, these were more prone to gas leaks compared to the flexible cuvettes. Stem cuvettes were covered with aluminium foil to exclude local woody tissue photosynthesis. The [CO₂] of the air leaving the cuvette that enclosed a stem segment was measured with an IRGA (IRGA, LI-7000, Li-COR, Lincoln, TE, USA) and was compared with the [CO₂] of the air leaving a reference cuvette, which enclosed a PVC tube with approximately the same dimensions as the average stem diameter of the stem sections enclosed for \( E_{stem} \) measurements. \( E_{stem} \) (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)) was calculated according to Long & Hallgren (1985) and was expressed per unit of surface area:

\[
E_{stem} = \left( \frac{f_a}{A_s} \right) \times \Delta \text{CO}_2 \times 22.4 \text{ mol l}^{-1}
\]  
(1.7)

where \( f_a \) is the flow rate through the cuvette (l s⁻¹), \( A_s \) is the surface area of the stem segment (m²) and \( \Delta \text{CO}_2 \) is the difference in [CO₂] of measurement and reference air (\( \mu \text{mol mol}^{-1} \)).
1.4.2 Isotopic techniques

Approaches for studying the fate of xylem-transported CO₂ is based on the use of isotopes, which are atoms of an element with the same atomic number, but with different atomic masses (Dawson & Brooks, 2001). These isotopes can be applied for tracing fluxes in the soil plant atmosphere continuum (Brüggemann et al., 2011), because they have identical physicochemical properties to the substance of interest and are detectable at low concentrations (Schurr, 1998). Overall, the commonly used carbon isotopes can be divided in three groups (Table 1.2; Shirley & Lederer, 1980), one containing stable isotopes (¹²C and ¹³C) and two groups of unstable isotopes which decay either by emission of a β⁺ (a positron, i.e. an anti-electron) like ¹¹C or by emission of a β⁻ (an electron) which includes ¹⁴C (Minchin & Thorpe, 2003). In this thesis we used ¹¹C and ¹³C to trace xylem CO₂ transport and therefore use of these carbon isotopes will be further discussed in detail.

Table 1.2  The different carbon isotopes commonly used to study carbon pools and fluxes in plants. Plant applications indicate the main application, but this list is extensive. T₁/₂: half-life. EC: electron capture (adapted from Shirley & Lederer, 1980).

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>% Abundance or T₁/₂</th>
<th>Decay mode</th>
<th>Plant applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹¹C</td>
<td>20.38 min</td>
<td>β⁺, 99.76%, EC 0.24%, no γ</td>
<td>Short term photosynthate tracing studies</td>
</tr>
<tr>
<td>¹²C</td>
<td>98.89%</td>
<td>-</td>
<td>CO₂ flux studies</td>
</tr>
<tr>
<td>¹³C</td>
<td>1.11%</td>
<td>-</td>
<td>Photosynthate tracing</td>
</tr>
<tr>
<td>¹⁴C</td>
<td>5730 y</td>
<td>β⁻, no γ</td>
<td>Long term photosynthate allocation studies</td>
</tr>
</tbody>
</table>

1.4.2.1 ¹³C concepts, use and measurements

Since the first report on ¹³C isotopic values of plant materials (commonly expressed as δ¹³C) by Craig (1953; 1954) ¹³C has seen an increased use as an important tool for understanding and quantifying C pools and fluxes in terrestrial ecosystems (Norby, 2009). Overall, studies on stable isotopes can be categorized in those working at naturally occurring levels (called ‘natural abundance’), reported around -27‰ and -12‰ for C₃ and C₄ plants (Farquhar et al., 1989), respectively, and others that work
outside the natural range of values (called "enriched" levels) with isotopically labeled substances (Dawson et al., 2002). Because working at natural abundance requires repeatable and distinct δ values and no significant fractionation of tracers or mixing of sources (Dawson et al., 2002), many stable isotope studies rely on the enriched isotope approach. Hence the addition of an enriched substance acts as a powerful tracer for following the flow of a specific element (Nadelhoffer & Fry, 1994).

At lower tracer enrichment levels, δ\(^{13}\)C (‰) can be expressed relative to an internationally accepted standard:

\[
\delta^{13}\text{C} = 1000 \times \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right)
\]

Where \(R_{\text{sample}}\) and \(R_{\text{standard}}\) are the abundance ratios of the heavier to the lighter isotope (i.e. \(^{13}\text{C}/^{12}\text{C}\)) of the sample and standard, respectively. Vienna Pee Dee Belemnite (VPDB) is used as standard for \(^{13}\text{C}\) with an \(R_{\text{standard}}\) of 0.0112372. In this thesis, \(\delta^{13}\text{C}\) of enriched samples was expressed relative to \(\delta^{13}\text{C}\) values of baseline samples to obtain the enrichment of the labeled tissues.

At high tracer enrichment levels, commonly at \(\delta^{13}\text{C}\) levels > 500‰, the isotopic composition of labeled substance should be expressed in atom\% (A, %), which is defined as:

\[
A = \frac{X_{^{13}\text{C}}}{X_{^{13}\text{C}} + X_{^{12}\text{C}}} = 100 \times \left(\frac{R_{\text{sample}}}{R_{\text{sample}} + 1}\right)
\]

where \(X_{^{13}\text{C}}\) and \(X_{^{12}\text{C}}\) are the numbers of \(^{13}\text{C}\) and \(^{12}\text{C}\) atoms, respectively, present in the sample.

**Tracing xylem-transported CO\(_2\) with \(^{13}\text{C}\)**

To date, \(^{13}\text{C}\) tracers have mainly been used to trace the fate of photosynthates allocated within plants and to the soil, stored within ecosystems and lost by respiration to the atmosphere (Brüggemann et al., 2011; Epron et al., 2012). Epron et al. (2012) synthesized data from short term labeling studies (called pulse labeling), in which the tracer was applied at canopy level by enclosing one or several trees in chambers or using free air carbon isotope enrichment systems. They observed that the time lag between peak \(^{13}\text{CO}_2\) efflux from the soil and label application at leaf level, varied with
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Tree height, and was longer for coniferous trees compared to deciduous trees (Fig. 1.2, Epron et al., 2012). Hence, results from $^{13}$C tracer studies have provided substantial information on the fate of labeled carbon after assimilation within the leaves (Fig. 1.15).

In this PhD thesis, $^{13}$C was used to trace the flow of xylem CO₂ transport within field-grown trees, detached branches, and leaves (Fig. 1.16). Previously, xylem pulse labeling studies were limited to detached branches (McGuire et al., 2009) or leaves (Stringer & Kimmerer, 1993; Hibberd & Quick, 2002). To our knowledge one study has used $^{13}$C to

Fig. 1.15 Fate of labelled carbon in a tree. The isotopically labeled carbon is applied at the leaves (1) and fills a soluble carbohydrates pool that is further allocated to respiration (2), storage (3), structural growth (4) and defense compounds (5). The soluble carbohydrate pools are also filled by remobilization from transient storage (6) or are potentially used for emission of organic compounds (7). Soluble carbohydrates are transferred both basipetally via the phloem (8) or acropetally from storage in perennial organs to new shoots (9). A fraction of the labelled carbon is also transferred to the rhizosphere, either indirectly via hyphae of mycorrhiza (10) or directly via exudation (11). There it can be transferred into microbial biomass (12) and further recycled due to the high turnover rate of microbes (13). Letters, l, w, r, and m indicate the compartment (leaf, woody organs, roots, and microbes, respectively) (after Epron et al., 2012).
trace the fate of xylem-transported CO$_2$ in field grown trees. Powers & Marshall (2011) dissolved $^{13}$C-Na$_2$CO$_3$ into a small volume of water and injected it into the xylem of a 4 m tall Thuja occidentalis tree at breast height. They observed five days after label injection isotopic enrichment of 6.1‰ and 7.7‰ in E$_{stem}$ and phloem tissues, respectively, however bulk foliage samples were not enriched. In this thesis, the transpiration stream of field-grown Populus deltoides trees was pulse labeled using an infusion technique (Fig 1.16 A, B). Holes were drilled in the stem base and containers containing $^{13}$CO$_2$-labeled solutions were connected to the stem with CO$_2$ impermeable tubing. The $^{13}$CO$_2$-labeled solutions were prepared by dissolving compressed 100% CO$_2$ gas at 99$^{13}$C in an aqueous solution, as described by McGuire et al. (2009).

Moreover in two other experiments, we used a similar dissolved $^{13}$C label to determine the impact of sap flow rate dynamics on the assimilation of xylem-transported CO$_2$ in detached Populus deltoides branches (Fig 1.16C) and detached Populus deltoides x canadensis ‘Robusta’ leaves (Fig. 1.16D).

**Measuring the fate of xylem-transported $^{13}$CO$_2$**

In this thesis $\delta^{13}$C of woody and leaf tissue samples and the isotope composition of stem and branch CO$_2$ efflux was measured, to determine the fate of xylem-transported CO$_2$. Solid woody and leaf tissue samples collected during the $^{13}$C infusion experiments were oven-dried, grinded, weighed in tin capsules, and flash-combusted before being
analyzed. Gas samples were collected from the efflux cuvettes installed on the field-grown *Populus deltoides* trees using syringes and injected in evacuated vials for storage before analysis. Both tissue and gas samples were analyzed by an Element Analyzer (EA) coupled to an Isotope Ratio Mass Spectrometry (IRMS).

### 1.4.2.2 $^{11}$C concepts, use and measurements

While $^{11}$C was used in photosynthesis research before $^{14}$C became available (Benson, 2002), it has seen only limited use (Minchin & Thorpe, 2003), because of its very short-half-life (20.4 min) and the specialized facility its use requires (Norby, 2009). Because of its short half-life, $^{11}$C has been used in most plant applications exclusively to trace short term processes, for instance the carbon partitioning within leaves of *Glycine max* (L.) Merr. (Dirks *et al.*, 2012) or carbon import within root systems of *Hordeum vulgare* L. (Beer *et al.*, 2010) or *Zea mays* L. (Jahnke *et al.*, 2009) after pulse-labeling the leaves with $^{11}$CO$_2$. On the other hand, the fast decay of $^{11}$C allows the application of a sequences of independent pulses, as there is no build up of tracer from previous pulses (Minchin & Thorpe, 2003).

In vivo observation of $^{11}$C tracer movement in plants is based on the detection of γ-rays, due to the annihilation of β$^+$ particles emitted by $^{11}$C with an electron in plant tissue, or on the direct detection of β$^+$ particles during $^{11}$C decay. Commonly, γ-ray detection is used for imaging tracers in humans, animals, and in the bulk of plants, while β$^+$ imaging is usually performed for thinner tissues, like leaves (Dirks *et al.*, 2012). In this PhD thesis direct detection of the β$^+$ particles with autoradiography was used to image xylem CO$_2$ transport in leaves.

**Tracing xylem CO$_2$ transport with $^{11}$C**

We used $^{11}$C as proxy for xylem-transported CO$_2$ entering the leaf to obtain a more detailed spatial analysis of its distribution among leaf tissue components than obtained with $^{13}$C. Similar as for the $^{13}$C experiment on excised leaves, we infused $^{11}$C in detached *Populus deltoides x canadensis* ‘Robusta’ leaves via the petiole. We dissolved gaseous CO$_2$ containing a small amount of $^{11}$CO$_2$ produced on site with a cyclotron into a TRIS buffer solution. Leaves were allowed to take up the labeled solution with a specially designed label application system.
To our knowledge, none have yet used $^{11}$C to track xylem CO$_2$ transport. One study has observed movement of $^{11}$C labeled methyl jasmonate in the xylem in *Nicotiana tabacum* L. (Thorpe *et al.*, 2007). However, Thorpe *et al.* (2007) administered the $^{11}$C label to the leaf blade, instead of to the petiole, and observed that after export from the labeled region, $^{11}$C methyl jasmonate moved in the xylem vasculature.

**Measuring the fate of xylem-transported $^{11}$CO$_2$**

For autoradiographic analysis, we exposed the leaf to an imaging phosphor plate for approximately 5 min. We used these high-efficient phosphor screens instead of the classic autoradiographic films because these capture signals more efficiently and are reusable. Subsequently, we scanned the latent image on the film with a plate reader to visualize the distribution of the $^{11}$C tracer in the leaf.

1.5 Conclusions

Recognition that tree stems can contain substantial amounts of respired CO$_2$ has led to new insights regarding the use of efflux-based measurements to estimate respiration and the role of a recycling mechanism in trees. While previous research almost exclusively attributed internal CO$_2$ in tree stems to aboveground respiration, recent studies expand these observations to the soil, because a fraction of belowground respired CO$_2$ dissolves in the xylem and is transported aboveground towards the canopy.

In the first part of this chapter we combined today's literature on internal CO$_2$ research to obtain a clearer picture on the significance of internal CO$_2$ in plant physiology. We discussed the different sources of internal CO$_2$ and focused primarily on the belowground ones. In particular, root respiration is recently acknowledged as an important source for internal CO$_2$ in stems. Therefore, further research on the fraction of respired CO$_2$ remaining inside the root system and thereby contributing to high internal [CO$_2$] is crucial to elucidate the contribution of root-respired CO$_2$ to stem internal CO$_2$.

Aboveground, respired CO$_2$ within trees diffuses radially into the atmosphere, is assimilated in chlorophyll containing woody tissues, or is transported upwards to the canopy, where it might serve as additional substrate in photosynthetic reactions.
However, we still lack a clear picture of the fate of xylem-transported CO₂ under field conditions. The relation between internal CO₂ and E_{stem} has been established previously, but results on excised branches, leaves or stem segments under controlled conditions should be translated to field experiments. New field observations will lead to a better understanding of the tree carbon economy and might provide new visions on the coupling between the carbon and water cycle in trees, given the potential role of xylary chloroplasts in xylem cavitation repair. In particular, aboveground diffusion of belowground respired CO₂ from the tree stem into the atmosphere might incur a revolution in the way scientist will partition and measure above- and belowground respiration in the near future.

In the second part of this chapter, we discussed the different techniques available for tracing and measuring the fate of xylem-transported CO₂. Previous studies mainly applied non-isotopic methods to quantify the amount of internal CO₂ that is transported via the xylem or that diffused radially into the atmosphere. However, the use of stable or unstable isotopes might give us new insights in the fate of xylem-transported CO₂, similar as currently obtained for the fate of labelled carbon applied at leaf or canopy level. By pulse-labeling the xylem and tracking the fate of the label, scientists will be able to quantify upward below- and aboveground xylem CO₂ transport and estimate radial diffusion of xylem-transported CO₂ into the atmosphere and assimilation rates in woody and leaf tissues.
- Chapter 2 -
Abstract

In recent studies, assimilation of xylem-transported CO₂ has gained considerable attention as a means of recycling respired CO₂ in trees. However, we still lack a clear and detailed picture on the magnitude of xylem-transported CO₂ assimilation, in particular within leaf tissues. To this end, detached poplar leaves (Populus x canadensis Moench ‘Robusta’) were allowed to take up dissolved ¹³CO₂ serving as a proxy of xylem-transported CO₂ entering the leaf from the branch. The uptake rate of the ¹³C was manipulated by altering the vapor pressure deficit (VPD) (0.84, 1.29, and 1.83 kPa). Highest tissue enrichments were observed under the highest VPD. Among tissues, highest enrichment was observed in the petiole and the veins, regardless of the VPD treatment. However, results derived from ¹³C leaf tissue enrichment were limited in spatial resolution. Therefore, ¹¹CO₂ was used and its detection by positron autoradiography showed a more detailed spatial distribution in particular in secondary veins. There ¹¹C is presumably assimilated in chlorophyll containing bundle sheath tissues, positioned in between the phloem and the xylem vasculature. Therefore, in addition to ¹³C, ¹¹C based autoradiography can be used to study the fate of xylem-transported CO₂ at leaf level, allowing the acquisition of data at a yet unprecedented resolution. Results from these labeling studies at leaf level might help us to better
understand the role of xylem CO₂ assimilation within bundle sheath cells in plant functioning.

2.1 Introduction

In plants, carbon assimilation is a relatively straightforward process with a well-established theoretical background (Trumbore, 2006). Atmospheric CO₂ enters the leaf via the stomates and diffuses to the parenchyma tissue where chlorophyll containing cells use light to reduce this CO₂, thereby synthesizing a wide range of carbon compounds, primarily sugars (Taiz & Zeiger, 2006). These sugars are the basis of plant growth via the catabolic and anabolic reactions of metabolism (Amthor, 1989). Therefore, assimilation of atmospheric CO₂ is generally considered as the sole pathway by which plants sustain their carbon economy.

However over the past decade, observations of assimilation of respired CO₂ in trees have led to newly emerging views on tree carbon budgets. While since the early 20th century stem-respired CO₂ was generally believed to entirely diffuse into the atmosphere (Johansson, 1933) it is now acknowledged that a substantial amount of respired CO₂ remains inside the tree, contributing to internal CO₂ concentrations ([CO₂]) substantially higher than the atmospheric one (Teskey et al., 2008) (Chapter 1). This internal CO₂ is partially transported through the transpiration stream towards the canopy, where it either diffuses into the atmosphere (McGuire & Teskey, 2004; McGuire et al., 2007) or is assimilated by chlorophyll containing woody and leaf tissues (McGuire et al., 2009; Bloemen et al., 2013b; 2013a) (Chapter 1). Recycling of respired CO₂ in woody and leaf tissues is assumed to partially pay for local carbon demands and to positively contribute to the carbon income of plants (Aschan & Pfanz, 2003).

Previous studies traced the fate of xylem-transported CO₂ by using ¹³C-labeled CO₂ (referred to as ¹³CO₂ in the text) added to the transpiration stream and by analyzing ¹³C tissue enrichment in field-grown trees (Powers & Marshall, 2011; Bloemen et al., 2013b) and detached branches (McGuire et al., 2009; Bloemen et al., 2013a). Powers & Marshall (2012) observed no substantial leaf enrichment after injecting a dissolved ¹³C-carbonate label into a Thuja occentalis tree at breast height. Bloemen et al. (2013b) observed for Populus deltoides Bartr. ex. Marsh trees that the ¹³C label infused at the base of the stem...
was transported with the transpiration stream throughout the tree and that at leaf level the label was mainly assimilated by the petioles. McGuire et al. (2009) and Bloemen et al. (2013a) introduced a dissolved $^{13}\text{CO}_2$ label into detached branches and observed a similar enrichment of the petioles relative to the other leaf tissue components. However, leaf tissue enrichment was observed to be strongly dependent on the transpiration rate, with increased enrichment levels at higher transpiration rates (Bloemen et al., 2013a). Nonetheless, branch and stem $^{13}\text{C}$ infusion as a proxy of xylem CO$_2$ transport underestimates the actual amount of xylem CO$_2$ that is transported to the leaves, because this method does not account for CO$_2$ derived from local respiration between the point of infusion and the leaf. Moreover, we lack detailed results on the extent of xylem-transported CO$_2$ assimilation within the leaf tissues under varying transpiration rates.

To grasp the full picture, isotopic proxies of xylem CO$_2$ transport can be used at leaf level. While $^{13}\text{C}$ based techniques require destructive tissue analysis to estimate assimilation of xylem-transported CO$_2$ within a specific tissue, the use of radiocarbon tracers is non-destructive and might be a promising tool in xylem-transported CO$_2$ research. As explained in Chapter 1, these unstable carbon isotopes, which decay by emission of a charged particle, can be subdivided in heavy isotopes, including $^{14}\text{C}$, and in light isotopes, which include $^{11}\text{C}$ (Minchin & Thorpe, 2003; Matsuhashi et al., 2006). In the past decades, most experiments have used $^{14}\text{C}$ as radiocarbon tracer, given its longer half-life (5730 years) and lower experimental cost compared to the use of $^{11}\text{C}$ (half-life of 20.4 min) (Minchin & Thorpe, 2003). Previously, $^{14}\text{C}$ autoradiography has been applied to detached leaves (Stringer & Kimmerer, 1993; Hibberd & Quick, 2002), hydroponically grown tree seedlings and cuttings (Zelawski et al., 1970; Vapaavuori & Pelkonen, 1985; Vuorinen et al., 1989) and herbaceous plants (Amiro & Ewing, 1992; Hibberd & Quick, 2002) for studying xylem CO$_2$ fixation at a detailed spatial resolution. On the other hand, $^{11}\text{C}$-based studies have exclusively traced the carbon export from labeled leaves, by applying $^{11}\text{C}$ as gaseous $^{11}\text{C}$-labeled CO$_2$ (referred to in the text as $^{11}\text{CO}_2$) (Minchin & Thorpe, 2003; Thorpe et al., 2007; Dirks et al., 2012). The higher energy of the positron emitted during $^{11}\text{C}$ decay than that from electron emission during $^{14}\text{C}$ decay allows a more efficient in vivo detection of $^{11}\text{C}$ decay relative to $^{14}\text{C}$ decay.
Therefore, $^{11}$C labeling could provide us detailed information on a whole array of short-term plant processes, amongst which xylem-transport of CO$_2$ in leaves might be of particular importance.

The aim of this study was to investigate in detail where xylem-transported CO$_2$ assimilation occurred at leaf level. Detached poplar leaves (Populus x canadensis Moench ‘Robusta’) were allowed to take up an aqueous solution enriched with dissolved $^{13}$CO$_2$. We manipulated the uptake rates of xylem CO$_2$ by altering the vapor pressure deficit (VPD) of the atmosphere under controlled conditions and analyzed the enrichment of the different leaf tissue components. We hypothesized that a larger enrichment would be observed under high VPD. However, the spatial resolution of the results derived from the $^{13}$C labeling was too limited. Therefore, an additional detached leaf of the same species was allowed to take up an aqueous solution enriched with dissolved $^{11}$CO$_2$ to obtain a detailed picture of xylem CO$_2$ assimilation in leaves using autoradiography.

### 2.2 Materials and methods

#### 2.2.1 Plant material

For this study, 20 cm long poplar cuttings (Populus x canadensis Moench ‘Robusta’) were planted early April 2012 in 4 l pots containing a commercial potting mixture (DCM, Grobbendonk, Belgium) and slow-releasing fertilizer (Basacote Plus 6M, Compo Benelux nv, Deinze, Belgium), and were grown within a growth chamber. Air temperature ($T_{air}$) was controlled day and night at 25°C and photosynthetic active radiation (PAR) was provided with densely packed fluorescent lamps (TLD 80, Philips Lighting NV, Eindhoven) from 8:00 h until 22:00 h. The cuttings were watered every two days.

#### 2.2.2 Baseline sampling

Prior to $^{13}$C labeling, leaf baseline samples of the same cuttings were used to determine the natural abundance carbon isotopic composition ($\delta^{13}$C, ‰) to which $\delta^{13}$C values of treated tissues would be compared. Baseline samples were collected on all treatment days and were immediately frozen in liquid nitrogen and then stored in a freezer at -18°C before further processing.
2.2.3 Experimental setup $^{13}$C labeling

The control and $^{13}$CO$_2$ treatment solutions were prepared as described by McGuire et al. (2009). Solutions were either enriched with 99 atom% (A) $^{13}$CO$_2$ (treatment) or with $^{13}$CO$_2$ at natural abundance and atmospheric concentration (control). To prepare the solutions, 2 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) and with 1 mM sodium bicarbonate to control pH at a level of around 7. One of these bottles was set aside and used for providing the KCl solution that treatment and control leaves were allowed to transpire before and after the labeling treatment. For another bottle, 160 ml of the solution was displaced with 99 A $^{13}$CO$_2$ gas from a compressed gas cylinder (Buchem BV, Minden, The Netherlands). Subsequently, the gas was then circulated with a pump through water in a closed loop for at least 3 h. For the control bottle, gas with $^{13}$CO$_2$ at natural abundance and atmospheric concentration was circulated through the control solution for the same amount of time. After bubbling, pH dropped by 0.5 units, slightly affecting the final dissolved inorganic carbon concentration ($[\text{CO}_2^*]$) (Erda et al., 2013). $[\text{CO}_2]$ measured with a microelectrode (Model Mi-720, Microelectrodes, Bedford, NH, USA) and dissolved CO$_2$ concentration ($[^{13}\text{CO}_2^*]$, mM) of the enriched solutions were 15.07 % and 10.51 mM, respectively.

Leaves were excised under water right before the start of the experiment to avoid xylem embolism formation. The cut end of the petiole was sealed with a closed-cell foam gasket in a 3 ml syringe (BD, Franklin Lakes, NJ, USA) filled with KCl solution and connected to a syringe and valve system enabling the supply of either KCl solution or treatment or control CO$_2$ solutions. Syringes were placed in a plexiglas box (0.56 x 0.45 x 1.6 m, H x W x L), which was specifically designed for this experiment. This box allowed the control of temperature, PAR and relative humidity (RH). RH inside the box was altered during three treatment days by controlling the water vapor content of the air entering the box. To this end, a gas cooling unit (Van der Heyden, Brussels, Belgium) was used to dehumidify the incoming air. Subsequently, the incoming air was bubbled in a beaker containing deionized water placed in a temperature controlled water bath (Colora Messtechnik GmbH, Frankenthal, Germany) to alter the water vapor content of the air to a set level. RH was varied from day to day and RH levels of 42.4, 59.5, and 73.6% were
imposed, corresponding with a high (1.83 kPa), mid (1.29 kPa) and low (0.84 kPa) vapour pressure deficit (VPD) treatment, respectively, hereafter referred to as high, mid, and low VPD. Box temperature was maintained at 25°C for the different days, while three SON-T lights (type 400 W/2220 E40 1SL, Philips, Eindhoven, The Netherlands) installed at approximately 35 cm above the box provided PAR of 530 μmol m⁻² s⁻¹ measured at the top of the box. Per day, a set of 22 syringes containing 16 treatment and six control leaves in total were randomly installed in the box. The control leaves were used to determine whether any of the \(^{13}\)CO₂ diffused from the treatment leaves to the atmosphere and was subsequently assimilated by the control leaves during the course of the experiment.

Pre-labeling, each leaf initially transpired the KCl solution for 1 h. The solution was then switched for 2 h to the treatment and control solutions. The time of labeling was determined by a preliminary experiment in which leaves from additional Populus x canadensis cuttings were placed in a dye solution. The dye was clearly visible in the veins after 15 min, indicating that 2 h was sufficient for xylem-transported CO₂ to be distributed in the leaves. After 2 h labeling, solutions for the control and treatment leaves were switched back to KCl solution for another 30 min, to allow complete assimilation of the xylem-transported label in leaf tissues.

2.2.3.1 Tissue sampling

After the 2 h labeling, the uptake of \(^{13}\)C (mg) was recorded and calculated as described in McGuire et al. (2009):

\[
^{13}\text{C}_{\text{uptake}} = \left[\text{CO}_2^*\right] \cdot A \cdot w
\]

where \([\text{CO}_2^*]\) was calculated using Henry’s coefficients (Butler, 1991; McGuire & Teskey, 2002) and \(w\) is water uptake of the leaf (ml).

After the final 30 min uptake of KCl solution, leaves were removed from the syringes and immediately frozen in liquid nitrogen and transferred to a freezer at -18°C to stop all metabolic activity.
Each leaf was thawed individually and the portion of the petiole below the foam gasket was removed. All leaves were separated into subsamples of petiole, primary vein, secondary veins and mesophyll. Baselines samples were processed similarly. All leaf subsamples were dried to a constant weight in an oven at 65°C and the dry weight was recorded for scaling the data from stable isotope analysis samples to leaf level. Finally all samples were ground to powder in a ball mill (Mixer Mill MM 200, Retsch, Haan, Germany) for $^{13}$C analysis.

### 2.2.3.2 Isotopic analysis

Ground tissue samples of four leaves per treatment were weighed to μg precision in tin capsules and analyzed by Element Analyzer (ANCA-SL, SerCon, Crew, UK) coupled to an Isotope Ratio Mass Spectrometry (20-20, SerCon, Crew, UK) (EA-IRMA). Baking flour with a $\delta^{13}$C value of -27.01 ‰ (certified by IsoAnalytical, UK) was used as a laboratory reference and all $\delta^{13}$C are expressed relative to VPDB.

Enrichment of the labeled tissues ($\delta^{13}$C$_t$, ‰) was calculated as the difference between the $\delta^{13}$C value of the labeled sample ($\delta^{13}$C$_s$, ‰) and the $\delta^{13}$C value of the baseline sample ($\delta^{13}$C$_b$, ‰) of similar tissue (Bloemen et al., 2013b): 

\[
\delta^{13}\text{C}_t = \delta^{13}\text{C}_s - \delta^{13}\text{C}_b
\]

(2.2)

A of the labeled tissues ($A_t$, %) was calculated as:

\[
A_t = \left( \frac{\delta^{13}\text{C}_t/1000}{1 + \left( \frac{\delta^{13}\text{C}_t/1000}{0.0112372} \right)} \right) \times 0.0112372
\]

(2.3)

Where 0.0112372 is the ratio of $^{13}$C/$^{12}$C of the VPDB standard.

Dry mass of the leaf tissue component subsamples was used to determine the mass proportions of the different leaf components. These mass proportions were used to calculate the amount of $^{13}$C assimilated in each leaf tissue component ($^{13}$C$_t$, mg) based on $A_t$:

\[
^{13}\text{C}_t = A_t \times \text{DM} \times C
\]

(2.4)
where DM is the dry mass of the leaf tissue component (mg) and C is the carbon content of the tissue component (%). The calculated $^{13}\text{C}_t$ was corrected for potential assimilation by the leaves of $^{13}\text{C}$ present in the air in the box, based on observed differences in baseline and control leaf tissue enrichment.

### 2.2.4 Experimental setup $^{11}\text{C}$ labeling

We used positron autoradiography on leaves from the same *Populus x canadensis* cuttings to provide a greater detail on the spatial distribution of tracer assimilation in leaves. Because $^{11}\text{C}$ is a short living isotope, this part of the experiment was performed close to the cyclotron (18/9 Mev, IBA, Belgium). The close distance allowed fast transport of the produced $^{11}\text{C}$ to the INFINITY imaging lab of Ghent University, Gent, Belgium. There, $^{11}\text{CH}_4$ produced from the $(p, \alpha)$ nuclear reaction in the cyclotron on a nitrogen target was oxidized in a synthetic train to yield $^{11}\text{CO}_2$ as described by Landais & Finn (1989). The captured $^{11}\text{CO}_2$ gas was immediately bubbled in 500 mM TRIS buffer containing 0.05 M KOH at a set pH of 7.2 to obtain $^{11}\text{CO}_2$ solution and the leaf was allowed to transpire this solution during the experiment.

Labeling was performed in a cylindrical airtight labeling chamber (135 mm inner diameter and 68 mm height), which was kept just below atmospheric pressure to avoid potential diffusion of $^{11}\text{CO}_2$ out of the chamber. RH and $T_{\text{air}}$ measured inside the labeling chamber and averaged (±SD) over the entire labeling period were 28.4 ± 0.8% and 30.2 ± 0.6°C, respectively. A light source provided a PAR of 926 μmol CO$_2$ m$^{-2}$ s$^{-1}$ at the leaf surface. After leaf excision under water, the leaf was installed in the labeling chamber and the cut end of the petiole was fixed with polysiloxan material (Xantopren, Heraeus Kulzer, GmbH, Hanau, Germany) in a plastic pipette tip filled with non-labeled aqueous TRIS buffer solution and connected to two 3 ml syringes with 0.6 mm tubing. The labeling chamber was closed and one syringe was used to supply the $^{11}\text{CO}_2$ solution at the start of the experiment, while the other syringe was used to replace the non-labeled solution with the labeled solution and to reduce the transfer time of $^{11}\text{CO}_2$ label from the other syringe to the pipette tip, given the short half-life of $^{11}\text{C}$. Two ml of $^{11}\text{CO}_2$ solution was supplied to the leaf with an activity at labeling of 7.42 MBq. The leaf was labeled for 1 h, after which autoradiography was performed.
2.2.4.1 Autoradiography

Distribution of the radioactivity was imaged by positron autoradiography, exposing the leaf by direct contact with an imaging phosphor plate for 5 min, after which the plate was scanned digitally (Cyclone Plus Phosphor imager, Perkin Elmer, Waltham, MA, USA). The autoradiographic image was quantified using OptiQuant (Perkin Elmer, Waltham, MA, USA). Different regions of interest (ROI) were selected in the autoradiogram corresponding with the petiole, primary vein and single secondary veins. A background ROI outside the leaf was used to subtract background scatter from the leaf data. Digital lighting units, which are used to express the ¹¹C decay rate based on autoradiogram analysis, were summed per ROI and divided by the surface area of the ROI.

