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Does soil CO_2 uptake by tree roots contribute to stem CO_2 efflux?

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Acknowledgements

It is almost a year ago that I tasted real scientific research for the first time. At once, these were my first steps in the USA. The perfect opportunity to kill two birds with one stone. Packed with some starting papers, fresh belief and two good friends I arrived at Atlanta International Airport somewhere in mid-July. As our time in Athens was short and there was a lot of work to do, there are a lot of people to thank. People from both the USA and Belgium.

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Ghent, June 2014

Summary

The carbon balance of forest ecosystems, a delicate balance between the carbon flux of ecosystem respiration and photosynthesis, fulfills an important role in the global carbon cycle. The aboveground component of the ecosystem respiration is typically based on carbon dioxide (CO_2) efflux measurements. However, these measurements are not an accurate estimation of local woody tissue respiration because recent research has revealed that a portion of the CO_2 from respiring living cells can dissolve in the xylem sap and be transported upward throughout the tree by the transpiration stream. This way, CO_2 can diffuse to the atmosphere on a location different from the site of origin.

A substantial part of internal CO_2 originated belowground. Generally, the soil solutions was believed to be the biggest source of belowground respired CO_2, but recently it was suggested that root respiration substantially contributes to internal CO_2. The scope of this thesis was to investigate the contribution of soil CO_2, taken up by tree roots of loblolly pine (*Pinus taeda*) and northern red oak (*Quercus rubra*). Our hypothesis was that almost no dissolved CO_2 from the soil solution will be taken up in the root xylem, and therefore the contribution of soil CO_2 to internal CO_2 is negligible under natural conditions.

One year old pine and oak trees were irrigated with four different CO_2 enriched aqueous solutions to vary the soil CO_2 concentration in the range of 0.3-8.5%. After irrigation, stem CO_2 efflux measurements were performed to investigate the effect of elevated soil CO_2 concentrations. Finally, tree roots were exposed to an aqueous solution enriched with ^{13}C for a soil CO_2 concentration above the natural range (0.5-1.5%). For each tree, a root and stem segment was removed and frozen in liquid nitrogen. Gas extracted from the air in the headspace of the vials containing these samples was than analyzed to confirm the uptake of soil CO_2 under these conditions.

For all treatments, increased stem CO_2 efflux values were measured. The mean contribution of the extra efflux to the total stem CO_2 efflux was 31.0% for pines and 38.6% for oaks, indicating that soil CO_2 contributes to internal CO_2. The $^{13}CO_2$ experiment confirmed the uptake of soil CO_2 for a concentration substantially higher than the natural range. As the internal CO_2 concentration of roots is generally reported to be higher than in the surrounding soil, we suggest that for a lower soil CO_2 concentration another effect dominates. Therefore, for natural conditions the actual uptake of soil CO_2 is negligible and root respiration is the biggest belowground CO_2 source. However, fluctuations in the soil CO_2 concentration can substantially affect the stem CO_2 efflux, especially in small trees.

Samenvatting

De koolstofbalans van bosecosystemen, een delicaat evenwicht tussen fotosynthese en ecosysteemrespiratie, speelt een belangrijke rol in de globale koolstofcyclus. Waarden voor de bovengrondse autotrofe component van de ecosysteemrespiratie zijn typisch gebaseerd op koolstofdioxide (CO_2)-efflux metingen. Echter, deze metingen vormen geen accurate schatting van de lokale respiratie aangezien recent onderzoek heeft aangetoond dat CO_2 afkomstig van respirerende levende cellen kan oplossen in het xyleemsap en met de transpiratiestroom opwaarts getransporteerd kan worden doorheen de boom. Zo kan CO_2 diffunderen naar de atmosfeer in een deel van de stam verschillend van de oorspronkelijke plaats van respiratie.

Een groot deel van de interne CO_2 is afkomstig van ondergronds. Doorgaans werd de bodemoplossing als grootste ondergrondse CO_2-bron beschouwd, maar recentelijk werd gesuggereerd dat wortelrespiratie in grote mate bijdraagt tot intern CO_2. Het doel van deze studie was de bijdrage van bodem CO_2, opgenomen door boomwortels van wierrookden (*Pinus taeda*) en Amerikaanse eik (*Quercus rubra*), te onderzoeken. Onze hypothese was dat er bijna geen opgeloste CO_2 uit de bodemoplossing zal opgenomen worden in het wortelxyleem waardoor de bijdrage tot intern CO_2 verwaarloosbaar wordt onder natuurlijke omstandigheden.

Eén jaar oude dennen en eiken werden achtereenvolgens geïrrigeerd met vier verschillende CO_2-aangerijkte oplossingen om de bodem CO_2-concentratie te laten variëren tussen 0.3-8.5%. Na irrigatie werd de stam CO_2-efflux gemeten om het effect van een verhoogde bodem CO_2-concentratie na te gaan. Ten slotte werden de wortels blootgesteld aan een met ^{13}C-aangerijkte oplossing met een CO_2-concentratie boven de natuurlijke range (0.5-1.5%), waarna telkens een wortel- en stamsegment verwijderd en gevriesdroogd werd. Gasextractie van lucht in contact met deze segmenten werd vervolgens geanalyseerd om de opname van CO_2 uit de bodemwateroplossing te valideren.

Voor alle behandelingen werd een verhoogde stam CO_2-efflux opgemeten. De gemiddelde contributie van de extra efflux ten opzichte van de totale stam CO_2-efflux bedroeg gemiddeld 31.0% voor den en 38.6% voor eik, wat suggereert dat bodem CO_2 bijdraagt tot intern CO_2. Het $^{13}CO_2$ experiment bevestigde de opname van bodem CO_2 voor een CO_2-concentratie hoger dan de natuurlijk range. Aangezien de CO_2-concentratie in wortels doorgaans hoger is als in de omringende bodem, suggereren we dat bij lagere CO_2-concentraties een ander effect geobserveerd wordt. Daarom zal voor natuurlijke condities de effectieve opname van bodem CO_2 verwaarloosbaar zijn en zal wortelrespiratie de grootste ondergrondse bron zijn van gerespireerde CO_2. Echter, fluctuaties in de bodem CO_2-concentratie kunnen een belangrijk effect hebben op de stam CO_2-efflux, in het bijzonder in kleine bomen.

Contents

List of symbols and abbreviations

Abbreviations

CO_2	Carbon dioxide
DIC	Dissolved inorganic carbon
FACE	Free air carbon dioxide enrichment
GPP	Gross primary production
IRGA	Infrared gas analyzer
NEP	Net ecosystem production
NPP	Net primary production
O_2	Oxygen
PAR	Photosynthetic active radiation
SE	Standard error

Symbols

Symbol	**Description**	**Unit**
$[CO_2]$	Gaseous CO_2 concentration	%
$[CO_2*]$	Dissolved CO_2 concentration	mM
$[O_2]$	Gaseous O_2 concentration	%
$\delta^{13}C$	Carbon isotopic composition ($^{13}C/^{12}C$)	‰
$\delta^{13}C_{\_b}$	Carbon isotopic composition ($^{13}C/^{12}C$) in baseline samples	‰
$\delta^{13}C_{\_t}$	Carbon isotopic composition ($^{13}C/^{12}C$) in treated samples	‰
A_{13C}	Atom%	%
A_{13C_b}	Atom% of the baseline samples	%
A_{13C_c}	Atom% enrichment of the treated samples (difference between A_{13C_t} and A_{13C_b})	%
A_{13C_t}	Atom% of the treated samples	%
E_{CO2}	CO_2 efflux	µmol CO_2 m^{-2} s^{-1}
E_{branch}	Branch CO_2 efflux	µmol CO_2 m^{-2} s^{-1}
E_{soil}	Soil CO_2 efflux	mg C m^{-2} h^{-1}
E_{stem}	Stem CO_2 efflux	µmol CO_2 m^{-2} s^{-1}
E_{stem_b}	Baseline stem CO_2 efflux	µmol CO_2 m^{-2} s^{-1}

E_{stem_c}	Corrected stem CO_2 efflux	µmol CO_2 m^{-2} s^{-1}
E_{stem_t}	Treatment CO_2 efflux	µmol CO_2 m^{-2} s^{-1}
F_s	Sap flow	mL h^{-1}
P_n	Photosynthesis	µmol CO_2 m^{-2} s^{-1}
P_{wt}	Woody tissue photosynthesis	µmol CO_2 m^{-2} s^{-1}
R_a	Autotrophic component of respiration	mg C m^{-2} h^{-1}
R_{eco}	Total ecosystem respiration	mg C m^{-2} h^{-1}
R_{branch}	Branch respiration	mg C m^{-2} h^{-1}
R_h	Heterotrophic component of respiration	mg C m^{-2} h^{-1}
R_{leaf}	Leaf respiration	mg C m^{-2} h^{-1}
R_{root}	Root respiration	mg C m^{-2} h^{-1}
R_{soil}	Soil respiration	mg C m^{-2} h^{-1}
R_{stem}	Stem respiration	mg C m^{-2} h^{-1}
R_{wt}	Woody tissue respiration	mg C m^{-2} h^{-1}
T	Temperature	°C

Introduction

The carbon cycle is probably one of the most commonly known biogeochemical cycles. The awareness that fossil fuel burning has perturbed the carbon cycle, with feedbacks to global climate, has inspired researchers and funding agencies worldwide to invest in carbon cycle research (Luyssaert et al., 2007). Focus often lays on forest ecosystems (e.g. Figure 1) because they account for the majority of terrestrial net primary production (NPP) and therefore have an important role in the global carbon cycle (Jobbágy & Jackson, 2000; Geider et al., 2001). For example, Houghton et al. (2001) estimated that every year roughly one-sixth of all atmospheric carbon dioxide (CO_2) passes through an ecosystem (Trumbore, 2006). However, even though forests sequester a large amount of atmospheric carbon and are sometimes considered to mitigate global warming (Schimel et al., 2001), it is not as straightforward to tell whether a forest ecosystem is a carbon sink or source. Some models even predict positive feedback loops to global warming whereby the terrestrial carbon cycle becomes a source of atmospheric CO_2 (Lindroth et al., 1998; Cox et al., 2000; Heimann & Reichstein, 2008). Heimann & Reichstein (2008) concluded that the wide spread of results between different models indicates our lack of knowledge about the ecosystem processes presented in these models. To increase our understanding of the global carbon cycle and to assess the impact and feedbacks of the climate change on forest ecosystems a good understanding of the different processes that contribute to forest carbon balances is required (Bolstad et al., 2004; Agneessens, 2012). In the first part of Chapter 1, the main carbon fluxes of forest ecosystems are discussed, with special attention to the importance of autotrophic respiration (R_a).

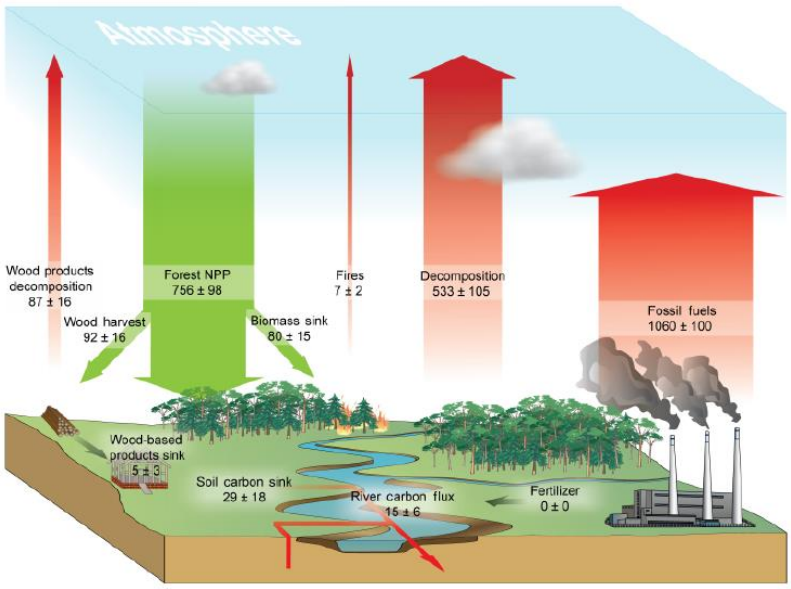

Figure 1. The forest carbon cycle (Tg C yr^{-1}) for 25 member states of the European Union, from data or model results. Heterotrophic respiration was calculated as the residual term. Uncertainties show the standard deviation of the variability across approaches. (Luyssaert et al., 2010)

Photosynthetic uptake of carbon represents the largest carbon flux in forests, but a substantial amount of this CO_2 is released back to the atmosphere during R_a. Teskey and McGuire (2007) underlined the importance of accurate tree stem respiration (R_{stem}) measurements, for instance for the quantification of carbon budgets (Bolstad et al., 2004), the estimation of net ecosystem productivity (NEP) (Maier et al., 2004) and the evaluation of carbon allocation patterns (Giardina et al., 2003). For years, local woody tissue respiration (R_{wt}) rates were based on CO_2 efflux (E_{CO2}) measurements from stems and branches to the atmosphere (E_{stem} and E_{branch}, respectively). However, recent research revealed the importance of internally transported CO_2 derived from respiration (e.g. Teskey and McGuire, 2002). Because of barriers to diffusion in the inner bark and xylem only a part of the CO_2 produced by respiring cells diffuses locally to the atmosphere. Some of the CO_2 can dissolve in the xylem sap and can be transported upwards in the tree through the higher stem and the canopy, where it can diffuse to the atmosphere or it can be fixed in photosynthetic cells in woody tissues or leaves. These new findings led to the awareness that E_{CO2} is often poorly correlated to the actual rate of R_{wt} (Teskey et al., 2008). A better understanding of the internal pathways will therefore help improving our knowledge about tree carbon balances. The second part of Chapter 1 will focus on measurements of R_{wt}. In Chapter 2, the different sources and sinks of CO_2 inside tree stems are discussed.

The main focus of this thesis is the contribution of belowground respired CO_2 to internal CO_2 transport. Teskey and McGuire (2002) mentioned the contribution of CO_2 derived from tree root respiration (R_{root}) and probably from uptake of soil solution. Teskey and McGuire (2007) measured high CO_2 concentrations ($[CO_2]$) at the base of the stem, but no indication of high R_{wt} was found, suggesting that the CO_2 was being transported upward from the root system. However, the relative roles of root respired CO_2 and soil dissolved inorganic carbon (DIC) are not clear. Chapter 3 will further focus on the uptake of CO_2 from the soil solution. A literature overview is given and a framework for this experiment is developed. A section of Chapter 3 will also discuss how isotope experiments (^{13}C) can help to reveal CO_2 pathways inside trees and thereby help to increase our understanding of tree carbon balances.

The scope of this experiment is to quantify the contribution of soil DIC uptake to internal CO_2 transport under natural conditions. In Chapter 4, materials and methods are discussed. Potted loblolly pine (*Pinus taeda*) and northern red oak (*Quercus rubra*) seedlings were exposed to CO_2 enriched soil solutions and E_{stem} rates were measured with an infrared gas analyzer (IRGA) to investigate the influence of soil CO_2 on E_{stem}. The study was performed in the growth chambers of the Warnell School of Forestry and Natural Resources (UGA, Athens GA, USA). We hypothesized that under natural occurring soil $[CO_2]$ almost no DIC in the soil solution enters the xylem of the root

system and therefore it is not the main source of the respired CO_2 transported upward in the xylem into the shoot. In order to confirm our findings, the experiment was repeated with a $^{13}CO_2$ enriched solution and the isotopic composition of stem and root tissue samples was measured.

In Chapter 5, the results of this study are presented, which will be discussed in Chapter 6. The results are compared with previous studies and interpreted using information from literature. A last paragraph will suggest some directions for further research. To end, in Chapter 7, a conclusion is formulated and the most important results of this study are summarized.

1. Stem respiration as a key factor of forest carbon budgets

1.1. The main carbon fluxes of forest ecosystems

Forest carbon balances are dominated by two carbon fluxes: photosynthesis (P_n) and total ecosystem respiration (R_{eco}). The gross uptake of CO_2 for P_n is expressed as the gross primary production (GPP) of an ecosystem and can be described as a single process with a well-known theoretical underpinning (Trumbore, 2006). In contrary, R_{eco} is a collective term representing a variety of metabolic pathways, both above- and belowground, by which CO_2 returns from the ecosystem to the atmosphere. The difference between both fluxes is called net ecosystem productivity (NEP) and the fraction of GPP resulting in growth when only R_a is taken into account, is called net primary production (NPP) (Schulze et al., 2000). Figure 1-1 gives an overview of the main CO_2 pathways in forest ecosystems. In literature, R_{eco} is generally divided in autotrophic (R_a) and heterotrophic respiration (R_h), consisting of an above- and a belowground component. R_a is the release of CO_2 due to the energy cost associated with the growth and maintenance of foliage, wood and roots (Luyssaert et al., 2007). The aboveground component of R_{eco} consists of the respiration of leaves, stems and branches and therefore is autotrophic (Trumbore, 2006). The belowground component consist of an autotrophic part (the respiration of the roots, their mycorrhizal fungal symbionts and the associated rhizosphere micro-organisms) and an heterotrophic part (CO_2 released during the decomposition of nonliving organic matter by soil micro-organisms) (Hanson et al., 2000). In this study, we will further focus on R_a, especially on measurements of woody tissue respiration (R_{wt}).

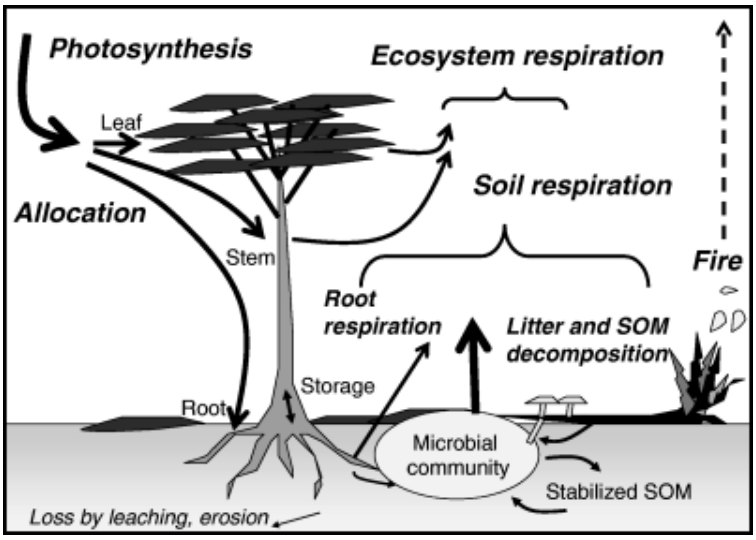

Figure 1-1. Overview of the main carbon pathways through forest ecosystems (Trumbore, 2006). Also non-respiratory losses (e.g. fires) are indicated, but these are not taken into account in the net ecosystem productivity (NEP).

In general, the P_n and R_{eco} flux are assumed to be of the same magnitude (Schimel, 1995), but small imbalances can lead to significant interannual variation in atmospheric CO_2 concentrations ($[CO_2]$) (Trumbore, 2006). Because of this delicate balance the NEP has a strong diurnal, seasonal, annual and spatial variability (Valentini et al., 2000; Bolstad et al., 2004). Several studies showed that R_{eco} is one of the key factors determining whether a forest ecosystem acts as a carbon source or sink (Lindroth et al., 1998; Valentini et al., 2000; Janssens et al., 2001). Therefore, they underlined the requirement of accurate R_{eco} as well as component flux measurements to improve our understanding of ecosystem responses to environmental variation and to further optimize the development of tree, ecosystem and global carbon cycling models (Running & Coughlan, 1988; King, 2006; Houghton, 2007; Reich et al., 2008). At this time, we still lack basic information on the key determinants of respiration rates in plant organs (Atkin et al., 2010).

Generally, R_{eco} is partitioned among its respiration components based on component and R_{eco} measurements. Bolstad et al. (2004) measured component (soil (R_{soil}), leaf (R_{leaf}) and stem (R_{stem})) and R_{eco} fluxes in northern hardwood (*Acer saccharum*, *Tilia americana*, *Fraxinus pennsylvanica*) and aspen (*Populus tremuloides*) forest stands from 1999 through 2002 to create site-specific respiration models. Their measurements indicated that R_{eco} varied substantially on an annual cycle and was in general dominated by R_{soil}. On an annual basis R_{stem} was the second largest respiration source, followed by R_{leaf}. Previous reports indicated that R_a can consume up to 50-70% of the carbon fixed by leaves (Ryan, 1991; Ryan et al., 1997; Waring et al., 1998), where R_{stem} and branch respiration (R_{branch}) represents 33 to 37% of the total carbon loss by R_{eco} (Granier et al., 2000; Janssens et al., 2001) and thus are a very important, even dominant, component of ecosystem carbon budgets (Saveyn, 2007). Therefore, an understanding of R_{stem} rates is important to quantify the carbon cycle of forests (Damesin, 2003; Bolstad et al., 2004; Saveyn, 2007).

1.2. Measurements of woody tissue respiration

Since the realization of the importance of R_{wt} in the 60's and 70's (Stockfors & Linder, 1998) gradually more studies have focused on this subject. Generally, R_{wt} rates were estimated based on measurements of CO_2 efflux (E_{CO2}). Two basic methods were commonly used for these measurements (Saveyn, 2007). First, a destructive method uses detached stem and branch sections that are transported to the laboratory and placed in a cuvette, where E_{CO2} is measured under controlled conditions (Yoda et al., 1965; Zabuga & Zabuga, 1990; Bowman et al., 2005). In a second method, permanent cuvettes are attached to living trees in the field and E_{CO2} rates are measured continuously. However, in both methods E_{CO2} is directly used to estimate R_{wt}. Therefore, it is assumed that the only source of E_{CO2} to the atmosphere is R_{wt} and thus that the only sink of CO_2 produced by

the living cells in the segment enclosed by the cuvette is radial diffusion to the atmosphere. However, ever since previous methods were used, scientists have been questioning whether the measured E_{CO2} accurately reflects the actual R_{wt}.

The first reports of high internal [CO_2] inside tree stems (see Section 2.1) have led to the speculation that these [CO_2] could have an effect on R_{wt} measurements (Hari et al., 1991; Levy et al., 1999). In addition, researchers have found it very difficult to find consistent relations between rates of R_{wt} and environmental conditions, tissue sizes and types, tree ages or species (Teskey & McGuire, 2002). For example, Lavigne et al. (1996) reported a variation of 100% in R_{stem} rates within stands of *Abies balsamea*. Teskey and McGuire (2002) stated that already many explanations have been put forward for the absence of these relationships, but an explanation that is often overlooked is the possible transport of CO_2 dissolved in the xylem sap (Negisi, 1979; Martin et al., 1994). It has been hypothesized that CO_2 inside tree stems may not solely originate from respiration by the living cells, but also from the import of respired CO_2 from the root system and the lower stem by the transpiration stream (Carrodus & Triffett, 1975; Hari et al., 1991; Levy et al., 1999). This way, the transpiration stream can affect E_{CO2} by locally act as a CO_2 sink, carrying away some of the CO_2 produced by the living tissues enclosed in the cuvette (Boysen-Jensen, 1933; Johansson, 1993), or as a CO_2 source, releasing some of the CO_2 transported upwards in the tree (Levy et al., 1999; Teskey & McGuire, 2007). Sprugel (1990) suggested that CO_2 transported in the xylem stream may be a significant unaccounted component of R_{wt} measurements.

Teskey and McGuire (2002) were the first to investigate the link between the xylem [CO_2] and E_{CO2}. They found a direct, linear relationship, suggesting that an increase in the xylem [CO_2] results in an increase of E_{CO2}. They concluded that, because of its high solubility in water, respired CO_2 can dissolve in the xylem sap and be transported with the transpiration stream. Diffusion of this CO_2 to the atmosphere at a different location in the tree can result in an underestimation of R_{wt} at CO_2 sources and an overestimated in other parts of the tree (Teskey et al., 2008; Atkin, 2011). This phenomenon could be largely responsible for the inconsistencies and unexplained variation that is common among studies of R_{wt}. In 2005, they performed a similar experiment by experimentally manipulating the xylem [CO_2] in saplings of *Platanus occidentalis* and *Liquidambar styraciflua* and examining the effect on the stem CO_2 efflux (E_{stem}). They confirmed their previous results and concluded that E_{stem} is a combination of CO_2 produced by locally respiring cells and CO_2 transported to the site of measurement from an unknown location lower in the stem, the root system or the soil atmosphere (Teskey & McGuire, 2005). Teskey and McGuire (2007) calculated that E_{stem} consists of locally respired- (55%) and upward transported CO_2 (45%), hereby again confirming that E_{stem} does not accurately estimate R_{stem}.

Today, more and more scientists belief that measurements of E_{CO2}, alone, are not sufficient to accurately quantify R_{wt}. Internal CO_2 transport is thereby more widely accepted and attention is drawn to develop new and more accurate methods to determine R_{wt}. Recently, a new mass balance approach was proposed (McGuire & Teskey, 2004) to estimate R_{stem} accounting for both internal and external fluxes. McGuire and Teskey (2004) calculated an error in efflux-based estimates of R_{stem} of up to 76% compared to estimates that include both internal and external fluxes. In general, more research started to develop to increase our understanding of the different CO_2 pathways inside tree stems and the factors that influence E_{CO2}. A more complete assessment of the internal CO_2 fluxes in trees will therefore improve our understanding of the carbon balance of trees (Teskey et al., 2008). Of course, the carbon budget of forest ecosystems does not only consist of tree carbon balances. Internal CO_2 transport will not only affect rates of E_{stem}, but also of soil CO_2 efflux (E_{soil}). During daytime, contribution of autotrophic sources to E_{soil} may be underestimated as a result of internal transport of root respired CO_2 through the transpiration stream (Aubrey & Teskey, 2009; Agneessens, 2012; Grossiord et al., 2012; Bloemen et al., 2014). Grossiord et al. (2012) observed a 17% underestimation of the autotrophic contribution to E_{soil} on a daily timescale and concluded that the magnitude of internal transport is a key issue in the quantification of belowground carbon allocation and forest ecosystem carbon budgets. However, E_{soil} is not the focus of this thesis and will further not be discussed.

2. CO_2 inside tree stems

In the most simplistic models, CO_2 enters the tree system in the process of photosynthesis (P_n) by leaf uptake and escapes back to the atmosphere when living cells are respiring. However, it is now known that the path of CO_2 inside tree stems is not that straightforward, as there are different CO_2 sources and sinks inside trees. Figure 2-1 gives an overview of the pathways of respired CO_2 in trees that have been distinguished in literature.

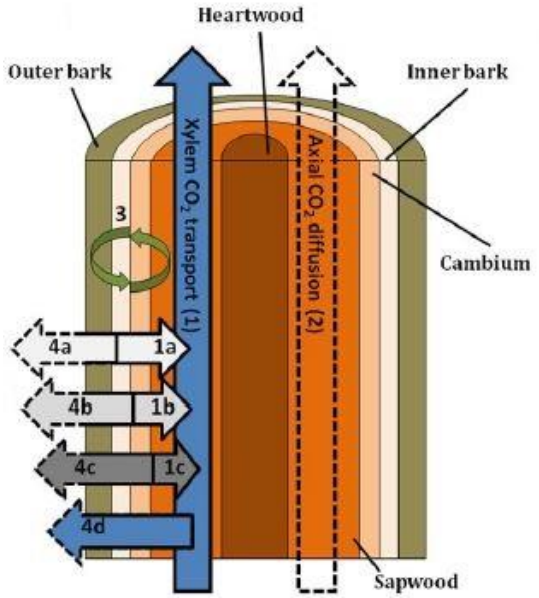

Figure 2-1. Schematic overview of the different sources and sinks of internal CO_2 inside a tree segment. (1) transport of respired CO_2 with the transpiration stream, (2) axial diffusion of respired CO_2, (3) CO_2 assimilation by woody tissue photosynthesis and (4) radial diffusion of respired CO_2 to the atmosphere. Radial diffusion is further divided into (a) fluxes from inner bark tissues, (b) fluxes from cambium tissues, (c) fluxes from xylem tissues and (d) the flux from the transpiration stream. In general, solid lines represent combined dissolved and gaseous CO_2 transport and dashed lines solely gaseous CO_2 transport. (Bloemen, 2013; adapted from Teskey et al., 2008)

Figure 2-1 does not directly include CO_2 uptake from the soil solution, although a part of the upward transported CO_2 could be taken up from the soil. Diffusion to the atmosphere also includes diffusion to the soil environment. In the following paragraphs, the sources and sinks of internal CO_2 are further discussed. Hereby, Figure 2-1 will function as a visual guideline.

2.1. Internal CO_2 concentrations

The first to analyze gas extracted from a tree stem was Bushong (1907). He withdrew gas from a hole drilled in a *Populus diatribes* stem and reported a CO_2 concentration ($[CO_2]$) of 7.2%. After Bushong (1907), several other researchers have sporadically reported stem $[CO_2]$ in the gas phase of xylem sap. The reported values are all within the range of 2-26% (e.g. (MacDougal & Working, 1933; Hari et al., 1991; Levy et al., 1999; Teskey & McGuire, 2002, 2007; McGuire & Teskey, 2004). Teskey et al.

(2007) and Saveyn (2007) give an overview of the reported stem [CO_2] so far. A general observation from all these reports is that the composition of gases inside tree stems greatly differs from that of the ambient air. The most remarkable and in this context the most interesting difference is the high [CO_2] within tree stems. In contrast to the previously indicated internal values, at this moment the [CO_2] of ambient air, measured at Mauna Loa Observatory (Hawaii)[1], is around 400 ppm (or 0.04%). The significant difference between the in- and outside of tree stems indicates that there are substantial barriers to diffusion of CO_2 from the stem tissue to the atmosphere (see Section 2.3.2) (MacDougal, 1926; Teskey & McGuire, 2005). Although some CO_2 released by respiring cells in tree stems diffuses directly to the atmosphere, on a daily basis 15-55% can remain within the tree (Teskey et al., 2008).

2.2. Sources of internal CO_2

2.2.1. Soil solution and rhizosphere

The sources of CO_2 in soils are autotrophic (R_a) and heterotrophic respiration (R_h), according to the type of substrate used by living organisms to sustain their metabolism (Bloemen, 2013). Strictly, the only autotrophic source is root respiration (R_{root}) (Schuur & Trumbore, 2006) and the respiration of their symbiotic mycorrhizal fungi, but generally this flux also includes the respiration of the microbial community associated with the rhizosphere, the region of the soil influenced by the root secretions. Although correctly the rhizomicrobial respiration is part of the heterotrophic component, these two sources are generally combined as one autotrophic flux (Kuzyakov, 2006; Trumbore, 2006). The heterotrophic component describes the release of CO_2 due to the decomposition of soil organic material by soil micro-organisms. In general, the [CO_2] in soils is much higher than that of the ambient air. In a recent research by De Bel (2014), [CO_2] ranged between 0.4 and 4.8%, with an average of 1.1%. These results are in line with previous reports, where [CO_2] ranged between <0.1% en 2% (Yavitt et al., 1995; Hamada & Tanaka, 2001; Jassal et al., 2005) and generally increased with depth (Pumpanen et al., 2003). Rarely, concentrations are higher than 4-5% (Amundson & Davidson, 1990). Teskey & McGuire (2007) also observed an average [CO_2] of 1.2%. These result are rather consistent, whereby in general the natural soil [CO_2] range can be defined between 0.5-1.5%. Nevertheless, it should be kept in mind that these values could possibly increase because elevated atmospheric [CO_2] are reported to increases soil [CO_2] (Andrews & Schlesinger, 2001; Jastrow et al., 2005).

An equilibrium exists between CO_2 in the gas phase of the soil and in the soil water (Saveyn, 2007). In the liquid phase, CO_2 is present in different forms ([CO_2]$_{aq}$, ([H_2CO_3]), [HCO_3^-], and [CO_3^{2-}], in general

[1] http://co2now.org/

[CO_2*]), collectively referred to as dissolved inorganic carbon (DIC). Although the [CO_2] of tree roots is generally much higher than in the surrounding soil (De Bel, 2014), indicating that the net flux of CO_2 from R_{root} is outward into the soil, several studies have shown that (small) amounts of DIC can be absorbed by tree roots (see Section 3.1.2) (Livingston & Beall, 1934; reviewed by Enoch & Olesen, 1993; Ford et al., 2007). The DIC that enters the xylem sap can be transported with the transpiration stream towards the stem, branches and leaves (number 1 in Figure 2-1). Therefore DIC can be considered as a source of internal CO_2 (Figure 2-2).

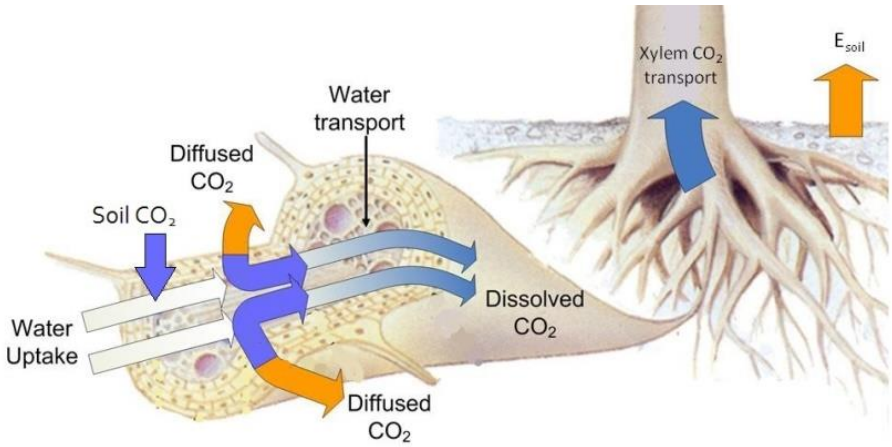

Figure 2-2. Schematic of the belowground CO_2 source of trees. Root respired CO_2 can diffuse into the soil environment, where it can partly dissolve into the soil solution. This CO_2 can be taken up by tree roots and transported with the transpiration stream, along with the CO_2 that did not diffuse to the soil. (adapted from Cruiziat et al., 1990)

2.2.2. Respiration of tree stems and roots

Stem respiration (R_{stem}) and R_{root} are probably the largest sources of internal CO_2 in trees (Teskey et al., 2008; number 1 in Figure 2-1), as a substantial part of locally respired CO_2 can be retained in the xylem (Steppe et al., 2007; Teskey et al., 2008). In tree stems (Figure 2-3A), respiring living cells can generally be found in the outer layer of the inner bark and the vascular cambium, a thin layer of cells between the phloem and the xylem (Teskey et al., 2008). In the xylem, living cells can only be found in the ray cells located in the sapwood (Cutler et al., 2009) and these are the most likely source of the high internal [CO_2] (Teskey et al., 2008) of tree stems. Because cells in the inner bark are considered to have a higher respiratory activity compared to xylem ray cells (Teskey et al., 2008; Araki et al., 2010), the relative distribution can affect woody tissue respiration (R_{wt}) measurements. Values for the ray cell volume, the proportion of living cells in the inner bark and the relative distribution are not only species-, but also time specific, because both xylem and inner bark live cell volumes are reported to change with growth (Ryan, 1990; Stockfors & Linder, 1998; Ceschia et al., 2002). In general, hardwoods, especially conifers, have more ray volume than softwoods (Teskey et al., 2008).