2.2.5 Statistical analysis

For the ¹³C experiment, we analyzed δ¹³Ct of the treatment leaves, as well as ¹³Ct, using multi-factorial analysis of variance (ANOVA). VPD treatment (n=3) and tissue component (n=4, petiole, primary vein, secondary veins, and leaf mesophyll) were treated as fixed factors and individual leaf (n=4 per VPD treatment) was treated as a random factor. We also used a similar ANOVA model to compare δ¹³Ct in the treatment versus control leaves with leaf type (n=2; control and treatment), VPD treatment (n=3), and tissue component (n=4) treated as fixed factors and individual leaf (n=4) treated as random factor. Solution uptake was analyzed using a similar ANOVA model with only leaf type (n=2) and VPD treatment (n=3) considered as fixed factors and individual leaf as random factor. 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 inc., Cary, NC, USA) with α=0.05.

2.3 Results

2.3.1 ¹³CO₂ uptake

VPD had a significant effect on solution uptake in both treatment (P=0.003) and control (P=0.010) leaves. Therefore in treatment leaves, mean ¹³C uptake (± SE) during the 2 h ¹³CO₂ label uptake period under high (0.93 ± 0.09 mg ¹³C) and mid (0.81 ± 0.09 mg ¹³C)
VPD was significantly higher than under low VPD (0.54 ± 0.05 mg $^{13}$C). In control leaves, the mean uptake of non-labeled solution under high VPD (1.08 ± 0.17 ml) was significantly higher than under mid (0.64 ± 0.22 ml) and low (0.50 ± 0.08 ml) VPD.

2.3.2 $^{13}$C tissue enrichment

Due to differences in $^{13}$C uptake, significant differences in tissue $^{13}$C enrichment ($\delta^{13}$C$_t$) were observed among VPD treatments ($P<0.0001$, Fig. 2.1). The $\delta^{13}$C$_t$ values were significantly higher under high VPD than under mid and low VPD in the petiole, the primary and secondary vein tissues. In contrast, VPD had no significant effect on $\delta^{13}$C$_t$ ($P=0.60$) in leaf mesophyll, which is more distal from the $^{13}$C source than the other tissues. Among leaf tissue components, significant differences in $\delta^{13}$C$_t$ were observed (Fig. 2.1). Petioles were most enriched, regardless of the VPD treatment. For the most distal tissues (secondary veins and leaf mesophyll) no significant differences in $\delta^{13}$C$_t$ were observed. In control leaves, $\delta^{13}$C$_t$ ranged from -0.19 to 2.65‰ across the different VPD treatments, indicating that the isotope composition of control leaves was similar as for baseline leaves. Petiole and primary vein enrichment of control leaves were significantly lower than corresponding tissues of treatment leaves, for all VPD treatments. For leaf mesophyll no significant differences were observed between treatment and control leaves ($P=0.2779$) due to the low tissue enrichment in treatment leaves, regardless of the VPD treatment.
2.3.3 \(^{13}\text{C}\) assimilation

Significant differences in the amount of label assimilated per leaf tissue component (\(^{13}\text{C}_t\)) were observed among VPD treatments for leaf mesophyll (\(P<0.0001\)), primary vein (\(P=0.0002\)) and secondary veins (\(P=0.0001\)), with highest \(^{13}\text{C}_t\) observed under high VPD (Fig. 2.2). For the petiole, \(^{13}\text{C}_t\) was highest under high VPD, but only significantly higher as compared to \(^{13}\text{C}_t\) under low VPD treatment. Because leaf mesophyll constitutes on average 73.20 ± 1.09% of the dry mass of the leaves used in the \(^{13}\text{C}\) experiment, highest \(^{13}\text{C}_t\) was observed for this leaf tissue component, regardless of VPD treatment (Fig. 2.2). Under both high and mid VPD treatment, assimilation levels were significantly different among leaf tissue components, whereas under low VPD treatment, \(^{13}\text{C}_t\) was approximately equal for the different leaf tissue components. The potential amount of \(^{13}\text{C}_t\) derived from the uptake of \(^{13}\text{C}\) of the atmosphere ranged between -0.0002 to 0.0028 mg \(^{13}\text{C}\). In contrast to \(^{13}\text{C}_t\) of treatment leaves, VPD treatment or tissue component had no effect on \(^{13}\text{C}_t\) of control leaf tissues.
2.3.3 ¹¹C autoradiography: detailed analysis of xylem CO₂ assimilation

Positron autoradiography was used to assess the spatial distribution of xylem-transported ¹¹CO₂ assimilation (Fig. 2.3). Clear differences are observed between the leaf vasculature, including the petiole, and the leaf mesophyll tissue: most ¹¹CO₂ accumulated in the vasculature, while only a limited amount of ¹¹CO₂ reached the mesophyll. Summation of the digital lighting units for the petiole, primary vein and the secondary veins recorded in the autoradiogram (Fig. 2.4A) indicate that ¹¹CO₂ accumulated mainly in the petiole and in the major vein, and to a lesser extent in the secondary veins (Fig. 2.4B). Additionally, among secondary veins, there was an important distance-to-source effect on the ¹¹CO₂ distribution, with highest ¹¹C activity observed for the secondary veins closest to the petiole.
Fig. 2.3  Positron autoradiogram showing the distribution of xylem-transported $^{11}$CO$_2$ in an excised poplar leaf (Populus x canadensis Moench ‘Robusta’), 1 h after the $^{11}$CO$_2$ label was administered to the leaf via the petiole.

Fig. 2.4  Distribution of xylem-transported $^{11}$C among petiole, primary vein and secondary veins in an excised poplar leaf (Populus x canadensis Moench ‘Robusta’), 1 h after the $^{11}$CO$_2$ label was administered to the leaf via the petiole. The numbers indicated in the autoradiogram (A) are used to rank the secondary veins in (B). The digital lighting units were summed for the petiole, primary, and the different secondary veins, background subtracted and divided by the area corresponding with the surface area of the different tissue components and single secondary veins considered in (A).
2.4 Discussion

Leaves absorbed both $^{11}\text{CO}_2$ or $^{13}\text{CO}_2$ enriched solution which were both detected in all leaf tissues, but mainly in the petiole and primary and secondary veins. Results from our $^{13}\text{C}$ labeling experiment are in line with previous studies which traced xylem CO$_2$ transport at tree (Bloemen et al., 2013b) and branch (McGuire et al., 2009; Bloemen et al., 2013a) level. We observed that under high vapor pressure deficit (VPD) solution uptake was higher, resulting in a higher label uptake (0.126 ± 0.012 mg $^{13}\text{C}$) than under mid (0.110 ± 0.012 mg $^{13}\text{C}$) and low (0.073 ± 0.006 mg $^{13}\text{C}$) VPD. Similarly, Bloemen et al. (2013a) manipulated, in their study on $P.\text{ deltoides}$ branches, $^{13}\text{CO}_2$ uptake rates by altering the VPD under controlled conditions and found a similar effect of VPD on the transpiration rate and leaf tissue enrichment, both depending on the applied $^{13}\text{CO}_2$ label concentration. Among leaf tissue components, highest tissue enrichment was observed in the petiole and to a lesser extent in primary and secondary veins. Petiole enrichment was also highest among different leaf tissue components after label infusion in the stem base of field-grown $\text{Populus deltoides}$ trees. Moreover, Stringer & Kimmerer (1993) observed that most of a $^{14}\text{C}$ label introduced via the petiole of $P.\text{ deltoides}$ leaves was assimilated in the petiole. Once scaled with leaf tissue dry mass, we found that most $^{13}\text{C}$ was assimilated in leaf mesophyll because almost three-quarter of the dry mass of the $P.\text{ x canadensis}$ leaves used in this study consisted of mesophyll tissue. Also Bloemen et al. (2013a) found that most $^{13}\text{C}$ was assimilated in the leaf mesophyll as compared to other leaf tissue components, regardless of the applied $^{13}\text{CO}_2$ label concentration or VPD treatment.

However, the destructive nature of $^{13}\text{C}$ analysis limits its applicability for detailed detection of xylem CO$_2$ assimilation in leaves. Leaf tissue component samples need to be separated, dried, and milled before $\delta^{13}\text{C}$ determination by isotope ratio mass spectrometry. Moreover, single secondary leaf veins are pooled to obtain enough sample material for $^{13}\text{C}$ analysis. Therefore, the spatial resolution of a $^{13}\text{C}$-based analysis is constrained to the leaf tissue component level. To circumvent this limitation in spatial resolution, we additionally used autoradiographic tracing of $^{11}\text{C}$ to study xylem CO$_2$ assimilation in leaves. In the case of radiocarbon tracing of xylem CO$_2$, previous studies have solely used $^{14}\text{C}$ in combination with autoradiography (Zelawski et al., 1970;
Vapaavuori & Pelkonen, 1985; Vuorinen et al., 1989; Amiro & Ewing, 1992; Stringer & Kimmerer, 1993; Hibberd & Quick, 2002). To date, $^{11}$C has been used almost exclusively for in vivo studies on short-term transfer of photosynthates from leaves to sinks via the phloem (Minchin & Thorpe, 2003; Kawachi et al., 2011b). One study yet observed $^{11}$C movement in the xylem by applying a gaseous $^{11}$C labeled phytohormone to Nicotiana tabacum leaves (Thorpe et al., 2007). We fed a dissolved $^{11}$CO$_2$ label to the petiole and autoradiography showed that the infused $^{11}$C label was transported throughout the leaf and mainly confined to petiole and leaf vein tissue (Fig. 2.3), which confirmed the results obtained with our $^{13}$C leaf labeling. However, in the positron autoradiogram, $^{11}$C distribution in smaller tertiary or even quaternary leaf veins could be detected, while in the $^{13}$C analysis these smaller veins were included in leaf mesophyll due to practical reasons. Moreover, $^{11}$C autoradiography allows a detailed analysis of label distribution, even within a single leaf tissue component. For instance, we observed among secondary veins differences in radioactivity, with highest label activity in the veins closest to the petiole, implying a variation in the assimilation of xylem-transported CO$_2$ (Fig. 2.4). In addition, positron autoradiography showed that xylem-transported $^{11}$CO$_2$ was able to reach the leaf mesophyll. This was unclear from the data derived from the $^{13}$C tissue analysis from a statistical standpoint, because of the lack of significant enrichment in the leaf mesophyll of the treatment leaves compared to the control ones, regardless of VPD treatment. Therefore, we believe that $^{11}$C tracing in plants might help us to better understand plant processes in the context of future studies on xylem CO$_2$ assimilation, by allowing a more detailed visualization of the fate of xylem-transported CO$_2$ in plants. However, the isotopes’ radioactive nature, its short-half life and the experimental cost related to its production limit the wide scale application of $^{11}$C.

The role of xylem-transported CO$_2$ as carbon source for photosynthetically active cells surrounding the xylem vasculature in petiole and leaf veins is intriguing. One of the main functions of leaf venation is to efficiently distribute water and solutes among the entire leaf surface (Roth-Nebelsick et al., 2001; Brodribb et al., 2010). From the leaf veins, xylem-transported CO$_2$ enters the bundle sheath cells (Sack & Holbrook, 2006) where chloroplasts use this respiratory CO$_2$ as substrate (Leegood, 2008). Photosynthetic activity in these bundle sheath cells has long been related to the origins of the C$_4$
pathway. However, Hibberd & Quick (2002) suggested that in C₃ plants a C₄-like carbon concentrating mechanism might exist in which these chlorophyll containing cells are supplied with respiratory carbon from the vascular system and not with atmospheric carbon from stomata (Chapter 1). They found that a ¹⁴C label supplied to the roots and stems of tobacco and celery was assimilated in photosynthetic cells adjacent to the xylem tissue (Hibberd & Quick, 2002). Similarly, Stringer & Kimmerer (1993) performed a labeling experiment on *P. deltoides* leaves and observed assimilation of xylem-transported CO₂ in the veins, mainly by Ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco) and in a lesser extent by Phosphoenolpyruvate carboxylase (PEPc). Janacek et al. (2009) found for Arabidopsis that respired CO₂ is the sole substrate source for cells adjacent to the leaf veins and that sugars and carbon skeletons synthesized in these cells are important for phloem transport and the Shikimate pathway, which is described as the biosynthetic route to aromatic amino acids (Herrmann & Weaver, 1999 and references therein). Moreover, the location of the bundle sheath cells, adjacent to the phloem strands, facilitates a direct loading of synthesized sugars into the phloem tissue. Therefore, assimilation of respired CO₂ in bundle sheath cells of leaves might have important implications for the physiology of the entire plant.

In particular for plants with heterobaric leaves, which is the case for poplar, dissolved CO₂ transport via the dense vein network might be an important means for redistributing substrate for photosynthetic reactions (Bloemen et al., 2013a). In these leaves, lateral diffusion of gaseous CO₂ is restricted due to the strong compartmentalization of the leaf by bundle sheath extensions (McCleland, 1992; Pieruschka et al., 2005). Lynch et al. (2012) observed that CO₂ refixation in heterobaric leaves might contribute to the difference in isotopic composition of different leaf sections, because isotopic signatures of respired CO₂ are either depleted or enriched relative to the substrate (Ghashghaie et al., 2003) or the tissues (Bowling et al., 2008), respectively. They observed an isotopic depletion in leaf tissue δ¹³C near the main vein as compared to tissue near the leaf margin (Lynch et al., 2012) which they related to a potential assimilation of respired CO₂. However, the role of assimilation of respired CO₂ in cells surrounding the vasculature in leaves might extend beyond maintaining the plant carbon status at leaf level. Griffiths et al. (2013) hypothesized that bundle sheaths
might play a central role in cavitation repair in leaves, by contributing to the considerable energetic demand for cavitation repair (Zwieniecki & Holbrook, 2009; Secchi & Zwieniecki, 2011). Refilling of embolized vessels requires a source of water and the release of energy stored in parenchymatous cells (Secchi & Zwieniecki, 2011). Given its close proximity to the xylem vasculature, bundle sheath cells might provide the sugars respired by living cells which are used to meet the energetic requirements to refill vessels under tension (Zwieniecki & Holbrook, 2009). This is consistent with their previously observed role in protecting the leaf mesophyll against water deficit (Terashima, 1992; Leegood, 2008). At branch and stem level, assimilation of xylem-transported CO$_2$ in xylary chloroplasts has been found to maintain hydraulic conductivity by repairing embolized xylem vessels (Schmitz et al., 2012) (Box 1.2). However, the hypothesis that CO$_2$ assimilation in the bundle sheath might play a crucial role in the local plant water status still needs to be tested experimentally.

In conclusion, stable and unstable isotope carbon tracing of xylem CO$_2$ transport reveals a wealth of information on the quantification and imaging of xylem CO$_2$ assimilation and might serve as useful tool to unravel its role in plant functioning. While in $^{14}$C tracing studies radiation safety is of important concern in particular regarding the disposal of radioactive waste, our results show that the short-living $^{11}$C tracer can be used to visualize xylem CO$_2$ transport in plants. Moreover, in vivo real-time imaging of xylem CO$_2$ transport in entire plants is potentially possible, given the recent advances in positron emission tomography (PET) $^{11}$C detection (e.g. Beer et al., 2010; Kawachi et al., 2011b). Therefore, a $^{11}$C labeling approach, recently used to trace carbon partitioning in leaves (Dirks et al., 2012), might be a next step in studying xylem CO$_2$ transport in plants. This will broaden our current knowledge on plant physiology and deepen our understanding on the significance of xylem CO$_2$ transport in maintaining the carbon and water status of plants.
Chapter 3
Xylem [CO$_2$] and transpiration rate affect xylem-transported CO$_2$ assimilation


**Abstract**

The effect of transpiration rate on internal assimilation of CO$_2$ released from respiring cells has not previously been quantified. In this study, detached branches of *Populus deltoides* were allowed to take up $^{13}$CO$_2$-labeled solution at either high (high label, HL) or low (low label, LL) $^{13}$CO$_2$ concentrations. The uptake of the $^{13}$CO$_2$ label served as a proxy for the internal transport of respired CO$_2$, while the transpiration rate was manipulated at the leaf level by altering the vapor pressure deficit (VPD) of the air. Simultaneously, leaf gas exchange was measured, allowing comparison of internal CO$_2$ assimilation to that assimilated from the atmosphere. Subsequent $^{13}$C analysis of branch and leaf tissues revealed that woody tissues assimilated more label under high VPD, corresponding with higher transpiration, than under low VPD. More $^{13}$C was assimilated in leaf tissue than in woody tissue under the HL treatment, whereas more $^{13}$C was assimilated in woody tissue than in leaf tissue under the LL treatment. The ratio of $^{13}$CO$_2$ assimilated from the internal source to CO$_2$ assimilated from the atmosphere was highest for the branches under the HL and high VPD treatment, but was relatively small regardless of VPD x label treatment combination (up to 1.9%). Our results show that assimilation of internal CO$_2$ is highly dependent on the rate of transpiration and xylem sap [CO$_2$]. Therefore, it can be
expected that the relative contribution of internal CO$_2$ recycling to tree carbon gain is strongly dependent on factors controlling transpiration, respiration, and photosynthesis.

3.1 Introduction

A portion of CO$_2$ 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 & Kozlowski, 1979; Lendzian, 2006; Sorz & Hietz, 2006; Steppe et al., 2007), resulting in internal CO$_2$ concentrations ([CO$_2$]) that substantially exceed atmospheric [CO$_2$] (Teskey et al., 2008; McGuire et al., 2009) (Chapter 1).

The importance of accounting for internal transport of CO$_2$ to accurately assess estimates of respiration based on measurements of CO$_2$ efflux has been previously described (e.g. Levy et al., 1999; Teskey & McGuire, 2007; Saveyn et al., 2008b). CO$_2$ originating from respiration both below- (Aubrey & Teskey, 2009; Grossiord et al., 2012; Bloemen et al., 2013b) and aboveground (Teskey & McGuire, 2007) can dissolve in xylem sap and be transported upward via the transpiration stream from the stem base to the top of the canopy (Bloemen et al., 2013b), and can diffuse into the atmosphere via aboveground tissues remote from the site of respiration (Teskey & McGuire, 2007) (Chapter 1).

Along the transport pathway, this internal CO$_2$ can be assimilated by active chloroplasts within the stem, branches and leaves (McGuire et al., 2009; Saveyn et al., 2010), thereby contributing to the whole-plant carbon gain. In woody tissues, chloroplasts occur both in the xylem and inner bark which justifies the use of the term woody tissue photosynthesis instead of bark or corticar photosynthesis when referring to stem CO$_2$ assimilation (Saveyn et al., 2010). In the leaves, it is believed that xylem-transported CO$_2$ is mainly fixed in the cells surrounding the xylem vasculature in the petioles and near the veins (Stringer & Kimmerer, 1993; McGuire et al., 2009). McGuire et al. (2009) found that assimilation of xylem-transported $^{13}$C-labeled CO$_2$ (referred to as $^{13}$CO$_2$ in the text), supplied in high concentrations as a tracer, averaged 6% of the assimilation of atmospheric CO$_2$ by the leaves in detached sycamore branches. Given that respired CO$_2$ is re-distributed by the transpiration stream (Chapter 1), it might be expected that at a
high transpiration rate and/or at high xylem sap [CO₂] a larger amount of CO₂ could be transported in the xylem towards photosynthetically active tissues in woody organs and leaves. Therefore, transpiration rate and xylem sap [CO₂] might be critical factors to consider when assessing assimilation of internally transported CO₂ in the context of whole-plant carbon cycling.

However, to our knowledge, the combined effect of transpiration and xylem sap [CO₂] on the assimilation of xylem-transported CO₂ 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 ^13^CO₂ while the transpiration rate was manipulated by varying the atmospheric vapor pressure deficit (VPD). Simultaneous leaf gas exchange measurements allowed us to compare the contributions of assimilation of internally transported CO₂ and atmospheric CO₂. We hypothesized that more ^13^C label would be assimilated in chlorophyll-containing woody and leaf tissues under the high [^13^CO₂] solution treatment and at high transpiration because a greater quantity of ^13^C-labeled substrate would be transported in those conditions.

### 3.2 Material and methods

#### 3.2.1 Plant material

Branches were cut from *P. deltoides* trees that were part of a 7-y-old plantation in Whitehall Forest, a research facility of the University of Georgia near Athens, Georgia, USA. Branches (length >2m) 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 lab, lateral segments of these branches that were between 20-50 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.

#### 3.2.2 Baseline sampling

Prior to the start of the labeling experiment, baseline samples were taken from the same trees to determine the natural abundance carbon isotopic composition (δ^13^C) to which
δ13C 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 immediately frozen in liquid nitrogen and then moved to a freezer at -9°C for storage before processing.

3.2.3 Experimental setup

The control solution (10 l in total) enriched with 13CO2 at natural abundance and atmospheric concentration was prepared as described in Chapter 2. The 13CO2 treatment solutions were enriched with 99 atom% (A) 13CO2 either at a high (high label, hereafter referred to as HL) or a low (low label, hereafter referred to as LL) concentration to account for the variation in internal [CO2] generally observed within trees. 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 A 13CO2 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. Solutions were amended with sodium bicarbonate to control pH and achieve and dissolved CO2 concentration ([13CO2*]) target according to modeled [CO2*] at a set pH (Erda et al., 2013). [CO2] and [13CO2*] concentration were 8.9%, and 3 mM for the LL solution and 18.5%, and 13 mM for the HL solution. Based on preliminary measurements, we assumed that the [CO2] of the HL and LL solutions represented high and low xylem sap [CO2], respectively. The pH of the HL and LL solutions were 6.4 and 4.8, respectively, which is within the range measured previously in P. deltoides (Aubrey & Teskey, 2009; Aubrey et al., 2011). On the first day (16 July), the experiment was performed with ten branches using the LL solution, while 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 CO2. 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 CO2 to the atmosphere. Bottles were placed in two growth chambers (Model GC36, Environmental Growth Chambers, Chagrin Falls, OH, USA) where different vapor pressure deficit (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-labeled solution were placed in each chamber to determine if any of the $^{13}$CO$_2$ 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$^{-2}$ s$^{-1}$) 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 labeled solution had reached the leaves. Similar as in Chapter 2, this time period was determined through a preliminary dye experiment. For the P. deltoides branches, the dye was clearly visible in the leaves within 30 min at low VPD, indicating that one hour was more than sufficient for xylem-transported CO$_2$ to reach the leaves under both VPD treatments.

### 3.2.4 Leaf gas exchange measurements

Leaf net photosynthesis ($A_{net}$, μmol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance ($g_s$, mol H$_2$O m$^{-2}$ s$^{-1}$), and transpiration ($E$, mmol H$_2$O m$^{-2}$ s$^{-1}$) were measured on a fully expanded lower leaf of all treatment and control branches with a portable photosynthesis system (model Li-6400, Li-Cor, Inc., Lincoln, Nebraska, USA). Measurements were repeated four times over an eight hour period at prevailing growth chamber temperature, light, and humidity conditions and a set atmospheric [CO$_2$] 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.
3.2.5 Tissue sampling for $^{13}$C 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 $^{13}$C (mg) was calculated as described in Chapter 2 (Equation 2.1).

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 (LA) of each branch section was measured with a LA meter (Model Li-3100, Li-Cor Inc., Lincoln, Nebraska, USA). Mean total LA per branch (±SE) was 1109.1 ± 56.7 cm². 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 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 powder in a ball mill (8000-D Mixer Mill, SPEX SamplePrep, Metuchen, New Jersey, USA) for carbon isotope analysis.

3.2.6 Isotopic analysis of samples

Ground tissue samples of four leaves per treatment were weighed to μg precision in tin capsules and analyzed by Element Analyzer (model 1500 CHN, Carlo-Erba, Italy) coupled to an Isotope Ratio Mass Spectrometry (Delta V, Thermo-Finnigan, Bremen, Germany) (EA-IRMS) at the Stable Isotope and Soil Biology Laboratory (SISBL), Odum School of Ecology, University of Georgia, Athens, Georgia, USA. Bovine with a $\delta^{13}$C value
of -21.24 ‰ (certified by the National Institute of Standards and Technology, USA) was used as a laboratory reference and all δ¹³C are expressed relative to VPDB.

Enrichment of the labeled branch tissues (δ¹³Ct, ‰) and A of the labeled tissues (Aₜ, %) was calculated as described in Chapter 2 (Equations 2.2 and 2.3).

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

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 Aₜ, the amount of ¹³C assimilated in each tissue component (¹³Ct, mg) was calculated for each branch as:

\[
¹³C_t = A_t \times DM \times C
\]

(3.1)

where DM is the dry mass of the tissue per section (mg) and C is the carbon content of the tissue component (%). Mean ¹³Ct was calculated for each label × VPD treatment combination.

### 3.2.8 Ratio of ¹³C assimilation to atmospheric CO₂ assimilation

To obtain the total amount of atmospheric carbon assimilated via photosynthesis (A atm, g) during the period of label uptake, \( A_{\text{net}} \) (μmol CO₂ m⁻² s⁻¹) averaged for the entire measurement period was scaled to the branch level:

\[
A_{\text{atm}} = A_{\text{net}} \times LA \times 10^{-6} \times A(C) \times t
\]

(3.2)

where LA is the total leaf area of all branch sections (m²), A(C) is the atomic weight of carbon (12 g mol⁻¹) and t is the time of CO₂-enriched solution uptake (s).

To determine the ratio of total amount of ¹³C assimilated in a branch (¹³C assim, g) to A atm (¹³C assim / A atm, %), ¹³Ct was summed for all the sections and tissues and divided by A atm:

\[
\text{Ratio} = \frac{¹³C_{\text{assim}}}{A_{\text{atm}}}
\]

(3.3)
3.2.9 Data processing and statistical analysis

We analyzed δ^{13}C_t of the woody and leaf tissue components of the treatment branches, as well as ^{13}C_t, using multi-factorial analysis of variance (ANOVA). For the woody tissues, ^{13}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^{13}C_t in the treatment versus control branches with branch type (n=3; control, HL, and LL), ^{13}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 random factor. Ratios of ^{13}C_{assim} to A_{atm} were analyzed using an ANOVA model similar to that used for δ^{13}C_t, but with ^{13}C label concentration (n=2) and VPD treatment (n=2) used as fixed factors and individual branch (n=5) treated as random factor. Solution uptake was analyzed using a similar ANOVA model with only branch type (n=3) and VPD treatment (n=2) considered as fixed factors. Average A_{net}, g_s and E were analyzed using a repeated measures ANOVA model with ^{13}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 (AICc) 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 inc., Cary, NC, USA) with α=0.05.

3.3 Results

3.3.1 Uptake of CO₂ enriched solution

Solution uptake was influenced by VPD (P<0.0001), but not by label treatment (P=0.179). Mean uptake of the ^{13}CO₂ enriched solution for all treatment branches at high VPD was 144.0 ± 11.0 ml which was significantly higher than the uptake at low VPD (61.5 ± 5.2 ml). Similar results were observed for the amount of solution taken up by
the control branches (148.8 ± 11.8 ml and 82.5 ± 6.3 ml under high and low VPD treatment, respectively).

3.3.2 Carbon isotope composition of woody and leaf tissue components

The $^{13}$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 × branch section × tissue component interaction, $P<0.0001$, and a label concentration × VPD treatment × 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. 3.1). 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. 3.1). Averaged across xylem and inner bark tissues, mean $\delta^{13}$C$_{t}$ of the woody tissue was higher under the high label (HL) treatment than under the low label (LL) treatment at the mid- (305.22 ± 24.23‰ vs. 122.18 ± 22.93‰) and upper-branch section (223.88 ± 14.89‰ vs. 21.50 ± 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 ± 0.07‰ and 2.72 ± 0.84‰ and was substantially lower than the $\delta^{13}$C$_{t}$ observed for the woody tissue components of the treatment branches ($P<0.0001$).
Fig. 3.1  Mean tissue enrichment ($\delta^{13}C_t$, ‰) of xylem (A) and inner bark (B) of three equal-length sections of five branches under high (black) or low (hatched) atmospheric vapor pressure deficit (VPD) averaged for high and low $^{13}$CO$_2$ concentration solution. $\delta^{13}C_t$ was calculated by subtracting the $\delta^{13}C_t$ value of baseline samples of non-labeled tissue from the $\delta^{13}C_t$ value of the enriched sample of the same tissue. Different lower case letters indicate significant differences (P<0.05) in $\delta^{13}C_t$ 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. Bars indicate standard error of the mean.

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, $\delta^{13}C_t$ 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 × tissue component × branch section × label concentration interaction, $P=0.0166$). For example, under the HL treatment, the average $\delta^{13}C_t$ of petiole (Fig. 3.2A),
primary vein (Fig. 3.2B), secondary veins (Fig. 3.2C), and leaf mesophyll (Fig. 3.2D) 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. 3.2A). 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. 3.2). Under the low VPD treatment, the $\delta^{13}C_t$ of the petiole (Fig. 3.2A) 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. 3.2B,C,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 $\delta^{13}C_t$ 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 as 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 ± 0.07‰ and 3.39 ± 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 ($P<0.0001$).
Fig. 3.2 Mean tissue enrichment (δ\(^{13}\)C, ‰) 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 either high \(^{13}\)CO\(_2\) concentration solution (high label, HL) under the high (black) or low (white hatched) atmospheric vapor pressure deficit (VPD) treatment, or low \(^{13}\)CO\(_2\) concentration solution (low label, LL) under the high (grey) or low (grey hatched) VPD treatment. δ\(^{13}\)C\(_t\) was calculated by subtracting the δ\(^{13}\)C value of baseline samples of non-labeled tissue from the δ\(^{13}\)C value of the enriched sample of the same tissue. Different lower case letters indicate significant differences (P<0.05) in δ\(^{13}\)C\(_t\) of tissues among the four label x VPD treatment combinations within each branch section. Different upper case letters indicate significant differences in δ\(^{13}\)C\(_t\) among different branch sections within tissue component and label and VPD treatment combinations. Bars indicate standard error of the mean.
3.3.3 $^{13}$C assimilation

Generally, more $^{13}$C was assimilated ($^{13}$C$_t$) in leaf tissue than in woody tissue under the HL treatment, whereas the opposite pattern was observed under the LL treatment (Table 3.1). The $^{13}$C$_t$ 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., a VPD treatment $\times$ tissue component $\times$ branch section $\times$ label concentration interaction, $P=0.0137$). Under the HL treatment, $^{13}$C$_t$ of the inner bark, petiole, and mesophyll was higher under the high VPD treatment than under the low VPD treatment, whereas $^{13}$C$_t$ 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 3.1). Averaged across VPD and label concentration treatments most assimilation of $^{13}$C in woody tissue occurred in the inner bark ($65.07 \pm 4.57\%$). Averaged across VPD and label concentration treatments, most $^{13}$C assimilation in leaves occurred in the mesophyll compared to the petiole, primary vein and secondary vein (Table 3.1). Under the HL treatment, $^{13}$C$_t$ of the petioles and mesophyll was higher at high VPD than at low VPD in all branch sections, whereas a VPD effect on $^{13}$C$_t$ 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, while it is assumed that the remainder diffused to the atmosphere. Regardless of VPD and label treatment, $^{13}$C$_t$ of the woody and leaf tissue of the control branches was lower than $^{13}$C$_t$ of woody and leaf tissue of the treatment branches ($P<0.0001$).
Table 3.1  Mean $^{13}$C assimilation ($^{13}$Ct, mg) (SE) in woody and leaf tissue components of five branches allowed to transpire solutions with dissolved $^{13}$CO$_2$ at high (high label: HL) or low (low label: LL) concentrations and at high or low atmospheric vapor pressure deficit (VPD). Significant differences between all label × VPD treatment combinations for both woody and leaf tissue components are indicated ($P<0.05$). $^{13}$Ct and standard errors displayed as 0.00 (0.00), respectively, were not zero, but have been truncated due to rounding.

<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>Branch</th>
<th>Xylem</th>
<th>Inner bark</th>
<th>Leaf</th>
<th>Primary vein</th>
<th>Secondary vein</th>
<th>Mesophyll</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Xylem branch</td>
<td></td>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yulem</td>
<td></td>
<td>Inner bark</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylem</td>
<td></td>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylem</td>
<td></td>
<td>vein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylem</td>
<td></td>
<td>Secondary</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Xylem</td>
<td></td>
<td>vein</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Xylem</td>
<td></td>
<td>Mesophyll</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylem</td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL × High VPD</td>
<td>0.39$^a$</td>
<td>0.93$^a$</td>
<td>0.75$^b$</td>
<td>0.01$^a$</td>
<td>0.00$^a$</td>
<td>0.82$^c$</td>
<td>2.9$^b$</td>
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</tr>
<tr>
<td></td>
<td>(0.11)</td>
<td>(0.16)</td>
<td>(0.14)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.16)</td>
<td>(0.47)</td>
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</tr>
<tr>
<td>LL × Low VPD</td>
<td>0.11$^b$</td>
<td>0.22$^c$</td>
<td>0.01$^c$</td>
<td>0.00$^a$</td>
<td>0.00$^a$</td>
<td>0.14$^c$</td>
<td>0.48$^c$</td>
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</tr>
<tr>
<td></td>
<td>(0.02)</td>
<td>(0.05)</td>
<td>(0.02)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.02)</td>
<td>(0.06)</td>
<td></td>
</tr>
<tr>
<td>HL × High VPD</td>
<td>0.60$^a$</td>
<td>1.15$^a$</td>
<td>1.63$^a$</td>
<td>0.18$^a$</td>
<td>0.15$^a$</td>
<td>5.11$^a$</td>
<td>8.11$^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.12)</td>
<td>(0.14)</td>
<td>(0.20)</td>
<td>(0.03)</td>
<td>(0.01)</td>
<td>(0.59)</td>
<td>(0.64)</td>
<td></td>
</tr>
<tr>
<td>HL × Low VPD</td>
<td>0.41$^a$</td>
<td>0.69$^b$</td>
<td>0.69$^b$</td>
<td>0.09$^a$</td>
<td>0.05$^a$</td>
<td>1.76$^b$</td>
<td>3.69$^b$</td>
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<tr>
<td></td>
<td>(0.08)</td>
<td>(0.13)</td>
<td>(0.10)</td>
<td>(0.01)</td>
<td>(0.00)</td>
<td>(0.28)</td>
<td>(0.33)</td>
<td></td>
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</tbody>
</table>

### 3.3.4 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 leaf net photosynthesis ($A_{net}$) and stomatal conductance ($g_s$) averaged over all measurements were similar under both VPD treatments (Table 3.2). However, for E, $A_{net}$, and $g_s$, both treatment responses changed through time (i.e., a VPD treatment × time interaction for E, $P<0.0001$; a VPD treatment × time interaction for $A_{net}$, $P=0.0363$; and a VPD treatment × time interaction for $g_s$, $P<0.0001$). Under both the high and the low VPD treatment, E and $A_{net}$ tended to decrease slightly over time. At the last measurement time $A_{net}$ 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.
Table 3.2  Average (SE) transpiration (E, mmol H$_2$O m$^{-2}$ s$^{-1}$), net photosynthesis ($A_{net}$, μmol CO$_2$ m$^{-2}$ s$^{-1}$), and stomatal conductance ($g_s$, mol H$_2$O vapor m$^{-2}$ s$^{-1}$) measured on leaves of branches allowed to transpire solutions with dissolved $^{13}$CO$_2$ at high (high label: HL) or low (low label: LL) concentrations and at high or low vapor pressure deficit (VPD). Data are averages of four measurements performed at two-hour intervals during an eight hour 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 $^{13}$C label treatments. Standard errors displayed as 0.00 (0.00), respectively, were not zero, but have been truncated due to rounding.

<table>
<thead>
<tr>
<th>Label treatment</th>
<th>LL</th>
<th>HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPD</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>E</td>
<td>2.57$^{a}(0.02)$</td>
<td>1.14$^{b}(0.03)$</td>
</tr>
<tr>
<td>$A_{net}$</td>
<td>8.98$^{a}(0.15)$</td>
<td>7.90$^{a}(0.22)$</td>
</tr>
<tr>
<td>$g_s$</td>
<td>0.15$^{a}(0.00)$</td>
<td>0.13$^{a}(0.00)$</td>
</tr>
</tbody>
</table>

3.3.5 Assimilation of internally transported CO$_2$ vs. atmospheric CO$_2$ assimilation

The ratio of total $^{13}$C label assimilation to atmospheric CO$_2$ assimilation ($^{13}$C$_{assim}$/A$_{atm}$) was affected by the VPD and label concentration treatments. The ratio was higher under high VPD than under low VPD (Fig. 3.3), 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 not significantly different between HL and LL treatments (label concentration × VPD treatment interaction, $P=0.046$, Fig. 3.3).
Fig. 3.3  Mean ratio of total assimilation of $^{13}$C label in branches (both woody and leaf tissues) to total atmospheric CO$_2$ assimilation by leaves ($^{13}$C$_{assim}$/A$_{atm}$, %), of five branches allowed to transpire solutions with dissolved $^{13}$CO$_2$ at high or low concentrations and at high (black or low (hatched) vapor pressure deficit (VPD). Different lower case letters indicate significant differences ($P<0.05$) in the ratio of $^{13}$C assimilation to atmospheric CO$_2$ assimilation between VPD treatments within $^{13}$CO$_2$ concentration treatment. Different upper case letters indicate significant differences between $^{13}$CO$_2$ concentration treatments within VPD treatment. Bars indicate standard error of the mean.

3.4 Discussion

Our results show that transpiration and xylem sap [CO$_2$] both affect the quantity of xylem-transported CO$_2$ that is assimilated by woody and leaf tissues. Higher $^{13}$C tissue enrichment ($\delta^{13}$C$_t$) and a larger quantity of assimilated $^{13}$C ($^{13}$C$_t$) were found in branch and leaf tissues of the branches subjected to higher transpiration rate and higher xylem sap CO$_2$ concentration ([CO$_2$]). A similar effect of transpiration rate on $\delta^{13}$C$_t$ and $^{13}$C$_t$ was observed for leaves as described in Chapter 2. The results for the branches were not surprising since the amount of label taken up by the branches was greater under high transpiration rate and high label (25.4 ± 2.4 mg $^{13}$C) relative to the other VPD x label treatment combinations (up to 10.7 ± 1.7 mg $^{13}$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 $\delta^{13}$C$_t$ found in the control branches relative to the treatment branches confirmed that stomatal uptake of $^{13}$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 solely due to the assimilation of xylem-transported $^{13}$C label.

Besides its role in plant water supply, the transpiration stream is also considered as an important sink for internally-transported CO$_2$ derived from plant respiration below- (Grossiord et al., 2012; Aubrey & Teskey, 2009; Bloemen et al., 2013b) and aboveground (Teskey & McGuire, 2007) (Chapter 1). It has been suggested that a substantial fraction of root-respired CO$_2$ 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 into the atmosphere from stem and branch surfaces (McGuire et al., 2007; Teskey & McGuire, 2007; Saveyn et al., 2008b).

We observed that at higher transpiration, more $^{13}$CO$_2$ was transported in the branches, thereby increasing the amount of $^{13}$C-labeled substrate available for assimilation in woody and leaf tissue. Assimilation occurs both in the xylem and inner bark of woody tissues (Pfanz et al., 2002; Aschan & Pfanz, 2003). While McGuire et al. (2009) estimated that CO$_2$ assimilation in the xylem accounted for 42% of woody tissue photosynthesis; we found an average contribution of 35% in small branches. The highest $\delta^{13}$C$_t$ was observed in the inner bark of the lower branch sections, likely due to its proximity to the $^{13}$C source. However, in contrast to previous observations (McGuire et al., 2009), $\delta^{13}$C$_t$ of the xylem showed only small variation over the length of the branch. It is possible that our longer period of label application (10 h), compared to 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 $^{13}$C label applied to branches was assimilated in the bottom section of the branch xylem, while in our experiment we observed only a small variation in assimilation in the xylem along the branch. The concentration of the $^{13}$C label provided at the lowest branch section was very high in our experiment, which suggests that the enzymes which fix the $^{13}$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 upward and assimilated higher in the branch. Thus, the longer exposure time in our experiment would result in transfer of more label to the higher branch sections, and 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 $^{13}$C infusion was observed for the same species in a whole-tree experiment in the field (Bloemen et al., 2013b) (Chapter 4). However, in whole trees a larger proportion of $^{13}$C tracer diffused to the atmosphere (up to 94%, Bloemen et al., 2013b) than in this study (up to 55%), likely 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, higher $\delta^{13}$C$_t$ was observed compared to other tissues at both high and low transpiration rate and independent of label concentration. Stringer & Kimmerer (1993) found that 53.7% of a $^{14}$C label applied to P. deltoides leaves via the xylem was assimilated in the petiole. Hibberd & Quick (2002) noted that photosynthetic cells in the petioles increased plant carbon gain, and related petiole photosynthesis to the possible existence of a C$_4$-like mechanism in C$_3$ plants. In our study, transpiration rate determined enrichment in veins and leaf mesophyll only in the HL treatment. At higher transpiration, a larger fraction of the $^{13}$C label was fixed in leaf veins and mesophyll tissue. High levels of $^{13}$C$_t$ were found in branches subjected to the HL treatment under high VPD and in contrast to previous studies (Stringer & Kimmerer, 1993; McGuire et al., 2009), more $^{13}$C was assimilated in the leaf mesophyll (5.1 ± 0.6 mg) than in the petioles (1.6 ± 0.2 mg).

Xylem-transported CO$_2$ has generally been considered as an important potential carbon source only for cells that surround the xylem and phloem in petioles and veins (Stringer & Kimmerer, 1993; McGuire et al., 2009). Our results show that at higher xylem sap [CO$_2$], internally transported CO$_2$ 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 & Evert, 1984). Thus, internally transported CO$_2$ can be delivered very effectively to every part of the leaf which may compensate for the lack of lateral CO$_2$ diffusion due to compartmentalization of the mesophyll in leaves with heterobaric anatomy (Pierschka et al., 2005; Leegood, 2008), which is the case for poplar leaves (McClendon, 1992).
Only a few isotope studies on detached leaves (Stringer & Kimmerer, 1993) and branches (McGuire et al., 2009) have reported on the assimilation of internally-transported CO₂ relative to the assimilation of atmospheric CO₂. McGuire et al. (2009) estimated that internal CO₂ assimilation averaged 6% of atmospheric uptake in branches, while Stringer and Kimmerer (1993) estimated that in leaves, input of CO₂ via the transpiration stream could account for 2.2% of atmospheric uptake. We observed that both a higher transpiration rate and higher xylem sap [CO₂] increased the contribution of xylem-transported CO₂ to branch carbon gain. There are other factors, not considered in this study, which might increase the ratio of internal compared to atmospheric CO₂ assimilation (McGuire et al., 2009). For example, the branches used in this experiment had a non-leaf mass to leaf mass ratio smaller than one, while 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 CO₂ than observed in this study. Our ¹³C based estimates of internal CO₂ assimilation relative to atmospheric uptake do not account for the re-assimilation of locally respired CO₂, which is composed almost entirely of ¹²CO₂. Finally, in contrast to our labeling experiment, in the field the amount of dissolved respired CO₂ available for internal CO₂ assimilation will vary, depending on factors affecting respiration and transpiration rate. All these factors could potentially affect the importance of assimilation of internal CO₂ in woody tissue, however our results indicate that the overall amount of xylem-transported CO₂ that is assimilated is small compared to uptake of atmospheric CO₂.

Previous studies have described the potential benefits of internal CO₂ assimilation from a water-use perspective. While leaves open their stomata to assimilate CO₂, thus subjecting plants to the ambient drying power of the atmosphere, CO₂ for internal assimilation is supplied endogenously by respiration, thereby avoiding excessive water loss (Pfanz et al., 2002; Aschan & Pfanz, 2003). Our results showed that at high transpiration rate, the assimilation of xylem-transported CO₂ increased in importance compared to low transpiration rate. Therefore, we suggest that the assimilation of xylem-transported CO₂ in woody and leaf tissues is strongly dependent on transpiration,
which is directly related to stomatal conductance. Beyond a certain threshold, stomatal conductance will exponentially decrease with increasing VPD (Massmann & Kaufmann, 1991; McCaughey & Iacobelli, 1994; Monteith, 1995; Oren et al., 1999) reducing the assimilation of atmospheric CO₂ (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 CO₂ will reduce, but xylem-transport of respired CO₂ 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 CO₂ 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) (Box 1.2), thereby reducing moisture stress and stomatal closure.

Our results show that assimilation of xylem-transported CO₂ is affected by both the rate of transpiration and xylem [CO₂], which is dependent on respiration. Therefore, the contribution and importance of internal CO₂ assimilation to overall plant carbon gain is likely to change with changes in the factors that control rates of transpiration, respiration, and photosynthesis.
Xylem transport of belowground respired CO₂ affects aboveground carbon assimilation and CO₂ efflux


**Abstract**

Upward transport of CO₂ via the transpiration stream from below- to aboveground tissues occurs in tree stems. Despite potentially important implications to our understanding of plant physiology, the fate of internally transported CO₂ derived from autotrophic respiratory processes remains unclear. We infused a ¹³CO₂-labeled aqueous solution into the base of 7-y-old field-grown eastern cottonwood (*Populus deltoides* Bartr. ex Marsh) trees to investigate the effect of xylem-transported CO₂ derived from the root system on aboveground carbon assimilation and CO₂ efflux. The ¹³C label was transported internally and detected throughout the tree. Up to 17% of the infused label was assimilated, while the remainder diffused to the atmosphere via stem and branch efflux. The largest amount of assimilated ¹³C was found in branch woody tissues, while only a small quantity was assimilated in the foliage. Petioles were more highly enriched in ¹³C than other leaf tissues. Our results confirm a recycling pathway for respired CO₂ and indicate that internal transport of CO₂ from the root system may confound the interpretation of efflux-based estimates of woody tissue respiration and patterns of carbohydrate allocation.
4.1 Introduction

The exchange of carbon between terrestrial ecosystems and the atmosphere has been the subject of many studies (e.g. Baldocchi et al., 2005; Luyssaert et al., 2007; Luyssaert et al., 2010). Carbon dioxide (CO₂) is assimilated in ecosystems by photosynthesis, expressed as gross primary productivity, and is returned to the atmosphere by a variety of metabolic processes both above- and belowground, which comprise total ecosystem respiration (Trumbore, 2006). Belowground, the respiratory processes that contribute to soil CO₂ efflux ($E_{soil}$) are functionally divided into autotrophic (CO₂ released by roots and associated rhizosphere organisms) and heterotrophic (CO₂ released during decomposition of non-living organic matter) components (Hanson et al., 2000). Different techniques are applied to quantify $E_{soil}$, but all conventional methodology is based on the assumption that root-respired CO₂ diffuses through the soil and upward to the atmosphere (Kuzyakov, 2006).

A number of studies have investigated the relationship between tree canopy photosynthesis and belowground autotrophic respiration, which are connected through the transport of photosynthates from the leaves to the roots via the phloem (e.g. Högberg et al., 2001; Subke et al., 2009; Kuzyakov & Gavrichkova, 2010). In contrast, few studies have focused on the reciprocal relationship: the coupling of autotrophic respiration and carbon assimilation resulting from the internal transport of dissolved CO₂ from belowground. Ford et al. (2007) demonstrated in Pinus seedlings that small amounts of soil dissolved inorganic carbon (DIC) can be taken up by roots, transported upward in the transpiration stream, and assimilated by foliage (Chapter 1). Aubrey & Teskey (2009) observed that a large amount of root-respired CO₂ was transported upward in the transpiration stream to aboveground tissues of Populus trees. Therefore, the transport of root-respired CO₂ in the transpiration stream has potentially important implications for measuring belowground respiration and assessing the role of transpiration in forest carbon cycling (Hanson & Gunderson, 2009).

The concentration of CO₂ in the xylem is usually many times greater than that of the atmosphere (Teskey et al., 2008) (Chapter 1). This internal CO₂ can be transported upward through the plant via the transpiration stream (Teskey & McGuire, 2002; McGuire & Teskey, 2004) where it is released radially into the atmosphere (Teskey &
McGuire, 2005; Steppe et al., 2007) or assimilated within the plant (Chapter 1). Small-scale experiments that introduced carbon isotope labeled solutions into detached leaves or branches revealed that CO₂ transported in the transpiration stream can be assimilated in leaf veins, petioles (Stringer & Kimmerer, 1993) (Chapter 2), and woody branch tissue (McGuire et al., 2009) (Chapter 3). Consequently, xylem-transported root-respired carbon in trees could provide substrate for carbon assimilation which has previously been overlooked. In addition, when large quantities of root-respired CO₂ are transported internally and diffuse into the atmosphere remote from the site of production, efflux-based approaches fail to accurately estimate respiration of both roots and aboveground woody tissues (Chapter 1).

Few studies have examined the fate of CO₂ transported internally from belowground. Ford et al. (2007) and Ubierna et al. (2009) applied ¹³C-labeled solution to soil around seedlings in pots and around large field-grown trees, respectively, and they found that labeled carbon contributed only 0.8% to whole seedling carbon gain (Ford et al., 2007) and 1 to 3% to stem CO₂ efflux (Ubierna et al., 2009). However, these studies did not address the transport and fate of CO₂ derived internally from root respiration. Our objective was to label the xylem sap at the base of large trees in situ with ¹³CO₂ to determine the fate of internally transported carbon. We hypothesized that as the label moved upward in the transpiration stream, a portion would be assimilated by chlorophyll-containing woody and leaf tissues and a portion would diffuse into the atmosphere from stems and branches.

4.2 Materials and methods

4.2.1 Overview

To investigate the role of xylem-transported root-respired CO₂ as a potential carbon substrate for photosynthesis, we infused ¹³C labeled solution into the xylem at the base of four intact eastern cottonwood (Populus deltoides Bartr. ex Marsh) trees and subsequently determined its presence in various tissues throughout the trees. The ¹³C label served as a proxy for dissolved CO₂ entering the stem from the roots. The experimental trees were part of a 7-y-old plantation in Whitehall Forest, a research
facility of the University of Georgia near Athens, Georgia, USA. Details of the experimental plant material are described in Table 4.1.

Table 4.1 Diameter at breast height (DBH, cm), tree height (m), total amount of $^{13}$CO$_2$-labeled solution infused (l), amount of $^{13}$C infused (g) and cumulative sap flow (l) during the infusion period of four *P. deltoides* trees. Trees 1 and 2 were infused with low label solution; trees 3 and 4 were infused with high label solution.

<table>
<thead>
<tr>
<th>Tree</th>
<th>DBH</th>
<th>Height</th>
<th>Label solution infused</th>
<th>$^{13}$C infused</th>
<th>Cumulative sap flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.7</td>
<td>9.8</td>
<td>41.0</td>
<td>0.71</td>
<td>152.6</td>
</tr>
<tr>
<td>2</td>
<td>11.9</td>
<td>7.2</td>
<td>40.0</td>
<td>0.70</td>
<td>157.9</td>
</tr>
<tr>
<td>3</td>
<td>13.9</td>
<td>11.1</td>
<td>45.0</td>
<td>6.76</td>
<td>189.5</td>
</tr>
<tr>
<td>4</td>
<td>9.7</td>
<td>8.6</td>
<td>45.0</td>
<td>6.76</td>
<td>139.1</td>
</tr>
</tbody>
</table>

4.2.2 Baseline tissue sampling for isotopic analysis

Prior to infusion, on 23 June 2010, samples of woody tissue and leaves were taken from all experimental trees to determine the natural abundance carbon isotopic composition ($\delta^{13}$C) to which $\delta^{13}$C of labeled tissues would be compared. For this sampling, a mid-canopy branch of each tree was detached and a stem core was taken with an increment borer at approximately the same canopy position. Samples were immediately frozen in liquid nitrogen and then moved to a freezer at -9°C for storage before processing for carbon isotope analysis.

4.2.3 $^{13}$C label infusion

$^{13}$CO$_2$-labeled solutions both at low $^{13}$CO$_2$ concentrations and high $^{13}$CO$_2$ concentrations were prepared as described in Chapter 3. For this experiment, 20 l containers were filled with solution, of which 1 to 3 l was displaced with $^{13}$C-labeled CO$_2$ (referred to as $^{13}$CO$_2$ in the text) gas from a cylinder of compressed 100% CO$_2$ at 99 atom% (A)$^{13}$C (ICON Services, Summit, NJ, USA) depending on the desired $^{13}$CO$_2$ concentration. Mean pH, gaseous CO$_2$ concentration ([CO$_2$]), and dissolved CO$_2$ concentration ([$^{13}$CO$_2$*]) were 5.30 ± 0.05, 3.77 ± 0.20%, and 1.4 ± 0.1 mM for the solutions at low $^{13}$CO$_2$ concentration (low label treatment, LL) and 6.90 ± 0.02, 6.97 ± 0.05%, and 12.0 ± 0.5 mM for the solutions at high $^{13}$CO$_2$ concentration (high label treatment, HL), respectively. The pH
and [CO₂] of the enriched solutions were within the range measured previously in *P. deltoides* (Aubrey *et al.*, 2011).

The trees were infused with the labeled solutions in the field. Preliminary experiments showed that infusing solution directly into a lateral root resulted in limited solution uptake, since it was only a small part of the root system, so infusion was performed at the base of the stem instead. A 19 mm diameter hole was drilled 4.5 cm deep into the xylem on two sides of each tree approximately 10 and 15 cm above ground level. A threaded brass hose-barb fitting was inserted 1.5 cm into each hole. The holes were drilled deeper than needed for installation of the fittings to increase the contact surface area of the solution with the sapwood. Both fittings were connected to the 20 l reservoir of labeled solution with 12.8 mm inner-diameter CO₂-impermeable tubing (Bev-a-Line IV, Thermoplastic Processes, Georgetown, Delaware, USA). The solution reservoirs were placed 1.5 m above the holes to provide a small pressure head. Two trees were labeled simultaneously for two days starting at noon (trees 1 and 2 on 25-27 June 2010; trees 3 and 4 on 3-5 July 2010). Trees 1 and 2 were infused with the LL solution and trees 3 and 4 were infused with the HL solution. Based on prior measurements of sap flow, we estimated that it would take c. two days for the solution to reach the top of the canopy. Therefore, we waited two additional days after infusion ceased before harvesting the trees for tissue sampling to allow adequate time for assimilation of the 1³C label by woody and leaf tissue.

### 4.2.4 Environmental conditions

Weather during infusion and measurement periods was hot and mostly sunny without precipitation. Mean maximum air temperature and minimum relative humidity (RH) were 30.7°C and 42% and 35.4°C and 45% during the June and July measurement periods, respectively.

### 4.2.5 Biomass determination and tissue sampling for isotopic analysis

Early on the third morning after the infusion ended, the trees were felled and tissues of the tree organs (stem, branch, and leaf) were sampled for carbon isotope analysis according to Fig. 4.1. Each tree was divided vertically into four strata: one below the canopy and three of equal length within the canopy (lower-, mid-, and upper-canopy).
From each stratum, three 20 cm segments of the main stem were collected, one each from the bottom, middle, and top portion of the stratum (at 10, 50, and 90% of the total stratum length, respectively). Two branches from each canopy stratum were divided into three equal-length sections (A, B, and C, Fig. 4.1). A 20 cm segment of woody tissue and all leaf tissue were collected from each branch section. All samples were placed in plastic bags and immediately transferred to an ultralow freezer at -25°C to stop metabolic activity. Samples were later moved to a walk-in freezer and stored at -9°C. All remaining tissue not sampled for isotopic analysis was collected, separated according to Fig. 4.1, and dried for biomass determination. Length and diameter of every stem and branch was measured and surface area was calculated.

Fig. 4.1 Scheme for biomass harvest and tissue sampling for isotopic analysis of four P. deltoides trees infused with $^{13}$CO$_2$-labeled solutions. Trees were subdivided into four strata: one below-canopy stratum and three canopy strata of equal length (lower-, mid-, and upper-canopy). Two branches from each canopy stratum were sampled. Branches were divided into three sections (A, B, and C) of equal length according to distance from the stem. The arrow indicates the location of infusion of the labeled solution.

4.2.6 Processing of tissue samples

Each tissue sample was thawed individually before subsamples were taken for carbon isotope analysis. Woody tissue was subsampled as follows: for stem sections where diameter was >5 cm, a radial xylem core was taken with an increment borer at the middle of each section (3 per canopy stratum) for analysis. At the same position on the section, a 2 cm ring of outer (non-living) bark was removed. The live inner bark of this
entire ring was then sampled for analysis. For stem sections where diameter was <5 cm, a 2-to-10 cm long (depending on diameter) subsample was taken from the middle of the section. Outer bark was removed and the entire subsample was separated into inner bark and xylem for analysis. Branch samples (2 branches per canopy stratum; 3 sections per branch) were subsampled in the same manner as the stems <5 cm diameter. Leaves were sampled as follows: a subsample of 20 mature leaves was removed from each sample branch section and dissected into petiole, primary (central) vein, secondary veins, and remaining leaf mesophyll. A second subsample of 10 mature leaves was taken and processed as whole leaves. Subsamples of tissues harvested prior to labeling were processed in a similar manner to provide baseline carbon isotope values. All subsamples were dried to constant weight in an oven at 65°C and then ground to powder in a ball mill (8000-D Mixer Mill, SPEX SamplePrep, Metuchen, New Jersey, USA) for $^{13}$C analysis.

4.2.7 Isotopic analysis of tissue samples

Ground tissue samples were weighed to μg precision in tin capsules and analyzed by Element Analyzer (model 1500 CHN, Carlo-Erba, Italy) coupled to an Isotope Ratio Mass Spectrometry (Delta V, Thermo-Finnigan, Bremen, Germany) (EA-IRMS) at the Stable Isotope and Soil Biology Laboratory (SISBL), Odum School of Ecology, University of Georgia, Athens, Georgia, USA. Bovine with a $\delta^{13}$C value of -21.24 ‰ (certified by the National Institute of Standards and Technology, USA) was used as a laboratory reference and all $\delta^{13}$C are expressed relative to VPDB. Enrichment of the labeled branch tissues ($\delta^{13}$C, ‰) and A of the labeled tissues (A, %) was calculated as described in Chapter 2 (Equations 2.2 and 2.3).

4.2.8 Scaling isotope measurements of tissue component samples to organ and whole tree levels

Biomass measurements of the subsamples were used to determine the mass proportions of the woody (inner bark and xylem) and leaf (petiole, primary vein, secondary veins, and mesophyll) tissue components for each organ (stem, branch, leaf) at every canopy stratum and branch section of each tree. These proportions were used to calculate the total biomass of the woody and leaf organs by tissue component for every canopy
stratum and branch section combination (Fig. 4.1) for each tree. Based on $A$, the amount of $^{13}$C assimilated in each tissue component ($^{13}$C, mg) was calculated as:

$$^{13}C_t = A_t \cdot DM \cdot C$$

(4.1)

where $DM$ is the dry mass of the tissue component per stratum and section (mg) and $C$ is the carbon content of the tissue component (%).

To determine the amount of $^{13}$C assimilated by the three organs (stem, branch, leaf) and the whole tree, the values of $^{13}$C for the individual tissue components of each stratum and section were summed to organ and tree level. The sum of the amount of $^{13}$C assimilated by the whole tree ($^{13}$Cassim, mg) and the amount of $^{13}$C lost to the atmosphere ($^{13}$Cefflux, mg) were assumed to be equal to the amount of $^{13}$C taken up by the tree ($^{13}$Cuptake, mg), based on the following mass balance equation:

$$^{13}C_{\text{uptake}} = ^{13}C_{\text{assim}} + ^{13}C_{\text{efflux}}$$

(4.2)

Thus, for each tree we estimated $^{13}$Cefflux by subtracting $^{13}$Cassim from $^{13}$Cuptake. $^{13}$Cuptake was calculated by multiplying the dissolved $^{13}$CO$_2$ concentration of the labeled solution by the amount of solution taken up by the tree as described in McGuire et al. (2009).

4.2.9 Measurements of sap flow

Sap flow was determined by scaling sap flux density measured with homemade thermal dissipation probes (TDP) (Granier, 1985) with sapwood area. Two TDP sensors were installed in the stem on opposite sides of each tree at a height of 0.45 m. Thermocouple depth was 10 mm and vertical separation of the sensor needles was 50 mm. Zero sap flow conditions were assumed between 03:00 and 05:00 h. Sap flow calibration parameters developed for $P$. deltoides at this site (Sun et al., 2011) were applied to the sap flow measurements as recommended by Steppe et al. (2010). Sap flow was recorded with a datalogger (23X, Campbell Scientific, Logan, Utah, USA) at 5 min intervals.

4.2.10 Gas sampling for isotopic analysis

Gas samples were collected from a cylindrical cuvette installed around a section of the stem and a section of a branch of each tree to confirm that $^{13}$C label diffused from woody tissues after uptake of the $^{13}$C enriched solution. These cuvettes were originally
constructed to measure stem and branch CO$_2$ efflux, but efflux data will not be presented in this report. Stem cuvettes were installed just below the base of the canopy at an average height of 1.05 m. Branch cuvettes were installed close to the stem in the lower third of the canopy at an average height of 1.70 m. Both stem and branch cuvettes were 15 cm long, constructed of 0.18 mm thick Mylar film (Ridout Plastics, San Diego, California, USA) and sealed to the stem with adhesive closed-cell foam gasket material and non-caustic silicone sealer (RTV162, MG Chemicals, Surrey, British Columbia, Canada). Compressed air at atmospheric composition was supplied from a cylinder to each cuvette at 500 ml min$^{-1}$ with a mass flow controller (FMA5514, Omega Engineering Inc., Kingston, Ontario, Canada). For gas sampling, a needle attached to a 35 ml syringe was inserted through the foam gasket of the cuvette and a sample was withdrawn and injected directly into a 10 ml evacuated vial (Vacutainer, BD, New Jersey, USA) that had been pre-filled with 1 ml distilled deionized water, to limit the diffusion of $^{13}$CO$_2$ through the septa seal when stored upside down. The septa of the vials were sealed with parafilm and samples were stored upside down to minimize gas diffusion through the septa seal. Starting 24 h after the beginning of label infusion, gas samples were collected every 2 h over a 24 h period and an additional sample was taken at 72 h. This timing was selected to detect changes in the enrichment of the air inside the cuvettes that might be related to the timing of label infusion and/or sap flow rate. Baseline samples of the air inside the cuvettes were taken before the start of infusion to determine natural abundance isotope concentrations.

4.2.11 Isotopic analysis of gas samples

Gas samples were analyzed at the Stable Isotope and Soil Biology Laboratory (SISBL). The enrichment of the air inside the cuvettes due to $^{13}$C label diffusion from the stem and branch tissues ($\delta^{13}$C$_a$, ‰) was calculated by subtracting the $\delta^{13}$C value of the baseline sample before label infusion ($\delta^{13}$C$_b$, ‰) from the $\delta^{13}$C value of the samples during label infusion ($\delta^{13}$C$_a$, ‰), as performed for calculating $\delta^{13}$C$_t$ based on Equation 2.2.

4.2.12 Data processing and statistical analysis

$\delta^{13}$C$_t$ of each organ (stem, branch, and leaf) and $^{13}$C$_t$ were analyzed using multi-factorial analysis of variance (ANOVA). At the stem level, $^{13}$C label concentration ($n=2$), canopy
stratum \((n=4)\), and tissue component \((xylem and inner bark)\) were treated as fixed factors, and individual tree \((n=4)\) was treated as a random factor. A similar ANOVA model was used at the branch and leaf level with a few differences. At the branch level, the number of canopy strata was reduced by 1 \((n=3, \text{ eliminating the below-canopy stratum})\), an additional tissue component \((\text{leaf})\) was included, and branch section \((n=3)\) was included as a fixed factor. At the leaf level, tissue components consisted only of leaf parts \((\text{petiole, primary vein, secondary veins, and mesophyll})\). A similar ANOVA model was used to compare enrichment of different organs \((\text{stem, branch, and leaf})\). In this case, we confined our measurements to those taken within the canopy and calculated the weighted average of enrichment across tissue components as a function of biomass per canopy stratum. Treatment means were compared using Fisher’s Least Significant Difference test. \(\delta^{13}\text{C}_a\) was analyzed using a repeated measures analysis of variance (ANOVA) with \(^{13}\text{C}\) label concentration \((n=2)\), tissue \((n=2)\), and time \((n=14)\) treated as fixed factors and individual tree \((n=4)\) treated as the random subject factor. Akaike Information Criterion corrected for small sample sizes \((\text{AIC}_c)\) was used to determine the covariance structure that best estimated the correlation among individual trees over time. All analyses were performed using the mixed model procedure \((\text{PROC MIXED})\) of SAS \((\text{Version 9.1.3, SAS Inc., Cary, NC, USA})\) with \(\alpha=0.05\).

4.3 Results

4.3.1 \(^{13}\text{C}\) label uptake

Each of the four trees took up between 40 and 45 l of \(^{13}\text{CO}_2\)-labeled solution during the two-day infusion period, which accounted for 23.8 to 32.4% of total sap flow. From visual observation it was clear that most of the solution was taken up during periods of high sap flow during the day while little was taken up at night. Soil moisture was high during the experiment, precluding downward flow into the roots at night. Although solution uptake for all trees was similar, the average amount of dissolved \(^{13}\text{C}\) label taken up was nearly 10-fold greater under the high label \((\text{HL})\) treatment \((6.76 \pm 0.00 \text{ g})\) compared with the low label \((\text{LL})\) treatment \((0.71 \pm 0.01 \text{ g})\) (Table 4.1).
4.3.2 Carbon isotope composition of woody tissue and leaves

The baseline $\delta^{13}C$ of tissues sampled before labeling ranged from -27.74‰ to -29.71‰. Tissue enrichment results ($\delta^{13}C_t$) showed that the label was transported from the base of the stem and assimilated in all tissues of the tree organs. The $\delta^{13}C_t$ of the organs (calculated by weighted average of the tissue components) was influenced by label concentration, canopy stratum, and organ; however, these factors were not independent of each other (i.e., label concentration $\times$ organ interaction, $P<0.0001$ and organ $\times$ canopy stratum interaction, $P<0.0001$). The $\delta^{13}C_t$ of the stem and leaves was higher under the HL treatment than under the LL treatment at all canopy strata. In the branches, a significant difference in $\delta^{13}C_t$ between label treatments was observed only at mid- and upper-canopy strata. Under the high-label treatment, $\delta^{13}C_t$ of the branches was higher than that of the stem and leaves at all canopy strata (Fig. 4.2). Mean $\delta^{13}C_t$ of branches was higher at mid-canopy than at upper- and lower-canopy strata, whereas stem and leaf $\delta^{13}C_t$ was similar among canopy strata under the HL treatment (Fig. 4.2).