However, scaling the amount of living cells with tissue volume generally illustrates that more living cells are present in the xylem than in the inner bark (Ryan, 1990; Ceschia et al., 2002).

Besides R_{stem}, recent research showed the importance of R_{root} as a source of internal CO_2 (Teskey & McGuire, 2002, 2007; Aubrey & Teskey, 2009; Grossiord et al., 2012; Bloemen et al., 2014). Root respired CO_2 can dissolve into the xylem sap and can be transported from the roots to the stem and the canopy. Aubrey and Teskey (2009) estimated that twice the amount of the CO_2 derived from belowground R_a enters the xylem stream as diffuses into the soil environment. The structure of tree roots (Figure 2-3B) is approximately similar as described for stems and so is the location of the respiring living cells (Bloemen, 2013). Although high root [CO_2] were previously reported (Clements, 1921; Rakonczay et al., 1997), Greenway et al. (2006) mentioned that there is not much information on root [CO_2]. In 2013 a study, in combination with this thesis, was performed to investigate [CO_2] in the roots of *Fagus grandifolia* and *Liriodendron tulipifera* by measuring [CO_2] on three different distances from the stem base (De Bel, 2014). The results (Figure 2-4) showed an increase in internal [CO_2] in root sections closer to the stem base, implying that root respiration contributes to internal CO_2 and xylem CO_2 transport.

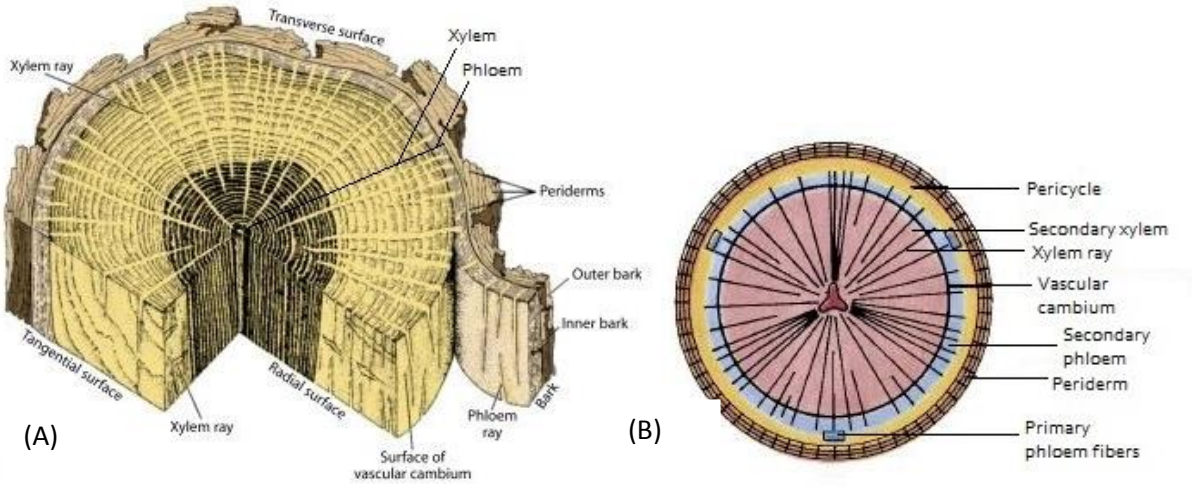

Figure 2-3. (A) Anatomical structure of a Red oak (*Quercus rubra*) stem. The dark area in the center is heartwood and the lighter part of the wood is sapwood. (B) Anatomical structure of a root in a woody eudicot at the end of the first year's growth. (adapted from Raven et al., 2005)

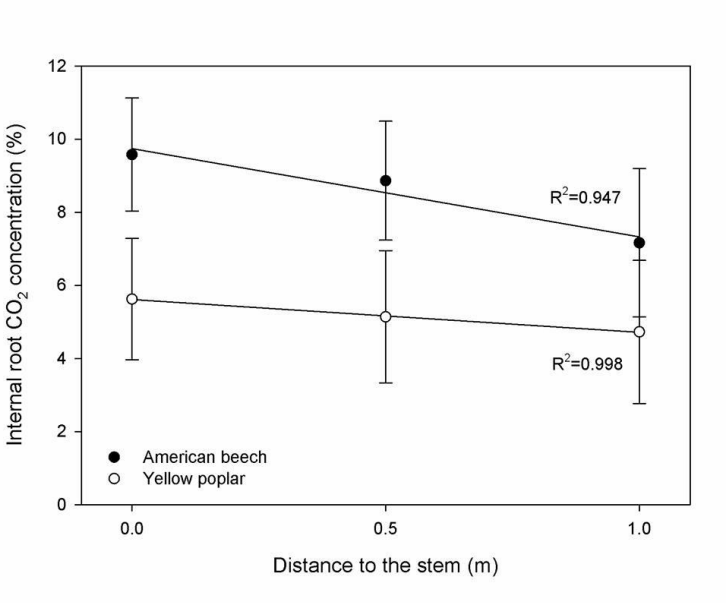

Figure 2-4. Internal CO_2 concentrations in roots of *Fagus grandifolia* and *Liriodendron tulipifera* in function of the distance to the tree stem. The linear regression lines illustrate the trend of the results and imply that root respiration contributes to internal CO_2 and xylem CO_2 transport. (De Bel, 2014)

Up to a certain point R_{root} and R_{stem} are depending on the amount and the distribution of the living cells. However, there also are other factors that can be important. Overall, these factors are temperature (T), moisture, carbohydrate supply, nitrogen content, morphology, age, [CO_2] and oxygen concentration ([O_2]) (Bloemen, 2013).

2.3. Sinks of internal CO_2

2.3.1. Transport with the transpiration stream

Gaseous CO_2 inside trees can be found in the intercellular spaces, the pore spaces within the cell walls and the lumen of cells (Hari et al., 1991). When in contact with the liquid phase, an equilibrium between gaseous CO_2 and the different forms of DIC exists. DIC can be transported throughout the tree by the transpiration stream (Teskey et al., 2008; number 1 in Figure 2-1) and when assessing tree carbon budgets, this internal transport of CO_2 needs to be taken into account. Boysen-Jensen (1933) and Johansson (1993) were the first to speculate about this pathway and recently more evidence was found (Levy et al., 1999; Teskey & McGuire, 2002; McGuire & Teskey, 2004; Aubrey & Teskey, 2009). For instance, Bloemen et al. (2013) used a $^{13}CO_2$ enriched aqueous solution to label the xylem sap at the base of large *Populus deltoides* trees. The ^{13}C label was transported internally and detected throughout the tree, confirming internal transport of CO_2 from the root system. In fact, the respiration stream is also a source of internal CO_2 because the transported CO_2 can efflux to the atmosphere or be assimilated by photosynthetic tissue on a location upward in the tree (Bloemen et al., 2013b).

According to Henry's law, the amount of respired CO_2 that can dissolve in the xylem sap is depending on T, pH and the $[CO_2]$ surrounding the xylem sap (Stumm & Morgan, 2012). The latter is usually high because of the resistance to radial diffusion of the respired CO_2 in tree stems (see Section 2.3.2). More information on Henry's law can be found in Section 4.2.4. In Figure 2-5, the influence of T and pH on xylem dissolved $[CO_2^*]$ is plotted. An increased pH increases the solubility of CO_2 in the xylem sap (Teskey et al., 2008; Cerasoli et al., 2009; Stumm & Morgan, 2012). An increased T will have an opposite effect, although less pronounced. Overall, both factors are similar as the factors that influence CO_2 efflux (E_{CO2}) (see Section 2.3.2).

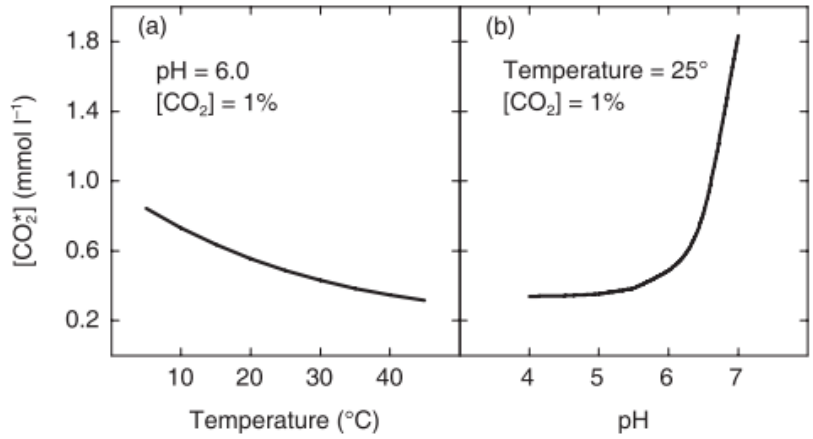

Figure 2-5. (a) Modeled effect of temperature on the total concentration of dissolved inorganic carbon ($[CO_2^*]$) in the xylem sap at a constant gaseous CO_2 concentration (1%) and pH (6.0). (b) Modeled effect of pH on the total concentration of dissolved inorganic carbon in the xylem sap at a constant gaseous CO_2 concentration (1%) and temperature (25°C). (Teskey et al., 2008)

2.3.2. Radial diffusion of respired CO_2 to the atmosphere

Initially, respired CO_2 can either diffuse into the gaseous phase of the inter- and intracellular gas spaces or into the aqueous phase around the living cells. The process of diffusion (number 4 in Figure 2-1) is described by the law of Fick (Pfanz & Aschan, 2001):

$$E_{CO_2} = \frac{\Delta[CO_2]}{R} \qquad (2\text{-}1)$$

where E_{CO2} is the CO_2 efflux to the atmosphere, $\Delta[CO_2]$ is the difference in $[CO_2]$ between the gas spaces of the stem and the atmosphere and R is the sum of a series of diffusion resistances. At 20 °C and 1013 hPa the diffusion coefficient of CO_2 in air (1.6E-05 $m^2\ s^{-1}$) is substantially higher than in water (1.6E-09 $m^2\ s^{-1}$). Therefore, the diffusion of CO_2 through gas spaces will be much more efficient than in the aqueous phase (Nobel, 1999). Aside of these diffusion coefficients, the resistance to

diffusion of tree stems and branches will determine E_{CO2}, depending on the radial location of the respiring cells within the tree.

Barriers to diffusion

Nowadays, the existence of high barriers to E_{CO2} is well recognized (Eklund & Lavigne, 1995; Teskey & McGuire, 2002). Depending on the location of respiration, respired CO_2 will have to overcome a series of resistances before it can diffuse to the atmosphere. Teskey and McGuire (2005) demonstrated the effect of these barriers by wounding a tree (by drilling holes through the stems), whereby the stem CO_2 efflux (E_{stem}) at the place of wounding highly increased. For a gas molecule diffusing from the xylem sap to the atmosphere, the first barrier is the xylem itself. Sorz and Hietz (2006) found that minimum diffusion coefficients for O_2 in water-saturated xylem are lower than these in straight water, indicating that the xylem cell walls act as a major barrier to gas diffusion. The next barrier is the cambium, located between the xylem an the inner bark. Whereas xylem [CO_2] have been reported to be as high as 26%, [CO_2] in bark tissues range between 0.06 and 0.17% (Cernusak & Marshall, 2000; Wittmann et al., 2006). This large difference could be the result of a significant barrier for gas movement (Hook et al., 1972; Kramer, 1979), although the permeability of the cambium is believed to change seasonally (Joseph & Kelsey, 2004). From the inner bark to the atmosphere, the molecule crosses the cortex, the phloem, the periderm and the outer bark (Ziegler, 1957). These layers have a protective function against water loss, pathogens and insects but also limit the exchange of gasses with the atmosphere (Lendzian, 2006; Teskey et al., 2008). It is however believed that diffusion through the inner- and outer bark is facilitated by lenticels, cracks and wounds (Rennenberg et al., 1997).

E_{CO2} rates are reported to vary substantially among and within trees, diurnally and seasonally and scaling factors that have been supposed only partially explained the observed variability (McGuire & Teskey, 2004; Teskey & McGuire, 2007; Steppe et al., 2007). However, variability in diffusion resistances could be a part of the explanation (McGuire & Teskey, 2004; Steppe et al., 2007; Teskey et al., 2008). Sprugel (1990) and Cernusak and Marshall (2000) already mentioned that differences in CO_2 permeability of bark layers could predominantly be responsible for the high variation reported in branch respiration (R_{branch}) and R_{stem}. Steppe et al. (2007) quantified the resistance to radial CO_2 diffusion for tree stems of *Populus deltoids* clones and found a substantial variation among trees, even for individuals of the same clone. Therefore, they concluded that a parameter for resistance to E_{CO2} should be included in R_{wt} models. In addition, the water content of tree stems can have a significant effect on E_{CO2} rates (Saveyn et al., 2007a), possibly leading to diurnal and seasonal variation, where the stem tissue may become more permeable to gas exchange when the volumetric water content decreases (Brown & Hook, 1972). Sorz and Hietz (2006) measured the radial diffusion

of O_2 in trees at different water contents and found that diffusion coefficients increased sharply if the gas volumes were increased from 15 to 40%. In general, resistance to diffusion is mainly depending on the amount of lignins, suberins, lipids and waxes and on the thickness of the layers gas molecules need to cross (Schönherr, 1982; Lendzian, 2006). Moreover, the resistance appears to differ substantially with tree species and age (Teskey et al., 2008).

Factors that influence CO_2 efflux measurements

In addition to barriers to diffusion, there are also several other biotic and abiotic factors that influence E_{CO2}. Teskey & McGuire (2007) concluded that the calculation of R_{stem} based on E_{stem} showed complex interrelationships between internal- and external CO_2 fluxes that in turn were affected by other factors including sap flow (F_s), stem [CO_2] and T. Here, some other factors distinguished in literature are briefly discussed. Moreover, these factors also influence the relative distribution of CO_2 between the different pathways inside trees (McGuire & Teskey, 2004; Bloemen et al., 2013a). It should be noted that a factor never investigated before is the contribution of soil CO_2 uptake.

Recent studies showed a relationship between the internal stem [CO_2] and E_{stem} (McGuire & Teskey, 2002; Teskey & McGuire, 2005). However, this relationship is not the same under all conditions or in all species (Teskey et al., 2008), (partially) explained by variation in diffusion resistances. Xylem sap [CO_2] are affected by F_s rates, which are reported to have a typical diurnal pattern (Maier & Clinton, 2006; McGuire et al., 2007; Saveyn et al., 2008). Teskey & McGuire (2002) reported an inverse coupling between F_s rates and the xylem sap [CO_2]. In general, when F_s is absent during the night, [CO_2] maximized. At daytime, [CO_2] decreased because of the diluting effect of increasing F_s (Bloemen, 2013). Hereby, E_{stem} followed the pattern of the xylem [CO_2], where higher F_s resulted in an increased internal CO_2 transport and a related decrease in E_{stem} (McGuire et al., 2007). F_s on its own can be affected by the amount of incoming photosynthetic active radiation (PAR). A typical light response curve shows an increase in P_n with increasing PAR, until the light saturation point is reached. Damesin (2003) reported a decrease in E_{stem} when PAR increased. Probably higher P_n resulted in an increased F_s, whereby more CO_2 was internally transported and less CO_2 effluxed to the atmosphere. A second reason could be that, because woody tissue photosynthesis (P_{wt}) is rather light limited (Aschan & Pfanz, 2003), an increased PAR resulted in more assimilation of internal CO_2 and a reduced E_{stem}.

Another important factor for respiration rates is T (Bolstad et al., 2004). Usually, E_{CO2} rates are scaled using simple exponential T functions (McGuire et al., 2007; Saveyn, 2007). McGuire et al. (2007) investigated the effect of T on the different CO_2 fluxes inside detached *Platanus occidentalis* branch

segments (Figure 2-6). T does not only directly influence R_{branch}, but also affects the solubility of CO_2 in water (Teskey & McGuire, 2007), what could be an explanation for the variation in the proportion of E_{branch} (E_a in Figure 2-6). At a higher T, CO_2 is less soluble in water whereby a smaller percentage will dissolve in the xylem sap and more CO_2 will efflux to the atmosphere. Nevertheless, because of barriers to diffusion also the xylem [CO_2] increased with increasing T and when sap is flowing, more CO_2 will be transported (McGuire et al., 2007). Along with T, the pH of the xylem sap can also affect the solubility of CO_2 (Figure 2-5, see Section 2.3.1). A final factor is the water status of the living tree tissue. Saveyn et al. (2007) hypothesized that the water status of tree stems could play an important role in the rates of growth and maintenance processes. They observed a well correspondence between fluctuations in E_{stem} and the water potential of the tree. In addition, Saveyn et al. (2007b) further investigated the influence of the water status on E_{stem} rates by inducing drought stress. They found that during drought, E_{stem} rates were more closely correlated with variations in stem diameter then with T.

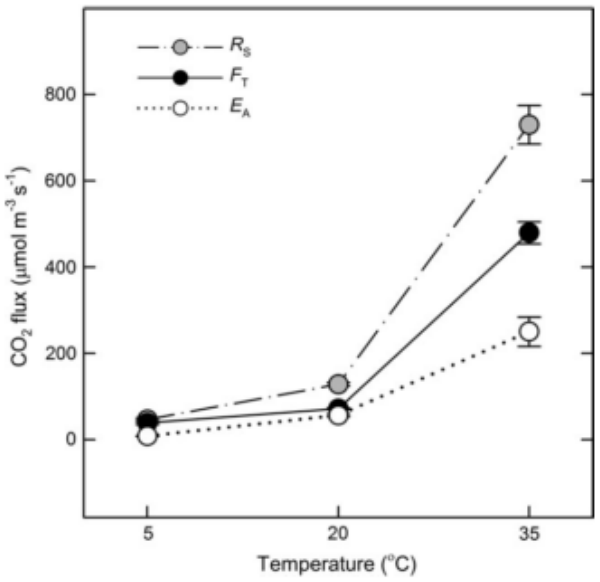

Figure 2-6. Effect of temperature on mean values of the components of the respiratory CO_2 flux: efflux to the atmosphere (E_A), xylem transport flux (F_T) and total flux (R_S) of detached branch segments of sycamore perfused with water at controlled flow rates. Error bars represent the standard error of the means. (McGuire et al., 2007)

2.3.3. Radial diffusion of respired CO_2 to the soil environment

The soil [CO_2] is generally much higher than in the atmosphere (see Section 2.2.1). Therefore, the concentration gradient between roots and the soil is different from the gradient between stems and the atmosphere, limiting the radial diffusion of respired CO_2 to the soil (Qi et al., 1994). In addition, growing roots form a waxy layer, called suberin, to prevent the escape of O_2 to the soil environment (De Simone et al., 2003). It is speculated that the effect could be the same for CO_2 (Qi et al., 1994;

Colmer, 2003; Greenway et al., 2006). Because high [CO_2] in roots have been reported previously (Clements, 1921; Rakonczay et al., 1997) and Teskey and McGuire (2007) demonstrated that a substantial portion of the CO_2 within tree stems originated from the root system, it is supposed that physical barriers to CO_2 diffusion in roots are high (Saveyn, 2007; Aubrey & Teskey, 2009).

2.3.4. Axial diffusion of respired CO_2

A rather indistinct pathway is the axial diffusion of gaseous respired CO_2 (Bloemen, 2013; number 2 in Figure 2-1). The large gas volumes inside trees allow axial and radial gas movements (MacDougal & Working, 1933; Brown & Hook, 1972; Armstrong & Armstrong, 2005). Especially when F_s is absent, a concentration gradient can develop inside the tree body. During lighted periods of zero F_s, vertical internal CO_2 gradients can develop due to the local decreases in internal CO_2 by P_{wt} (Saveyn et al., 2008). This way, and according to the law of Fick (Sorz & Hietz, 2006), CO_2 molecules can be transported along the CO_2 gradient, away from the site of respiration. Although sufficient evidence for the existence of this process was recently obtained (Armstrong & Armstrong, 2005; Saveyn et al., 2008; Etzold et al., 2013), Bloemen (2013) concluded that we lack detailed information to determine the influence and the significance of axial CO_2 diffusion for E_{stem} based measurements of R_{stem}.

2.3.5. Fixation in photosynthetic tissues

P_n takes place in specific organelles, called chloroplasts, and is mostly associated with the canopy. Although, there are at least five different chloroplast containing woody tissues described in literature (Saveyn, 2007): the rhytidome, the ray parenchyma, the tissues adjacent to the cork cambium, the phloem and the wood around the pith (Pfanz and Aschan, 2001; Figure 2-7). It is estimated that the chlorophyll content of young twigs can be as much as 50-70% of the chlorophyll content of the adjacent leaves (Solhaug et al., 1995; Pfanz et al., 2002). Kharouk et al. (1995) found that the bark of young *Populus tremuloides* and *Populus tremula* trees contained up to 42% of the chlorophyll content of the tree. Therefore, when sufficient light is available, P_n can also take place in tree stems and branches (reviewed by Aschan and Pfanz, 2003; Pfanz and Aschan, 2001). However, both light transmission and fixation capacity usually decreases as stems age increases and a thicker layer of outer bark is developed (Teskey et al., 2008). P_{wt} recycles respired CO_2 that otherwise would have been lost to the atmosphere (Pfanz et al., 2002; Teskey & McGuire, 2002; Saveyn et al., 2010), a process called refixation (Cernusak & Hutley, 2011; number 3 in Figure 2-1). Because of high stem [CO_2], P_{wt} is rather light limited. Although some studies indicate that the amount of CO_2 assimilated by P_{wt} may occasionally exceed E_{CO2} (Damesin, 2003; Berveiller et al., 2007), in general rates of P_{wt} are relatively low compared to atmospheric assimilation rates (Pfanz et al., 2002). However, also in leaves internal CO_2 could become an important carbon source, especially when CO_2 uptake from the atmosphere is reduced during periods of limited water supply.

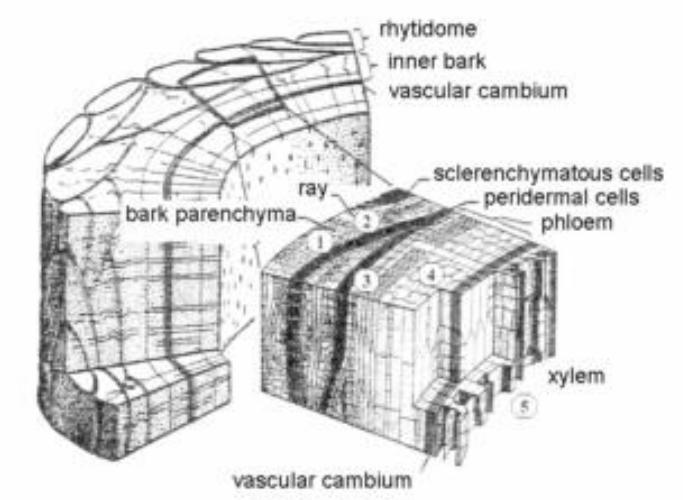

Figure 2-7. Localization of chloroplast containing cells in a tree stem: (1) the rhytidome, (2) the ray parenchyma, (3) the tissues adjacent to the cork cambium, (4) the phloem and (5) the wood and the region around the pith. (adapted from Pfanz & Aschan, 2001; Saveyn, 2007)

Internal CO_2 transport also affects refixation because not only local respired CO_2, but also CO_2 transported in the transpiration stream can be assimilated by photosynthetic cells in woody tissues and leaves (McGuire et al., 2009; Bloemen et al., 2013). McGuire et al. (2009) supplied detached *Platanus occidentalis* branches with a $^{13}CO_2$ labeled aqueous solution and Bloemen et al. (2013) infused a $^{13}CO_2$ labeled aqueous solution into the base of *Populus deltoides* trees to investigate the effect of CO_2 transported with the transpiration stream on aboveground carbon assimilation. Both studies observed the assimilation of the labeled solution, predominantly by woody tissue and to a lesser extent in the foliage, where most of the label was found in the petioles. In addition, Levy et al. (1999) found that 0.5-7% of the CO_2 fixed by leaf photosynthesis originated from xylem transport.

3. Contribution of soil CO_2 to internal CO_2 in trees

3.1. Soil CO_2 uptake by trees

3.1.1. Water uptake by tree roots

Plants lose tremendous amounts of water by leaf transpiration. To meet the water requirements, the root system absorbs water from the soil environment. In general, absorption takes place directly through the epidermis, especially in young roots. The uptake is facilitated by the root hairs, tubular extensions of epidermal cells located several millimeters above the root tip, who greatly increase the root surface area available for absorption. Once absorbed, water will move through the cortex to the vascular cylinder, from where it is transported upward in the tree. Three possible pathways are distinguished in literature (Figure 3-1), depending on the degree of differentiation of the root tissue (Raven et al., 2005): apoplastic (via the cavities between protoplast and cell walls), symplastic (from protoplast to protoplast via plasmodesmata) and transcellular (from cell to cell, across the plasma membranes and tonoplasts). However, at the endodermis water is forced to cross the plasma membranes and protoplasts because of water-impermeable Casparian strips.

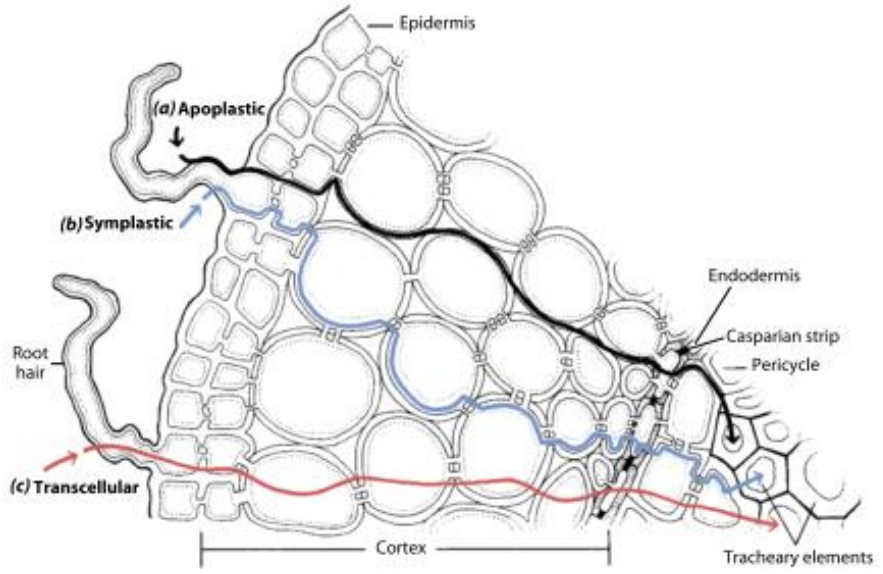

Figure 3-1. Possible pathways for the movement of water from the soil, across the epidermis and cortex and into the vascular cylinder of the root. (a) Apoplastic movement (black line) occurs via cell walls; (b) symplastic movement (blue line) is from protoplast to protoplast via plasmodesmata; and (c) transcellular movement (red line) occurs from cell to cell, with the water passing through the plasma membranes and tonoplasts. This root lacks an exodermis (a subepidermal layer of cells with Casparian strips). (Raven et al., 2005)

After secondary growth, roots can form a periderm, a protective exodermis mainly constructed of suberin (Taiz & Zeiger, 2010) that makes more mature regions of roots relatively impermeable to

water. Therefore, water uptake by the root system mainly occurs in less suberized root tips (Kramer & Boyer, 1995; Zwieniecki et al., 2002). The suberine layer also reduces the efflux of O_2 (De Simone et al., 2003) and may serve a similar function for the diffusion of CO_2 (Teskey et al., 2008).

3.1.2. Soil CO_2 uptake by tree roots

It is know that the contribution of belowground CO_2 to the internal CO_2 in trees is substantial and that much of this CO_2 is coming from root respiration (R_{root}) (Teskey & McGuire, 2007). However, the contribution of dissolved inorganic carbon (DIC) taken up from the soil solution by tree roots is still not entirely clear. Several studies have already shown that DIC can be absorbed by tree roots (Livingston & Beall, 1934; Skok et al., 1962; Amiro & Ewing, 1992; Viktor & Cramer, 2005), although mostly this contribution is reported to be only a small fraction of the belowground contribution (Ford et al., 2007; Teskey & McGuire, 2007; Jones et al., 2009). In the following paragraphs results of several studies are briefly discussed and finally, in Section 3.3, the objectives for this thesis are formulated.

The first indirect indications on soil DIC uptake came from early studies about the effect of enriched soil CO_2 solutions on plant growth (reviewed by Enoch & Olesen, 1993). The earliest irrigation experiments with CO_2 enriched water were performed by Birner & Lucanus (1866). Their results showed that the extra supply of CO_2 slightly increased the production of organic material, but they were not able to conclude whether the additional CO_2 was absorbed by the roots and transported to the leaves or diffused from the solution into the air and then was absorbed by the leaves. The focus of later studies shifted more and more to the uptake of DIC. For instance, *Salix aquatica* is reported to absorb ^{14}C labeled DIC and transport it throughout the plant (Vapaavuori & Pelkonen, 1985; Vuorinen et al., 1989). A detailed overview of all these studies is beyond the scope of this thesis. However, Enoch & Olesen (1993) reviewed more than 100 studies and concluded that although CO_2 uptake via roots into the transpiration stream could theoretically contribute up to 5% of the carbon gain of trees, it usually contributes less than 1%.

More recently DIC uptake, and even transport and photosynthetic fixation was investigated for herbaceous plants (Hibberd & Quick, 2002) and trees (Ford et al., 2007; Ubierna et al., 2009). Hibberd & Quick (2002) supplied ^{14}C labeled DIC ($NaHCO_3$) to the roots of hydroponically grown tobacco and detected ^{14}C in insoluble material (e.g. starch) and cells associated with the vascular system. Ford et al. (2007) exposed potted *Pinus taeda* seedlings to ^{13}C labeled soil DIC to test their ability to take up and fix soil DIC. After analysis they observed that seedlings exposed to labeled DIC were significantly more $^{13}CO_2$ enriched compared to reference seedlings, whereby the fixed carbon was almost evenly distributed between above- and belowground tissue (55 and 45%, respectively).

Similarly, Ubierna et al. (2009) also applied a ^{13}C labeled solution to the soil around potted seedlings and around large field-grown trees. All three studies confirmed that DIC can be taken up and fixed by trees, although labeled carbon contributed only to a very limited extend (<1%) to whole seedling carbon gain (Ford et al., 2007).

To our knowledge, little studies have investigated the influence of soil CO_2 on the xylem CO_2 concentration ($[CO_2]$) or the stem CO_2 efflux (E_{stem}). McGuire & Teskey (2004) mentioned that the effect of CO_2 transported from the soil could be a potential source of error in estimating carbon fluxes inside trees. Especially in the morning, when transpiration begins, water with high $[CO_2]$ can be transported upward and affect E_{stem} (Levy et al., 1999). Moore et al. (2008) identified the source of CO_2 diffusing from *Pinus taeda* stems by using free air carbon dioxide enrichment (FACE) with a gas depleted in $^{13}CO_2$. In addition, they measured soil $[CO_2]$ and tree growth. They found a positive correlation between mean E_{stem} values and mean soil $[CO_2]$, for both ambient and elevated conditions, suggesting that a portion of E_{stem} was derived from the soil. Moreover, they estimated that the soil $[CO_2]$ explained between 12 and 50% of the variation in E_{stem} and that 19% of the average E_{stem} came from soil CO_2 uptake. They concluded that although it is a small flux, the uptake of DIC from the soil solution and the failure to account for this flux could lead to considerable overestimations of R_{wt} based on E_{stem} measurements. This is especially true in light of future elevated atmospheric $[CO_2]$.

3.2. The use of ^{13}C as tracer in ecophysiological experiments

In literature, a wide variety of methods to study internal CO_2 in trees is described. Especially since the discovery of xylem CO_2 transport and the internal CO_2 pathways, the use of carbon isotope methods have received more attention (Bloemen, 2013). Isotopes, which are atoms of an element with the same atomic number, but with a different atomic mass (Dawson & Brooks, 2001) are suitable 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). Dawson et al. (2002) distinguishes two categories of ^{13}C studies, namely those working at naturally occurring levels (natural abundance) and those working outside the natural range (enriched levels). Enriched substances can be used as a powerful tracer to follow the flow of a specific element (Michener & Lajtha, 2008).

During the past two decades, the use of stable isotope techniques in plant ecological research has increased (Dawson et al., 2002; Trumbore, 2006). Stringer & Kimmerer (1993) were the first who introduced isotopes directly into xylem to trace the internal transport of respired CO_2. Similarly, McGuire et al. (2009) introduced $^{13}CO_2$ in the xylem of excised *Platanus occidentalis* branches. More

recently, Bloemen et al. (2013b) infused a $^{13}CO_2$ labeled aqueous solution into the base of *Populus deltoids* trees to investigate the effect of xylem-transported CO_2 from the root system on aboveground carbon assimilation and E_{stem}. In this thesis an aqueous $^{13}CO_2$ enriched solution was applied to the soil and used to detect the uptake of DIC by tree roots.

3.3. Objectives

The uptake of soil DIC by tree roots is repeatedly confirmed, but the contribution to whole tree carbon gain is reported to be small. The scope of this thesis was to investigate the uptake of soil DIC by tree roots, especially the influence of the uptake on E_{stem} and the link with the soil [CO_2]. To this end, four aqueous solutions were prepared with different [CO_2]. Potted 1 year old loblolly pine (*Pinus taeda*) and northern red oak (*Quercus rubra*) seedlings were irrigated with these solutions to vary the soil [CO_2] between 0.3-8.5% and E_{stem} was measured under controlled conditions in a growth chamber. E_{stem} rates were compared with baseline measurements to detect any change in efflux. The [CO_2] concentration range was chosen to start with natural conditions (0.5-1.5%, see Section 2.2.1), ranging to unnaturally high values. This way, we wanted to investigate the effect of increasing soil [CO_2]. By altering the gradient between the [CO_2] in roots and soil we expected that more CO_2 would be taken up with an increasing gradient, although possibly the resistance in the root would be too high and no change of E_{stem} could be detected. In general, we hypothesized that almost no DIC in the soil solution enters the xylem of the root system under natural conditions and therefore it is not the main source of the CO_2 transported upward in the xylem into the stem and canopy. If the uptake of soil DIC and especially the effect on E_{stem} is limited, studies on belowground internal CO_2 sources should focus on R_{root}.

Finally, a similar experiment was performed, though where tree roots were exposed to a $^{13}CO_2$ enriched aqueous solution with a high [CO_2]. This way, we wanted to confirm the uptake of CO_2 under these conditions. Labeling of the trees was followed by a destructive tissue sampling at stem and root level, followed by an isotopic analysis of gas extracted from the headspace above these samples. Results on isotope analysis were compared with non-labeled trees to quantify ^{13}C enrichment relative to baseline values.

4. Materials and methods

4.1. Plant material and growth conditions

For this study, loblolly pine (*Pinus taeda*) and northern red oak (*Quercus rubra*) seedlings were grown in 4.26 L pots (model CP612R, Steuwe and Sons Inc, Tangent OR, USA) and were grown within a greenhouse at Whitehall forest, an experimental forest of the University of Georgia (Athens, GA, USA). Trees were watered on a daily basis and five 0.9 cm diameter holes were drilled in the base of the pots to increase drainage. At the start of this study, trees were 1 year old (Figure 4-1) and diameters ranged between 5.0-6.1 mm and 3.2-4.3 mm for pines and oaks, respectively.