![Mean $^{13}C$ enrichment ($\delta^{13}C_t$, ‰) of stem (black), branch (hatched), and leaf (grey) organs of two *Populus deltoides* trees infused with high label (HL) $^{13}CO_2$ solution showing interaction between organ and canopy stratum. Tissue components of the organs were sampled according to the scheme depicted in Fig. 4.1. $\delta^{13}C_t$ was averaged per organ and canopy stratum. Different lower-case letters indicate significant differences (Fisher’s least significant difference test, $P<0.05$) in $\delta^{13}C_t$ among organs within a canopy stratum. Different upper-case letters indicate significant differences in $\delta^{13}C_t$ of the same organ among different canopy strata. Bars indicate standard error of the mean.](image-url)
In the stems, δ\textsuperscript{13}C\textsubscript{t} was influenced by label concentration, tissue component, and canopy stratum. For example, δ\textsuperscript{13}C\textsubscript{t} of the inner bark was higher under the HL treatment (3.86 ± 0.57‰) than under the LL treatment (0.58 ± 0.21‰) at the upper- and mid-canopy strata, but cortex δ\textsuperscript{13}C\textsubscript{t} was not influenced by label treatment at the lower- or below-canopy strata. Under the HL treatment, δ\textsuperscript{13}C\textsubscript{t} of the inner bark was higher than that of the xylem at the upper- and mid-canopy strata, but not at the lower- or below-canopy strata (i.e., label concentration × tissue component × canopy stratum interaction, P=0.0108) (Fig. 4.3). The δ\textsuperscript{13}C\textsubscript{t} of both the inner bark and xylem under the HL treatment was higher at the upper- and mid-canopy strata than at the lower- and below-canopy strata. (Fig. 4.3).

![Graph showing δ\textsuperscript{13}C\textsubscript{t} values for different canopy strata](image)

**Fig. 4.3** Mean 13C enrichment (δ\textsuperscript{13}C\textsubscript{t}, ‰) of inner bark (black) and xylem (hatched) of the stem at four canopy strata of two *Populus deltoides* trees infused with high label (HL) 13CO\textsubscript{2} solution showing interaction between tissue component and canopy stratum. Tissue components of the stem were sampled according to the scheme depicted in Fig. 4.1. δ\textsuperscript{13}C\textsubscript{t} was averaged for the inner bark and xylem per canopy stratum. Different lower-case letters indicate significant differences (Fisher’s least significant difference test, P<0.05) in δ\textsuperscript{13}C\textsubscript{t} between inner bark and xylem within a canopy stratum. Different upper-case letters indicate significant differences in δ\textsuperscript{13}C\textsubscript{t} of the same tissue component among different canopy strata. Bars indicate standard error of the mean.

Overall, in the branches, δ\textsuperscript{13}C\textsubscript{t} was highest in the mid canopy stratum, intermediate in the upper canopy stratum and lowest in the lower canopy stratum (P=0.0013), and was also influenced by label concentration and tissue component; However, the effects of label concentration and tissue component were not independent of each other (i.e., label...
concentration × tissue component interaction, \( P<0.0001 \). The \( \delta^{13}\text{C}_t \) of the inner bark was higher under the HL treatment than under the LL treatment. It also differed among the branch tissue components (xylem, inner bark, and leaves) under the HL treatment but not under the LL treatment. Mean \( \delta^{13}\text{C}_t \) across all canopy strata was highest in the inner bark (10.81 ± 1.13‰), intermediate in the xylem (5.27 ± 0.55‰), and lowest in the leaves (1.04 ± 0.12‰) under the HL treatment.

Mean \( \delta^{13}\text{C}_t \) of the leaves was higher under the HL treatment than under the LL treatment at the lower-canopy stratum (1.57 ± 0.39‰ vs. -0.67 ± 0.12‰) and at the mid-canopy stratum (2.16 ± 0.34‰ vs. 0.53 ± 0.09‰) (i.e., label concentration × canopy stratum interaction, \( P=0.0007 \)). Under the HL treatment, petioles were significantly more enriched than the other leaf tissue components regardless of canopy stratum or branch section. However, under the HL treatment, \( \delta^{13}\text{C}_t \) of the petioles decreased with increasing distance from the stem (i.e., label concentration × tissue component × branch section interaction, \( P=0.0008 \)) (Fig. 4.4).

![Fig. 4.4](image)

Mean \( ^{13}\text{C} \) enrichment (\( \delta^{13}\text{C}_t \), ‰) of petiole (black) and other tissue components (combined primary vein, secondary veins, and mesophyll) (hatched) of the leaves of three equal-length branch sections in the canopy of two \( P. \) deltoides trees infused with high label (HL) \( ^{13}\text{CO}_2 \) solution showing interaction between tissue component and branch section. Tissue components of the leaves were sampled according to the scheme depicted in Fig. 4.1. \( \delta^{13}\text{C}_t \) was averaged for the petioles and other leaf tissue components per branch section. Different lower-case letters indicate significant differences in \( \delta^{13}\text{C}_t \) (Fisher’s least significant difference test, \( P<0.05 \)) between petioles and other leaf tissue components within a branch section. Different upper-case letters indicate significant differences in \( \delta^{13}\text{C}_t \) of either petioles or other leaf tissue components among different branch sections. Bars indicate standard error of the mean.
4.3.3 Amount of $^{13}\text{C}$ assimilated

Generally, the total amount of $^{13}\text{C}$ assimilated ($^{13}\text{C}_t$) was higher under the HL treatment than under the LL treatment (Table 4.2), but it also depended on canopy stratum, and organ; however, these individual effects were not independent of each other (i.e., label concentration × organ interaction, $P=0.0009$ and organ × canopy stratum interaction, $P=0.0002$). The highest $^{13}\text{C}_t$ was observed in the branches compared with stem and leaves under both label treatments (Table 4.2). Mean $^{13}\text{C}_t$ of the branches was higher at the lower-canopy stratum than at the mid- and upper-canopy strata ($P<0.0001$) under the HL treatment.

Within the stem, $^{13}\text{C}_t$ was solely dependent on tissue component ($P=0.014$). Averaged across canopy strata and label treatments, $^{13}\text{C}_t$ of the xylem was higher than $^{13}\text{C}_t$ of the inner bark (Table 4.2).

Within the branches, $^{13}\text{C}_t$ of inner bark and xylem were higher under the HL treatment than the LL treatment, but only in the lower- and mid-canopy strata. Averaged across canopy strata and branch section, $^{13}\text{C}_t$ was higher in the xylem than in the inner bark under both label treatments (Table 4.2). Under the HL treatment, $^{13}\text{C}_t$ of the xylem decreased with increasing distance from the stem. However, these individual effects were not independent of each other, i.e., $^{13}\text{C}_t$ of xylem and inner bark varied with label concentration, tissue component, canopy stratum, and branch section (canopy stratum × label concentration interaction, $P<0.0001$, canopy stratum × tissue component interaction, $P=0.0004$, and branch section × tissue component interaction, $P=0.0003$).

In the leaves, $^{13}\text{C}_t$ was affected by label concentration, and varied among tissues and branch sections. Averaged across canopy strata and branch sections, $^{13}\text{C}_t$ of the petioles was higher under the HL treatment than under the LL treatment (tissue component × label concentration interaction, $P=0.0274$) (Table 4.2). Under the HL treatment, $^{13}\text{C}_t$ of the petioles was higher than that of the other leaf tissue components at the more distal branch sections B and C, but not at the proximal branch section A, resulting in a branch section × tissue component interaction ($P=0.0002$).
### Table 4.2

Mean (SE) total biomass of tissue components (kg), amount of 13C fixed per tissue ($^{13}$C$_t$, mg), and amount of 13C assimilated relative to 13C uptake ($^{13}$C$_t$/$^{13}$C$_{uptake}$, %) of *Populus deltoides* trees infused with low label (LL, two trees) and high label (HL, two trees) $^{13}$CO$_2$ solutions. All data have been scaled to the whole tree level. Standard errors displayed as (0.00) were not zero, but have been truncated due to rounding.

#### Low label treatment

<table>
<thead>
<tr>
<th></th>
<th>Stem</th>
<th>Inner bark</th>
<th>Branch</th>
<th>Inner bark</th>
<th>Leaf</th>
<th>Prim. vein</th>
<th>Sec. vein</th>
<th>Mesophyll</th>
<th>Total biomass (kg)</th>
<th>$^{13}$C$_t$ (mg)</th>
<th>$^{13}$C$<em>t$/$^{13}$C$</em>{uptake}$ (%)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.37 (1.73)</td>
<td>2.56 (0.34)</td>
<td>8.54 (3.37)</td>
<td>2.75 (1.19)</td>
<td>0.76 (0.16)</td>
<td>0.25 (0.05)</td>
<td>0.18 (0.01)</td>
<td>5.87 (1.29)</td>
<td>36.28 (2.10)</td>
<td>18.00 (0.01)</td>
<td>4.20 (0.00)</td>
</tr>
<tr>
<td>$^{13}$C$<em>t$/$^{13}$C$</em>{uptake}$</td>
<td>2.52 (0.19)</td>
<td>0.59 (0.04)</td>
<td>8.69 (0.01)</td>
<td>2.94 (0.06)</td>
<td>1.18 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.05 (0.01)</td>
<td>1.40 (0.06)</td>
<td>17.40 (0.10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### High label treatment

<table>
<thead>
<tr>
<th></th>
<th>Stem</th>
<th>Inner bark</th>
<th>Branch</th>
<th>Inner bark</th>
<th>Leaf</th>
<th>Prim. vein</th>
<th>Sec. vein</th>
<th>Mesophyll</th>
<th>Total biomass (kg)</th>
<th>$^{13}$C$_t$ (mg)</th>
<th>$^{13}$C$<em>t$/$^{13}$C$</em>{uptake}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.34 (6.42)</td>
<td>2.56 (0.85)</td>
<td>7.82 (2.88)</td>
<td>2.48 (0.86)</td>
<td>0.70 (0.27)</td>
<td>0.27 (0.11)</td>
<td>0.16 (0.07)</td>
<td>5.83 (2.05)</td>
<td>35.16 (3.72)</td>
<td>64.00 (0.02)</td>
<td>24.00 (0.00)</td>
</tr>
<tr>
<td>$^{13}$C$<em>t$/$^{13}$C$</em>{uptake}$</td>
<td>0.94 (0.04)</td>
<td>0.36 (0.01)</td>
<td>2.10 (0.01)</td>
<td>1.72 (0.03)</td>
<td>0.22 (0.00)</td>
<td>0.01 (0.00)</td>
<td>0.05 (0.00)</td>
<td>0.20 (0.01)</td>
<td>17.40 (0.10)</td>
<td>20.00 (0.00)</td>
<td>20.00 (0.00)</td>
</tr>
</tbody>
</table>

Note: All data have been scaled to the whole tree level. Standard errors displayed as (0.00) were not zero, but have been truncated due to rounding.
4.3.4 Carbon isotope composition of air inside stem and branch cuvette

Isotopic signatures of the air inside the stem and branch cuvette confirmed that the $^{13}$C label was transported via the transpiration stream and diffused to the atmosphere from aboveground woody tissue. Enrichment of the air inside the cuvettes ($\delta^{13}$C$_a$) was influenced by label concentration and organ (stem and branch) and changed temporally; however, these individual effects were not independent of each other (i.e., organ $\times$ time $\times$ label concentration interaction, $P=0.0003$). Significant temporal variation in $\delta^{13}$C$_a$ was observed in the stem, but not in the branch under both LL (Fig. 4.5A) and HL (Fig. 4.5B) treatments. The highest $\delta^{13}$C$_a$ of the stem was observed at 36 h and 24 h from the start of label infusion under LL and HL treatments, respectively. At 72 h from the start of label infusion, $\delta^{13}$C$_a$ of the branch under both label treatments and $\delta^{13}$C$_a$ of the stem under the low-label treatment returned to baseline, whereas $\delta^{13}$C$_a$ of the stem under the HL treatment remained high relative to the baseline. $\delta^{13}$C$_a$ of the stem, averaged across the observation period ($45.40 \pm 6.75\%$) was significantly higher than that of the branch ($3.75 \pm 1.92\%$) under the low-label treatment. Similarly, $\delta^{13}$C$_a$ of the stem, averaged across the observation period ($41.96 \pm 3.75\%$) was significantly higher than that of the branch ($3.75 \pm 1.92\%$) under the HL treatment.

![Fig. 4.5](image-url) Enrichment of the air in cuvettes ($\delta^{13}$C$_a$, ‰) installed on the stem (black circles) and a branch (grey circles) of *P. deltoides* trees infused with low label (A, two trees) and high label (B, two trees) $^{13}$CO$_2$ solutions. Gas samples were taken hourly from 24 h to 48 h and at 72 h after the start of label infusion. Asterisks indicate significant ($P<0.05$) differences in $\delta^{13}$C$_a$ between the stem and branch at each observation. Bars indicate standard error of the mean.
4.3.5 Assimilation and efflux of $^{13}$C relative to the amount taken up

On average, $17.40 \pm 0.10\%$ and $5.60 \pm 0.02\%$ of the infused $^{13}$C label was assimilated in aboveground tree organs under LL and HL treatments, respectively (Fig. 4.6). $^{13}$C assimilation occurred mainly in the branches and, to a lesser extent, in the stem and leaves (Table 4.2, Fig. 4.6). Most of the $^{13}$C label was not assimilated and was therefore assumed to have diffused to the atmosphere both via the stem and the branches ($82.60 \pm 0.10\%$ and $94.40 \pm 0.02\%$ under LL and HL treatments, respectively).

Fig. 4.6 Overview of the fate of a $^{13}$C label infused at the base of *Populus deltoides* trees calculated by mass balance. Values are mean assimilation or efflux of the label (percent of total label infused in brackets) for two trees infused with low label solution (left panel) and two trees infused with high label solution (right panel). Total efflux was calculated by subtracting total label assimilated from total label infused.
4.4 Discussion

Our results provide the first experimental evidence that xylem-transported root-respired CO$_2$ can be assimilated in stems, branches, and leaves of large trees in the field. We found that up to 17% of a $^{13}$C label infused at the base of the stem was assimilated in woody and leaf tissues, providing evidence of an internal recycling mechanism for respired CO$_2$. Moreover, based on mass balance calculations, we estimated that most of the putative root-respired CO$_2$ diffused to the atmosphere from higher in the stem and branches, suggesting that efflux-based estimates of above- and belowground autotrophic component of respiration (R$_a$) are inaccurate. Based on the previous observation that a substantial quantity of CO$_2$ was transported from roots into shoots in xylem sap in this species (Aubrey & Teskey, 2009), the objective of this experiment was to determine the fate of root-respired CO$_2$ that dissolved in xylem sap. Our infusion of the $^{13}$C label at the base of the stem was designed to serve as a proxy for dissolved CO$_2$ that was transported in xylem sap from the root system into the stem. However, the study also provides insights into the potential fate of xylem-transported dissolved CO$_2$ that originates from respiring cells in stems and branches (Chapter 1).

Recycling of respired CO$_2$ by woody tissues has been assumed to positively contribute to the overall carbon gain of plants (Aschan & Pfanz, 2003; Teskey et al., 2008). Assimilation of CO$_2$ in woody tissues has been previously reported in the inner bark of branches and stems (Aschan et al., 2001; Aschan & Pfanz, 2003) where sufficient light for CO$_2$ assimilation is transmitted to chlorophyll-containing cells (Aschan & Pfanz, 2003). Our results showed that the inner bark of the stem and branches was the most enriched tissue, confirming the potential importance of the inner bark in assimilation of internally transported CO$_2$. In branches receiving the HL treatment, we found that the average $\delta^{13}C_t$ of the inner bark was twice as high as the xylem, which agrees with results of $^{13}$C labeling experiments on detached branches (McGuire et al., 2009) (Chapter 3). Notwithstanding the fact that the upper- and mid-canopy strata were more distal from the $^{13}$C infusion, the inner bark of woody tissues in these strata was more enriched than in the lower- and below-canopy strata. In the upper canopy, where young tissues are more metabolically active, corticular photosynthesis can be greater than in lower, older canopy sections (Cernusak & Marshall, 2000; Aschan et al., 2001; Pfanz et al., 2002). In
addition to reduced metabolism in lower parts of the canopy, light transmission is substantially lower, which decreases corticular CO₂ assimilation (Aschan & Pfanz, 2003). The importance of woody tissue CO₂ assimilation to overall carbon gain can only be evaluated when data are scaled to the whole tree. The xylem of stems and branches makes up a large proportion of total tree biomass in comparison to the inner bark (in our study, on average, 65.9% vs. 14.5%, respectively). Scaling δ¹³Cₜ with biomass demonstrated that, despite lower enrichment of xylem compared with inner bark, the greatest quantity of ¹³C label was assimilated in the xylem, rather than in the inner bark, in both branches and stems. The stems and branches of *P. deltoides* in this study had visibly green xylem. The occurrence of chlorophyll in xylem has been reported for woody species (Rentzou & Psaras, 2008; Saveyn et al., 2010) and chlorophyll-containing pith cells in young *Populus* were found to be capable of fixing ¹⁴CO₂ in the light (Van Cleve et al., 1993). Therefore, from a mechanistic perspective, assimilation of xylem-transported CO₂, which is in essence a carbon recycling process, should be considered when assessing the total quantity of carbon assimilated by trees.

Few studies have described the role of assimilation of xylem-transported CO₂ by leaves in the context of a recycling mechanism. Aubrey & Teskey (2009) estimated that up to 50% of root-respired CO₂ could be transported internally to aboveground tree organs, and based on these results, Hanson & Gunderson (2009) argued that if a large fraction of root-respired CO₂ reached the foliage via the transpiration stream, it could have a substantial impact on the availability of CO₂ for leaf photosynthesis. Powers & Marshall (2011) labeled the xylem of a *Thuja occidentalis* tree in situ and subsequently could not detect any label in the leaves. However, their failure to detect label in the leaves could be partly the result of the small quantity of label solution that was infused and partly due to their strategy of sampling just a small amount of foliage from the top of the canopy. In our study, a portion of the infused label reached the leaves and was assimilated, with the petioles being the most enriched. Previous isotopic labeling experiments on detached branches of *Platanus occidentalis* (McGuire et al., 2009) (Chapter 3) and detached leaves of *P. deltoides* (Stringer & Kimmerer, 1993) (Chapter 2) reported similar high values of carbon isotope enrichment in petioles compared with more distal leaf components. Apparently, the petioles scrubbed most of the label from
the transpiration stream before it reached the leaf mesophyll. Russin & Evert (1984) reported that in the petioles of *Populus* leaves, chlorophyll-containing cells are in close contact with the vasculature, suggesting that xylem-transported CO$_2$ may be a carbon source for photosynthetic reactions in adjacent tissues, as was reported by Stringer & Kimmerer (1993) and observed in other species (Hibberd & Quick, 2002; Berveiller *et al*., 2007b; Leegood, 2008). However, in our study, leaf assimilation of transported $^{13}$C label was limited. It appeared that most of the infused label either diffused to the atmosphere or was assimilated by woody tissues before it could reach the foliage.

In total, we found that up to 17% of the infused label was assimilated in woody tissue and leaves. However, our results likely underestimate the actual importance of assimilation of internally transported CO$_2$, because we could not account for any assimilation of internally sourced $^{12}$C, i.e., the $^{13}$C label represented only part of the internal carbon available for assimilation. The water taken up by roots and transported upward contained dissolved CO$_2$ (composed almost entirely of $^{12}$C) from root respiration. Based on the proportion of total sap flow that the label solution represented (23.8 to 32.4%), it can be expected that the amount of root-respired CO$_2$ assimilated was three to five times more than estimated with the label. Finally, CO$_2$ from aboveground $R_a$, which is composed almost entirely of $^{12}$CO$_2$, could be re-assimilated immediately in nearby photosynthetic cells or become part of the internal transport pool. McGuire & Teskey (2004) found that up to 55% of daily stem-respired CO$_2$ could be transported in the xylem stream, so it is likely that this source contributed substantially to the amount of internal CO$_2$ available as substrate for assimilation. Moreover, because the xylem was not saturated with $^{13}$CO$_2$ label after the 2 d infusion period, photosynthetic discrimination against $^{13}$C in woody tissue (Cernusak *et al*., 2001; Saveyn *et al*., 2010) is another likely source of underestimation. Longer-term labeling would likely have resulted in even greater $^{13}$C concentrations in all tissues where the label was assimilated.

Based on our mass balance calculations, we found that the largest fraction of the $^{13}$C label (83% and 94% under the LL and HL treatments, respectively) diffused to the atmosphere from the stem and branches. This observation suggests that as root-respired CO$_2$ is transported from belowground upward through the stem, most of it
diffuses to the atmosphere before reaching the canopy. We assumed that the quantity of $^{13}$C label that remained in the xylem in dissolved and gaseous states at the end of the experiment was negligible and that diffusion of the label from the leaves was inconsequential because the direction of CO$_2$ movement is from the atmosphere into leaves during the day and the amount of CO$_2$ efflux from leaves at night is only a small fraction of the amount taken up during the day.

As carbon dioxide (CO$_2$) dissolves in water, pH dependent equilibria are established between the aqueous (CO$_2$(aq)), bicarbonate (HCO$_3^-$) and carbonate (CO$_3^{2-}$) forms of CO$_2$ (Chapter 1). In the xylem, with pH levels reported in the range of 4.7-7.4 (Teskey et al., 2008), CO$_2$ is present only as CO$_2$(aq) and HCO$_3^-$ and not in its carbonate form (CO$_3^{2-}$). Measurements of the pH of the xylem sap in six trees at our site showed the average pH value to be 6.22 ±0.07.

Similarly, the $^{13}$C solutions applied in this study, in which the average pH was 5.30 and 6.90 for the low and high-labeled solutions, respectively, contained $^{13}$C only in its aqueous ($^{13}$CO$_2$ (aq)) and bicarbonate (H$^{13}$CO$_3^-$) forms. Other studies performed with carbon tracers have specifically supplied the bicarbonate or carbonate form of the stable or non-stable isotope to leaves (Stringer & Kimmerer, 1993), herbaceous plants (Hibberd & Quick, 2002), cuttings (Vapaavuori & Pelkonen, 1985) and small trees (Powers & Marshall, 2011) and they all concluded that there was an accumulation of the tracer in various tissues. Moreover, CO$_2$ and HCO$_3^-$ ($^{12}$C or $^{13}$C) can be assimilated by Ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco) and Phosphoenolpyruvate carboxylase (PEPc)-mediated carboxylation, respectively, and previous studies have reported that Rubisco (e.g. Pfanz et al., 2002; Saveyn et al., 2010) and PEPc (Hibberd & Quick, 2002; Berveiller et al., 2007) in woody and leaf tissues actively contribute to CO$_2$ refixation. Thus, it is reasonable to believe that the inorganic form of the supplied $^{13}$C label was assimilated by biological processes.

Previous experiments demonstrated that CO$_2$ dissolved in soil water contributed only a small amount to the overall carbon economy of plants (Ford et al., 2007; Jones et al., 2009). Moore et al. (2008) found that the isotopic composition of soil CO$_2$ explained a considerable amount of the variation in the isotopic composition of CO$_2$ that fluxed from stems, but (Ubierna et al., 2009) observed that neither belowground processes nor CO$_2$
transported in the transpiration stream had a detectable influence on stem CO2 efflux. By applying an isotope label to the soil, Ford et al. (2007) and Ubierna et al. (2009) tested the assumption that CO2 dissolved in soil water could be taken up by roots and transported in the xylem stream, while our study simulated the transport of CO2 originating from both root and soil sources by infusing a 13C label at the base of tree stems. The internal [CO2] measured at the bottom of the stem (Teskey & McGuire, 2007; Aubrey & Teskey, 2009) was substantially higher than the [CO2] in soil surrounding the roots, indicating that only a small quantity of the CO2 in the xylem at the base of the stem could have originated from the soil (Chapter 1). Therefore, most of the internal CO2 at the base of the stem must be derived from the root system. Ford et al. (2007) and Ubierna et al. (2009) were not able to simulate the transport of CO2 derived internally from root respiration, which has been shown to comprise 92% of the CO2 transported in xylem from belowground (Aubrey & Teskey, 2009). In addition, Ford et al. (2007) and Ubierna et al. (2009) were unable to determine the total amount of label that was taken up by the roots, but by infusing the label solution directly into the xylem, we were able to quantify the uptake of 13C, which allowed us to calculate 13C efflux as the difference between uptake and assimilation.

The magnitude of root-respired CO2 transported in the xylem and subsequently lost to the atmosphere may have important implications for how we assess above- and belowground metabolism (Aubrey & Teskey, 2009; Grossiord et al., 2012) (Chapter 7). Given that one-half of root-respired CO2 may follow an internal flux pathway that cannot be accounted for by soil CO2 efflux measurements (Aubrey & Teskey, 2009) and that up to 94% of the 13C label infused into trees in this study was lost to the atmosphere, we suggest that up to 47% of root-respired CO2 could diffuse into the atmosphere from aboveground woody tissues. That a substantial amount of CO2 diffusing out of the stem and branches actually originates from belowground autotrophic respiration has important implications for how we interpret efflux-based measurements of soil and woody-tissue respiration and patterns of carbohydrate allocation (Box 1.3). First, we suggest that the gas-exchange approach for estimating above- and belowground respiration is inaccurate and needs to be adjusted for internal transport of CO2. Second, our understanding of patterns of allocation of carbohydrates in trees should be
reconsidered because we are routinely under- and over-estimating the carbon needed to sustain below- and aboveground tissues, respectively, when estimates are derived from measurements of CO$_2$ efflux. In future research, additional measurements of metabolic processes (photosynthesis, root, and stem respiration) conducted simultaneously with measurements of internal CO$_2$ transport may reveal the implications of this recently recognized carbon flux pathway.
- Chapter 5 -
Woody tissue photosynthesis induces axial diffusion of resired CO₂ in dormant oak tree stems


**Abstract**

Previous findings on the transport of resired CO₂ via the transpiration stream led to new insights in how to interpret efflux-based stem respiration measurements. However, no studies have yet addressed whether efflux-based estimates represent actual stem respiration rates at times when no sap flow occurs, for instance during the dormant season. Stem CO₂ efflux ($E_{stem}$) was measured with a stem cuvette on dormant oak (*Quercus robur* L.) trees in a growth chamber. Different rates of axial CO₂ diffusion were induced by illuminating 10-cm-long stem sections at varying distances from the stem cuvette, while light was excluded from the remainder of the tree. Our results show how axial diffusion of resired CO₂ within dormant tree stems may lead to an underestimation of dormant season stem respiration. Largest non-temperature related reductions in $E_{stem}$ were observed when the stem section closest to the cuvette was exposed to light. Due to axial diffusion of CO₂, $E_{stem}$ decreased by 22% relative to $E_{stem}$ measured when light was excluded from the entire tree. Lower rates of axial diffusion were observed upon illumination of more remote stem sections. While during the growing season, transport of dissolved CO₂ overrules the potential impact of axial diffusion on $E_{stem}$, our results indicate that dormant season efflux-based estimates of stem respiration might be biased, particularly in ‘open’ forest stands with sufficient light.
penetration. Consequently, this may lead to wrong estimates of dormant season $E_{stem}$ coefficients ($Q_{10}$ and $E_{stem(ref)}$) which are generally used to estimate maintenance respiration throughout the year.

5.1 Introduction

Within forest ecosystems, CO$_2$ efflux from woody tissues to the atmosphere represents a major component of ecosystem respiration, accounting for about 7 to 50% of gross primary productivity (Ryan et al., 1994; Damesin et al., 2002). The wide range in these estimates partially reflects differences observed between different forest ecosystems (Ryan et al., 1995; Chambers et al., 2004; Yang et al., 2012), but is additionally resulting from our limited ability to accurately measure and model this respiratory flux at the tree level and to scale it to the stand level (Ryan et al., 2009).

However, recent advances have been made on using efflux-based measurements to accurately quantify woody tissue respiration during the growing season. Where past studies mainly highlighted the exponential relationship between stem temperature and stem CO$_2$ efflux ($E_{stem}$) (Ryan et al., 1995; Maier et al., 1998; Stockfors, 2000), most recent studies report on non-temperature related factors interfering with $E_{stem}$ (Yang et al., 2012). In particular, CO$_2$ originating from woody tissue respiration can diffuse into the atmosphere remote from the site of respiration, as dissolved CO$_2$ is transported internally away from the site of respiration via the transpiration stream (Teskey & McGuire, 2002) (Chapter 1). Teskey & McGuire (2007) found that internal transport of respired CO$_2$ might account for up to 70% of the CO$_2$ derived from stem respiration, which explains past observations of decreases in stem CO$_2$ efflux during periods of high transpiration (Negisi, 1974; Martin et al., 1994; McGuire & Teskey, 2004; Bowman et al., 2005; McGuire et al., 2007; Saveyn et al., 2007b). Moreover, Bloemen et al. (2013b) recently showed that a considerable fraction of $E_{stem}$ might be derived from belowground respired CO$_2$ transported with the transpiration stream (Chapter 4), indicating the need to measure continuously CO$_2$ efflux as well as transport of respired CO$_2$ via the transpiration stream (Trumbore et al., 2013).

When no sap flow occurs, other factors that may influence efflux-based estimates of stem respiration are often neglected. At night-time, lower temperatures and a better
overall tissue water status might lower and increase the overall respiration rate, respectively, as compared to the day-time when temperatures are higher and sap flow depletes the water reserves. Woody tissue photosynthesis in chlorophyll containing bark and xylem tissues can refix a part of the locally respired CO₂ (Pfanz et al., 2002), present in tree stems at CO₂ concentrations ([CO₂]) ranging from <1 to over 26% (Teskey et al., 2008). Wittmann et al. (2006) observed a reduction in CO₂ diffusion to the atmosphere under illumination because up to 97% of the respired CO₂ was locally assimilated by woody tissue photosynthesis. While an opaque stem cuvette is generally used to measure Eₘₚ, preventing local assimilation of respired CO₂, woody tissue photosynthesis in stem or branch sections remote from the site of measurement might account for observed non-temperature related variations in Eₘₚ during the dormant season (Saveyn et al., 2008a). Saveyn et al. (2008a) hypothesized that woody tissue photosynthesis in the stem sections above or below the stem cuvette might reduce the internal [CO₂] within these sections, inducing an axial diffusion of CO₂ away from the site of respiration. However, we still lack an accurate assessment of the impact of axial diffusion of respired CO₂ on Eₘₚ measurements.

Therefore, the aim of this study was to proof the hypothesis of Saveyn et al. (2008a) and to illustrate the extent to which axial diffusion of respired CO₂ could have an impact on Eₘₚ during dormant season measurements. Although diffusion in the axial direction is higher than in the radial direction, it is still considered insignificant relative to internal transport of CO₂ via the transpiration stream (Hölttä & Kolari, 2009). We measured under controlled conditions Eₘₚ with a stem cuvette on dormant oak (Quercus robur L.) trees and induced woody tissue photosynthesis at varying heights by illuminating different stem sections, while the remainder of the tree was excluded from light. We hypothesized that a fraction of the respired CO₂ would be transported axially instead of contributing to Eₘₚ and that the effect of axial diffusion of CO₂ on Eₘₚ would be more pronounced when stem sections closer to the cuvette were illuminated. This hypothesis led us to speculate that previous efflux-based measurements of dormant season stem respiration, generally used to partition respiration in its growth and maintenance component during the growing season may need reconsideration.
5.2 Materials and methods

5.2.1 Plant material and measurement conditions

Experiments were conducted under controlled conditions in a growth chamber (2 m x 1.5 m x 2 m, height x width x length) during the winter of 2011-2012. Measurements were performed on two 4-y-old oak (Quercus robur L.) trees, hereafter referred to as QR1 and QR2, with both an approximate height of 2 m and a diameter at stem base of 3.22 and 3.19 cm, respectively. Both trees were previously grown outdoors in 50 l containers, containing a potting mixture (LP502D, Peltracom nv, Gent, Belgium) and a fertilizer (Basacot Plus 6M, Compo Benelux nv, Deinze, Belgium). Each tree was moved to the growth chamber one week prior to the measurements, allowing them to adapt to the indoor conditions. To this end we measured stem CO₂ efflux (E_{stem}) before the actual light manipulation started and checked whether E_{stem} remained stable under the controlled constant environmental conditions in the growth chamber. Air temperature (T_{air}) was measured with a Type-T thermocouple (Omega, Amstelveen, The Netherlands), relative humidity (RH) with a capacitive RH-sensor (Model HIH-3605-A, Honeywell, Morristown, NJ, USA) and photosynthetic active radiation (PAR) with a quantum sensor (model Li-190, Li-COR, Lincoln, TE, USA). T_{air} and RH were kept constant during the entire experiment. Light was supplied by densely packed fluorescent lamps (TLD 36 W/85, Philips, Eindhoven, The Netherlands), producing a constant background PAR of 140 μmol m⁻² s⁻¹ at the top of the tree during the entire experiment.

5.2.2 Stem CO₂ efflux measurements

E_{stem} was measured on a stem section at 30 cm above the soil surface, with a stem diameter of 3.06 and 3.02 cm for QR1 and QR2, respectively. The cuvette was 13 cm long, constructed of a Polycarbonate film (Roscolab Ltd, London, UK) and sealed to the stem with adhesive closed-cell foam gasket material (Rs components benelux, Anderlecht, Belgium) and non-caustic silicone sealer (Rs components benelux, Anderlecht, Belgium) (Fig. 1.14A). Outside air was mixed in a 50 l buffer vessel in order to obtain a stable inlet air [CO₂] and was pumped to the stem cuvette with a membrane pump (model 2-Wisa, Hartmann and Braun, Frankfurt am Main, Germany) at an average flow rate of
1.1 l min⁻¹, as measured with a flow meter (model 5860S, Brooks Instruments, Ede, The Netherlands). The [CO₂] of the air leaving the stem cuvette was measured with an infrared gas analyzer (IRGA, LI-7000, Li-COR, Lincoln, TE, USA) and was compared with the [CO₂] of the air leaving a reference cuvette. This cuvette had the same dimensions as the measurement cuvette but enclosed a PVC tube of 3.2 cm outer diameter, which was approximately similar to the average stem diameter of the sections enclosed for Estem measurements. Every hour a zero measurement was performed to correct for possible drift of the IRGA during the measurements. Estem was calculated according to Long & Hallgren (1985) and was expressed per unit of surface area (Equation 1.7). The stem cuvettes were leak tested before the start of the experiment and sealed where needed. Stem temperature (Tstem) was measured 2 cm below and 2 cm above the stem cuvette with 1 cm long home-made thermocouple needle (type T, Omega Engineering, Omega, Amstelveen, The Netherlands) to verify constant Tstem during the experiment.

5.2.3 Axial CO₂ diffusion in tree stems

To account for the potential effect of axial CO₂ diffusion on Estem, we induced a [CO₂] gradient within the tree stem (Fig. 5.1). Where light was excluded from the whole tree and the stem cuvette by loosely wrapping the tree with aluminum foil, a 10 cm long stem section was exposed to a movable fiber optic light source (Model FL-4000, Walz Mess und Regeltechnik, Effeltrich, Germany), producing an average PAR of 850 μmol m⁻² s⁻¹ (Fig. 1.14B). A fiber optic light source was selected because it does not emit heat in contrast to other standard light sources while producing a homogenous light distribution. A PVC tube, 10 cm long, cut in half and at the inside covered with reflective foil, was used to illuminate the other side of the stem section at an average PAR of 55 μmol m⁻² s⁻¹. As a result, woody tissue photosynthesis occurred at this particular stem section, lowering the stem [CO₂] relative to the site of Estem measurements, resulting in an axial diffusion of CO₂ inside the stem (Fig. 5.1). We altered the axial diffusion of CO₂ by illuminating different stem sections along a height gradient: 5-15 cm, 15-25 cm, 25-35 cm, and 35-45 cm above the stem cuvette, hereafter referred to in the text as S₁, S₂, S₃, and S₄, respectively. At every position, the stem section was illuminated for 24 h while background PAR in the growth chamber was fixed at 140 μmol m⁻² s⁻¹ and data from 6 h prior and 12 h after the light exposure was additionally recorded as reference. Between
the periods of illumination of the different stem sections, a reference state was maintained for at least 24 h by loosely wrapping the previously light-exposed stem sections with aluminium foil, until a stable $E_{stem}$ reference reading was obtained.

Fig. 5.1  Schematic of radial and axial CO$_2$ fluxes in a dormant tree stem when measuring stem CO$_2$ efflux ($E_{stem}$) with a stem cuvette under different conditions. (A) During tree and stem cuvette light exclusion only outward radial diffusion of respired CO$_2$ occurs from the inner bark (1a), cambium (1b) and xylem ray cells (1c). (B) A stem section remote from the stem cuvette is illuminated, which triggers woody tissue photosynthesis (2), while light is excluded from the remainder of the stem and the cuvette. Stem CO$_2$ concentration ([CO$_2$]) decreases within this stem section relative to [CO$_2$] in the stem section enclosed in the stem cuvette, resulting in an upward axial diffusion of CO$_2$ (3) and a decreased contribution of radial diffusion of CO$_2$ to $E_{stem}$ (1a, 1b, and 1c). Adapted from Teskey et al. (2008).

5.2.4 Sap flow and stem diameter measurements

Sap flow rate ($F_s$) and variations in stem diameter ($D$) were measured to validate whether the trees were dormant. Variations in $D$ were measured using a linear variable displacement transducer (LVDT; model DF 5.0, Solartron Metrology, Leicester, UK). This LVDT was attached to the tree with a custom-made stainless steel holder installed at a height of 0.95 m above the soil. A small circular-shaped hole was made in the aluminium foil to ensure proper contact between the stem and the sensor head of the LVDT. $F_s$ was measured with a heat balance (HB) sensor (model SGB 17-WS, Dynamax Inc., Houston,
USA) at a height of 1.05 m above the soil. Sensor installation and $F_s$ calculation were performed according to the guidelines in the operation manual (van Bavel & van Bavel, 1990).

5.2.5 Measurements of chlorophyll concentration

At the end of each experiment, bark of the stem sections $S_1$ to $S_4$ was collected for determination of bark chlorophyll concentration. Per stem section, four samples were randomly collected, immediately frozen in liquid nitrogen and stored at -80°C. Samples were grinded (A11 basic analytic mill, IKA-Werke GmbH & Co. KG, Staufen, Germany) and chlorophyll was extracted by adding 7.5 ml acetone (80%) to 150 mg of sample. After 24 h extraction in the dark, samples were centrifuged and the supernatant was transferred to a glass cuvette and analyzed for chlorophyll concentration with a spectrophotometer (UVIKON XL, Bio-Tek Instruments, Winooski, VT, USA) at wavelengths of 663.6 and 646.6 nm. Chlorophyll concentrations were calculated according to Porra et al. (1989) and expressed per unit of bark fresh weight (mg chl g$^{-1}$ FW).

5.2.6 Data and statistical analysis

All data was recorded with a data logger (HP 34970A, Hewlett-Packard, Palo Alto, CA, USA) at a 1 min interval and averaged over 5 min intervals. For statistical analysis, we used a multi-factorial analysis of variance (ANOVA) model to compare the chlorophyll concentration of the different stem sections with stem section ($n=4$, $S_1$ to $S_4$) as fixed factor and individual tree ($n=2$) as random factor. For statistical analysis of $E_{stem}$, data was averaged over 1 h intervals and a repeated measures ANOVA was performed with the different experimental stages ($n=5$, reference and $S_1$ to $S_4$) and time ($n=42$) as fixed factors and individual tree as random factor ($n=2$). Akaike's information criterion corrected for small sample sizes (AICc) was used to determine the covariance structure that best estimated the correlation among individual trees over time. ANOVA analyses were performed using the mixed model procedure (PROC MIXED) of SAS (Version 9.1.3, SAS inc., Cary, NC, USA) with $\alpha=0.05$. 
5.3 Results

5.3.1 Microclimate, sap flow and stem diameter

Both QR1 and QR2 were subjected to a constant air temperature (T_air) and relative humidity (RH) regime during the entire experiment in the growth room (Table 5.1). Also stem temperature (T_stem) was constant, irrespective of the light treatment. For QR1, averaged T_stem below and above the stem cuvette during the reference period was 20.52 ± 0.05°C, which is in the range of the measured average T_stem when one of the stem sections was illuminated (20.42 ± 0.18°C to 20.75 ± 0.20°C). Average T_stem of QR2 during reference (20.08 ± 0.02°C) was similar to the average T_stem observed during illumination (range from 20.06 ± 0.05°C to 20.16 ± 0.15°C). Therefore changes in E_stem measured during stem section illumination are independent of variations in T_stem.

Table 5.1 Microclimatological conditions prevailing during the experiments on both oak trees (QR1 and QR2). Air temperature (T_air, °C), stem temperature (T_stem, °C) and relative humidity (RH, %) data are averages (± SD) over the entire measurement period.

<table>
<thead>
<tr>
<th></th>
<th>T_air</th>
<th>T_stem</th>
<th>RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>QR1</td>
<td>19.86 ± 0.34</td>
<td>20.61 ± 0.22</td>
<td>42.53 ± 5.08</td>
</tr>
<tr>
<td>QR2</td>
<td>20.37 ± 0.08</td>
<td>20.10 ± 0.12</td>
<td>40.81 ± 2.84</td>
</tr>
</tbody>
</table>

Measurements of sap flow rate (F_s) and variation in stem diameter (D) confirmed that both trees were dormant. Heat balance data indicated that there was no heat transfer by convection within the stem, excluding the occurrence of sap flow within the xylem. Variations in D on a diurnal and longer time scale were not observed (data not shown).

5.3.2 Stem CO2 efflux and axial diffusion of CO2

Overall, stem CO2 efflux (E-stem) was low, with an average reference value of 0.76 ± 0.02 and 1.04 ± 0.02 μmol m^-2 s^-1 for tree QR1 and QR2, respectively. Illumination of the defined stem segments induced axial diffusion of CO2 in the stem, which decreased E_stem relative to the reference value (Fig. 5.2). The distance between the illuminated stem section and the stem cuvette had a significant effect on the decrease in E_stem (P=0.0115). The largest decrease in E_stem was observed when S1 was exposed to light. In QR1 a reduction of 0.17 μmol m^-2 s^-1 was observed 24 h after light exposure (Fig. 5.2) or 22%
relative to the reference $E_{stem}$ (Fig. 5.3). In QR$_2$ a decrease of 0.14 μmol m$^{-2}$ s$^{-1}$ was detected or 13% relative to the reference (Fig. 5.3). In both trees, the response in $E_{stem}$ on S$_1$ illumination was fast, with a time lag of 1 h 30 min and 1 h 50 min between light exposure and response for QR$_1$ and QR$_2$, respectively. A similar time lag was observed at the end of the S$_1$ illumination period.

The more remote the light-exposed stem sections were, the smaller the impact of axial CO$_2$ diffusion on $E_{stem}$ was. Where illuminating S$_2$ reduced $E_{stem}$ by 0.10 μmol m$^{-2}$ s$^{-1}$ and 0.12 μmol m$^{-2}$ s$^{-1}$ in QR$_1$ and QR$_2$, respectively, a reduction of only 0.06 μmol m$^{-2}$ s$^{-1}$ in $E_{stem}$ was found when exposing S$_3$ and S$_4$ in both trees. These findings led us to conclude that the reduction in $E_{stem}$ in these trees due to axial CO$_2$ diffusion are small and can be ignored when stem sections at a distance of 30 cm or more from the stem cuvette are illuminated (Fig. 5.3). Due to the long distance between stem cuvette and S$_3$ and S$_4$, the
transient decrease in $E_{\text{stem}}$ due to axial diffusion was slow, with only a slightly lower value in $E_{\text{stem}}$ compared to the reference after the end of illumination (Fig. 5.2).

![Graph showing the reduction in stem CO$_2$ efflux ($E_{\text{stem}}$) measured with a stem cuvette for two dormant Quercus robur trees (QR1, black circles; QR2, grey circles), when illuminating 10-cm-long stem sections at different distances from the site of $E_{\text{stem}}$ measurement, while light is excluded for the remainder of the tree and stem cuvette. Reductions are expressed relative to $E_{\text{stem}}$ measurements performed when light was excluded from the entire tree.]

5.3.3 Bark chlorophyll concentration

No significant difference in bark chlorophyll concentration among $S_1$, $S_2$, $S_3$, and $S_4$ was observed ($P=0.31$) (Table 5.2). Chlorophyll concentrations ranged from $0.37 \pm 0.05$ to $0.45 \pm 0.02$ mg chl g FW$^{-1}$ in QR1 and from $0.35 \pm 0.04$ to $0.41 \pm 0.04$ mg chl g FW$^{-1}$ in QR2. These similar levels in chlorophyll concentration observed in $S_1$ to $S_4$ indicate that the capacity for woody tissue photosynthesis can be assumed equal in the different light-exposed stem sections.
Table 5.2  Bark chlorophyll concentrations (mg chl g\(^{-1}\) FW) of bark of both oak trees (QR\(_1\) and QR\(_2\)), randomly sampled after the experiment for the four different 10-cm-long stem sections (S\(_1\), S\(_2\), S\(_3\), and S\(_4\)) exposed to light. Data are averages (±SD) of four samples per stem section per tree.

<table>
<thead>
<tr>
<th>Stem segment</th>
<th>QR(_1)</th>
<th>QR(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1)</td>
<td>0.45 ± 0.08</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>S(_2)</td>
<td>0.44 ± 0.04</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>S(_3)</td>
<td>0.45 ± 0.04</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>S(_4)</td>
<td>0.37 ± 0.02</td>
<td>0.38 ± 0.02</td>
</tr>
</tbody>
</table>

5.4 Discussion

Recent advances in the field of tree respiration research fostered an important discussion regarding the use of efflux-based measurements to estimate the “actual” stem respiration (Trumbore et al., 2013). Where past studies assumed that stem CO\(_2\) efflux (E\(_{stem}\)) equaled stem respiration, it is now acknowledged that CO\(_2\) emitted by stems is derived from a multitude of sources affected by different factors (Yang et al., 2012; Trumbore et al., 2013) (Chapter 1). With this study we want to illustrate that axial diffusion of respired CO\(_2\) is an additional factor that should be accounted for when estimating stem respiration. Up till now, this flux was considered insignificant in comparison to xylem transport of respired CO\(_2\) during the growing season (Hölttä & Kolari, 2009), but its importance remained unclear for efflux-based dormant season estimates of stem respiration.

During tree dormancy, it is generally accepted that only maintenance stem respiration contributes to E\(_{stem}\) and that its dynamics are mainly driven by temperature (Amthor, 1989; Maier et al., 1998). However, we observed large non-temperature related variations in E\(_{stem}\) in dormant oak trees, similar as described by Saveyn et al. (2008a). By locally illuminating different stem sections near the stem cuvette, while excluding light from the remainder of the tree and the cuvette, we observed pronounced decreases in E\(_{stem}\) rates. More interestingly, we observed the largest reduction in E\(_{stem}\) when the stem section closest to the cuvette was exposed to light, with decreases up to 22% due to axial diffusion of respired CO\(_2\) in stems.
Bark chlorophyll concentrations in the dormant oak stems were within the range reported for trees (Pfanz et al., 2002). Assimilation of respired CO₂ within the chlorophyll containing tissues locally reduces the CO₂ concentration ([CO₂]) relative to internal [CO₂] at stem sections where woody tissue photosynthesis is excluded, as observed by Saveyn et al. (2008a). As a result, a [CO₂] gradient along the stem arose, inducing a spontaneous axial diffusion of respired CO₂ from the site of high to low [CO₂], according to Fick’s law of diffusion (Fig. 5.1; Jones, 1992; Saveyn et al., 2008a). As a consequence, a lower fraction of respired CO₂ diffused radially into the stem cuvette, observed as a decrease in Eₘₚ. Additionally, Fick’s law of diffusion states that the amount of CO₂ diffusing along the concentration pathway is dependent on its length. This explains why the largest decrease in Eₘₚ was observed during illumination of the stem section closest to the stem cuvette.

In general, axial diffusion of gases within plants induced by woody tissue photosynthesis has been described to play a role in root aeration in genera of Alnus, Salix as well as Betula pubescens and Populus tremula (Grosse et al., 1996; Armstrong & Armstrong, 2005). During illumination of aboveground plant parts, photosynthesis in chlorophyll containing woody tissues increases the internal O₂ concentration, inducing a diffusive transfer of O₂ to the roots (Armstrong & Armstrong, 2005). Moreover, axial diffusion of O₂ is facilitated relative to its radial diffusion due to the anatomy of the tree stem. Sorz & Hietz (2006) reported that for Quercus robur diffusion of O₂ in the axial direction was more than 20 times higher than in radial direction, due to the fact that the diffusing gas encounters less cell walls when travelling along the stem axis. For CO₂, a similar high resistance to radial diffusion exerted by the xylem, cambium and bark layers has been described and a radial resistance factor should be included when using efflux-based measurements to model stem respiration (Steppe et al., 2007). The fact that CO₂ might diffuse rapidly in axial direction due to woody tissue photosynthesis was first suggested by Saveyn et al. (2008a). Based on the species specific diffusion coefficients for oxygen diffusion for Quercus robur (6.9 x 10⁻⁸ m² s⁻¹ at a 15% moisture content, Sorz & Hietz (2006)) we can estimate the time for CO₂ to diffuse axially within stems, as done in Nobel (1999). For a 10 cm distance, oxygen axial diffusion would take around 4.6 h. Axial diffusion of CO₂ will probably take longer, given that molecules with a higher mass
tend to have a lower diffusion coefficient (Nobel, 1999), but the transfer time would be in the same order of hours. This estimate is in contrast with the observation by Gansert (2003), which stated that diffusion of gases in stems is very slow (1 m would take several years), depending on the CO₂ gradient. However Gansert (2003) assumed that movement of gases in stems mainly will occur via the aqueous phase, while an important fraction of the stem consists of gas voids (i.e. 25% gas by volume in stems of Quercus sp. (MacDougal et al., 1929; Teskey et al., 2008)), where diffusion occurs much faster than in water (Nobel, 1999).

Our experimental data also allows us to illustrate whether it is justified to neglect axial diffusion of respired CO₂ when estimating Estem during the growing season. Respired CO₂, either derived from below- or aboveground sources, is transported upward via the transpiration stream (Aubrey & Teskey, 2009; Bloemen et al., 2013b) and may confound efflux-based estimates of stem and branch respiration (Teskey & McGuire, 2002; McGuire & Teskey, 2004; McGuire et al., 2007). In a previous study on six 9-y-old field grown Quercus robur trees the maximal rate of internal CO₂ transport with the transpiration stream measured at the stem base was about 6 μmol CO₂ s⁻¹ through the stem cross section (Chapter 7). If we now assume that the maximal observed reduction in Estem in our dormant trees (0.17 μmol CO₂ m⁻² stem surface area s⁻¹) reflects the potential axial diffusion in these field-grown trees, and we scale this number with the stem surface area of the respective cuvette (0.026 m²), we obtain an axial diffusion rate of about 0.0045 μmol respired CO₂ s⁻¹. This diffusion rate is approximately three orders of magnitude lower than the internal CO₂ transport rate and therefore it is reasonable to postulate that axial diffusion of respired CO₂ and its impact on Estem is negligible during the growing season when sap flow occurs. On the other hand, the diffusion coefficient of gases in stems is expected to increase with an increase in stem gas volume (Sorz & Hietz, 2006), which occurs concomitant with a decrease in volumetric water content during the change from dormant to growing season (MacDougal et al., 1929; Pausch et al., 2000). Nonetheless, axial diffusion of respired CO₂ remains of lower magnitude during the growing season relative to the internal transport of CO₂ with the transpiration stream.
Accurate efflux-based estimates of stem respiration during dormancy are essential for partitioning respiration in a growth and a maintenance component according to the functional model of respiration (McCree, 1970; Amthor, 1989; Maier, 2001). In field experiments, the most widely used approach to estimate growth and maintenance stem respiration is the mature tissue method (e.g. Maier, 2001; Damesin, 2003; Gaumont-Guay et al., 2006), where temperature-corrected coefficients of dormant season stem respiration, i.e. $Q_{10}$ (coefficient that describes the change in $E_{stem}$ for a $10^\circ$C increase in $T_{stem}$) and $E_{stem}(ref)$ (a regression coefficient that estimates $E_{stem}$ at a reference $T_{stem}$), are combined with actual $T_{stem}$ data to estimate maintenance stem respiration during the growing season (Lavigne & Ryan, 1997). However, results from an additional experiment on QR$_2$, in which we determined $Q_{10}$ and $E_{stem}(20)$ under a changing temperature program, showed that the above efflux-based mature tissue method overly and underestimated the first and latter parameter, respectively. When exposing S$_1$ to light, we found $Q_{10}$ and $E_{stem}(20)$ coefficients of 2.25 and 1.29 μmol CO$_2$ m$^{-2}$ s$^{-1}$, respectively compared to the reference $Q_{10}$ (2.05) and $E_{stem}(20)$ (1.40 μmol CO$_2$ m$^{-2}$ s$^{-1}$) values. However, we assume that the results obtained during our experiment, where we illuminated stem sections only above the stem cuvette, may underestimate the potential effect of axial CO$_2$ diffusion on dormant season stem respiration under natural conditions. Larger differences in $Q_{10}$ and $E_{stem}(20)$ were obtained by Saveyn et al. (2008) where the entire Quercus robur stem surface area above and below a stem cuvette was exposed to low light levels under controlled conditions.

Under natural conditions even larger errors in dormant season $Q_{10}$ and $E_{stem}(ref)$ might arise, because in species that contain higher bark and/or xylem chlorophyll concentrations (Pfanz et al., 2002), a larger axial diffusive flux might build up in the stem and confound dormant season stem respiration estimates. On the other hand larger trees with larger stems will have a thicker dead outer bark than the trees used in our study, thereby reducing the light that reaches the chloroplasts and the potential bias due to axial diffusion of respired CO$_2$. Moreover, in larger trees the volume of wood relative to the stem surface area would be higher, probably resulting in larger radial contribution to $E_{stem}$ relative to axial diffusion. Nevertheless in larger dormant trees, axial diffusion of respired CO$_2$ is hypothesized to affect dormant season stem internal...
[CO₂], which contributes to $E_{stem}$ (Etzold et al., 2013). Therefore accounting for the effect of axial diffusion of CO₂ when estimating $Q_{10}$ and $E_{stem(ref)}$ respiration coefficients might be crucial to understand and model stem respiration, at least from a mechanistic perspective. However, further validation of the magnitude of axial diffusion of CO₂ in larger trees and in other species is needed.

Our study, hence, illustrates the importance of accurate dormant season stem respiration estimates to generate realistic maintenance respiration data. While in actively transpiring trees during the growing season the impact of internal transport of respired CO₂ on $E_{stem}$ has been established, our results show that axial diffusion of CO₂ induced by woody tissue photosynthesis might affect $E_{stem}$ measurements in dormant trees. Our above findings led us to recommend additional shading in dormant trees of upper and lower stem sections adjacent to the opaque stem cuvette in order to avoid axial diffusion of respired CO₂ away from the site of respiration. This newly proposed methodology will lead to more accurate $Q_{10}$ and $E_{stem(ref)}$ estimates, particularly in ‘open’ forest stands with sufficient light penetration. These more accurate estimates will enable a more accurate quantification of dormant season stem respiration, which, in turn, is crucial to better understand stand-level respiration dynamics throughout the year.
Role of woody tissue photosynthesis in tree drought stress resilience


**Abstract**

Within trees, a portion of the respired CO₂ is assimilated by woody tissue photosynthesis, but its physiological role in trees remains unclear, in particular under unfavourable conditions like drought stress. We measured leaf gas exchange (maximum leaf net photosynthesis ($A_{\text{max}}$) and transpiration rate (E)) and stem daily growth rate (DG) for *Populus deltoides x nigra* 'Monviso' trees under both well-watered and dry conditions. Half of the trees were subjected to a stem and branch light exclusion treatment to prevent woody tissue photosynthesis, while the others served as controls. We additionally determined bark chlorophyll concentration and measured cavitation in detached control and light-excluded branches to investigate the role of woody tissue photosynthesis in xylem embolism repair. We hypothesized that woody tissue photosynthesis will contribute to overall tree carbon gain both under sufficient water supply and during drought stress and that light-excluded trees will suffer faster and more dramatically from drought stress than (non light-excluded) control trees. Under well-watered conditions, light exclusion resulted in reduced stem DG relative to control trees. In response to drought, stem shrinkage of light-excluded trees was more pronounced as compared to control trees and $A_{\text{max}}$ and E decreased more rapidly during drought stress for light-excluded trees compared to control trees. Results from our
acoustic xylem cavitation measurements confirmed the potential role of woody tissue photosynthesis in xylem embolism repair. Therefore, our study indicates that woody tissue photosynthesis may be a key factor in the resilience of trees to drought stress by maintaining both the plant carbon economy and hydraulic function.

6.1 Introduction

Chlorophyll in stem and branch tissues has been found to play a role in carbon acquisition. By assimilating respired CO₂, which is present at high concentrations in trees (Chapter 1), these photosynthetically active tissues photo-reduce CO₂ similarly as described for green leaves (Pfanz et al., 2002; Berveiller et al., 2007a). This re-assimilated CO₂ would otherwise be lost to the surrounding atmosphere, which explains why woody tissue photosynthesis is often described within the context of a tree carbon recycling mechanism (Aschan & Pfanz, 2003; Cernusak & Hutley, 2011).

Woody tissue photosynthetic rates are relatively low compared to leaf photosynthetic rates and it rarely results in a positive net CO₂ assimilation rate (Wittmann et al., 2001). Wittmann et al. (2006) found that woody tissue photosynthesis was able to assimilate up to 97% of the dark respired CO₂ in young Betula pendula Roth. and Coe & McLaughlin (1980) reported a maximum re-assimilation of respired CO₂ of 31% for Acer rubrum branches, measured under dormancy. Other studies reported similar maximum re-assimilation values for other trees species (see Table 4 in Pfanz et al., 2002), but overall the impact of woody tissue photosynthesis on plant carbon economy under normal conditions is expected to be limited.

Nevertheless, woody tissue photosynthesis might play an important role during particular events. Different authors consider woody photosynthesis as a potentially important means of bridging the carbon balance between defoliation and re-foliation (Bossard & Rejmanek, 1992; Wittmann et al., 2001; Pfanz, 2008; Eyles et al., 2009) or during bud development (Saveyn et al., 2010). Other studies suggest that woody tissue photosynthesis might improve the stem carbon balance under limited water availability (Wittmann & Pfanz, 2008). In desert and semi-desert habitats, many drought-adapted plants have photosynthetic stems (Gibson, 1983; Nilsen & Sharifi, 1994), which
potentially provide a major fraction of the carbon used to sustain plant metabolism (Comstock & Ehleringer, 1990; Nilsen & Bao, 1990; Aschan & Pfanz, 2003). For temperate plants under moderate drought stress, decreased stomatal conductance can limit the assimilation of atmospheric CO₂ in the leaves (Flexas & Medrano, 2002; Chaves et al., 2003), whereas woody tissue photosynthesis is supplied by endogenously respired CO₂ (Pfanz et al., 2002; Aschan & Pfanz, 2003; Wittmann & Pfanz, 2008). Therefore, the fraction of tree carbon derived from woody tissue photosynthesis might become more important under drought conditions.

However, the exact role of woody tissue photosynthesis during drought stress is not yet well understood. Woody tissue photosynthesis has been shown to be less sensitive to drought stress than leaf photosynthesis (Nilsen, 1992). Moreover, Schmitz et al. (2012) hypothesized that woody tissue photosynthesis in xylary chloroplasts might be important for maintaining the hydraulic function of the vasculature, which is crucial during drought stress. Photosynthetic activity in these chloroplasts potentially fulfils local energetic and carbohydrate demands for repair of cavitated vessels (Zwieniecki & Holbrook, 2009; Secchi & Zwieniecki, 2011; Schmitz et al., 2012) (Box 1.2). Therefore, by maintaining the carbon balance as well as the plant water status, woody tissue photosynthesis might play a crucial dual role in tree drought stress resilience.

In this study, we investigated the importance of woody tissue photosynthesis in 1-y-old poplar (Populus deltoides x nigra ‘Monviso’) trees under both well-watered and drought stress conditions. We measured photosynthesis and transpiration at leaf level and diameter growth at stem level (both manually and automatically) while manipulating the light availability to stem and branches (control and 100% light-excluded trees). We hypothesized that woody tissue photosynthesis will contribute to overall tree carbon gain both under sufficient water supply and during drought stress. We also hypothesized that light-excluded trees will suffer faster and more dramatically from drought stress than (non light-excluded) control trees. Finally, we tested the hypothesis of Schmitz et al. (2012) on the significance of woody tissue photosynthesis in maintaining hydraulic functioning of drought-stressed trees, by acoustically measuring xylem cavitation in branches of light-excluded and control trees (Perks et al., 2004).
hypothesized that the hydraulic functioning of xylem tissue in light-excluded trees will be less than in control trees.

6.2 Material and methods

6.2.1 Plant material and experimental design

1-y-old cutting-derived trees of *Populus deltoides x nigra* ‘Monviso’ were used for this study. Twelve cuttings were planted at the end of June 2012 in 50 l containers filled with a potting mixture (LP502D, Peltracom nv, Gent, Belgium) and slow-releasing fertilizer (Basacot Plus 6M, Compo Benelux nv, Deinze, Belgium) and grown in the greenhouse facility at the Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium. Eight plants were selected based on uniform height (approximately 1.6 m) and stem diameter and were randomly assigned to two treatments. Four plants served as control, while the others were used for the light exclusion treatment, which started on 2 August 2012 (day of the year, DOY 214). The stem and woody branches of these light-excluded trees were loosely wrapped with aluminium foil following Saveyn *et al.* (2010), so gaseous diffusion from woody tissues to the atmosphere was not restricted. At this time, all trees were irrigated at least twice a week, ensuring adequate water supply. Subsequently, trees were irrigated for the last time on 30 September 2012 (DOY 273) to impose drought stress. No leaf fall occurred until one week after the start of the drought stress treatment. The fully developed leaf area before drought stress was determined by randomly sampling ten leaves per tree and multiplying this average leaf area with the number of leaves per tree. Leaf area ranged from 3.2 to 4.7 m² and from 2.8 to 3.9 m² for control and light-excluded trees, respectively, and average leaf area was not different between both treatments (*P*=0.20).

6.2.2 Microclimate and plant measurements

Relative humidity (RH) in the greenhouse was measured with a capacitive RH sensor (Type hih-3610, Honeywell, Morristown, NJ, USA), air temperature (T<sub>air</sub>) with a copper constantan thermocouple (Type T, Omega, Amstelveen, USA), and photosynthetic active radiation (PAR) with a quantum sensor (LI-190S, Li-COR, Lincoln, TE, USA). Sensors were installed at a height of approximately 2 m.
Plant measurements were performed from the beginning of July 2012 until mid-October 2012. Stem diameter variations were continuously measured with linear variable displacement transducers (LVDT; model DF5.0, Solartron Metrology, Bognor Regis, UK), installed with custom made stainless steel holders on one control tree and one light-excluded tree. Stem radial daily growth rate (DG) was calculated as the difference between two successive daily maximum values of the stem diameter. On 21 September 2012 (DOY 264), before the start of the drought stress, two additional trees (one control and one light-excluded tree) were instrumented with LVDT sensors. Continuous stem diameter measurements were complemented with weekly calliper-based manual measurements, performed between 10 h and 14 h. Two measurements were made at a height of 30 cm along perpendicular axes. Average DG derived from the manual measurements was calculated as the difference between two successive measurements divided by the elapsed time between both measurements.

At leaf level, maximum net photosynthesis ($A_{\text{max}}$ μmol CO$_2$ m$^{-2}$ s$^{-1}$) and transpiration rate (E, mmol H$_2$O m$^{-2}$ s$^{-1}$) were measured on all control and light-excluded trees on two fully expanded leaves per tree with a portable photosynthesis system (model Li-6400, Li-Cor, Inc., Lincoln, Nebraska, USA). Measurements were performed at 25°C, prevailing RH conditions, set atmospheric CO$_2$ concentration of 400 ppm and a PAR level of 1500 μmol m$^{-2}$ s$^{-1}$. The latter was determined via a preliminary experiment in which light response curves were obtained for one leaf on six trees. Leaf gas exchange measurements were performed biweekly during the period before drought stress between 10 h and 16 h, and at a 2 d interval during the drought stress treatment. At each measurement time, the average of five measurements recorded at 10 s intervals was used for the analysis.