The experimental study was performed in the growth chambers of the Warnell School of Forestry and Natural Resources (University of Georgia, Athens, GA, USA) during August and September 2013. In the growth chamber (GC36, Environmental Growth Chambers, Chagrin Falls, OH, USA), conditions were controlled in such way that trees transpired well and thus had a big water uptake. PAR was provided with fluorescent lamps during the day, whereby lamps were kept at a fixed distance from the trees in the beginning, but were raised after the first treatment (1.5%) to prevent leaf scorching of the oaks. During nights, lamps were switched off. Air temperature was maintained at 25 °C during the day and 20 °C during night. The humidity was 50% in the beginning, but again, to prevent leaf scorching of the oaks, this was adapted to 75% after the first $^{12}CO_2$ treatment. Trees were watered every 1-2 days to keep the pots draining and the water status of the trees approximately optimal. Also a fan was placed to blow off CO_2 that possibly escaped from packed treated trees. Therefore, one door was slightly opened during the day. It was made sure that the fan did not directly blow on the trees.

Figure 4-1. The 1 year old pine and oak seedlings used in this study.

4.2. $^{12}CO_2$ experiment

4.2.1. Experimental setup

At the start of the experiment 20 pines and 20 oaks were moved from a greenhouse to the growth chambers, where they could adapt to the new conditions during the first days. Trees were divided into two groups, each consisting of an equal amount of pines and oaks (five trees per species per group). During the experiment, these groups had the same treatments, but on two consecutive days to even out mechanical errors. The other trees were used as test trees and to determine soil CO_2 concentrations ($[CO_2]$). In the text, baseline stem CO_2 efflux measurements (E_{stem_b}) refer to measurements after irrigation with tap water. Treatment stem CO_2 efflux measurements (E_{stem_t}) were performed after irrigation with a specific $^{12}CO_2$ enriched solution. These solutions were prepared with gas of four different $[CO_2]$ at 1.5, 5, 10, and 20%. In the text we refer to these different treatments as the 1.5, 5, 10, and 20% treatment, respectively.

During four weeks (August 12 – September 5), every week another enriched aqueous solution was prepared and applied to the trees (in the order 1.5, 5, 20 and 10%). All trees were numbered and every week a random order was selected for both groups. A normal workweek started with E_{stem_b} measurements on Monday and Tuesday afternoon, when trees were irrigated with tap water. Wednesday and Thursday trees were irrigated with a specific $^{12}CO_2$ enriched solution in the morning and E_{stem_t} measurements were performed in the afternoon. Finally, Friday was used as a back-up day and to measure tree dimensions. Irrigation was done by removing trees from the growth chamber and pouring approximately 500 mL of $^{12}CO_2$ enriched solution in the pots. After this, tree pots were sealed, trees were moved back to the growth chamber to take up the soil solution and three hours later E_{stem} rates were measured. Twelve minutes were left between irrigation and sealing of two consecutive trees and between two E_{stem} measurements to ensure that the period between irrigation and E_{stem} measurement was similar for all trees. Finally, all pots were irrigated with tap water to flush out the remaining CO_2 from the applied enriched soil solution. During every treatment water uptake rates were measured and every week stem diameters were minimally measured once. At the end of the experiment, leaf areas of all trees were measured.

Sealing of the irrigated pots (Figure 4-2) was done to prevent CO_2 escaping via the soil surface and to prevent the draining of CO_2 enriched aqueous solution. This way, it was tried to maintain as much CO_2 into the soil as possible, stabilize the conditions/concentrations and to allow trees to take up as much CO_2 as possible. A thick plastic foam was used to cover the surface of the soil. Next, pots were put into a thick transparent plastic bag that covered the holes at the bottom of the pots and prevented draining. This bag was taped securely to the outside of the pot. Finally, a thin plastic bag

covered the whole pot and was sealed to the stem with a wire at the level of a white foam gasket wrapped around the stem. For some trees, stem diameters were very small, whereby the white foam did not tightly fit to the stem. Then, the top of the foam was sealed with some extra mounting putty (Loctite®, Düsseldorf, Germany).

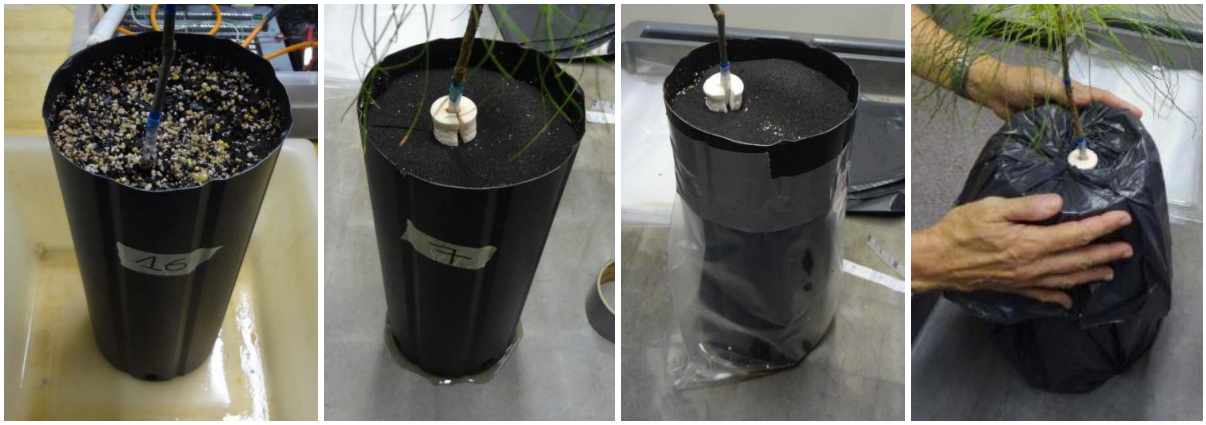

Figure 4-2. Sealing of the potted trees after irrigation with an enriched CO_2 solution. A thick plastic foam was used to cover the surface of the soil. A thick transparent plastic bag, taped securely to the outside of the pot, covered the holes at the bottom of the pots and prevented draining. Finally, a thin plastic bag covered the whole pot and was sealed to the stem with a wire at the level of a white foam gasket wrapped around the stem.

Before the start of the experiments a dye test was performed to make a rough estimation of the time needed by the trees to take up soil solution and transport it through the stem to the canopy (Figure 4-3). To this end, tree roots were exposed to a mixture of blue dye and water. On average, it took three hours before colored spots appeared on the leaves and needles. Therefore, it was decided to wait approximately three hours after irrigation of the trees before E_{stem} was measured.

Figure 4-3. Setup and result of the dye test used to make a rough estimation of the time needed by the trees to take up enough soil solution.

Water uptake rates (mL h^{-1}) of the trees were measured during each treatment as an estimation of sap flow (F_s) rates, first of all to determine if trees took up enough water to fill the sampled stem

section and second as a potential scaling factor. To calculate water uptake rates, trees were weighed with a balance (model VA16000, Acculab, Bohemia NY, USA) after irrigation and sealing. Sealing should minimize evaporation from the pots whereby the decrease in weight could be used as an estimation of the water loss by transpiration. Trees were weighted again before measuring E_{stem} and again before removing the sealing system. Water uptake was then compared with stem volumes (up to 1 cm above the upper part of the cuvette), assuming that 50% of the stem volumes was occupied by water (McGuire & Teskey, 2004; McGuire et al., 2007). All trees took up enough water to fill the stem segments.

4.2.2. Tree dimension measurements

Trees were marked with adhesive tape at the top and the bottom margin of the segment covered by the cuvette to assure that all measurements were performed at the same stem segment. Stem diameters of the top and bottom mark were measured manually nine times with a digital caliper (Model Absolute Digimatic CD-6CSX, Mituyo Corp, Kawasaki, Japan). Because stems were not perfectly round shaped an average of two diameters was taken per measurement. Diameter measurements were not always performed on the same day as CO_2 efflux (E_{CO2}) measurements. To overcome this issue, a linear regression of diameters in time was made for each tree separately, making it possible to estimate diameters on specific days. Day zero was chosen as the first measurement day, being Monday the 12^{the} of August.

Volumes (V) and surface areas (A) of the stem segments inside the cuvette were calculated using (4-1 and (4-2, assuming that stem segments approximated the shape of a truncated cone:

$$V = \pi * h * \left(\frac{R^2 + r^2 + R * r}{3}\right) \quad (4\text{-}1)$$

$$A = \pi * l * (R + r) \quad (4\text{-}2)$$

where h, R, r and l are the height of the cuvette (mm), radius of the bottom part, radius of the upper part (mm) and the length of the cones hypothenuse (mm), respectively. The height of the inner part of the cuvette is fixed for all trees and is 50 mm. Linear regressions were used to estimate dimensions on specific days.

At the end of the experiment, leaf areas were measured. For oaks, all leaves were removed from the canopy and leaf areas were automatically measured with a Li-3100 Area Meter (LI-COR inc., USA). For

pines, two needles were selected per tree, a big and a small one. For every needle, diameter, weight and length was measured. Needle diameters were a mean of three measurements, made with a hand lens micrometer (10X scale loupe; Model PEAK 02227-AB with standard reticle, SPI Supplies, West Chester PA, USA), and weights were measured after drying the needles in a gravity flow convection oven (Model Isotemp 637G; Fisher Scientific, Waltham, MA, USA) at 65 °C. Next, leaf areas (LA, mm^2) were calculated for each needle using following equation (corrected from Ford et al., 2007):

$$LA = L * \left(\frac{2 * \pi * r}{N} + 2 * r\right) \quad (4\text{-}3)$$

where L, r and N are the needle length (mm), the mean needle radius (mm) and N the number of needles per fascicle, respectively. By dividing LA by the needle weight, the specific leave area (SLA, $mm^2\ g^{-1}$) could be determined as given by:

$$SLA = \frac{LA}{w} \quad (4\text{-}4)$$

with w the weight of the needle (g). After removing, drying and weighting all the needles from a tree, total LA per tree could be estimated by taking a mean of the SLA for the big and the small needle.

4.2.3. Preparation of the soil solutions

The four $^{12}CO_2$ enriched aqueous solutions were prepared as described in McGuire et al. (2009). A 20 L polycarbonate container was filled with tap water and for the 10 and 20% treatment water was amended with sodium bicarbonate ($NaHCO_3$; 1.2 and 1.5 g L^{-1}, respectively) to adjust the pH of the solution and the amount of CO_2 that could dissolve. Gas from a cylinder of compressed CO_2 gas (1.5, 5, 10 and 20%, respectively) was bubbled through the solution for at least three hours before the start of the experiment. Solution pH and [CO_2] were measured after three hours, whereby [CO_2] were used to confirm that enough gas was bubbled and pH values were used to calculate dissolved CO_2 concentrations ([CO_2*]) in the soil solution (see Section 4.2.4). Mean pH values were 6.1 ± 0.1, 5.8 ± 0.1, 6.8 ± 0.1 and 6.8 ± 0.1 for the four solutions respectively and mean [CO_2] were 1.4 ± 0.1%, 3.3 ± 0.9%, 7.6 ± 0.6% and 14.5 ± 0.3%. All errors in this thesis represent the standard error (SE) of the means, except when the text indicates otherwise. [CO_2] was measured with a non-dispersive infra-red carbon dioxide sensor (model GMT220, Vaisala Inc, Woburn MA, USA) enclosed in a sealed PTFE tube (model 200-07-S-4, International Polymer Engineering, Tempe AZ, USA) after isolating some of the solution into a 500 mL vial.

4.2.4. Soil CO_2 concentration measurements

It was not possible to measure soil [CO_2] in pots of treated trees because this would probably damage the root system and influence E_{stem}. Therefore, four other trees were selected (two oaks and two pines) that were treated with the same solution as the treated trees, but no E_{stem} measurements were performed. These trees were sealed similar to the other trees, but without the thin plastic bag. A PTFE tube (model 200-07-S-4, International Polymer Engineering, Tempe AZ, USA) was inserted into the soil of the four pots and [CO_2] was measured with a non-dispersive infra-red carbon dioxide sensor (model GMT220, Vaisala Inc, Woburn MA, USA). Because every treatment was divided between two days, soil [CO_2] was measured multiple times and for each treatment an average was calculated. [CO_2] was converted to [CO_2*] (mM) using Henry's law (Stumm & Morgan, 1996; Teskey et al., 2008):

$$[CO_2^*] = \left(1 + \frac{K_1}{10^{-pH}} + \frac{K_1 * K_2}{(10^{-pH})^2}\right) * K_H * pCO_2 \qquad (4\text{-}5)$$

where [CO_2*] is the concentration of the total dissolved inorganic carbon in the solution, K_1 and K_2 the first and the second acidity constants, respectively, K_h the henry's constant, pCO_2 the partial pressure of CO_2 over the solution and pH the pH of the solution. Details of these calculations can be found in McGuire & Teskey (2002). Temperature dependent constants were calculated based on the temperature of the growth chamber, where the trees were stored after treatment. It should be noted that the pH of the enriched aqueous solutions was used. Therefore, [CO_2*] values are only an estimation because soil pH values will probably have been slightly different. In Table 4-1, soil [CO_2] and soil [CO_2*] values for the four different treatments are given.

Table 4-1. Gaseous ([CO_2]) and aqueous soil CO_2 concentrations ([CO_2*]) of the four different aqueous solutions.

	Soil [CO_2] (%)		**Soil [CO_2*] (mM)**	
Treatment	Pine	Oak	Pine	Oak
1.5%	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
5%	1.3 ± 0.2	1.3 ± 0.2	0.6 ± 0.1	0.6 ± 0.3
10%	5.1 ± 0.3	6.4 ± 0.4	7.0 ± 0.5	8.7 ± 0.4
20%	8.6 ± 0.8	8.5 ± 0.5	10.0 ± 0.9	10.1 ± 0.9

In between irrigation with the treatment solutions, baseline soil [CO_2] was determined. These measurements were performed similar as the treatment soil [CO_2] measurements, except that pots

were irrigated with tap water. For both species, baseline soil [CO_2] values slightly increased in time in the range of 0.1-0.5% for pines and 0.1-0.4% for oaks.

4.2.5. CO_2 efflux measurements and optimal setup

Preliminary tests

Because E_{stem} was low for the young trees (especially for the oaks) and changes were expected to be small, some preparatory tests were done to determine the optimal setup for the E_{stem} measurements. It is know that stem refixation of internal CO_2, especially in young trees, compensates for a portion of respiratory carbon loss (Teskey et al., 2008). Woody tissue photosynthesis (P_{wt}) can fix up to 100% of the respiratory CO_2 emissions (Cernusak & Marshall, 2000) and may occasionally exceed CO_2 release (Damesin, 2003; Berveiller et al., 2007). Therefore, for two test trees (one pine and one oak) the cuvette was wrapped in aluminum foil to eliminate P_{wt}. The change in E_{stem} was significant (E_{stem} ~ treatment, $p < 0.0001$) for both pine and oak. Secondly, to avoid efflux of CO_2 before the sampled stem segment, the cuvette was installed as close as possible to the soil and the stem segment beneath the cuvette was wrapped with parafilm. Compared with the foil setup, E_{stem} changed significantly (E_{stem} ~ treatment, $p < 0.001$) for both pine and oak. In a last test, temperature of the growth chamber was raised to 30 °C for an oak test tree because temperature is known to affect respiration rates (Teskey & McGuire, 2002, 2007; Saveyn et al., 2008) and the solubility of CO_2 in the transpiration stream. E_{stem} changed not significantly for the elevated growth chamber temperature (E_{stem} ~ treatment, $p = 0.07$).

CO_2 efflux measurements

Starting three hours after irrigation with a CO_2 enriched solution, E_{stem} rates were measured with a cuvette system connected to a LI-6400 infrared gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA). The final configurations for the IRGA were a block temperature of 25 °C, a flow rate of 300 µmol s^{-1} and a reference [CO_2] of 400 µmol L^{-1}. The cuvette was covered with aluminum foil to exclude light from the stem and the portion of the stem below the cuvette was wrapped with parafilm (Figure 4-4). Measurements were performed on the same time of the day (afternoon between 13-17h) because E_{stem} rates are known to change diurnally (see Section 2.2.2), although under controlled conditions this effect should have been minimized. Five measurements were done on each stem, at an interval of ten seconds, and averaged. The cuvette (Conifer Chamber, Li-Cor Biosciences, Lincoln, NE, USA) was installed around the stem segments and mounting putty (Loctite®, Düsseldorf, Germany) was used to seal the edges of the cuvette (Figure 4-5A). The cuvette was checked for leaks by blowing exhaled air over the edges with a small tube. When no rise of the [CO_2] inside the cuvette was detected, it was assumed that the cuvette was leak tight. To reduce the

chance of leaks the flow channel of the cuvette's tube for outgoing air was narrowed a bit (Figure 4-5B), as described in the application note 6 (LI-6400; Li-Cor Biosciences, Lincoln, NE, USA). This way, pressure inside the cuvette slightly increased which avoids the potential inflow of outside air into the chamber via small leaks.

Figure 4-4. Setup for the stem efflux measurements for both oak and pine. A part of the stem was covered with the cuvette. The cuvette was covered in aluminum foil to eliminate woody tissue photosynthesis. The stem segment beneath the cuvette was wrapped with parafilm.