6.2.3 Cavitation measurements

To test the hypothesis of Schmitz et al. (2012) on light-dependent repair of cavitated vessels by woody tissue photosynthesis, we performed a dehydration experiment in the lab on cut branches of one non-instrumented control and one light-excluded tree, while acoustically measuring xylem cavitation as described in Lo Gullo & Salleo (1993) and Rosner et al. (2006). Measurements were repeated three times: once 9 days before the start of the drought stress treatment (21 September 2012, DOY 265) and twice during
the drought stress treatment (i.e. 5 and 20 days after the start of the drought stress treatment, 5 and 19 October 2012, DOY 279 and 293, respectively). After cutting the branches, the outer end was covered with parafilm to prevent dehydration before the start of the measurement. In the lab, one acoustic emission (AE) sensor (VS150-M sensor and ASCO-P signal conditioner, Vallen systeme, Icking, Germany) was installed per branch. The AE sensors were clamped to the middle of the branches according to Rosner et al. (2009). Measurements were performed over a 60 h time span. The maximum peak amplitude per second was recorded using a data acquisition system (Type NI USB 6009, National Instruments, Austin, Texas, USA) and software (Labview 8.2, National Instruments, Austin, Texas, USA). The acoustic signals above a certain threshold were assumed to occur due to cavitation of xylem vessels. The threshold was set at the background noise level (35.5 and 31 dB) and was determined by waving the sensors in the air. The cumulated number of acoustic signals was determined over the 60 h measurement period.

6.2.5 Measurements of bark chlorophyll concentration

We determined the effect of the light exclusion treatment on bark chlorophyll concentration by randomly sampling the bark of stems of two additional control and light-excluded trees on two dates: at the day of light exclusion (2 August 2012, DOY 214) and 53 days after the start of light exclusion (24 September 2012, DOY 267). At the end of the measurement period, 99 days after the start of light exclusion (9 November 2012, DOY 313), bark was sampled from all control and light-excluded trees. Per stem, three samples were randomly collected, immediately frozen in liquid nitrogen and stored at -80°C. Samples were ground (A11 basic analytic mill, IKA-Werke GmbH & Co. KG, Staufen, Germany) and chlorophyll was extracted by adding 7.5 ml aceton (80%) to 150 mg of sample. After 24 h extraction in the dark, samples were centrifuged and the supernatant was transferred to a glass cuvette and analyzed for chlorophyll concentration as described in Chapter 5.
6.2.6 Statistical analysis

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

DG derived from manual and automated measurements, leaf gas exchange data ($A_{\text{max}}$ and E) and bark chlorophyll concentration were analyzed using a repeated measures multi-factorial analysis of variance (ANOVA). DG data from manual measurements were confined to those taken during the well-watered conditions with treatment ($n=2$) and date ($n=16$) treated as fixed factors and individual tree ($n=8$) as random factor. A similar model was used to analyze DG data from the automated measurements. In this case our data was derived from a lower number of individual trees ($n=4$) and confined to the measurements taken one week before and during the drought stress treatment (date, $n=16$). Similarly, $A_{\text{max}}$ and E measurements were confined to those taken during the drought stress treatment (date, $n=12$) with a higher number of tree replicates (individual tree, $n=8$). Bark chlorophyll concentration was analyzed with date ($n=3$), treatment ($n=2$) and repetition ($n=3$ per tree) as fixed factors, and tree ($n=2$ per treatment for the first two dates and $n=4$ for the last date) as random factor. Treatment means were compared using Fisher’s least significant difference test. Akaike Information Criterion corrected for small sample sizes (AICc) was used to determine the covariance structure that best estimated the correlation among individual trees over time. All analyses were performed using the mixed model procedure (PROC MIXED) of SAS (Version 9.1.3, SAS Inc., Cary, NC, USA) with $\alpha=0.05$.

6.3 Results

6.3.1 Daily growth rate

Light exclusion of the stem and woody branches had an impact DG under both well-watered and drought conditions. Before light exclusion, DG derived from manual stem diameter measurements was similar for both treatments (Fig. 6.1). However, during light exclusion, DG of light-excluded trees was systematically lower than those calculated for the control trees. Significant differences ($P<0.05$) in DG between both
treatments were observed at specific dates throughout the well-watered period. The average overall stem diameter increment over the period July-September was smaller for the light-excluded trees (4.00 ± 0.12 mm) than for the control trees (5.28 ± 0.57 mm), indicating a 24% impact of light exclusion on stem growth during the period July-September.

**Fig. 6.1** Radial daily stem growth rate (DG) under well-watered conditions derived from manual stem diameter measurements and averaged for control (black) and light-excluded (hatched) trees (n=4 per treatment). DG was calculated as the difference between two successive measurements divided by the elapsed time between both measurements. The dashed line indicates the start of light exclusion of the stem and woody branches. Asterisks indicate significant ($P<0.05$) differences in DG between both treatments at each observation. Bars indicate standard error of the mean.

During drought stress, LVDT-based measurements indicated that stems of control and light-excluded trees shrank, resulting in a negative DG (Fig. 6.2). Before the onset of drought stress, DG was within the same range as observed for estimates from manual measurements (Fig. 6.1). During drought stress, DG of control trees decreased gradually over time, as expected. However, DG of light-excluded trees first decreased and then slightly increased until five days after the start of the drought stress. Thereafter, DG again decreased to a level lower than observed for the control trees, indicating that at this time light-excluded trees were shrinking faster than control trees (Fig. 6.2).
6.3.2 Maximum net photosynthesis and transpiration rate

Light exclusion of the stem and woody branches had additionally an impact on leaf gas exchange during drought stress (Fig. 6.3). Before drought stress, similar rates of $A_{\text{max}}$ were observed in leaves of control and light-excluded trees. During the drought treatment, $A_{\text{max}}$ in light-excluded trees started to decrease about three days earlier than in control trees (4 versus 7 October, Fig. 6.3A) and this was approximately simultaneous to the onset of negative DG during drought stress (Fig. 6.2). A similar difference between control and light-excluded trees was observed in E (Fig. 6.3B). Where E of light-excluded trees gradually decreased from 1 October onwards, E of control trees tended to increase to a local maximum at 7 October before finally decreasing to lower rates. Finally, near the end of the drought stress period, similar $A_{\text{max}}$ and E rates were observed in both treatments.
Fig. 6.3 Maximum leaf net photosynthesis ($A_{\text{max}}$) (A) and transpiration rate ($E$) (B) before and during drought stress, averaged for leaves ($n=2$ per tree) of control (black circles) and light-excluded (grey circles) trees ($n=4$ per treatment). The dashed line indicates the timing of the last irrigation before the onset of drought stress. No significant differences ($P<0.05$) in $A_{\text{max}}$ neither $E$ between both treatments were observed. Bars indicate standard error of the mean.

6.3.3 Cavitation

Our results on the AE detection of xylem cavitation in branches of control and light-excluded trees indicate that woody tissue photosynthesis might play an important role in cavitation repair during drought stress. While the cumulative number of acoustic hits was similar in branches of control and light-excluded trees before being subjected to drought stress (Fig. 6.4), we found a larger cumulative number of acoustic hits in the control branches five days after the start of the drought stress treatment (Fig. 6.4). This indicated that more xylem vessels in control branches were hydraulically functional just
before being excised compared to light-excluded ones so that more vessels could still cavitate during the three-day dehydration of the excised branch. Finally, 20 days after the start of the drought stress treatment, a low cumulative number of hits were recorded (Fig. 6.4), regardless of the treatment, implying that the majority of the xylem vessels in the branches were already cavitated before the last branch dehydration experiment started.

![Fig. 6.4](image)

**Fig. 6.4** Cavitation in branches of control (black) and light-excluded (hatched) trees measured as the cumulative number of acoustic emissions (AE) over a 60 h dehydration period. Measurements were repeated three times: at 9 d before the start of the drought stress treatment (22-24 September 2012), and 5 and 20 d after the start of the drought stress treatment (5-7 and 19-21 October 2012), respectively. The larger the cumulative number of acoustic hits, the more xylem vessels were hydraulically functional just before being excised and the more vessels could thus still cavitate during dehydration.

### 6.3.4 Bark chlorophyll concentration

Stem and branch light exclusion clearly affected bark chlorophyll concentration. At the start of the light exclusion treatment, similar bark chlorophyll concentrations were detected in the bark of control and light-excluded trees (Table 6.1).

However, light exclusion resulted in a significant decrease in bark chlorophyll concentration, whereas bark chlorophyll concentration in control trees (no light exclusion) increased over time. In particular, 99 days after the start of the light exclusion treatment, when bark of all control and light-excluded trees was sampled, a large difference was observed in bark chlorophyll concentration between both treatments (Table 6.1).
Table 6.1  Chlorophyll concentration (mg Chl g FW⁻¹) of bark, randomly sampled from Populus deltoides x nigra ‘Monviso’ trees, on different dates (2 August 2012, 24 September 2012, and 9 November 2012) during the light exclusion experiment. On the first two dates, bark was sampled (n=3 per tree) from additional trees (n=2 per treatment) treated similarly as the measured control and light-excluded trees, while on the last date bark was sampled from all control and light-excluded trees (n=4 per treatment). Data are averaged (± SE) per treatment for all samples and all trees. Different letters indicate significant differences in chlorophyll concentration between treatments for a specific date (P<0.001).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of light exclusion</th>
<th>53 days after start light exclusion</th>
<th>99 days after start light exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.33 ± 0.03ᵃ</td>
<td>0.41 ± 0.01ᵃ</td>
<td>0.73 ± 0.02ᵃ</td>
</tr>
<tr>
<td>Light-excluded</td>
<td>0.38 ± 0.02ᵃ</td>
<td>0.24 ± 0.01ᵇ</td>
<td>0.28 ± 0.01ᵇ</td>
</tr>
</tbody>
</table>

6.4 Discussion

Since the first observation of chlorophyll containing woody tissues early 20ᵗʰ century, a large number of studies have been performed on the nature and magnitude of woody tissue photosynthesis in trees (Bossard & Rejmanek, 1992; Nilsen, 1995; Cernusak & Marshall, 2000; Wittmann et al., 2001; Pfanz et al., 2002; Berveiller et al., 2007a) (Chapter 1). This photosynthetic tissue uses respired CO₂ as substrate, thereby partially compensating for the loss of carbon by respiration while providing carbon compounds for tissue synthesis or to sustain plant metabolism (Cernusak et al., 2001). The overall supply of carbon by woody tissue photosynthesis as compared to the fraction of carbon derived from leaf level photosynthesis is assumed to be small (Aschan & Pfanz, 2003).

However, woody tissue might play an important role as a means to guarantee photosynthesize supply when trees are leafless due to environmental (e.g. due to herbivory or fungal disease) or phenological conditions (Pfanz, 2008; Eyles et al., 2009; Saveyn et al., 2010).

In our study, we observed that light exclusion of the stem and the woody branches of foliated Populus deltoides x nigra ‘Monviso’ trees resulted in a decreased DG and bark chlorophyll concentration, implying that even under well-watered growing conditions woody tissue photosynthesis can significantly contribute to the overall plant carbon income. Previously, Saveyn et al. (2010) combined a similar light exclusion treatment with stem diameter measurements and observed a reduction in stem diameter and stem
chlorophyll concentration for light-excluded trees of different native Californian species: *Prunus ilicifolia*, *Umbellularia californica* and *Arctostaphylos manzanita*. Based on isotope analysis of wood samples of covered and uncovered branch sections of *Eucalyptus miniata*, Cernusak & Hutley (2011) calculated that 11% of newly formed branch tissue was constructed from stem assimilates. Our estimates of stem increment during the period July-September for control and light-excluded trees indicated that the contribution of woody tissue photosynthesis to radial stem growth was around 24% for *Populus deltoides x nigra* 'Monviso'. Therefore, a large fraction of the sugars derived from stem photosynthesis might rapidly enter pools which are directly or indirectly used in stem growth. Moreover, results from the study of Powers & Marshall (2011) indicated that stem synthesized sugars might be loaded in the phloem and distributed to the other parts of the tree in the order of days. However, lower contributions might be observed for older trees or for species that contain lower concentrations of chlorophyll in their stem and branch tissues (Aschan & Pfanz, 2003). In foliated trees, xylem-transported CO₂ derived from below- and aboveground respiration is available as substrate for woody tissue photosynthesis, besides locally respired CO₂ (McGuire et al., 2009; Bloemen et al., 2013a; 2013b) (Chapter 1). During leafless periods, woody tissue photosynthesis solely relies on locally respired CO₂ (Chapter 5), which internally concentrates due to the high resistance to radial CO₂ diffusion exerted by woody tissues (Steppe et al., 2007). Therefore, woody tissue photosynthesis in foliated trees may benefit from this additional substrate source and use CO₂ respired in organs remote from the site of assimilation to maintain the local stem and branch carbon status.

More importantly, our study shows that woody tissue photosynthesis might play an important role in tree drought stress resilience. While leaf photosynthesis converts atmospheric CO₂ into carbohydrates, woody tissue photosynthesis relies on endogenously respired CO₂ for sugar synthesis, which makes this process less vulnerable to potentially lethal tissue dehydration under drought conditions (Comstock & Ehleringer, 1988; Nilsen, 1992; Wittmann & Pfanz, 2008). Moreover, the low peridermal water vapour conductance as compared to leaf conductance leads to a high water use efficiency (Wittmann & Pfanz, 2008). In particular in desert and semi-desert environments, where water scarcity is considered as the most important abiotic
constraint for plant growth (Aschan & Pfanz, 2003), plants are assumed to benefit from photosynthesis in green non-leaf tissues. We observed that the stem of light-excluded *Populus deltoides x nigra* 'Monviso' trees tended to shrink more drastically in response to severe drought stress than observed in stems of control trees. Growth processes are known to be very sensitive to drought stress (Hsiao, 1973; Steppe *et al*., 2006; Saveyn *et al*., 2007a) and are related to the water status of living stem tissues, of which the cell turgor pressure in the stem tissue is a good measure (Bradford & Hsiao, 1982). In living plant tissues, cell turgor and volume can be maintained by osmotic adjustment (i.e., the accumulation of solutes in the symplast, for instance sugars) (Woodruff *et al*., 2004; Saveyn *et al*., 2007a). Sugars synthesized in chlorophyll containing woody tissues are potentially used to maintain cell turgor, which might explain why stems of light-excluded trees tended to shrink more drastically relative to control trees. Stem growth cessation can additionally be explained by a decrease in carbohydrate supply under drought stress (Saveyn *et al*., 2007b; Steppe *et al*., 2008; Lavoir *et al*., 2009; De Schepper & Steppe, 2010; Simpraga *et al*., 2011). We observed that stem shrinkage under drought stress (i.e., a negative daily growth rate) occurred simultaneously with the pronounced decrease in $A_{\text{max}}$, regardless of the treatment. Leaf photosynthesis measured under drought conditions tended to decline more rapidly when woody tissue photosynthesis was impeded by light exclusion. In contrast to previous assumptions (Wittmann & Pfanz, 2008), we believe that the role of woody tissue photosynthesis in maintaining the carbon status of temperate species under drought stress conditions might extend beyond the stem level.

While past studies mainly described woody tissue photosynthesis from a plant water use efficiency perspective, recent results indicate that stem and branch CO$_2$ assimilation might directly affect the xylem hydraulic pathway in drought-stressed plants. In these plants, the decrease in hydraulic function due to xylem cavitation has been considered as the main source of productivity loss (Logullo & Salleo, 1993; Hacke *et al*., 2000; Zwieniecki & Holbrook, 2009). Cavitation of water transporting vessels typically results from the expansion of a vapor/gas void within the liquid phase, when xylem tension exceeds the inward pressure due to the curvature of the void’s gas/water interface (Zwieniecki & Holbrook, 2009; Secchi & Zwieniecki, 2011), generating a pressure-wave
induced ultra-sonic emission which can be detected acoustically (Milburn & Johnson, 1966; Tyree & Dixon, 1983; Rosner et al., 2006). Recovery from cavitation may occur under suitable conditions (McCully et al., 1998; Perks et al., 2004; Zufferey et al., 2011) but the actual mechanisms that allow plants to refill embolized xylem conduits and thereby maintain the hydraulic function is still under debate (Clearwater & Goldstein, 2005; Zwieniecki & Holbrook, 2009). Most recent studies hypothesize that sugars play a crucial role in the creation of an osmotic driving force to refill embolized xylem vessels (Secchi & Zwieniecki, 2011) and in the chemical sensing of cavitation (Zwieniecki & Holbrook, 2009).

In this context, woody tissue photosynthesis in chlorophyll containing xylem parenchyma cells might play a role in the local provision of these sugars, in addition to sugars derived from local phloem unloading (Nardini et al., 2011) (Box 1.2). In spite of our limited number of replicates, our acoustics measurements illustrate that woody tissue photosynthesis potentially plays an important role in drought-stressed cavitation repair in poplar, as observed previously for mangrove species (Schmitz et al., 2012). Xylem vessels in branches of control drought-stressed trees were probably hydraulically more functional compared to vessels in light-excluded trees, detected as high number of acoustics events during the dehydration experiment. In addition, we performed a qualitative analysis of the acoustic events, based on amplitude analysis, and clear differences were recorded in amplitude distribution between both branches. In the control branches, a number of events with a lower amplitude occurred near the end of the dehydration period measured five days after drought stress. Wolkerstorfer et al. (2012) observed similar late low-amplitude acoustic hits when measuring cavitation acoustically during a dehydration experiment and attributed these events to cell wall deformations in the wood, induced by changes in cell wall structure under dehydration (Jakob et al., 1994; 1995). Additional correction for these low-amplitude acoustic signals for our control branches as suggested by Wolkerstorfer et al. (2012) resulted in a reduced number of acoustic hits (i.e. 45675 hits), but the total number was still substantially higher than recorded for our light-excluded branches.

Long-term light exclusion treatment probably hindered refilling of the embolized xylem vessels due to the lack of local leakage of sugars from the parenchyma cells into the
xylem vessels. Similarly, Schmitz et al. (2012) observed that hydraulic conductivity of mangrove branches decreased following light exclusion. At leaf level, a similar xylem embolism repair mechanism might exist by chlorophyll containing bundle sheath cells in the petiole (Griffiths et al., 2013), which are potentially supplied with either malate from the roots (Hibberd & Quick, 2002) or dissolved respired CO$_2$ (Bloemen et al., 2013b) transported via the xylem. Stem hydraulics, which is strongly dependent on cavitation of xylem vessels, directly affects leaf water status because plants supply water to their leaves dependent on the degree of cavitation (Hacke et al., 2000). Therefore, the differences in the extent of cavitation repair observed between both treatments might explain why during our measurements transpiration in leaves of light-excluded and control trees responded differently to drought stress, in particular around five days after the start of the drought stress.

In summary, our results expand on our current knowledge on the role of woody tissue photosynthesis in plant functioning under well-watered and drought conditions. Our study shows that assimilation of respired CO$_2$ might play a dual role in tree drought stress resilience. The sugars synthesized in chlorophyll containing woody tissues might either be used to meet the metabolic carbon demand of drought-stressed plants or to maintain the hydraulic function of the xylem. In particular, the significance of woody tissue photosynthesis in providing local sugars for maintaining cell turgor or in xylem embolism repair merits further study. Therefore, future studies that aim at a full understanding of plant drought stress resilience should consider including measurements of woody tissue photosynthesis.
- Chapter 7 -
Tree girdling confirms that root-respiration contributes to xylem CO₂ transport


Abstract

There is recent clear evidence that an important fraction of root-respired CO₂ is transported upward in the transpiration stream in tree stems rather than fluxing to the soil. In this study, we aimed at quantifying the contribution of root-respired CO₂ to both soil CO₂ efflux (Eₘₜ) and xylem CO₂ transport by manipulating the autotrophic component of belowground respiration. We compared Eₘₜ and the flux of root-respired CO₂ transported in the transpiration stream (Fₜ) in girdled and non-girdled 9-y-old oak trees (*Quercus robur* L.) to assess the impact of a change in the autotrophic component of belowground respiration on both CO₂ fluxes. Stem girdling decreased xylem CO₂ concentration indicating that belowground respiration contributes to the aboveground transport of internal CO₂. Girdling also decreased Eₘₜ. These results confirm that root respiration contributes to xylem CO₂ transport and that failure to account for this flux results in inaccurate estimates of belowground respiration when efflux-based methods are used. This research adds to the growing body of evidence that efflux-based measurements of belowground respiration underestimate autotrophic contributions.


### 7.1 Introduction

In forests, soil CO\(_2\) efflux ($E_{\text{soil}}$) contributes between 30 and 80\% to total ecosystem respiration (Goulden \textit{et al.}, 1996; Davidson \textit{et al.}, 2006b) thereby representing the second largest carbon flux after photosynthesis (Davidson \textit{et al.}, 2002; Bond-Lamberty \textit{et al.}, 2004; Subke \textit{et al.}, 2011). As mentioned in Chapter 1, the different sources of belowground respiration contributing to $E_{\text{soil}}$ can be divided into those originating from living roots, their mycorrhizal fungal symbionts, and rhizosphere micro-organisms (autotrophic component of belowground respiration, $R_{a,b}$) and those from the decomposition of dead organic matter in the bulk soil (heterotrophic component of belowground respiration, $R_{h,b}$) (Edwards \textit{et al.}, 1970; Bowden \textit{et al.}, 1993). Accurate estimates of both $R_{a,b}$ and $R_{h,b}$ are needed to better understand the dynamics in $E_{\text{soil}}$, which is a crucial component in modeling the carbon cycle in forests (Scott-Denton \textit{et al.}, 2006).

By removing a circumferential band of bark and phloem from a tree stem during girdling, the downward transport of photosynthates to roots and associated rhizomicrobial organisms is interrupted (De Schepper \textit{et al.}, 2010), which reduces $R_{a,b}$ (Kuzyakov & Gavrichkova, 2010) while water and nutrient transport in the upward direction in the xylem can continue (Högberg \textit{et al.}, 2001; Frey \textit{et al.}, 2006). Among various forest ecosystems, $E_{\text{soil}}$ was reduced by girdling between 24 and 65\% relative to $E_{\text{soil}}$ measured in non-girdled control plots (see Table 1 in Högberg \textit{et al.}, 2009), illustrating the importance of $R_{a,b}$ contribution to forest $E_{\text{soil}}$. However, these estimates were potentially biased by root death (Bhupinderpal-Singh \textit{et al.}, 2003) or increased use of starch (Högberg \textit{et al.}, 2001; Frey \textit{et al.}, 2006) after girdling.

Previous studies of $E_{\text{soil}}$ might also have underestimated $R_{a,b}$ because the upward transport of root-respired CO\(_2\) with the transpiration stream ($F_{t}$) was not considered. With simultaneous measurements of sap flow and CO\(_2\) concentration ($[\text{CO}_2]$) in stems and $E_{\text{soil}}$ in an eastern cottonwood (\textit{Populus deltoides}) stand, Aubrey & Teskey (2009) estimated that twice the amount of CO\(_2\) derived from $R_{a,b}$ was transported internally via the xylem compared with that which diffused into the soil environment (Chapter 1). This xylem-transported CO\(_2\) contributes to high internal CO\(_2\) concentrations in tree stems (Chapter 1), which affects efflux from stems, and is not accounted for with
conventional efflux-based measurements of belowground respiration. Grossiord et al. (2012) observed similar internal transport of CO₂ derived from Rₘₐₜ in Eucalyptus, however in a smaller quantity than observed by Aubrey & Teskey (2009). Nevertheless, xylem transport of root-respired CO₂ raises important questions about our understanding of respiration (Hanson & Gunderson, 2009), which is potentially misestimated in studies that neglect to account for it (Aubrey & Teskey, 2009) (Chapter 1).

To account for the contribution of Rₘₐₜ to both Fᵣ and Eₘₑᵦ (Fig 1.12), we made measurements of sap flow, internal [CO₂], and Eₘₑᵦ at high temporal resolution in girdled and non-girdled oak (Quercus robur L.) trees. Stem girdling was used to manipulate the contribution of Rₘₐₜ to belowground respiration. We addressed the following hypotheses: (1) root-respired CO₂ in Quercus robur trees does not solely diffuse into the soil environment, rather, a portion dissolves in xylem sap and is transported upward in the transpiration stream; and (2) estimates of belowground respiration based on conventional Eₘₑᵦ measurements underestimate Rₘₐₜ in particular at high sap flow rates (Fₛ) during the daytime.

7.2 Material and methods

7.2.1 Study site

Two plots of 90 m² were established within the plantation, each containing six trees. In each plot, three trees were randomly selected for measurement. Average diameter at stem base and breast height (DBH) was 11.5 ± 0.9 and 6.7 ± 0.6 cm, respectively, in the first (non-girdled) plot, hereafter referred to as the control treatment, and 11.1 ± 0.3 cm and 6.1 ± 0.7 cm in the second (girdled) plot, hereafter referred to as the girdling treatment (Table 7.1). We focused on one control and one girdling plot, because we had limited equipment for the detailed continuous measurements of soil CO₂ efflux (Eₘₑᵦ), internal CO₂ concentration ([CO₂]), sap flow, and environmental parameters. These measurements at high temporal resolution were essential to understand short term responses in belowground dynamics after stem girdling. The day before girdling, six undisturbed soil samples (0-30 cm depth) were taken per plot to determine soil bulk
density and an additional eight soil subsamples per plot were taken with a soil corer and mixed to a composite sample to determine pH, organic carbon and soil texture for each plot. Analyses of soil bulk density and other soil properties were performed as described by D’Haene et al. (2008). In addition, near the end of September (23 September 2011, day of year (DOY) 266), soil samples were taken at 0-30 cm depth at two locations within 1 m of each tree from which roots were collected and dried to constant weight to determine average root biomass density per plot.

Table 7.1 Stem diameter at base (0.1 m, cm) and breast height (DBH, cm) and distance from the plot center (m) for three *Quercus robur* trees selected for measurement in the control and girdling treatment plots.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tree</th>
<th>Diameter at 0.1m</th>
<th>DBH</th>
<th>Distance from center</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>11.0</td>
<td>6.6</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.9</td>
<td>6.1</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.6</td>
<td>7.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Girdling</td>
<td>1</td>
<td>11.1</td>
<td>5.2</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.2</td>
<td>6.6</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.1</td>
<td>6.4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

In the centre of each plot, soil temperature and soil moisture were recorded at 7.5 and 22.5 cm with a type-T thermocouple (Omega, Amstelveen, The Netherlands) and soil moisture probe (SM-300, Delta-T, Cambridge, UK), respectively. Air temperature (T<sub>air</sub>; type-T thermocouple, Omega, Amstelveen, The Netherlands) and relative humidity (RH; Hygroclip, Rotronic AG, Bassersdorf, Switzerland) were measured on site and used to calculate vapour pressure deficit (VPD). All data were recorded at 1 min interval with a datalogger (CR1000, Campbell Scientific, Logan, Utah, USA). Atmospheric pressure was obtained from a weather station located 7 km from the site.

### 7.2.2 Girdling treatment

On 5 August 2011 (DOY 217), the girdling treatment was applied to the six trees in the girdled plot by carefully removing a 5.5 cm wide circumferential band of bark and phloem at a height of 50 cm from the stem without damaging the xylem tissue (Fig. 7.1). The exposed xylem was covered with clear plastic film to prevent tissue dehydration and to allow visual inspection for formation of new bark tissue, which was removed
when it occurred. In addition to the trees in the girdled plot, four trees in close proximity to the selected trees but outside the plot were girdled to avoid possible edge effects from ingrowing roots of non-girdled trees. The trees in the control plot were left intact.

**Fig. 7.1** Schematic of the experimental set up, indicating the distances and positions of the tree girdle and equipment installed on the stem and within the soil area occupied by the roots ($A_{\text{root}}$) to measure the flux of root-respired CO$_2$ transported in the transpiration stream ($F_t$) and soil CO$_2$ efflux ($E_{\text{soil}}$). The black and grey chamber(s) represent the measurement of $E_{\text{soil}}$ with automated and manual chambers, respectively. Displayed chambers were located at 40 cm from the stem. NDIR, non-dispersive infrared CO$_2$ sensor; TDP, thermal dissipation probe. The black and grey chambers were located at 40 cm from the stem. NDIR, non-dispersive infrared CO$_2$ sensor; TDP, thermal dissipation probe. The stem thermocouple, installed 3 cm above the NDIR sensor and the manual chambers for $E_{\text{soil}}$ measurements performed at 70 cm from the stem are not shown.

### 7.2.3 Soil CO$_2$ efflux measurements

For automated measurements of $E_{\text{soil}}$, a PVC chamber (20 cm diameter, 15 cm height, design adapted from Suleau *et al.* (2009)) ([Fig. 1.13A,B](#)), was inserted 2 cm into the soil 40 cm from the stem base of each tree (Fig. 7.1). A pump (KNF Neuberger GmbH, Freiburg, Germany) was used to circulate air from the chambers to an infrared gas analyzer (GMP 343, Vaisala inc., Helsinki, Finland) via a gas multiplexer system. At the start of a measurement cycle, the collar top of the first chamber was opened in half-open position for 1 min to allow flushing of the tubes before the chamber was closed for 4 min to determine the increase in [CO$_2$]. Chamber [CO$_2$] was recorded at a 12 s interval with a datalogger (CR1000x, Campbell Scientific, Logan, Utah, USA). Finally, the collar top was opened. The remaining five chambers were measured sequentially in a similar manner.
to complete the measurement cycle. One measurement cycle lasted 30 min and measurements were made alternately in chambers in the control and girdling treatments. Based on the increase in [CO₂] in the chambers over time, E<sub>soil</sub> was calculated and analyzed as described by Savage <em>et al.</em> (2008). The most linear section in increase in [CO₂] over time was identified and the rate of increase (slope) over time was calculated, but only accepted if the coefficient of determination of the linear regression (i.e., R²) >0.90. Slope estimates were scaled by chamber cross-sectional area and corrected for T<sub>air</sub> and atmospheric pressure (P) to yield the CO₂ efflux rate, according to Savage <em>et al.</em> (2008).

Automated measurements of E<sub>soil</sub> were complemented with manual measurements (EGM-4 analyzer connected to a SRC-1 chamber, PP systems, Amesbury, Massachusetts, USA) to account for variation in E<sub>soil</sub> within the rooting area among the different cardinal directions. These measurements were performed on three additional PVC collars (diameter 20 cm, height 12 cm) installed 40 cm from each stem (Fig. 7.1). Later in the growing season (from DOY 231, 19 August 2011 onwards) we performed additional manual measurements with collars installed 70 cm from each stem to account for spatial variation in E<sub>soil</sub>. Manual data were collected at a three-day interval. Both manual and automated measurements continued for 1.5 months after girdling.

7.2.4 Xylem transport of root-respired CO₂

The six selected trees in the control and girdled plots (three per plot) were instrumented to measure the transport of root-respired CO₂ via the transpiration stream (F<sub>t</sub>) simultaneously with E<sub>soil</sub>. We quantified F<sub>t</sub> based on measurements of sap flow rate (F<sub>s</sub>) and concentration of dissolved CO₂ in the xylem ([CO₂*]) based on Equation 1.5.

Xylem gaseous [CO₂] was measured in situ by inserting non-dispersive infrared (NDIR) CO₂ sensors (model GMM221; Vaisala Inc., Helsinki, Finland) in the base of each stem 5 cm above the soil (Fig. 7.1). Stem temperature (T<sub>stem</sub>) was recorded in all trees with a type-T thermocouple (Omega, Amstelveen, The Netherlands), installed at 3 cm from the NDIR sensor. We assumed that xylem [CO₂] measured at stem base represented the [CO₂] of xylem sap entering the stem from belowground. Xylem [CO₂] was corrected for T<sub>stem</sub> and atmospheric pressure based on Equation 1.2. Due to technical problems with
the sensors, data for the control trees was limited to the period from 30 July 2011 (DOY 211) till 13 August 2011 (DOY 225). \([\text{CO}_2^*]\) was calculated using Henry’s law coefficients from xylem \([\text{CO}_2]\), \(T_{\text{stem}}\) and biweekly measurements of xylem sap pH (McGuire & Teskey, 2002; Erda et al., 2013), according to Equation 1.3. To obtain xylem pH, sap was expressed from excised twigs of nearby non-girdled trees with a pressure chamber (PMS Instruments, Corvallis, Oregon, USA). The expressed sap was transferred with a pipette to a solid-state pH microsensor connected to a pH meter (Hi 9124, Hanna instruments Ltd., Bedfordshire, UK). Mean pH of biweekly samples from five trees during the measurement period was 6.8 ± 0.1. \(F_s\) was determined by scaling sap flow density (SFD) with sapwood area. SFD was measured using thermal dissipation probes (model TDP-10, Dynamax Inc., Houston, Texas, USA) installed in each stem at 35 cm height with a vertical needle separation of 40 mm (Fig. 7.1). Zero flow was calculated based on the mean temperature difference (\(\Delta T\)) between the needles from 03:00 to 05:00 h, assuming no or very limited nocturnal transpiration. Additional trees from the site were used to calibrate the sap flow sensors using a Mariotte-based verification system (Steppe et al., 2010) and generate a site-specific calibration parameter (\(k\)) for oak, as recommended by Bush et al. (2010), Steppe et al. (2010), and Sun et al. (2011). Stem \([\text{CO}_2]\), temperature, and SFD were recorded with a datalogger (CR1000, Campbell Scientific, Logan, Utah, USA) at 1 min interval.

Because \(E_{\text{soil}}\) is expressed on a \(\text{m}^2\) area basis, \(F_t\) was scaled \((F_{t,\text{scaled}}, \text{mg C m}^{-2} \text{h}^{-1})\) with the soil area occupied by the roots \((A_{\text{root}}, \text{m}^2)\) based on Equation 1.6.

To estimate \(A_{\text{root}}\), we excavated the entire root system of three additional trees at the same site with similar dimensions as the measured trees. Based on the radii of \(A_{\text{root}}\) for the three additional trees (0.8, 1.2, and 0.9 m) we estimated average \(A_{\text{root}}\) as equal to a circular area around the trees with radius 1 m (3.14 \(\text{m}^2\)), as shown in Fig. 7.1. Manual and automated \(E_{\text{soil}}\) point measurements performed within this area were assumed to represent average \(E_{\text{soil}}\) within \(A_{\text{root}}\).

7.2.5 Estimation of the autotrophic component of belowground respiration

We estimated \(R_{a,b}\) using two methods. The first estimate of \(R_{a,b}\) was made according to the approach used in previous girdling studies \((R_{a,b-\text{conv}})\), where \(R_{a,b}\) (mg C m\(^{-2}\) h\(^{-1}\)) is
assumed to be equal to the difference in average \( E_{\text{soil}} \) between the control (\( E_{\text{soil-c}} \)) and girdling (\( E_{\text{soil-g}} \)) treatments, hereafter referred to as the conventional approach:

\[
R_{a,b-\text{conv}} = (E_{\text{soil-c}} - E_{\text{soil-g}}) \tag{7.1}
\]

The second estimate of \( R_{a,b} \) (\( R_{a,b-\text{new}} \)) additionally accounts for internal transport of root-respired \( \text{CO}_2 \) by including the difference in average \( F_{t,\text{scaled}} \) between the control (\( F_{t,\text{scaled-c}} \)) and girdling (\( F_{t,\text{scaled-g}} \)) treatments, hereafter referred to as the new approach:

\[
R_{a,b-\text{new}} = (E_{\text{soil-c}} - E_{\text{soil-g}}) + (F_{t,\text{scaled-c}} - F_{t,\text{scaled-g}}) \tag{7.2}
\]

### 7.2.6 Soluble sugars and starch concentration of fine roots

We girdled three additional three additional trees at the site for sampling fine roots for sugar and starch analysis. These samples were collected on the girdled trees and three nearby non-girdled trees on the day of girdling and 11 (16 August 2011, DOY 228) and 40 days after girdling (14 September 2011, DOY 237). The sampling dates were selected to obtain data concurrent with the flux measurements and to assess the long-term impact of girdling on fine root soluble sugar and starch content. For sample harvesting, we carefully uncovered part of the root system and excised about 3 mg of fine roots, according to Regier et al. (2010). Samples were immediately frozen in liquid nitrogen, transported to the lab and stored in a freezer at -18°C until analyzed for glucose, fructose, sucrose, and starch as described by De Schepper et al. (2012). Our intention was to use the soluble sugar and starch data to reveal the relative effect of girdling on fine root sugar content and to identify the potential sources of root-respired \( \text{CO}_2 \). As for this purpose no absolute fine root soluble sugar and starch concentrations are needed, we normalized concentrations for each tree by dividing the post-treatment concentrations by the concentration on the day of girdling, facilitating inter-treatment comparison and accounting for variation among trees.

### 7.2.7 Statistical analysis

We compared daily totals of continuously measured \( F_t \) and \( E_{\text{soil}} \) for both treatments using repeated measures multi-factorial analysis of variance (ANOVA). Treatment (\( n=2 \), control and girdled) and date (\( n=14 \)) were treated as fixed factors, while the individual tree (\( n=3 \) per treatment) was considered as a random factor. Similarly, automated and
Tree girdling experiment

7.3 Results

7.3.1 Impact of tree girdling on soil CO$_2$ efflux and xylem CO$_2$ transport

The soil properties of the control and girdling treatment plots were similar (Table 7.2). Automated measurements showed that soil CO$_2$ efflux (E$_{soil}$) prior to girdling was similar in the control and the girdling treatment plots ($P=0.95$), with an average ($\pm$ SD) of 162.5 ± 18.4 and 162.6 ± 20.9 mg C m$^{-2}$ h$^{-1}$, respectively (Fig. 7.2A). Following girdling, automated measurements showed a strong decrease in E$_{soil}$ in the girdling treatment, while E$_{soil}$ in the control treatment remained stable over time. Within 5 d, girdling significantly reduced E$_{soil}$ ($P=0.02$) by 21.8 ± 3.7% relative to the control treatment (Fig. 7.2A). This pronounced initial decrease of E$_{soil}$ in the girdling treatment was followed by a slower decrease, such that 25 d after girdling, E$_{soil}$ in the girdling treatment was 34.9 ± 4.3% lower than the control treatment. The effect of girdling on E$_{soil}$ was confirmed by manual measurements of E$_{soil}$ 40 cm from the stem base (Fig. 7.2A). For manual measurements, significant differences in E$_{soil}$ between the control and girdling plots were observed for the last two measurement dates ($P=0.01$). Manual measurements of E$_{soil}$ tended to be slightly higher than automated measurements, but no significant differences were observed between the manual and automated measurements for the control ($P=0.92$) nor the girdling treatment ($P=0.51$). In addition, the manual E$_{soil}$ measurements made at 70 cm from the stem base suggested that measurements

manual measurements performed at 40 cm were compared, but with fewer temporal measurements ($n=5$). Finally, manual E$_{soil}$ measurements performed at 40 cm and 70 cm were compared, based on the measurements made later in the growing season (date, $n=10$). Relative sugar and starch concentration were analyzed using a similar ANOVA model, however with fewer temporal measurements (date, $n=3$) and soluble sugar type ($n=4$, fructose, glucose, sucrose, and starch) as an additional fixed factor. Akaike’s information criterion corrected for small sample sizes (AICc) was used to determine the covariance structure that best estimated the correlation among individual trees over time. All analyses were performed using the mixed model procedure (PROC MIXED) of SAS (Version 9.3, SAS Inc., Cary, NC, USA) with $\alpha=0.05$. 

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performed at 40 cm were representative of $E_{soil}$ in the rooting areas of the trees in the control and girdling treatments.

**Table 7.2** Root biomass density (g m\(^{-2}\)), pH, Organic carbon (%), and soil bulk density (g cm\(^{-3}\)) (± SD) of the control and girdling treatment plots measured to a depth of 30 cm. pH and organic carbon were determined for one composite sample per treatment, while root biomass density ($n=8$) and soil bulk density ($n=8$) were averaged per treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root biomass density</th>
<th>pH</th>
<th>Organic carbon</th>
<th>Soil bulk density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>286.7 ± 40.1</td>
<td>5.1</td>
<td>1.8</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Girdling</td>
<td>287.8 ± 59.6</td>
<td>4.7</td>
<td>2.0</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

Before girdling, xylem CO\(_2\) concentration ([CO\(_2\)]) measured at the stem base was similar in the control and girdling treatments, averaging 10.7 ± 1.2% and 10.1 ± 1.4%, respectively (Fig. 7.2B). Girdling reduced average xylem [CO\(_2\)] by approximately one fifth (i.e by 21.4 ± 1.1%) in the girdling treatment compared with the control treatment within five days after girdling.
7.3.2 Estimation of xylem CO₂ transport in control and girdled trees

Before girdling, sap flow rate (Fₛ) was slightly lower in the control plot (Fig. 7.3A) relative to the girdled plot (Fig. 7.3B), with 6 d averages of 0.29 ± 0.10 l h⁻¹ and 0.36 ±
0.12 l h⁻¹, respectively. After girdling, the three measured trees responded differently to the treatment: a pronounced decrease in $F_s$ was observed in one tree, while $F_s$ remained similar to pre-girdling rates in the other two trees.

The decrease in xylem [CO₂] in response to girdling (Fig. 7.2B) influenced the magnitude of scaled xylem transport of root-respired CO₂ ($F_{t,scaled}$) (Fig. 7.3C). After girdling, $F_{t,scaled}$ was lower compared with the control treatment, particularly on days with the highest sap flow. On these days, average daily total $F_{t,scaled}$ in the control treatment was significantly higher than in the girdling treatment ($P=0.03$) and at peak sap flow, average maximal $F_{t,scaled}$ in the control treatment ($44.7 \pm 0.9$ mg C m⁻² h⁻¹) more than doubled the average maximal $F_{t,scaled}$ in the girdling treatment ($16.5 \pm 2.4$ mg C m⁻² h⁻¹).

Fig. 7.3 Average sap flow rate ($F_s$) in (A) control and (B) girdled trees measured with sap flow sensors installed on the tree stem (see Fig. 7.1). (C) Scaled xylem transport of root-respired CO₂ ($F_{t,scaled}$) in control (black line) and girdled (black dashed line) trees. The vertical dashed line indicates the time of girdling. The shaded areas represent standard deviation. DOY: day of the year.
7.3.3 Estimation of the autotrophic component of belowground respiration

For estimating the autotrophic component of belowground respiration ($R_{a,b}$) we used data beginning two days after girdling. Diel variations were less pronounced when $R_{a,b}$ was calculated as in previous girdling studies ($R_{a,b-conv}$) compared with estimates obtained with the new approach ($R_{a,b-new}$) (Fig. 7.4A). During the daytime, an important fraction of root-respired CO$_2$ was transported upward in the xylem, which was not accounted for in $R_{a,b-conv}$. Especially on the days with highest sap flow (DOY 222-223, Fig. 7.4A), large differences were observed between $R_{a,b-conv}$ and $R_{a,b-new}$. $R_{a,b-conv}$ decreased in the middle of the day, while $R_{a,b-new}$ peaked during the day, when the highest sap flow occurred (Fig. 7.4A). We expressed $R_{a,b}$ estimates relative to belowground respiration estimated according to the conventional and new approach (Fig. 7.4B). According to the conventional approach, $E_{soil,c}$ is an estimate of total belowground respiration. Averaged over 5 d, we estimated that $27.0 \pm 4.5\%$ of belowground respiration was derived from $R_{a,b}$. With the new approach, $E_{soil,c} + F_{scaled-c}$ was used to estimate belowground respiration and we found that on average $32.8 \pm 8.1\%$ of belowground respiration was contributed by the autotrophic component (Fig. 7.4B). Larger differences were observed on the days with highest sap flow (DOY 222-223). On these days, at peak sap flow (from 12h-16h), $25.0 \pm 2.7\%$ and $45.4 \pm 1.5\%$ of belowground respiration was derived from $R_{a,b}$ calculated by the conventional and new approaches, respectively. During nighttime hours, small differences were observed in $R_{a,b}$ estimates between the two approaches, because low nocturnal sap flow rates (i.e. 1-2% of the 24h flux) had the potential to transport only a small quantity of root-respired CO$_2$. 
Fig. 7.4 (A) Estimates of the autotrophic component of belowground respiration ($R_{a,b}$) calculated by the conventional approach based solely on measurements of soil CO$_2$ efflux ($E_{soil}$) (black dashed line), or calculated by the new approach, based on the combination of measurements of $E_{soil}$ and the scaled flux of root-respired CO$_2$ transported in the transpiration stream ($F_{t,scaled}$) (black line). (B) Contribution of $R_{a,b}$ to belowground respiration, estimated according to the conventional approach (black symbols) and the new approach (grey symbols). Symbols represent four hour averages. DOY: day of the year.

7.3.4 Analysis of fine root samples

Relative to pre-girdling values, the girdling treatment had a significant overall effect on the soluble sugar and starch concentrations of fine roots compared to the control treatment ($P$=0.002) (Fig. 7.5). At 11 d after girdling, relative starch (Fig. 7.5A), sucrose (Fig. 7.5B) and fructose (Fig. 7.5C) concentrations decreased in fine roots of the girdled trees, while relative soluble sugar and starch concentrations increased in control trees. Relative concentrations between girdled and control trees were significantly different.
for glucose (Fig. 7.5D, $P=0.02$) and sucrose (Fig. 7.5B, $P=0.04$). At 40 days after girdling, relative sucrose and starch concentrations further decreased in girdled trees, to levels $40.2 \pm 2.7\%$ and $14.4 \pm 4.8\%$ lower than observed pre-girdling, respectively, while fructose and glucose concentrations returned to the same level and increased relative to the day of girdling, respectively. In the control trees, soluble sugar and starch concentrations were higher relative to the day of girdling, with the largest increases in starch ($44.8 \pm 2.4\%$) and sucrose ($43.3 \pm 11.1\%$), the latter being significantly higher compared to the girdled trees ($P=0.036$).
Fig. 7.5  Concentration of (A) starch, (B) sucrose, (C) fructose, and (D) glucose relative to concentration on the day of girdling in the fine roots of control (black) and girdled (hatched) trees at 11 (16 August 2011) and 40 (14 September 2011) days after girdling. Bars represent the mean of three trees per treatment and four samples per tree. Error bars represent standard deviation. The horizontal dotted line represents the relative concentration of fine root soluble sugars and starch on the day of girdling. Significant differences ($P<0.05$) per date between the two treatments are indicated by different letters.
7.4 Discussion

This study provides evidence that autotrophic component of belowground respiration ($R_{ab}$) contributes to xylem transport of respired CO$_2$. Root-respired CO$_2$ either diffuses from the root surface into the soil environment, thereby contributing to soil CO$_2$ efflux ($E_{soil}$), or dissolves in xylem sap and is transported upward in the tree with the transpiration stream ($F_t$). Girdling reduced both $E_{soil}$ and $F_t$, suggesting that efflux-based methods underestimate the contribution of $R_{ab}$ to belowground respiration.

The strong reduction in $E_{soil}$ after stem girdling has been described in detail in previous studies (Högberg et al., 2001; Johnsen et al., 2007; Högberg et al., 2009; review by Kuzyakov & Gavrichkova, 2010; Subke et al., 2011). Högberg et al. (2001) reported decreases up to 37% in $E_{soil}$ within 5 d after girdling Pinus sylvestris trees, while Subke et al. (2011) observed a similar decrease (35%) for Tsuga heterophylla trees within two weeks after girdling. Data from our high-frequency $E_{soil}$ measurements agree with these previous results. We found that girdling affected $E_{soil}$ after 2 d and reduced $E_{soil}$ after 5 and 25 d by 22% and 35%, respectively, relative to $E_{soil}$ measured in the control treatment.

Previous studies exclusively used the reduction in $E_{soil}$ induced by girdling to quantify the contribution of $R_{ab}$ to belowground respiration, based on the general belief that all root-respired CO$_2$ diffuses into the soil and subsequently into the atmosphere. However, recent studies have suggested a large-magnitude upward flux of dissolved CO$_2$ in xylem of trees derived from $R_{ab}$ (Aubrey & Teskey, 2009; Grossiord et al., 2012) (Chapter 1). Based on measurements of xylem CO$_2$ concentration ([CO$_2$]) and sap flow near the stem base of Populus deltoides trees, Aubrey & Teskey (2009) found that $F_t$ rivaled the flux that contributed to $E_{soil}$. Additionally, they calculated that only a small fraction of $F_t$ resulted from the uptake of CO$_2$ dissolved in the soil solution. In our study, we also simultaneously measured the $F_t$ to quantify $R_{ab}$. Girdling reduced [CO$_2$] at the stem base by 21% relative to xylem [CO$_2$] of control trees within 5 d, which was similar to the reduction observed for $E_{soil}$. This result agrees with previous observations and supports our first hypothesis that a fraction of CO$_2$ derived from $R_{ab}$ is transported in the transpiration stream.
Simultaneous high-frequency measurements of \( F_t \) and \( E_{soil} \) in girdling and control treatment plots allowed us to re-assess our understanding of \( R_{a,b} \). When we accounted for \( F_t \), \( R_{a,b} \) dynamics were more pronounced during the daytime and indicated a greater contribution of \( R_{a,b} \) to belowground respiration than previously reported. In particular, on days with high sap flow, a substantial quantity of root-respired \( \text{CO}_2 \) was transported upward with the transpiration stream, especially at peak sap flow, resulting in a large underestimation of \( R_{a,b} \) when calculated by the conventional efflux-based method (second hypothesis). Similarly, Grossiord et al. (2012) reported for *Eucalyptus* that the largest underestimation (24%) of the contribution of autotrophic sources to belowground respiration was observed between 11:00 h and 15:00 h, which was associated with peak sap flow.

Results of girdling studies may be prone to biases related to the interruption of assimilate flow to belowground tissues, which is assumed to reduce \( R_{a,b} \). First, girdling leads to an accelerated use of stored carbohydrates in roots (Högberg et al., 2001; Olsson et al., 2005; Högberg et al., 2009), i.e. starch in oak trees (Barbaroux & Breda, 2002; Maunoury-Danger et al., 2010). In this case, \( R_{a,b} \) would be underestimated because tree roots would still be respiring after girdling. Girdling also leads to enhanced decomposition of roots and their associated mycorrhizae by soil heterotrophs (Högberg et al., 2001; Bhupinderpal-Singh et al., 2003; Ekberg et al., 2007), resulting in greater fungal abundance and a possible shift in the fungal community from symbiotic to saprophytic fungi in the long term (Subke et al., 2004). These rhizosphere effects could confound the appearance of reduced \( R_{a,b} \) in girdled plots (Kuzyakov & Larionova, 2006), leading to underestimation of \( R_{a,b} \). However, enhanced decomposition of roots and associated mycorrhizae by heterotrophs and changes in fungal abundance and community structure have been observed to occur at multi-season (Högberg et al., 2001; Subke et al., 2004; Ekberg et al., 2007) and multi-year (Ekberg et al., 2007) scales. Therefore, we assume that these effects were negligible during our relatively short experimental period. Finally, girdling affects the secretion of root exudates into the soil, whose microbial consumption has been found to contribute to \( R_{a,b} \) and potentially affect \( R_{h,b} \) (negative or positive priming effect). After girdling, we observed a relative decrease in fine root starch concentration of the girdled trees relative to concentrations at the day
of girdling (i.e. by 11% 40 d after girdling). At the same time, starch in fine roots of control trees increased (i.e. by 45% 40 d after girdling), probably due to late-season loading to maintain winter carbon reserves, as has been observed in previous studies (Barbaroux et al., 2003; Regier et al., 2010 and references therein). Increased use of starch in roots in response to girdling was observed in other field studies and has been suggested to lead to conservative estimates of R_{ab} (Högberg et al., 2001; Frey et al., 2006). In addition, a fraction of the metabolic demand related to starch usage after girdling might be related to starch remobilization (Rodgers et al., 1995; Jordan & Habib, 1996), which suggests that the increased glucose levels we observed after girdling (Fig. 7.5D) could have resulted from hydrolysis of starch (Maunoury-Danger et al., 2010). Therefore, in our study, estimates of R_{ab} contributions to F_t and E_soil in the girdled plot might be lower due to the increased use of stored carbohydrates that potentially fueled R_{ab} after girdling. There was also potentially an effect of starch mobilization from larger roots to fine roots, as observed by Aubrey et al. (2012) in a canopy scorching experiment. All these different processes contribute to the large standard deviations in fine root sugar concentrations observed among trees and might explain why the reduction in starch was not significant in girdled trees as compared to control trees. In spite of increased starch use, both xylem sap [CO_2] and E_soil decreased rapidly in response to girdling, presumably due to the interruption of the translocation of photosynthates to the root system. Sucrose is commonly recognized as the main translocated sugar (e.g. Zimmerman, 1957; Rolland et al., 2006) and has a different nature compared to the other sugars. We observed a significant 40% reduction in sucrose concentration in fine roots in response to girdling. This result confirms previous findings that an important fraction of R_{ab} may be dependent on the rapid turnover of recent photosynthates within roots (Epron et al., 2011; 2012 and references therein). Thus, autotrophic metabolic activity that contributes both to F_t and E_soil is likely fueled by a combination of recent photosynthates and stored carbohydrates, as has been observed in other root respiration studies (Lynch et al., 2013). Because root-respired CO_2 both diffuses into the soil and is transported in the transpiration stream (Aubrey & Teskey, 2009) regardless of whether it is derived from recent photosynthates or stored carbohydrates, we expect that the biases due to girdling-related increases in the use of reserves were similar for both fluxes.
Second, girdling may affect whole-tree sap flow (Domiec & Pruyn, 2008; De Schepper et al., 2010), inducing different rates of water and nutrient uptake between girdled and non-girdled trees. By removing the bark, the outer xylem tissue is exposed to air and might dry out and become non-functional for water transport. Moreover, a reduction in stomatal conductance of leaves after girdling related to feedback inhibition of photosynthesis (see De Schepper et al., 2010 and references therein) could reduce sap flow. During our study, we tried to avoid desiccation of exposed xylem tissues by wrapping the girdled stem section in plastic film. However, sap flow in girdled trees was reduced by 20% on average relative to control trees, which was similar to the reduction in sap flow observed in a canopy scorching study by Aubrey et al. (2012). The decrease in sap flow likely affected both the conventional \( R_{a,b-\text{conv}} \) and new \( R_{a,b-\text{new}} \) estimate of \( R_{a,b} \). Due to a reduction in sap flow, a fraction of root-respired CO\(_2\) might have contributed to \( E_{\text{soil}} \) instead of being transported with the transpiration stream (Aubrey et al., 2012). In addition, previous studies have shown that internal [CO\(_2\)] builds up in stems when sap flow decreases, because the transpiration stream is not removing respired CO\(_2\) from the tissues (Teskey & McGuire, 2002; McGuire et al., 2007; Saveyn et al., 2007b); a similar phenomenon may occur in roots, which would also contribute to increased efflux due to the larger concentration gradient.

Because root-respired CO\(_2\) both diffuses into the soil environment and dissolves in the transpiration stream, the two fluxes must be investigated simultaneously to fully understand root metabolism dynamics. For instance, we observed non-temperature related depressions in \( R_{a,b-\text{conv}} \) at high \( F_s \) when upward transport of root-respired CO\(_2\) with the transpiration stream was large (DOY 222 and 223, Fig. 7.4). Daytime depressions in root CO\(_2\) efflux related to the rate of transpiration have been observed for other species (Bekku et al., 2009; 2011) and Subke et al. (2009) observed unexpected decreases in daytime \( E_{\text{soil}} \) related to depressions in the autotrophic component of \( E_{\text{soil}} \) (Box 1.1). However, these studies lacked measurements of \( F_t \) to fully elucidate the observed dynamics in \( E_{\text{soil}} \) and the tight coupling between the two fluxes. Daytime depressions in root CO\(_2\) efflux could be similar to what has been observed for stems, where daytime depressions in stem CO\(_2\) efflux (\( E_{\text{stem}} \)) were related to increased internal transport of CO\(_2\) away from the site of respiration due to increased sap flow (Teskey &
McGuire, 2002; Gansert & Burgdorf, 2005; McGuire et al., 2007) or changes in stem turgor pressure (Saveyn et al., 2007a).

We have shown that efflux-based studies may underestimate the actual contribution of $R_{ab}$ to belowground respiration, as has been suggested previously (Aubrey & Teskey, 2009; Grossiord et al., 2012). Especially at high sap flow rates, a substantial amount of root-respired CO$_2$ is transported upward with the transpiration stream, resulting in large underestimations in belowground respiration when estimated $R_{ab}$ is based solely on $E_{soil}$ measurements. Therefore, future studies on the contribution of $R_{ab}$ to belowground respiration should use approaches for measuring CO$_2$ fluxes that include the internal transport of respired CO$_2$ (Bloemen et al., 2013b; Trumbore et al., 2013) and consider potential factors that control the flux of root-respired CO$_2$ with the transpiration stream, like sap flow. These measurements are crucial for improving the accuracy of estimates of $R_{ab}$ and our ability to understand belowground respiration dynamics from a mechanistic point of view.
General conclusions and future perspectives

The main objective of this PhD study was to improve our understanding of internal CO₂ in trees and its role in the tree carbon cycle. In this concluding chapter, the main findings of the performed research are discussed. Subsequently, remaining unanswered questions and suggestions for promising future avenues of research on the role of internal CO₂ in tree functioning are identified and discussed.

8.1 General research outcomes and scientific contributions

Reconsidering efflux-based estimates of above- and belowground woody tissue respiration

One of the most long-standing assumptions in plant research is that CO₂ efflux equals respiration, because of the hypothesized immediate diffusion of tissue respired CO₂ into the atmosphere or soil environment (Chapter 1). A corollary is that CO₂ efflux chambers are being used to estimate above- and belowground respiration and their contribution to ecosystem respiration. However, recent observations of the existence of an internal CO₂ transport in trees of large magnitude (Teskey & McGuire, 2002; 2007; Aubrey & Teskey, 2009; Grossiord et al., 2012), which redistributes above- and belowground respired CO₂ by the transpiration stream, questions the use of efflux-based measurements for quantifying tissue respiration.
Results from the girdling study (Chapter 7) confirm that a fraction of root-respired CO$_2$ is not accounted for when using efflux-based measurements of soil respiration. The reduction in internal CO$_2$ after girdling indicates that a substantial fraction of root-respired CO$_2$ is transported within the tree, leading to inaccurate efflux-based estimates of belowground respiration. Hence, as proposed in Chapter 7, accounting for the internal transport of root-respired CO$_2$ ($F_t$) besides its contribution to soil CO$_2$ efflux ($E_{soil}$) leads to a more accurate estimate of the autotrophic component of belowground respiration ($R_{a,b}$). In particular during the daytime at peak sap flow, a large fraction of the root-respired CO$_2$ is transported away from the roots inside the tree, instead of contributing to $E_{soil}$.

Once aboveground, the largest fraction of $F_t$ is expected to diffuse into the atmosphere, as indicated by the results from the tree labeling study (Chapter 4). Aboveground diffusion of root-respired CO$_2$ implies that efflux-based measurements overestimate the actual metabolic cost of stem and branch respiration, while the amount of carbohydrates needed to sustain root respiration has been underestimated. Moreover, belowground photosynthate allocation in trees is potentially much larger than previously assumed (Aubrey & Teskey, 2009). Therefore, internal transport of respired CO$_2$ compels us to rethink commonly held assumptions on the tree carbon economy. However, it does not imply that absolute estimates of the ecosystem carbon exchange between forests and the atmosphere need to be revisited.

*Carbon isotopes provide us with information on the fate of xylem-transported CO$_2$ at an unprecedented scale*

While above- and belowground sources of xylem-transported CO$_2$ have been identified (Chapter 1), its fate in aboveground tree organs remains largely unknown. Small-scale studies have introduced a carbon isotope label into detached leaves and branches (Chapter 1) as performed in Chapters 2 and 3, respectively, but few have studied the fate of xylem-transported CO$_2$ in field-grown trees. To this end, $^{13}$C label infusion was used to simulate respired CO$_2$ entering the stem of poplar trees from belowground while isotope analysis was used to track its fate (Chapter 4). During transport, the dissolved $^{13}$C label present in inorganic form was assimilated in organic form by Ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco) and phosphoenolpyruvate carboxylase
(PEPc) mediated carboxylation. Between 6-17% of the infused $^{13}$C label was assimilated in the tree, mainly in the branches and the stem and in a lesser extent in the leaf petioles. Hence, recycling of respired CO$_2$ in trees potentially contributes substantially to the tree carbon income. In the branches most of the $^{13}$C label was assimilated in the inner bark (Chapter 4), where most of the chlorophyll is located, corroborating the results of the detached branch labeling study (Chapter 3). At leaf level, assimilation of the $^{13}$C label in the petioles underpins the possible existence of a spatially separated C$_4$-like pathway in temperate trees, as suggested previously for herbaceous species (Chapter 1). In the leaf blade, detailed analysis of $^{11}$C label distribution showed that near the leaf veins a large fraction of respired CO$_2$ entering the leaf via the petiole is potentially assimilated in chlorophyll containing cells of the bundle sheath tissue (Chapter 2). In contrast to the $^{13}$C label, the $^{11}$C label was possibly still present in the transpiration stream during label distribution analysis and therefore the data from the $^{11}$C labeling gives us different information on internal CO$_2$ transport relative to the results from $^{13}$C labeling. According to Michaelis Menten enzyme kinetics, the more $^{11}$C label available as substrate for PEPc and Rubisco mediated carboxylation, the higher the amount of $^{11}$C label can be expected to be assimilated in tissues like bundle sheaths. These tissues are positioned in between the phloem and the xylem vasculature, and might directly load synthesized sugars into the phloem sap. In order to reveal these mechanisms, $^{11}$C-based labeling in combination with positron emission tomography (PET) (Beer et al., 2010; Kawachi et al., 2011a) might allow a real time in vivo detection of sugar transfer from bundle sheaths into the phloem.

Assimilation of xylem-transported CO$_2$ depends on xylem CO$_2$ concentration and transpiration rate

The $^{13}$C labeling experiments performed under controlled conditions showed that xylem-transported CO$_2$ assimilation depends on transpiration rate (Chapters 2 and 3) and xylem CO$_2$ concentration ([CO$_2$] (Chapter 3). Therefore, it is important to account for factors controlling transpiration and respiration when estimating the contribution of internal CO$_2$ recycling to the tree carbon income. For example, under high sap flow rates (F$_s$) more root-respired CO$_2$ could be transported upwards, allowing higher assimilation rates by chlorophyll containing tissues in aboveground tree organs. At leaf level, results
from previous studies suggested that a large fraction of the xylem-transported CO₂ reaching the leaves was removed from the transpiration stream by the petioles (Stringer & Kimmerer, 1993; McGuire et al., 2009), which was confirmed by our ¹³C labeling experiments on leaf (Chapter 2), branch (Chapter 3), and tree (Chapter 4) level. In addition, strong ¹³C enrichment of the mesophyll was observed under the high ¹³C label (HL) and high vapor pressure deficit (VPD) treatment (Chapter 3), which implies that a substantial amount of internal CO₂ might be transported into the leaf mesophyll under high transpiration and respiration rates. Therefore under specific conditions, xylem CO₂ transport might represent an important pathway for providing carbon substrate to photosynthetically active mesophyll tissues in the leaf blade.

**Axial diffusion of respired CO₂ confounds dormant season stem respiration estimates**

Under dormancy, assimilation of locally respired CO₂ by woody tissue photosynthesis is hypothesized to confound efflux-based estimates of stem respiration (Chapter 1). By lowering internal [CO₂] locally, it is suggested to induce axial diffusion of respired CO₂ away from the site of respiration, hence reducing stem CO₂ efflux (Eₛₘₑₜ) under temperature stable conditions (Saveyn et al., 2008a). Illuminating stem sections remote from the stem cuvette reduced Eₛₘₑₜ (Chapter 5), with highest reduction in Eₛₘₑₜ observed when illuminating the stem section closest to the stem cuvette. Therefore, internal movement of respired CO₂ during the dormant season by axial diffusion might have important implications for estimating stem respiration, in particular because dormant season stem respiration estimates are used to quantify maintenance stem respiration throughout the year. Hence, it is advised to exclude woody tissue photosynthesis in neighboring stem sections by shielding stem sections above and below the stem cuvette to obtain accurate efflux-based stem respiration estimates. The shielding height above and below the stem cuvette will depend on the extent of axial diffusion of respired CO₂ in tree stems, which is related to stem (water content, woody density, photosynthetic capacity) and forest stand (open forest vs. closed forest) characteristics, and tree dimensions.

*Woody tissue photosynthesis plays a role in tree drought stress resilience*
Results from the branch labeling study indicated that internal CO$_2$ assimilation in woody and leaf tissues is small relative to atmospheric CO$_2$ fixation via the leaves (Chapter 3). However, assimilation of internal CO$_2$ is hypothesized to play a more pronounced role in sustaining the tree carbon balance when leaf photosynthesis is limited, for instance during drought stress (Chapter 1). Under drought stress, stomatal closure is one of the earliest responses affecting leaf photosynthesis, by limiting the uptake of atmospheric CO$_2$ (Chaves et al., 2002; Flexas & Medrano, 2002; Grassi & Magnani, 2005). Woody tissue photosynthesis is supplied by endogenously respired CO$_2$, thereby avoiding excessive water loss. The stem and branch light exclusion treatment that prevented woody tissue photosynthesis to occur in poplar trees resulted in reduced growth and altered leaf gas exchange rates (Chapter 6), demonstrating the impact of local stem processes like woody tissue photosynthesis on the overall plant status. The observed relationship between woody tissue photosynthesis and leaf level processes is presumably related to its hypothesized possible role in maintaining plant hydraulics under limited water supply (Schmitz et al., 2012). However, the extent to which light-dependent embolism repair might play a role in maintaining the hydraulic function under drought stress remains unclear (Box 1.2). Moreover, the ultimate fate of sugars synthesized by woody tissue photosynthesis remains unknown. Nonetheless, based on the results in Chapter 6, it can be expected that species that are able to perform woody tissue photosynthesis might have a higher drought stress resilience as compared to non-photosynthesizing species.