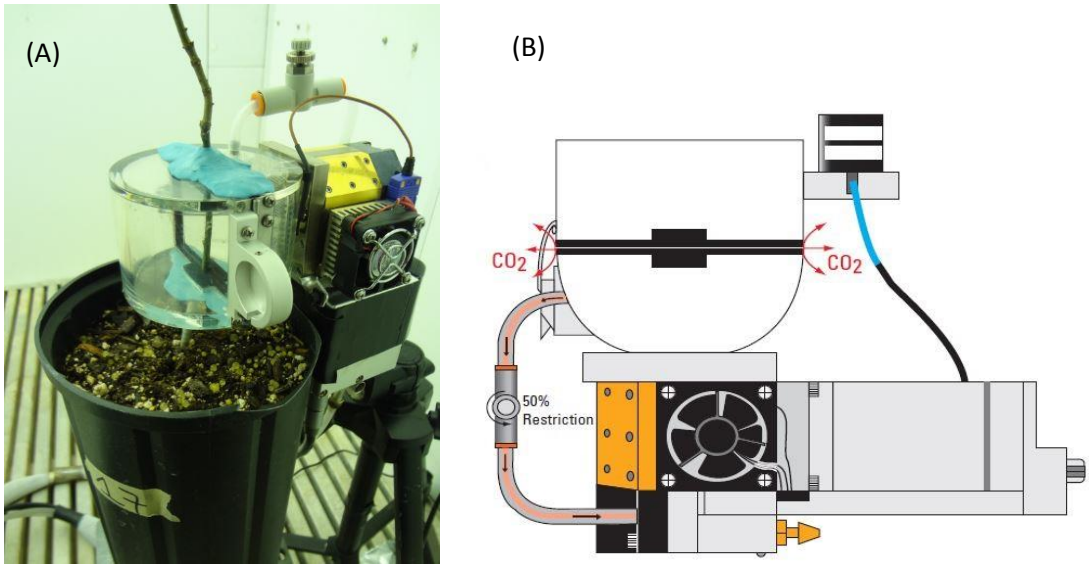

Figure 4-5. Exhaust path of the Opaque Conifer Chamber with the optional Adjustable Exhaust Tube Assembly. The needle valve regulates flow through the exhaust tube. (A) A picture from the setup of this experiment. Mounting putty was used to seal the edges of the cuvette. (B) An illustration from application note 6 (LI-6400; Li-Cor Biosciences, Lincoln, NE, USA).

E_{stem} (µmol m^{-2} s^{-1}) values were calculated as described in Coombs et al. (1985):

$$E_{stem} = \frac{f}{A} * \Delta[CO_2] \quad (4\text{-}6)$$

where f is the mole flow of air (mol s^{-1}) through the cuvette surrounding the stem segment, A the surface area of the stem segment (mm^2) and ΔCO_2 the $[CO_2]$ difference between the reference air stream and the air leaving the cuvette (µmol mol^{-1}). It should be noted that A can be substituted by V (mm^3), the volume of the stem segment, as described in McGuire & Teskey (2004), but for small stems usually A is used (Saveyn, 2007). Because E_{stem_b} rates were not measured at the same days as E_{stem_t} rates and baseline ΔCO_2 values increased approximately linear in time, linear regressions of baseline ΔCO_2 values were made per tree. Predicted ΔCO_2 values were then used to estimate E_{stem_b} rates on specific days. Both E_{stem_b} and E_{stem_t} values increased in time due to tree growth. To account for the extra E_{stem} due to growth, corrected CO_2 efflux rates (E_{stem_c}) were calculated based on E_{stem_b} measurements with following equation:

$$E_{stem\_c} = E_{stem\_t} - E_{stem\_b} \quad (4\text{-}7)$$

The contribution of E_{stem_c} to E_{stem_t} (%) expresses the potential contribution of soil CO_2 uptake to E_{stem} and was calculated using:

$$contribution\ of\ E_{stem\_c}\ to\ E_{stem\_t} = \left(\frac{E_{stem\_c}}{E_{stem\_t}}\right) * 100\% \quad (4\text{-}8)$$

4.3. $^{13}CO_2$ experiment

4.3.1. Experimental setup

To confirm the uptake of soil CO_2 by tree roots, a similar experiment was performed with a $^{13}CO_2$ enriched aqueous solution, where $^{13}CO_2$ served as a proxy for soil CO_2. The same setup was used as outlined for the $^{12}CO_2$ experiment. Trees were randomly divided in three groups, namely baselines, treatments and controls. Every group consisted half of trees from the growth chamber, already used in the previous experiments, and half of new trees from the greenhouse. This way, it can be checked if previous treatments had an influence on the results. All groups consisted of an equal amount of pines and oaks. The baseline group (six trees per species) was used to determine the natural abundance carbon isotopic composition ($\delta^{13}C_{\_b}$) which was compared to the carbon isotopic

composition of the treatment trees ($\delta^{13}C_{\_t}$), the group (six trees per species) irrigated with the $^{13}CO_2$ enriched solution. The control group (three trees per species) was irrigated on the same day as the treatment group, but these were irrigated with tap water. They were used to check for other pathways of $^{13}CO_2$ (Ford et al., 2007).

Baseline tissues were sampled on 10 September 2013. Trees were removed from the growth chamber, a part of 8 cm on each stem was marked and diameters of the top and bottom mark were taken with a caliper. Next, stems were cut on the marks and the stem segments were put in a vial (Model BD Vacutainer 366430, Becton-Dickinson, Franklin Lakes NJ, USA). Gas samples from the air in the headspace of the vials was used to determine $\delta^{13}C$. The samples were cut in half to ensure enough diffusion from the segments. Before sealing, sample vials were flushed with nitrogen gas (N_2) to flush out all ambient air, so gas buildup in the vials came solely from the samples. After sealing, the vials were immediately frozen in liquid nitrogen to stop all metabolic activity and incubated at 3 °C to allow the [$^{13}CO_2$] of the xylem water to equilibrate with the gas in the sample tubes. Also root samples were taken. Therefore, trees were taken out of the pots and sand was removed. Mid root system segments of similar thickness were randomly selected in function of the root physiology. Root samples were washed thoroughly to wash of $^{13}CO_2$ that was not taken up by the roots, but stuck on the root surface, then cut in half, stored in a sealed vial, flushed with N_2 and frozen in liquid nitrogen.

Irrigation with $^{13}CO_2$ enriched water was performed on 12 September 2013. Control trees were prepared before the irrigation of the treatment group to avoid an elevated [$^{13}CO_2$] in the room. Next, treatment trees were randomly selected and stems were girdled just beneath the canopy by removing a 1 cm ring of bark from the circumference of the stem (Figure 4-6) to avoid downward phloem transport of $^{13}CO_2$ possibly taken up from the air by leaves. The exposed xylem was then covered with parafilm. After girdling, trees were irrigated with 500 mL of $^{13}CO_2$ enriched solution, sealed as described for the $^{12}CO_2$ experiment and put back in the growth chamber, where treated and control trees were randomly distributed as described in Ford et al. (2007). Trees were left in the growth chamber for approximately three hours. After incubation, the same process as for the baseline tissue sampling was repeated on the control and treatment trees. Root samples were taken, but these samples were intensively washed before analysis to wash of $^{13}CO_2$ that was possibly stuck on the root surface. For each tree, water uptake rates were measured and compared with stem volumes to estimate if enough water was taken up. When all samples were taken, also leaf areas were determined. All samples were incubated for a few days to allow the [$^{13}CO_2$] of the water in the tissue and the gas in the vial to equilibrate before an isotopic analysis was performed.

Figure 4-6. Girdling of the tree stems to avoid downward phloem transport of leaf assimilated $^{13}CO_2$. Preparation of the $^{13}CO_2$ enriched solutions was similar to the $^{12}CO_2$ solutions. A 20 L polycarbonate container was filled with tap water and $^{13}CO_2$ gas from a cylinder of compressed 100% CO_2 at 99 atom% $^{13}CO_2$ (ICON Services, Summit, NJ, USA) was used to displace approximately 3 L of the water in the container. The gas was circulated through the solution in a closed loop with a pump. After three hours, solution pH and [CO_2] were measured (6.3 and 8.0%, respectively).

4.3.2. Isotopic analysis

Samples were analyzed by isotope-ratio mass spectrometry (IRMS) at the Stable Isotope and Soil Biology Laboratory (SISBL), Odum School of Ecology, University of Georgia, Athens GA, USA. During sampling the vials were not opened. A needle was used to pierce the rubber septum and the gas was withdrawn into a syringe. The gas was then injected into the mass spectrometer for analysis. If there was enough gas in the vial, each vial was sampled twice and an average was taken. When there was not enough gas in the vial only one measurement was performed. Freezing the samples in liquid nitrogen killed the tissue in the vials. Since the tissue did not respire anymore, any excess $^{13}CO_2$ came from what was dissolved in the trees sap due to root uptake. According to Dawson et al. (2002), for natural abundance studies, the stable isotope composition of a particular material or substance ($\delta^{13}C$, ‰) is expressed as a ratio relative to an internationally accepted standard as given by:

$$\delta^{13}C = 1000 * \left(\frac{R_{sample}}{R_{standard}} - 1\right) \quad (4\text{-}9)$$

where R is the abundance ratio of the isotopes ($^{13}C/^{12}C$). In this study, the PeeDee Belemnite standard was used (0.0112372). Because for this experiment values for enriched materials exceeded 500‰, it is more convenient to refer to the isotopic composition of these materials by using ‘atom %’

(A_{13C}, %; (4-10), the percentage contribution of the heavy isotope to the total number of atoms of an element in a sample (Dawson et al., 2002):

$$A_{13C} = 100 * \left(\frac{R_{sample}}{R_{sample} + 1} \right) \quad (4\text{-}10)$$

where R is the abundance ratio of the isotopes ($^{13}C/^{12}C$). Enrichment of the labeled tissues (A_{13C_c}, %) was calculated as the difference between the A_{13C} value of the labeled tissues (A_{13C_t}) and the A_{13C} value of the baseline tissues (A_{13C_b}) ((4-11) as described by Bloemen et al. (2013). Hereby, A_{13C_b} values were a mean of those measured for pines and oaks, for stem and roots, respectively.

$$A_{13C\_c} = A_{13C\_t} - A_{13C\_b} \quad (4\text{-}11)$$

where R is the abundance ratio of the isotopes ($^{13}C/^{12}C$).

4.4. Data and statistical analysis

Data were processed using Excel (Version 2010, Microsoft, Redmond, WA, USA). It should be noted that during the experiment two oaks died and corresponding data were removed from the dataset. E_{stem_c} was calculated by subtracting E_{stem_b} from E_{stem_t}. When E_{stem_c} values were negative, these were not used in further calculations. Linear regressions and visualization were performed using Sigmaplot (Version 11.0, Systat Software Inc., San Jose, CA, USA).

Statistical analysis was performed using the statistical software R (Version 3.0.2, R Foundation) with $\alpha = 0.05$. Comparison of the primary test results of the E_{stem} measurements was performed with a one-way analysis of variance (ANOVA) using the linear model (lm) of R. Tree dimensions in function of time were analyzed using a mixed-effects model for a repeated-measures ANOVA with species (n = 2; pine and oak) treated as a fixed factor and individual tree (n = 18) treated as the random subject factor. A similar ANOVA model was used to analyze the effect of tree dimensions on E_{stem_b}, the effect of time on ΔCO_2 and the effect of soil [CO_2] and soil solution [CO_2*] on E_{stem_c}. Mean F_s values were compared between treatments using a mixed-effects model for a repeated-measures ANOVA with species (n = 2) and treatment (n = 4; 1.5, 5, 10 and 20%) treated as fixed factors and individual tree (n = 18) treated as the random subject factor. A similar ANOVA model was used to analyze the effect of stem segment volumes on E_{stem} and to compare E_{stem} measurements between treatments and setups, although now setup (n = 2; baseline or treatment) was treated as an extra fixed factor. Finally, A_{13C} values were compared using a mixed-effects model for a repeated-measures ANOVA with

species (n = 2), tissue type (n = 2; root and stem), place of storage during the experimental period (n = 2; greenhouse and growth chamber) and treatment (n = 3; baseline, control and treatment) treated as fixed factors and individual tree (n=12) treated as the random subject factor. All mixed effect analyses were performed using a the linear mixed effect model (nlme) of R. For overall comparisons, the random subject factor tree was nested in species.

5. Results

5.1. $^{12}CO_2$ enrichment experiment

5.1.1. Tree dimensions

Diameters of the top and the bottom part of the stem segments enclosed by the cuvette were measured nine times during the time span of the experiment. In Figure 5-1, the means of both diameters, for all trees, are plotted in time. Mean diameters increased in time for both pines (diameter ~ time, $p < 0.0001$) and oaks (diameter ~ time, $p < 0.0001$). R^2 values of a linear regression indicate that diameters grew approximately linear during the time of the experiment. In general, pines grew much faster than oaks. Linear regressions for every tree separately resulted in mean R^2 values of 0.99 and 0.74 for pines and oaks, respectively.

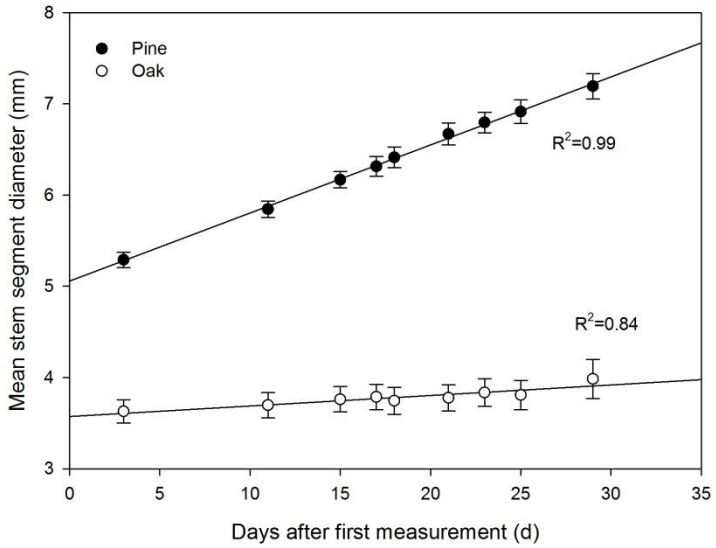

Figure 5-1. Mean diameters of the stem segments in time. R^2 values indicate that diameters grew approximately linear during the time of the experiment. Error bars represent the standard error of the means.

Diameters were used to calculate the volumes and the surface areas of the stem segments enclosed by the cuvette (data not shown), assuming that these segments can be approximated by truncated cones. Both mean stem segment volumes and mean stem segment surface areas also increased linear in time, with R^2 values of 0.99 and 0.81 for volumes and 0.99 and 0.84 for surface areas, for pines and oaks, respectively. Pine volumes and surface areas increased faster (volume ~ time, $p < 0.0001$ and area ~ time, $p < 0.0001$) than for oaks (volume ~ time, $p < 0.0001$ and area ~ time, $p < 0.0001$). Linear regressions for all trees separately resulted in mean R^2 values of 0.99 and 0.58 for volumes and 0.99 and 0.74 for surface areas, for pines and oaks, respectively.

5.1.2. Water uptake

In general, over the four weeks of the experiment, pines had higher sap flow (F_s) rates than oaks (4.3 ± 0.3 mL h^{-1} and 1.2 ± 0.1 mL h^{-1}, respectively; sap flow ~ species, $p < 0.0001$ for all treatments). For both species, mean F_s values are plotted in function of the four treatments in Figure 5-2. F_s rates varied substantially between trees of the same species, resulting in big error bars for the mean values. Statistically, mean F_s values of different treatments differed significantly (sap flow ~ treatment, $p < 0.0001$ and $p = 0.03$ for pines and oaks, respectively). Especially for oaks, F_s values for the 1.5% treatment are remarkably out of line in relation to the other treatments.

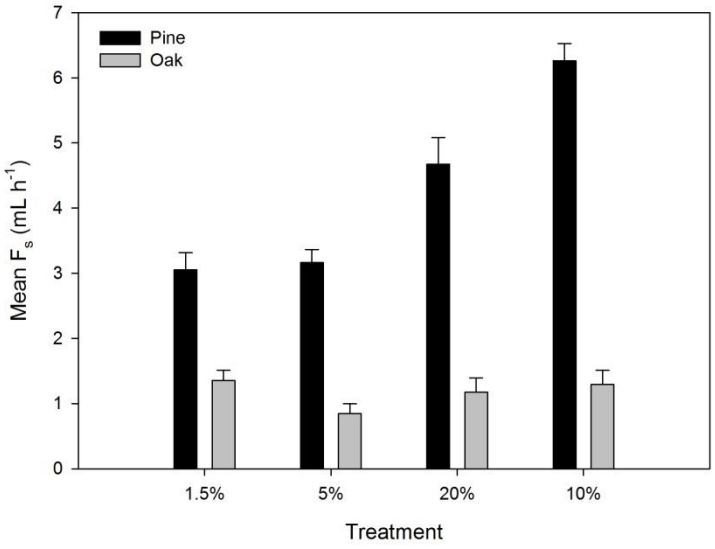

Figure 5-2. Mean sap flow rates (F_s) for pines and oaks, measured on the days of treatment measurements. Error bars represent the standard error of the means. The 10% treatment is plotted after 20% because in time this was performed during the fourth week.

5.1.3. Baseline stem CO_2 efflux

The IRGA detected the CO_2 concentration difference ($\Delta[CO_2]$, ppm) between the air stream that passed the cuvette and a reference air stream. In Figure 5-3A, mean baseline $\Delta[CO_2]$ values are plotted in time. For both pine and oak, $\Delta[CO_2]$ increased over time ($\Delta[CO_2]$ ~ time, $p < 0.0001$ and $p < 0.0001$ for pine and oak, respectively). Linear regressions of all trees separately resulted in mean R^2 values of 0.90 and 0.45 for pines and oaks respectively and were used to estimate baseline stem CO_2 efflux (E_{stem_b}, Figure 5-3B) rates on the days of treatment stem CO_2 efflux (E_{stem_t}) measurements using (4-6.