### 8.2 Directions for future research

**Detailed analysis of the fate of xylem-transported CO$_2$: moving towards a laser-based approach**

Recent advances in isotopic labeling techniques have made it possible to elucidate patterns and processes in tree carbon cycling at a yet unprecedented resolution (e.g. Marron et al., 2009; Wingate et al., 2010; Bahn et al., 2012; Epron et al., 2012). Of particular interest is the development of Laser Absorption Spectroscopy (LAS), which allows continuous real-time measurements of the concentration and isotopic composition of respiratory CO$_2$ efflux, based on the absorption of near and mid-infrared
light at selected wavelengths by individual isotopologues (e.g. $^{12}\text{C}^{16}\text{O}_2$, $^{13}\text{C}^{16}\text{O}_2$, and $^{18}\text{O}^{12}\text{C}^{16}\text{O}$) (Werner et al., 2012). Within the context of tree carbon cycling, LAS-based measurements of the isotopic composition of CO$_2$ efflux in combination with cuvettes have been used exclusively to trace the allocation of photosynthates from the leaves to aboveground (Dannoura et al., 2011; Epron et al., 2012) and belowground (Plain et al., 2009; Barthel et al., 2011; Epron et al., 2011) tissues and to assess in situ the velocity of downward carbon transfer, after pulse labeling the canopy (Dannoura et al., 2011; Epron et al., 2012). In this PhD study (Chapter 4) and in the study by Powers & Marshall (2011), respiratory CO$_2$ efflux samples were taken manually, providing data on the upward transfer of $^{13}$C label at low resolution. LAS could provide us continuous data on the upward xylem transport of a $^{13}$C label within the tree, based on the sampling of respiratory efflux or internal CO$_2$, the latter using porous tubing installed in the stem. Moreover, the use of high temporal resolution isotope tracing after canopy pulse labeling could provide us solid evidence whether root-respired CO$_2$ transported in the xylem is derived mainly from recently assimilated photosynthates or stored carbon reserves, which remained elusive in our girdling study (Chapter 7). Hence, the use of LAS-based approaches to trace isotopic proxies of xylem-transported CO$_2$ within trees will further deepen our understanding of the significance of internal CO$_2$ in the tree carbon cycle.

**Unraveling the significance of xylem transport of CO$_2$ and malate as part of a C$_4$-like mechanisms in C$_3$ plants**

Results from tissue enrichment analysis after $^{13}$C labeling (Chapters 2, 3, and 4) showed that respired CO$_2$ can be transported to chlorophyll containing tissues in the stem, leaves, and branches. However, an important fraction of the photosynthetic cells might be supplied with carbon transported in organic form (e.g. malate) (Angert et al., 2012; Trumbore et al., 2013), which is derived from phosphoenolpyruvate carboxylase (PEPc) mediated carboxylation of respired CO$_2$. Following transport, malate is decarboxylated enzymatically and the CO$_2$ is re-assimilated by photosynthetically active cells adjacent to the xylem vasculature. These characteristics of C$_4$ photosynthesis have been shown to occur in stems and petioles of tobacco, a typical C$_3$ plant (Hibberd & Quick, 2002). It is unclear to what extent such a C$_4$-like mechanism exists in trees.
Berveiller & Damesin (2008) have reported high activities of PEPc and decarboxylation enzymes in mature *Fagus sylvatica* stems. The assimilation of xylem-transported $^{13}$C labeled CO$_2$ in petioles of poplar observed in our study (Chapter 4) and by Stringer & Kimmerer (1993) might support the hypothesized role of petioles in a C$_4$-like mechanism in trees (Hibberd & Quick, 2002). However, an additional labeling experiment, based on the infusion of malate in the transpiration stream of field-grown trees, might shed a new light on the distinction between C$_3$ and C$_4$ photosynthesis in plants.

**Exploring the role of woody tissue photosynthesis in light-dependent embolism repair**

As mentioned in Box 1.2, Schmitz *et al.* (2012) suggested that by recycling respired CO$_2$ in parenchymatous xylem ray cells (Fig. 1.4), xylary chloroplasts play a role in the light-dependent repair of embolized xylem vessels in branches of mangrove species. In Chapter 6, it is suggested that a similar mechanism might exist in the stems of young poplar trees, based on acoustic cavitation measurements during a drying out experiment of light-excluded and non light-excluded branches. Maintaining plant hydraulics based on xylary chloroplast sugars is consistent with the hypothesized role of sugars in the refilling of embolized vessels (Salleo *et al.*, 2004; Zwieniecki & Holbrook, 2009; Nardini *et al.*, 2011) or in triggering a biological response to embolism (Secchi & Zwieniecki, 2011). These sugars are assumed to be derived from starch depolymerization (Salleo *et al.*, 2004) or from phloem unloading into xylem parenchyma cells (Nardini *et al.*, 2011). The occurrence of chloroplasts in the xylem parenchyma, which lie nearby the xylem vessels susceptible to cavitation, suggests that sugars used in embolism repair might be derived locally (Schmitz *et al.*, 2012). However, the significance of light-dependent embolism repair in trees remains unclear. Their role could be limited to small branches and trees, because the levels of light penetrating the inner tree tissues is generally considered as a limiting factor (Pfanz *et al.*, 2002 and references therein). However, PEPc which is uniformly present in adult tree stems (Berveiller *et al.*, 2007b), might mediate CO$_2$ assimilation resulting in osmotically active compounds under low light or dark conditions. Eventually, understanding the significance of xylary chloroplasts in
embolism repair and sensing might help us to understand these mechanisms and allow us to explain why chloroplasts are present at such deep stem layers like the pith.

**The occurrence of high internal CO₂ concentrations in roots**

Within the root system, CO₂ concentrations ([CO₂]) are assumed to be higher than observed for the soil (**Chapter 1**), indicating that a portion of root-respired CO₂ accumulates within the root system (**Box 1.1**). In particular in larger roots, where suberized root tissues might represent a substantial barrier to radial diffusion of respired CO₂, a substantial fraction of respired CO₂ might accumulate, as observed for roots of aquatic plants. However, combined measurements of root internal CO₂ and soil CO₂ concentrations are not performed yet and it is unknown whether the accumulation of a part of root-respired CO₂ is a phenomenon omnipresent among root classes and tree species. Drilling a hole in a root section next to the measurement site should lead to fluxing out of CO₂ into the soil environment and a reduced internal [CO₂]. In addition, stable isotopes might provide us with detailed information on the occurrence of high internal [CO₂] in roots. First, dynamics in the isotopic composition of internal and rhizosphere CO₂ after infusion of ¹³CO₂ labeled gas in the root might prove that the root tissues exert a significant resistance to the diffusion of root-respired CO₂ into the soil environment. Moreover, additional tracing of ¹³C in the root xylem after ¹³CO₂ infusion in the root might enable a detailed quantification of the amount of root-respired CO₂ that is transported in the xylem or diffuses radially into the soil environment.

**Unraveling the role of CO₂ recycling in tree functioning under changing climate regimes**

There is ample evidence that the terrestrial component of the carbon cycle is responding to climate variations and trends on a global scale (Heimann & Reichstein, 2008). Recycling of internal CO₂ in trees could be one of those plant strategies to cope with changing climate regimes (**Chapter 6**). For instance, the frequency and the intensity of extreme events like drought and heat waves are predicted to increase (Reichstein *et al.*, 2013). In response to these extreme events, plants will close their stomates to a larger degree, thereby negatively affecting leaf gas exchange and plant growth (Bauweraerts *et al.*, 2013). Internal CO₂ recycling is less susceptible to the drying power of the atmosphere and water loss via the stem is limited, leading to an increased plant water
use efficiency under climate extremes (Chapter 1). On the potential role of internal CO$_2$ recycling in tree resilience to climate extremes, much can be learned from internal CO$_2$ recycling in drought-adapted species inhabiting desert environments. These plants have a limited leaf area and stem photosynthesis is considered as an integral component of the plant carbon balance (e.g. Gibson, 1983; Comstock & Ehleringer, 1990; Nilsen & Sharifi, 1997), stressing the significance of internal CO$_2$ recycling for their carbon income. Therefore, accurate climate change experiments with combined drought, increased atmospheric CO$_2$, heat wave and stem and branch light manipulation treatments are needed to test the postulated role of CO$_2$ recycling in tree functioning under changing climate regimes.

The importance of respired CO$_2$ transport and recycling in large or mature trees

The results from the experiments performed in this PhD study have indicated the potential importance of respired CO$_2$ transport and recycling in younger trees (1-9 years old). However, the question is what to expect of the importance of these processes in larger or mature trees? First in larger trees, xylem CO$_2$ transport with the transpiration stream might represent a flux of larger magnitude as the one observed in younger trees (Chapter 7) as larger trees possess a larger sapwood area. Within the stem of aging trees, more respired CO$_2$ will accumulate as more suberized, older tissues in the bark are expected to exert a larger barrier to radial diffusion, which leads to higher dissolved CO$_2$ concentrations. Moreover, a fraction of the internal CO$_2$ could be derived from several-year-old storage pools as recently observed for older tropical trees by Muhr et al. (2013). Second, larger trees will have a larger root system, allowing a larger import of more root-respired CO$_2$ with the transpiration stream into the stem. Teskey & McGuire (2007) observed for *Platanus occidentalis* that trees with a larger diameter had a higher internal [CO$_2$] at stem base. This probably indicates that larger trees with larger root systems might accumulate more CO$_2$ in their root system. As root systems age, an increasing proportion becomes suberized (Kramer & Kozlowski, 1966). This will increase the amount of respired CO$_2$ that remains within the root system (Chapter 1) rather than fluxing into the soil environment. Finally, light-driven woody tissue photosynthesis will possibly be lower in aging tree tissues. The amount of light penetrating the rhytidome and periderm is an important limiting factor for light-driven
woody tissue photosynthesis. Older bark layers, where lenticels have lost their light conducting function by rhytidomal thickening, are expected to permit less light penetration up to chlorophyll containing cells in the stems (Wittmann et al., 2001). However, additional measurements of light conductance by mature tissues are needed. Nonetheless, a fraction of the respired CO₂ might still be assimilated by non light-driven woody tissue photosynthesis, as high PEPc activity has been reported in stems of aging trees of varying species (Berveiller & Damesin, 2008).


References


Craig H. 1954. $^{13}$C in plants and the relationships between $^{13}$C and $^{14}$C variations in nature. *Journal of Geology* 62: 115-149.


References


**Li MR, Jones MB. 1995.** CO$_2$ and O$_2$ Transport in the Aerenchyma of *Cyperus papyrus* L. *Aquatic Botany* **52**: 93-106.


Norby RJ. 2009. Introduction to a Virtual Special Issue: probing the carbon cycle with $^{13}$C. *New Phytologist* 184: 1-3.


Summary

Since our childhood we are learned that trees are “The green lungs of our planet” because they take up CO\(_2\) and emit O\(_2\) during photosynthesis. However, respiration is a second vital plant process, during which CO\(_2\) is emitted into the atmosphere. At the global scale, both fluxes of CO\(_2\) uptake and release are several orders of magnitude larger than the anthropogenic one and approximately balance. In contrast to our understanding of photosynthesis, we (i.e. the scientific community) lack the ability to accurately quantify and model the contribution of the different above- and belowground sources to the respiratory flux from forest ecosystems into the atmosphere. In our attempts to quantify the respiratory fluxes from a single source, efflux-based methods are being used, based on the general believe that all locally respired CO\(_2\) is released immediately into the atmosphere at the site of respiration thereby contributing to the local CO\(_2\) efflux.

However, recent literature has shown that a fraction of the respired CO\(_2\) remains within the tree instead of diffusing into the atmosphere. As a result internal CO\(_2\) concentrations ([CO\(_2\)]) in different trees species are reported in a range (<1 to over 26%) significantly higher than the atmospheric one (c. 0.04% at 2013). While aboveground respired CO\(_2\) has previously been recognized as an important source of internal CO\(_2\), most recent reports suggest that also a fraction of root-respired CO\(_2\) is transported within the tree, instead of diffusing into the soil environment. Nevertheless, internal CO\(_2\) in trees remains a missing link in our understanding of the tree carbon cycle. Therefore, this PhD study aimed at increasing our knowledge on the fate and importance of respired CO\(_2\) in the tree carbon economy based on results obtained during experimental studies.

In particular, the fate of xylem-transported CO\(_2\) remains poorly understood. In this PhD study, a \(^{13}\)C-labeled CO\(_2\) (referred to as \(^{13}\)CO\(_2\)) tracer experiment was performed to study the fate of xylem-transported CO\(_2\) in full size field-grown trees. Dissolved \(^{13}\)CO\(_2\) was
infused at the stem base of *Populus deltoides* Bartr. ex Marsh trees and isotope analysis was used to reveal whether the isotopic proxy was assimilated in the tree or diffused to the atmosphere. Results from the isotopic analysis from the gas and tissue samples demonstrate not only the upward transport of the label, but also the assimilation of the $^{13}$CO$_2$ in the stem, canopy and leaf tissues. The largest fraction of the $^{13}$CO$_2$ label diffused to the atmosphere. Therefore, a substantial amount of belowground respired CO$_2$ might diffuse from stem and branch surface into the atmosphere, leading to misestimates of above- and belowground respiration. Additional experiments, based on the introduction of $^{13}$CO$_2$ in the xylem of poplar branches from the same species and leaves (*Populus x canadensis* Moench ‘Robusta’), were performed under controlled conditions. Xylem CO$_2$ transport was manipulated either by altering the uptake rate or the xylem $^{13}$CO$_2$ concentration of the infused solution. For the woody and leaf tissues of the branches allowed to take up the $^{13}$C label, inner bark and petioles, respectively, were most enriched, regardless of the uptake rate of xylem $^{13}$CO$_2$. However, the total assimilation of $^{13}$CO$_2$ relative to the uptake of atmospheric CO$_2$ was small (up to 1.9%). In leaves, detailed distribution of xylem-transported CO$_2$ was obtained using positron autoradiographic detection of $^{11}$C. This high-resolution data indicated that assimilation of xylem-transported CO$_2$ mainly occurred near the leaf veins, which is presumably related to the occurrence of bundle sheath cells surrounding the leaf veins.

Assimilation of internal CO$_2$ has been considered as an important means for improving the tree carbon balance under reduced atmospheric CO$_2$ uptake, like under drought stress. To this end, it was tested to which extent woody tissue photosynthesis contributed to the overall tree carbon gain of *Populus deltoides x nigra* ‘Monviso’ trees under well-watered and drought-stressed conditions. Light exclusion was used to prevent woody tissue photosynthesis to occur and stem growth rate and leaf gas exchange were assessed both for control and light-excluded trees. Moreover, the hypothesis that woody tissue photosynthesis might play a role in light-dependent embolism repair was tested. Under well-watered conditions, reduced stem growth was observed for light-excluded trees. Under drought-stressed conditions, trees lacking woody tissue photosynthesis shrunk more pronounced and showed a faster reduction in leaf gas exchange. Moreover, acoustic measurements confirmed that woody tissue
photosynthesis could play a role in embolism repair in trees by synthesizing sugars used for embolism sensing and refilling. Therefore, assimilation of internal CO₂ might be one of the overlooked strategies of plants to cope with drought.

Similarly, woody tissue photosynthesis might have an important role in leafless dormant trees. When sap flow is absent, reduction of the local stem internal [CO₂] by woody tissue photosynthesis can induce axial diffusion of respired CO₂ away from the site of respiration, hence confounding dormant season efflux-based stem respiration estimates. In this PhD study, the significance of the axial diffusion of respired CO₂ induced by woody tissue photosynthesis for estimating dormant season stem respiration was evaluated. Stem CO₂ efflux (E_{stem}) was measured under temperature-controlled conditions with a light-excluded stem cuvette, while axial CO₂ diffusion was induced by illuminating remote stem sections at varying distances from the stem cuvette. Due to axial diffusion by woody tissue photosynthesis, E_{stem} measured with the stem cuvette was reduced by 22% relative to control conditions when no woody tissue photosynthesis occurred. Highest reductions were observed when woody tissue photosynthesis was induced in the stem section closest to the stem cuvette, while the effect was negligible when stem sections were illuminated at 30 cm and more from the stem cuvette. Temperature-corrected dormant-season stem respiration estimates are used for partitioning growing season stem respiration into a growth and maintenance component. Therefore, failure to account for the effect of woody tissue photosynthesis on dormant season E_{stem} might bias component respiration estimates throughout the year.

The recent discovery that a fraction of root-respired CO₂ contributes to internal CO₂ rather than fluxing into the soil environment cast doubt on common efflux-based approaches for quantifying the autotrophic component of belowground respiration. Therefore, a re-estimation of the autotrophic component of belowground respiration at ecosystem level is necessary. In this PhD study, the contribution of root-respired CO₂ to both soil CO₂ efflux (E_{stem}) and xylem CO₂ transport (F₁) from root respiration was estimated in an oak tree (Quercus robur L.) plantation based on the manipulation of the autotrophic component of belowground respiration (R_{a,b}) by removing a circumferential band of bark during tree girdling. Next to E_{soil}, xylem [CO₂] at stem base decreased in
response to girdling, corroborating the previous observations on $F_t$ in trees. Accounting for both the reduction in $E_{\text{soil}}$ and scaled $F_t$ led to more accurate estimates of $R_{a,b}$. In particular, large differences with the previous efflux-based estimates of the autotrophic component of belowground respiration were observed during the day, when root-respired CO$_2$ is transported away from the site of respiration with the transpiration stream.

In conclusion, results from this PhD study demonstrate that internal CO$_2$ plays an important role in the tree carbon cycle, regardless whether it is transported with the transpiration stream, assimilated in chlorophyll containing tissues or diffused to the atmosphere. Hence, measurements of internal CO$_2$ and related ecophysiological parameters should be more widely applied to improve our ability to understand and model the flow of carbon within trees.
Samenvatting

Sinds onze kindertijd wordt ons verteld dat bomen “De groene longen van onze planeet” zijn, aangezien ze CO₂ opnemen en O₂ terug afgeven tijdens fotosynthese. Echter, respiratie is een tweede vitaal plant proces, waarbij CO₂ wordt afgegeven aan de atmosfeer. Globaal gezien is de opname en afgifte van CO₂ door planten vele malen groter dan de antropogene CO₂ flux en beide zijn ongeveer in balans. In tegenstelling tot onze kennis over fotosynthese ontbreekt het ons (met name de wetenschappers) aan kennis om zeer accuraat de bijdrage te kwantificeren en te modelleren van de verschillende boven- en ondergrondse bronnen tot de gerespireerde CO₂ flux van bos ecosystemen tot de atmosfeer. In onze pogingen om deze bijdragen te kwantificeren gebruiken we efflux-gebaseerde methodes, volgens het algemeen aanvaard geloof dat al het lokaal gerespireerd CO₂ direct diffundeert naar de atmosfeer en bijgevolg bijdraagt aan CO₂ efflux.

Echter, recent gepubliceerde resultaten geven aan dat een deel van het gerespireerd CO₂ in de boom blijft in tegenstelling tot directe diffusie naar de atmosfeer. Bijgevolg, worden voor verschillende boomsoorten intern CO₂ concentraties ([CO₂] gerapporteerd in een grootteorde (<1 to over 26%) significant hoger dan de atmosferische [CO₂] concentratie (c. 0.04% in het jaar 2013). Waar voorheen bovengronds gerespireerd CO₂ werd erkend als een belangrijke bron van intern CO₂, suggereren de meest recente resultaten dat zelfs een deel van het ondergronds gerespireerd CO₂, afgeleid van de wortels, kan getransporteerd worden in de boom en zo bijdragen aan intern CO₂. Niettegenstaande, intern CO₂ in bomen blijft een ontbrekende schakel in onze kennis over de koolstof cyclus van bomen. Daarom had dit doctoraatsonderzoek als doel onze kennis te verbeteren over de rol van intern CO₂ in de koolstof economie van bomen, gebaseerd op de resultaten van experimentele studies.

Het ontbreekt ons vooral aan kennis over de bestemming van xyleem getransporteerd CO₂. In dit doctoraatsonderzoek werd een ¹³C-gelabeld CO₂ (verder vernoemd als ¹³CO₂)
tracer experiment uitgevoerd met als doel het bestuderen van de bestemming van xyleem getransporteerd CO₂ in grote bomen onder veld omstandigheden. Een infusie van een ¹³CO₂ label in de basis van de stam van *Populus deltoides* Bartr. Ex Marsh bomen werd gebruikt om te bestuderen of xyleem getransporteerd CO₂ wordt geassimileerd in de boom of diffundeert naar de atmosfeer. Resultaten van de isotopen analyses van gas-, blad- en houtstalen gaven aan dat het label werd getransporteerd in de boom en dat assimilatie van ¹³CO₂ optrad in de stam en de kruin. De grootste fractie van de ¹³CO₂ label diffundeerde naar de atmosfeer. Dit geeft aan dat een substantieel deel van het ondergronds gerespireerd CO₂ mogelijk diffundeert van stam en tak oppervlakken naar de atmosfeer, wat aangeeft dat methodes gebaseerd op het meten van CO₂ efflux de hoeveelheid boven- en ondergrondse respiratie verkeerd inschatten. Extra labeling experimenten werden uitgevoerd op takken van dezelfde populierensoort en op bladeren (*Populus x canadensis* Moench 'Robusta') onder gecontroleerde condities. Het transport van xyleem CO₂ werd gemanipuleerd door het veranderen van de opnamesnelheid of door het wijzigen van xyleem ¹³CO₂ concentratie van de oplossing. Voor de blad- en houtige weefsels waren het binnenste van de bast en de bladpetiolen het meest aangerijkt in ¹³C, respectievelijk, ongeacht de hoeveelheid opgenomen oplossing of de ¹³CO₂ concentratie. Relatief ten opzichte van de opname van atmosferische CO₂ door de bladeren was de totale assimilatie van ¹³CO₂ klein (maximaal 1.9%). De autoradiografische detectie van ¹¹C in bladeren zorgde voor een gedetailleerde analyse van distributie van xyleem getransporteerd CO₂. Deze hoge resolutie data geeft aan dat de assimilatie van xyleem getransporteerd CO₂ ter hoogte van de bladeren en de bladnerven hoogstwaarschijnlijk gerelateerd is aan het voorkomen van bundel schede cellen langsheen de bladnerven.

Assimilatie van intern CO₂ in bomen wordt over het algemeen beschouwd als een mogelijke strategie ter verbetering van de koolstof balans van bomen wanneer opname van atmosferisch CO₂ beperkt is, zoals onder droogtestress. In dit doctoraatsonderzoek werd daarvoor nagegaan wat de bijdrage was van stam en tak fotosynthese tot de koolstof opname door *Populus deltoides x nigra* 'Monviso' bomen, onder goed bewaterde en droogte stress condities. Beschaduwen van de stam werd gebruikt voor het uitsluiten van stam en tak fotosynthese. Stamgroei en gasuitwisseling ter hoogte van de bladeren
werden opgemeten voor controle en beschaduwde bomen. Daarenboven werd de hypothese over de lichtafhankelijke rol van stam en tak fotosynthese in het herstel van gecaviteerde xyleem vaten getest. Onder goed bewaterde condities werd een beperktere stamgroei geobserveerd voor de beschaduwde bomen relatief ten opzichte van de stam groei bij de controle bomen. Onder droogtestresscondities kromp de stam van beschaduwde bomen meer uitgesproken en daalde gasuitwisseling op bladniveau sterker. Daarenboven bevestigten akoestische metingen dat stam en tak fotosynthese mogelijk een rol speelt in het herstel van gecaviteerde xyleem vaten, door de synthese van suikers gebruikt voor het hervullen en het opsporen van gecaviteerde vaten in de boom. Mogelijks is assimilatie van intern CO₂ dus één van de over het hoofd geziene processen waarmee planten met droogte omgaan.

Op een gelijkwaardige manier zou stam en tak fotosynthese een belangrijke rol kunnen spelen in bladloze dormante bomen. Wanneer sap stroom afwezig is, kan een daling in lokale stam intern CO₂ concentratie door stam en tak fotosynthese een axiale diffusie van gerespireerd CO₂ teweeg brengen weg van de plaats van respiratie. Bijgevolg heeft dit een sterk effect op de efflux gebaseerde schattingen van stam respiratie tijdens het dormante seizoen. In dit doctoraatsonderzoek werd nagegaan wat het belang is van de axiale diffusie van gerespireerd transport door stam en tak fotosynthese voor de schatting van stam respiratie. Stam CO₂ efflux werd gemeten onder temperatuur gecontroleerde condities met een beschaduwde stam cuvette terwijl een verschillende mate van axiale diffusie van gerespireerd CO₂ in de stam werd geïnduceerd door het belichten van naburige stam segmenten op een verschillende afstand van de stam cuvette. Relatief ten opzichte van controle omstandigheden waarbij geen tak of stam fotosynthese optrad werd een daling in stam CO₂ efflux waargenomen tot 22%. De grootste daling trad op wanneer stam fotosynthese plaats vond in het stam segment het dichtst bij de stam cuvette, terwijl het effect van stam fotosynthese op stam CO₂ efflux verwaarloosbaar was op afstand van 30 cm en meer, gemeten vanaf de stam cuvette. Temperatuur gecorrigeerde schattingen van stam respiratie gedurende het dormante seizoen worden gebruikt om respiratie tijdens het groei seizoen onder te verdelen in een groei en een onderhouds component. Bijgevolg, metingen van stam respiratie die
het effect van stam fotosynthese niet in acht nemen zullen mogelijk leiden tot verkeerde inschattingen van de respiratie componenten gedurende de rest van het jaar.

De recente ontdekking dat een deel van het wortel gerespireerd CO₂ bijdraagt tot intern CO₂ in plaats van te diffunderen in de bodem trekt de conventionele efflux gebaseerde methodes voor het schatten van de autotrofe component van ondergrondse respiratie in twijfel. Bijgevolg, een hernieuwde schatting van de autotrofe component van ondergrondse respiratie op ecosysteem niveau is noodzakelijk. In dit doctoraatsonderzoek werd de bijdrage van wortel gerespireerd CO₂ aan zowel bodem CO₂ efflux als xyleem CO₂ transport geschat voor eiken (*Quercus robur* L.) plantage. Deze schatting werd uitgevoerd op basis van de manipulatie van de autotrofe component van ondergronds respiratie door het ringen van de stam (het verwijderen van een strook de bast). Door het ringen van de stam daalde naast bodem CO₂ ook de xyleem [CO₂] ter hoogte van de basis van de stam, wat de vorige waarnemingen van xyleem transport van ondergronds gerespireerd CO₂ in bomen onderbouwt. Bijgevolg zal het in rekening brengen van zowel de daling in bodem CO₂ efflux als de daling in de geschaalde flux van intern CO₂ transport na ringen leiden tot betere schattingen van de autotrofe component van ondergrondse respiratie. Een verschil tussen de nieuwe en oude schattingen van de autotrofe component van ondergrondse respiratie zal voornamelijk gedurende de dag waargenomen worden, wanneer een deel van het wortel gerespireerd CO₂ getransporteerd wordt weg van de plaats van respiratie via de sapstroom in de boom in plaats van bij te dragen aan bodem CO₂ efflux.

In conclusie, de resultaten van dit doctoraatsonderzoek geven aan dat intern CO₂ een belangrijke rol speelt in de koolstofcyclus van bomen, ongeacht of het nu getransporteerd wordt met de sapstroom, geassimileerd wordt in chlorofyl houdende weefsels of diffundeert naar de atmosfeer. Bijgevolg moeten metingen van intern CO₂ en gerelateerde ecofysiologische parameters meer uitgevoerd worden wat onze capaciteit om de stroom van koolstof in bomen te begrijpen en te modelleren zal verbeteren.
Curriculum vitae

Personal details

Name: Jasper Toon Tine Bloemen
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Education/work experience

               Brugge centrum, Brugge, Belgium

2004-2007 Bachelor degree in Bioscience Engineering (Environmental technol.),
          Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
          (distinction)

2007-2009 Masters degree in Bioscience Engineering (Environmental technol.),
          Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
          (Great distinction)
          Master thesis: Performed at the Department of Applied Ecology and
          Environmental Biology, Laboratory of Plant Ecology, Ghent University,
          Ghent, Belgium
          Dutch title: “Dynamische interactie tussen fotosynthese, transpiratie en
          BVOS emissie in bosecosystemen”
          Promotor: Prof. dr ir. Kathy Steppe
Curriculum Vitae

2010-present  PhD training program in Applied Biological Sciences, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Courses followed in the framework of this program:

- Ecophysiology
- Bio-imaging
- ANOVA: analysis of variance
- Advanced Academic English
- Introduction to SAS
- Applying for a post doctoral job

Professional experience

2009 - 2013  Research assistant at the Department of Applied Ecology and Environmental Biology, Laboratory of Plant Ecology, Ghent University, Gent, Belgium

Other Scientific activities

Jan - June 2008  Université Laval, Québec, Canada, Study exchange within the framework of a bilateral agreement framework (Atlantis program)

Nov - Jan 2009  Research visit at the laboratory of prof. Andrew D. Richardson Harvard University, Boston, MA, US. Data analysis of soil and ecosystem respiration measurements. Introduction to the use of webcams to monitor phenology

June - July 2010  University of Georgia, Daniel B. Warnell school of Forestry and Natural Resources, Athens, Georgia, USA. Growth chamber and field experiments with $^{13}$C isotopes

Sept 2012  INRA Research center, Champenoux, France. Training school

Stable Isotopes in ecosystem research in the framework of the SIBAE COST action (ES0806)
June 2013  Member of the Editorial Board of the 9th International Workshop on Sap Flow, Ghent, Belgium, 4-7 June 2013

July-Aug 2013  Universität Innsbruck, Institute of Ecology (lab of Prof. Michael Bahn) Innsbruck, Austria. Introduction to the use of a quantum cascade laser system to trace stable isotopes in tree CO₂ efflux. Short Term Scientific Mission (STSM) within the framework of the SIBAE COST action (ES0806)

2009-2013  Review tasks for Tree Physiology, Flora, and Annals of Botany

Grants and prizes

June 2010  Grant from the Scientific Research Committee (CWO) for the research visit at the University of Georgia, Athens, Georgia, USA

April 2012  Grant from the Fund for Scientific Research (FWO) for attending the EGU general assembly conference, Vienna, Austria

April 2012  Grant from the Scientific Research Committee (CWO) for attending the EGU general assembly conference, Vienna, Austria

Sept 2012  COST Travel grant for attending the course on stable isotopes in the framework of the SIBAE COST action (ES0806), Nancy, France

Dec 2012  Grant from the Scientific Research Committee (CWO) for attending the AGU Fall meeting, San Francisco, California, USA

May 2013  Grant from the Fund for Scientific Research (FWO) for attending the SIBAE COST (ES0806) meeting, Wroclaw, Poland

July-Aug 2013  COST STSM grant for the research visit at Universität Innsbruck Innsbruck, Austria
Educational activities

2010 - present  Guest lecturer practical part courses "Plant water relations", "Ecology", and "Terrestrial ecology".

2010 - 2011  Tutor of the Master thesis of Sim Minnaert
Dutch title: “Invloed van licht en warmte op BVOC emissie bij schaduwbladeren van beuk (Fagus sylvatica L.)”

2011 - 2012  Tutor of the Master thesis of Laura Agneessens
Dutch title: “Autotrofe bodemrespiratie in bosecosystemen: hoe belangrijk is intern CO₂ transport via sapstroom?”

2011 - 2012  Tutor of the Master thesis of Yentl Dupon
Dutch title: “Temperatuursonafhankelijk variatie in stam CO₂ efflux bij eiken (Quercus robur L.) tijdens dormantie”

2011 - 2012  Tutor Bachelor project
Dutch title: “CO₂ in bomen: even verder denken dan fotosynthese”

Scientific output

International publications with peer review


*equal contributions


*Proceedings*

**Scientific Reports**


**Participation in conferences, symposia or workshops**


Bloemen J. (2011) Quantification of Belowground respiration and fate of root-derived CO2 in trees. Workshop Importance of phenology, carbon partitioning and allocation for tree and forest function, Class of Excellence prof. John S. King, 19 May 2011 (oral presentation)


Bloemen J. and Steppe K. (2013) ¹³C as a tracer for respired CO₂ transport in the xylem at leaf, branch and tree level. COST-SIBAE meeting, Wroclaw, Poland, 14-17 May 2013 (oral presentation)


Now everyone dreams of a love lasting and true
But you and I know what this world can do
So let's make our steps clear that the other may see
And I'll wait for you
If I should fall behind
Wait for me