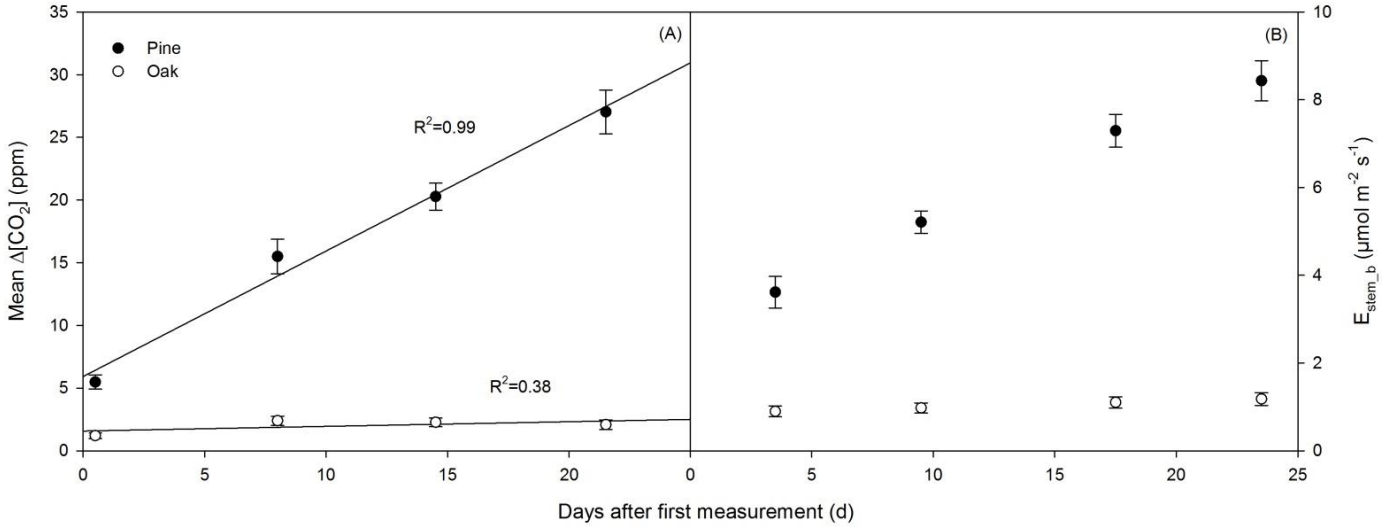

Figure 5-3. (A) Mean baseline CO_2 concentration difference ($\Delta[CO_2]$) as measured with the IRGA for pines and oaks. For every datapoint, the mean consist of $\Delta[CO_2]$ values for all trees over two consecutive days. (B) Mean predicted surface area weighted baseline stem CO_2 efflux (E_{stem_b}) rates. Error bars represent the standard error of the means.

The increase in $\Delta[CO_2]$, and therefore in predicted $\Delta[CO_2]$ based E_{stem_b} values, can be attributed to tree growth. For this experiment, Figure 5-4 shows the relation between surface area weighted E_{stem_b} values and tree stem dimensions, over the experimental period. Diameters are a mean of the upper and lower diameter of the stem segment enclosed by the cuvette. Statistical analysis confirmed the influence of mean stem diameters and stem segment volumes on E_{stem_b} for both species (E_{stem_b} ~ diameter, $p < 0.0001$ and E_{stem_b} ~ volume, $p < 0.0001$ for pine and oak, respectively).

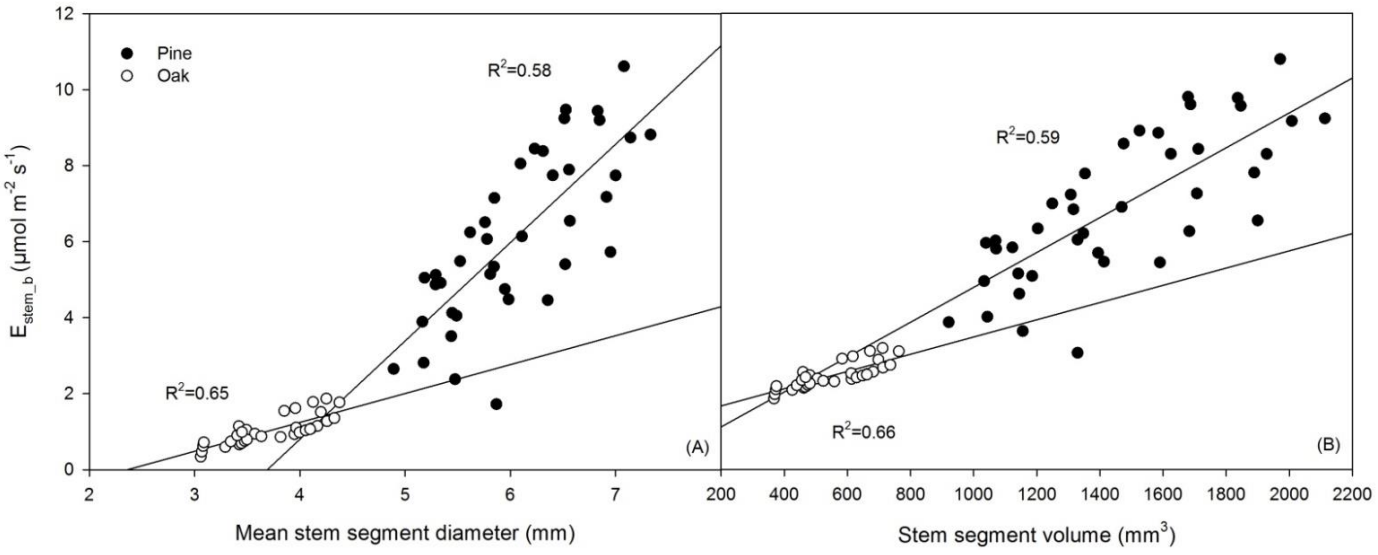

Figure 5-4. (A) The influence of stem segment diameters and (B) stem segment volumes on the baseline surface area weighted stem CO_2 efflux rates (E_{stem_b}) for pines and oaks.

5.1.4. Treatment stem CO_2 efflux

After irrigation with the four $^{12}CO_2$ enriched solutions and the related E_{stem_t} measurements, both mean E_{stem_b} and E_{stem_t} can be plotted in function of the four treatments (Figure 5-5). For all treatments mean E_{stem_t} values were higher than mean E_{stem_b} values (E_{stem} ~ setup, $p < 0.0001$, for both pine and oak) and the difference between both increased for increasing treatment, where E_{stem_t} was highest for the highest treatment (20%). For both species, change in E_{stem_b} and E_{stem_t} between treatments was statistically significant (E_{stem_b} ~ treatment, $p < 0.0001$ for both species and E_{stem_t} ~ treatment, $p < 0.0001$ for both species). Moreover, variation between species was bigger than the variation in between trees of the same species.

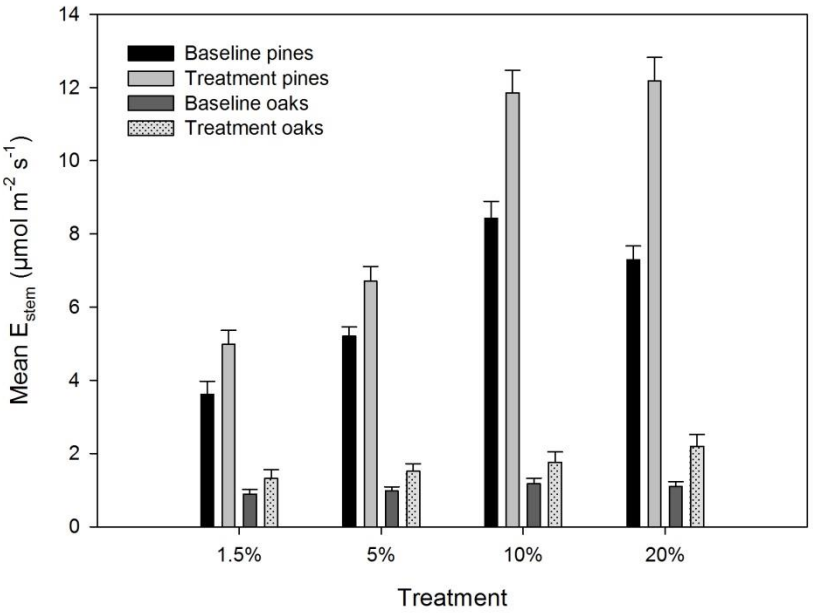

Figure 5-5. Mean surface area weighted baseline and treatment CO_2 efflux rates (E_{stem}) for the four treatments, for pines and oaks. Error bars represent the standard error of the means. In time, the 10% treatment was performed a week after the 20% treatment.

Error bars in Figure 5-5 slightly increase in time as a result of increasing variations in E_{stem} rates. These variations are related to the variation in the volume of the stem segments inside the cuvette. Figure 5-6 shows E_{stem} rates for both species in function of the stem segment volume. A distinction is made between different treatments because also treatment influences E_{stem_t}. Every section of the figure has the same scale to visualize tree growth. In general, segment volumes increased for every treatment (in the order: 1.5, 5, 20 and 10%) because of growth. In addition, the difference between pine and oak volumes also increased, indicating the higher growth rate of pines during the experiment. As the spread on the volumes became bigger, also the spread on E_{stem} increased, resulting in bigger error bars for mean E_{stem} rates. For almost all trees E_{stem_t} is higher than E_{stem_b} and

the difference between both values increased if the treatment CO_2 concentration ($[CO_2]$) increased. Statistically, there only was an influence of the stem segment volumes on E_{stem_t} for oaks (E_{stem_t} ~ volume, $p > 0.3$ for pines and $p < 0.05$ for oaks for the four treatments separately).

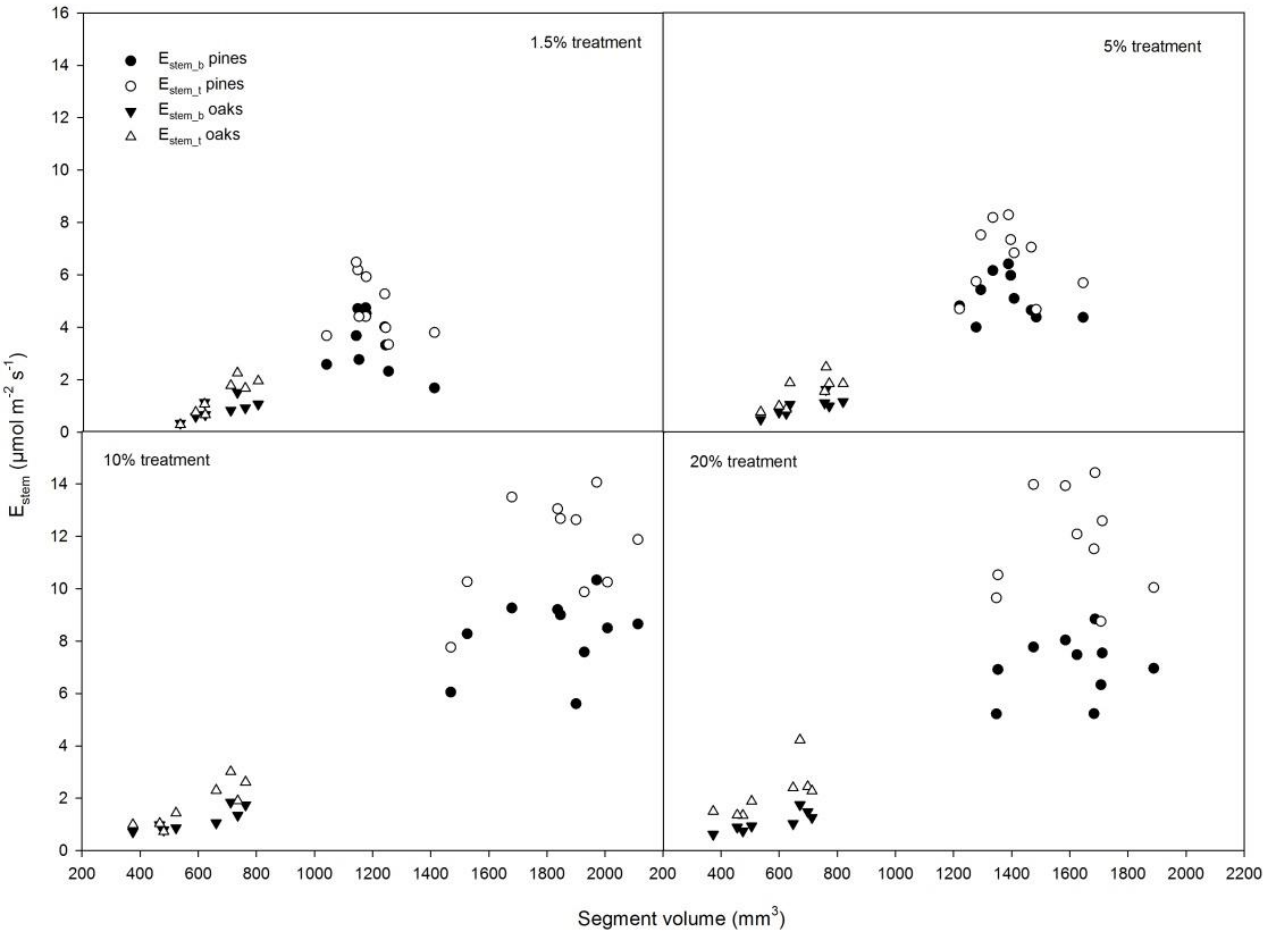

Figure 5-6. Surface area weighted baseline and treatment stem efflux rates (E_{stem}) in function of the volume of the stem segment inside the cuvette. All plots have the same scaling to visualize tree growth. Pines have bigger volumes and bigger volume variation than oaks, resulting in a bigger variation in E_{stem}.

The four different treatments resulted in a different soil $[CO_2]$ and soil solution CO_2 concentration ($[CO_2*]$). To investigate the influence of these concentrations on E_{stem} in Figure 5-7A the mean corrected stem CO_2 efflux (E_{stem_c}) is plotted in function of the mean soil $[CO_2]$. This graph holds the key message of this experiment as it shows the increase of E_{stem_t} that cannot be attributed to tree growth because E_{stem_t} rates were corrected for fluctuations in E_{stem_b}. In addition, it shows the potential influence of the soil $[CO_2]$, whereby E_{stem_c} increased for an increasing soil $[CO_2]$. Figure 5-7B holds the same information, but now soil $[CO_2]$ values are converted to soil solution $[CO_2*]$ values using (4-5. Statistically, the influence of the soil $[CO_2]$ and the soil solution $[CO_2*]$ on E_{stem_c} was significant for both species (E_{stem_c} ~ soil $[CO_2]$, $p < 0.0001$ for both species and E_{stem_c} ~ soil solution

[CO_2*], $p < 0.0001$ for both species). For all treatments, E_{stem_c} values significantly differed between species (E_{stem_c} ~ species, $p < 0.001$ for all treatments). Moreover, variation between species was bigger than the variation in between trees of the same species.

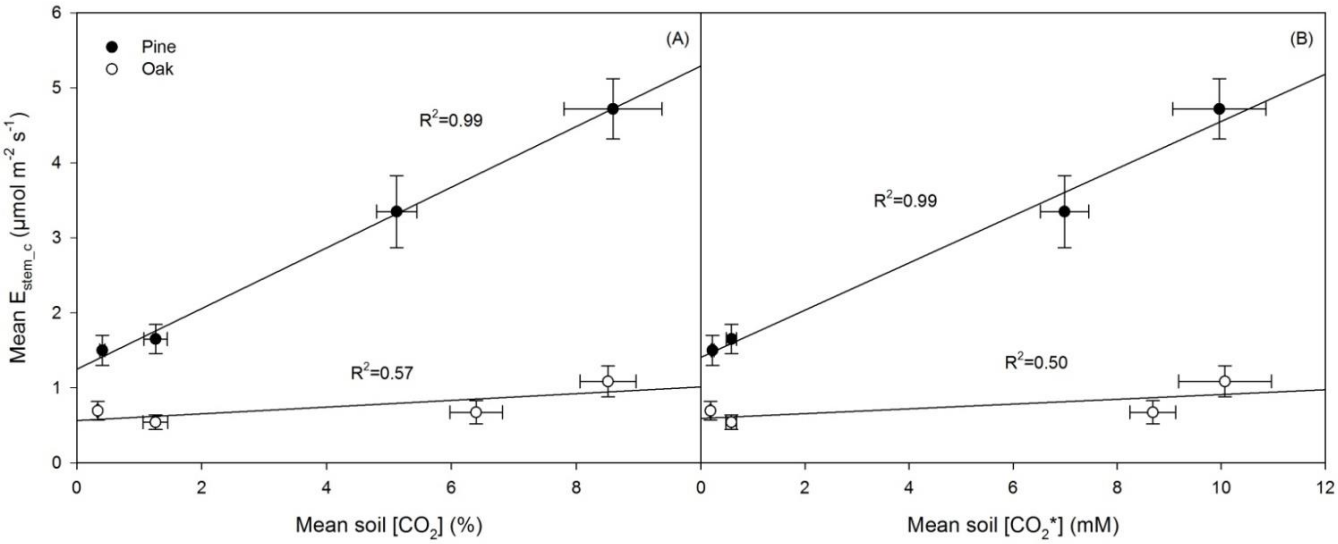

Figure 5-7. (A) Mean corrected surface area weighted stem CO_2 efflux rates (E_{stem_c}) in function of the soil CO_2 concentration ([CO_2]) and (B) in function of the soil solution CO_2 concentration ([CO_2*]). Both vertical and horizontal error bars represent the standard error of the means.

E_{stem_c} rates thus represent an extra efflux with respected to baseline rates. Although, it is not sure whether the increase of E_{stem} after irrigation with a $^{12}CO_2$ enriched solution is due to the uptake of soil CO_2 and thus E_{stem_c} consists of CO_2 originated belowground, the percentage of the contribution of E_{stem_c} to E_{stem_t} can be calculated as described in (4-8 (Table 5-1).

Table 5-1. Contribution of the soil CO_2 expressed as the percentage of the corrected stem CO_2 efflux compared to the total stem CO_2 efflux after irrigation. Values are a mean of the contributions for all trees of that species.

Species	Treatment	Contribution (%)	Standard error (%)
Pine	1.5%	31.7	3.8
	5%	23.7	2.5
	10%	28.1	3.2
	20%	39.7	2.3
Oak	1.5%	39.3	5.0
	5%	33.9	3.2
	10%	32.4	5.2
	20%	48.3	3.1

These values show that for all treatments enriched soil [CO_2] resulted in an increased E_{stem}. Mean

values are 31.0% for pines and 38.6% for oaks. However, the increase in E_{stem} could have other explanations than soil CO_2 uptake by tree roots.

5.2. $^{13}CO_2$ enrichment experiment

The mean natural abundance of $^{13}CO_2$ (A_{13C_b}) in trees of the baseline group are shown in Table 5-2. Measurements were performed for both stem and root tissues. A significant difference exists between A_{13C_b} of root and stem tissues in pines (A_{13C_b} ~ tissue, $p < 0.0001$), but not in oaks (A_{13C_b} ~ tissue, $p < 0.4$). Between the two species, the difference between mean A_{13C_b} values was significant for both root (A_{13C_b} ~ species, $p = 0.0001$) and stem (A_{13C_b} ~ species, $p < 0.0001$) tissues, where A_{13C_b} is generally higher in oaks.

Table 5-2. Mean natural abundance of $^{13}CO_2$ (A_{13C_b}) in pine and oak for root and stem tissues, respectively.

Species	Tissue type	A_{13C_b} (%)	Standard error (%)
Pine	Stem	1.075	0.00040
	Root	1.077	0.00044
Oak	Stem	1.083	0.00046
	Root	1.082	0.00042

After irrigation with the $^{13}CO_2$ enriched solution, mean treatment tissue atom% values (A_{13C_t}), compared to A_{13C_b} values, were significantly higher for both pine (A_{13C} ~ treatment, $p < 0.0001$) and oak (A_{13C} ~ treatment, $p < 0.0001$). $^{13}CO_2$ enrichment (A_{13C_c}) of stem and root tissue was determined using (4-11 (Figure 5-8). The difference between A_{13C_c} in stem and root tissues was significant for both pines (A_{13C_c} ~ tissue, $p < 0.0001$) and oaks (A_{13C_c} ~ tissue, $p < 0.0001$), where A_{13C_c} was generally higher in roots. However, although A_{13C_c} is slightly higher in oaks compared to pines, for both tissue types, in between species the difference between mean enrichment of both tissue types did not differ significantly (A_{13_c} ~ species, $p = 0.1$ for roots and $p < 0.1$ for stems). Finally, it was checked if there was a difference between A_{13C_c} values of trees that were already used in the $^{12}CO_2$ experiment and trees that were imported from the greenhouse after the $^{12}CO_2$ experiment. This difference was significantly for oak (A_{13C_c} ~ location, $p = 0.004$), but not for pine (A_{13C_c} ~ location, $p = 0.7$). However, for both species enrichment was higher for plants from another location and therefore this difference is probably irrelevant.

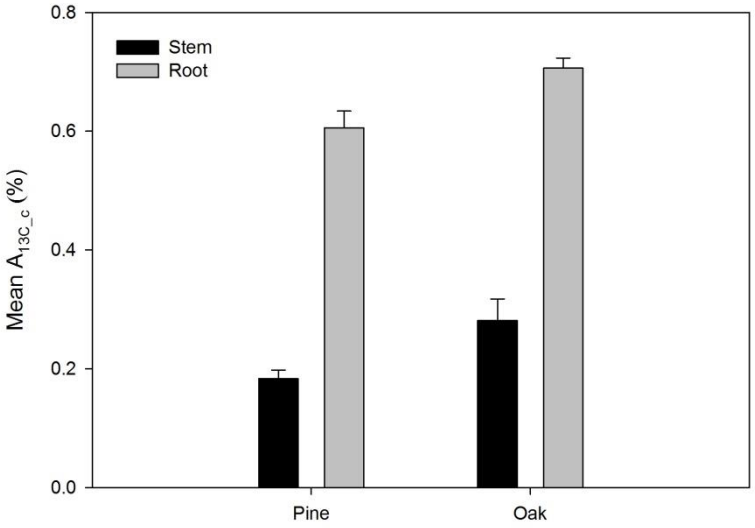

Figure 5-8. Mean enrichment of the stem and root tissues (A_{13C_c}) for pines and oak, respectively. The enrichment is calculated as the difference between mean A_{13C_t} and mean A_{13C_b}. Error bars represent the standard error of the means.

To make sure that there were no other pathways for $^{13}CO_2$ than root uptake, mean A_{13C} of the control trees was compared with A_{13C_b}. Statistically, the difference between mean A_{13C} for baseline and control trees was significant for pines (A_{13C} ~ treatment, $p = 0.03$), but not for oaks (A_{13C} ~ treatment, $p = 0.1$). However, mean control A_{13C} values were lower than A_{13C_b} values for both tissues in both species, indicating that there was no other pathway for $^{13}CO_2$ than root uptake.

6. Discussion

6.1. $^{13}CO_2$ enrichment

For loblolly pine, mean natural abundance carbon isotopic composition ($\delta^{13}C_{\_b}$) in this study was -27.1‰, what is in line with the values reported previously by Ford et al. (2007) for the same species. To our knowledge, no values are reported in literature for northern red oak. The data indicate that there is a natural difference between both species in natural abundance carbon isotopic composition (A_{13C_b}), where baseline oak tissue samples are more enriched in ^{13}C compared to pine, for both root and stem tissues. There also was a significant difference between A_{13C_b} of both tissue types in pine, but this difference was found not to be significant for oaks. Moreover, because for both species A_{13C_b} is higher for another tissue type, it can be assumed that under natural conditions A_{13C_b} is approximately the same in stems and roots. The control group was used to check for other possible pathways of $^{13}CO_2$ whereby control A_{13C} values were slightly lower than A_{13C_b} values and therefore it can be assumed that there was no other pathway for $^{13}CO_2$ than root uptake.

The treatment group trees confirmed the uptake of soil CO_2 after trees were irrigated with a $^{13}CO_2$ enriched solution. The CO_2 concentration ($[CO_2]$) of the treatment solution (8.0%) fell into the higher range of the concentrations used in the $^{12}CO_2$ experiment. From the data of the $^{12}CO_2$ experiment it can be estimated that the soil $[CO_2]$ approximated 6%. At this concentration, soil CO_2 uptake was observed, as can be concluded from the mean $^{13}CO_2$ enrichment (A_{13C_c}) values of 0.4 and 0.5% for pines and oaks, respectively. It seems that oak tissues are generally more ^{13}C enriched than pine tissues (although this difference was not significant) and for both species there is a difference between root and stem, where root tissues are generally more ^{13}C enriched (Figure 5-8). ^{13}C enrichment (A_{13C}) was determined by analyzing gas extracted from the headspace of the vials containing tissue samples. Therefore, enrichment of the gas phase in contact with the xylem sap was analyzed and results did not hold any information on the assimilation of the $^{13}CO_2$. The higher ^{13}C enrichment values in root tissues are probably related to tissue location. As roots were in the soil, where the source of the $^{13}CO_2$ enriched solution was applied, direct uptake in the roots was possible. To eliminate a high contribution of ^{13}C, that was sticking to the outer surface of the root segments, segments were washed thoroughly. As xylem sap is transported upwards from the roots into the stem and the canopy some $^{13}CO_2$ can be assimilated by photosynthetic active cells and more $^{12}CO_2$ will enter the xylem by respiration of living cells along the xylem path.

6.2. Baseline stem CO_2 efflux and the influence of growth

Baseline stem CO_2 efflux (E_{stem_b}) rates were measured four times during the period of the experiment. Mean surface area weighted values ranged between 3.6 ± 0.4-8.4 ± 0.5 µmol m^{-2} s^{-1} for

pines, and 0.9 ± 0.1-1.2 ± 0.2 µmol m^{-2} s^{-1} for oaks. As CO_2 efflux (E_{CO2}) rates are reported to vary substantially on an annual basis and in function of temperature (see Section 2.3.2), it should be noted that at the time trees were removed from the greenhouse it was summer and in the growth chamber conditions were controlled at 25 °C. Maier (2001) reported monthly stem CO_2 efflux (E_{stem}) rates for 10 year old *Pinus taeda* trees, whereby values were normalized to 20 °C based on a Q_{10} value of 1.7. Similarly, efflux rates measured during this thesis can be rescaled using following equation:

$$R_2 = R_1 * 1.7^{\left(\frac{T_2 - T_1}{10}\right)} \quad (6\text{-}1)$$

where R_1 and R_2 are the E_{stem} rates (µmol m^{-2} s^{-1}) at temperature T_1 and T_2 (°C), respectively. Rescaled to 25 °C, mean E_{stem} rates during the summer months were in the range of 5-6 µmol m^{-2} s^{-1}. In another study, Maier & Clinton (2006) reported E_{stem} rates in the range of 2-10 µmol m^{-2} s^{-1} during the end of April, for 8 year old loblolly pines. All E_{stem_b} rates measured during this thesis are within the ranges reported in previous studies. For *Quercus rubra*, to our knowledge no values on E_{stem} are reported in literature.

Mean surface area weighted E_{stem_b} rates increased linearly in time for both species (Figure 5-3). However, the increase was substantially higher for pines compared to oaks. Because all conditions were approximately the same for every baseline measurement, the increase in E_{stem} was attributed to tree growth. Mean stem segment diameters and volumes, for both pine and oaks, also increased linearly with time (Figure 5-1). For both species, for the experimental period of this study, a linear relation existed between E_{stem_b} rates and the stem segment dimensions (Figure 5-4), supporting the influence of growth on E_{stem_b}. In literature, there are many conflicting reports about the relationships between E_{CO2} and tree stem diameters (McGuire et al., 2007). Moore et al. (2008) and McGuire et al. (2007) found a linear relation between surface area weighted E_{CO2} values and diameters in stems of *Pinus taeda* and branches of *Platanus occidentalis*, respectively. However, the main reason for the change in E_{CO2} with increasing stem diameter is probably the change of the proportion of respiring living cells in woody tissues with increasing tree age (Teskey et al., 2008). Negisi (1979) found higher E_{CO2} rates for younger trees than for older trees in detached *Pinus densiflora* stem or branch sections of similar diameter. Bosc et al. (2003) found a decreasing proportion of phloem tissue in the total mass of *Pinus pinaster* stems and branches and this change was reflected in E_{CO2} that varied with age, regardless of the diameter. In addition, Pruyn et al. (2002) found that sapwood of younger trees had a higher respiratory potential than that of older trees. In young loblolly pine seedlings, nearly all the

wood is sapwood (Schultz, 1997) and therefore stem diameter increase is almost entirely attributed to increasing sapwood volumes. During this study mean tree diameter growth rates were 2.7 cm yr^{-1} for pines and 0.5 cm yr^{-1} for oaks. However, because of the short experimental period these values are a rough estimation. In literature, growth data are difficult to interpret because of variations in conditions and ages. For 10 year old trees, mean yearly diameter increments are reported to be 2.1 cm yr^{-1} for *Pinus taeda* (Katsvanga et al., unpublished results)[2] and 0.6 cm yr^{-1} for *Quercus rubra* (Sandi & Nicolescu, 2011). Although these growth rates are for older trees, it can be concluded that in general pines grow faster than oaks. In addition, oak trees showed symptoms of stress as colored and dry spots appeared at the surface of the leaves during the first week in the growth chamber. Two oaks even died, indicating that stress could possibly have further reduced growth rates of the oak trees. To this end, growth chamber conditions were adjusted from the second week onwards.

6.3. Stem CO_2 efflux rates

Results of the treatment stem CO_2 efflux (E_{stem_t}) rates, measured after trees were irrigated with the four different $^{12}CO_2$ enriched aqueous solutions, showed that mean E_{stem_t} rates were higher than mean E_{stem_b} rates for all treatment solutions in both species (Figure 5-5). Moreover, both mean E_{stem_t} and mean E_{stem_b} rates increased over time. For all treatment solutions separately, a relation was found between stem segment volumes and both E_{stem_t} and E_{stem_b} (Figure 5-6), indicating that the increase of E_{stem_t} in time could also be attributed to growth. However, for pines the relation between E_{stem_t} and stem segment volumes was not significant. Moreover, an increased difference between mean E_{stem_b} and mean E_{stem_t} was observed with increasing applied solution [CO_2], indicating that there also is an effect of the treatment solution [CO_2] on E_{stem}. In addition, E_{stem_t} peaked during the third week of the experiment for the 20% treatment, even though highest E_{stem_b} rates were measured during the fourth week for the 10% treatment. In general, the differences between treatment and baseline measurements were more pronounced for pines than for oaks. The reason for this could be that oak trees grew slower than pines, but also because of morphological and anatomical differences as for example barriers to diffusion are known to vary substantially between tree species and even for trees of the same species (see Section 2.3.2).

6.3.1. Contribution of soil CO_2 as a source of stem CO_2 efflux

All soil [CO_2] were elevated after treatment irrigation. The soil baseline [CO_2] ranged between 0.1-0.5% for pines and 0.1-0.4% for oaks. Low soil [CO_2] in the beginning of the experimental period and the increase during the experimental period are a result of small and growing trees, respectively. Growth of the root system will have resulted in an increased root respiration (R_{root}) and a related

[2] http://www.fao.org/docrep/ARTICLE/WFC/XII/0496-B4.HTM

increase in the soil [CO_2]. Because pines grew faster, also soil [CO_2] increased slightly faster in time. After treatments, tree pots were flushed with tap water to flush out the treatment CO_2. In case some CO_2 remained in the soil, E_{stem_b} measurements could have been overestimated. However, this would only result in an underestimation of the corrected stem CO_2 efflux (E_{stem_c}) and therefore general results would not have been affected. To estimate the potential [CO_2] that could be taken up by the tree roots, the soil [CO_2] was converted to the soil solution CO_2 concentration ([CO_2*]). These conversions merely are an approximation because they were based on the pH values of the prepared CO_2 enriched solutions and the resulting soil pH values could have been slightly different. However, because soils were richly irrigated with these solutions it can be assumed that once poured on the soil the solutions represented the largest fraction of the soil water. For both species a relation was observed between the mean E_{stem_c} rates in function of the soil [CO_2] (Figure 5-7A) and in function of the soil solution [CO_2*] (Figure 5-7B). In this graphs, variations in E_{stem_b} related to growth are eliminated and therefore E_{stem_c} represents the fraction of E_{stem} not related to growth. Although units of both axes differ, both figures show the same general trend. For pines there is a clear linear relation with the soil [CO_2] (R^2=0.99) and the soil solution [CO_2*] (R^2=0.99), but these relations are less pronounced for oaks (R^2=0.57 and 0.50, respectively). These results imply that an elevated soil [CO_2] affects E_{stem}.

In Section 2.2.1, the general [CO_2] of naturally occurring soils was defined in the range of 0.5-1.5%. If we assume that E_{stem_c} is the extra E_{stem} due to uptake of CO_2 from the soil solutions, as mentioned in the previous paragraph, we observe a limited impact of soil CO_2 on E_{stem} for natural conditions. This implies that under natural conditions R_{root} is the main source of internal CO_2 originated belowground. However, it should be kept in mind that E_{stem_c} represents the stem efflux in addition to E_{stem_b} values and that because trees were still very small in the beginning of the experiment E_{stem_b} values were small. Therefore, we calculated the percentage contribution of E_{stem_c} to E_{stem_t}, resulting in a mean contribution of 31.0% for pines and 38.6% for oaks. These values indicate that there is a substantial contribution of E_{stem_c} to E_{stem_t}, even for a natural [CO_2], implying that a substantial part of the internal CO_2 that originated belowground comes from CO_2 taken up from the soil solution. However, it is difficult to find a pattern in the contribution values of E_{stem_c} over the different treatments and the variation between trees is big. Generally, the contribution of E_{stem_c} seems to be higher for oaks.

The potential contribution of soil CO_2 to E_{stem} was analyzed by Moore et al. (2008). Their calculations were based on the findings of Teskey & McGuire (2005) who found a linear relation between the stem [CO_2] and E_{stem} in *Liquidambar styraciflua*. Based on the results of Teskey & McGuire (2005), Moore et al. (2008) estimated the difference in E_{stem} due to a 1% increase in the soil [CO_2] at 0.277 µmol m^{-2} s^{-1}. They took the mean soil [CO_2] (2.6%) and the mean E_{stem} (3.8 µmol m^{-2} s^{-1}) from their

study and applied the estimated contribution for a 1% increase of the soil [CO_2] to calculate that 19% of the mean E_{stem} is contributed by CO_2 taken up from the soil and not respired by local woody tissues. A similar approach can also be applied on this experiment as shown in Table 6-1. The first column gives the average soil [CO_2] for pines, oaks and the mean of both species, respectively. In the second column the potential extra efflux related to the mean soil [CO_2] in column one is given (based on 0.277 µmol m^{-2} s^{-1} for a 1% soil [CO_2] increase). The last column gives the contribution of this extra efflux to the mean E_{stem_t} (%). Although, the mean value averaged for both species is within the same range as reported by Moore et al. (2008), large differences were observed between pines and oaks. In addition, Moore et al. (2008) based their calculation on data from a study on a different species with different characteristics, where trees were severed at the base and placed in water with different [CO_2]. Therefore, these values only are a rough estimation, however they also indicate a higher contribution for oaks. Moreover, Ubierna et al. (2009) commented on this study by estimating that a 19% contribution of soil CO_2 would only alter the $\delta^{13}C$ of the stem efflux by 1.3‰, which lies within the measurement error (2‰), and suggested that soil CO_2 uptake by roots and transport into the stem deserves a second look.

Table 6-1. Potential contribution of CO_2 taken up from the soil solution by tree roots, estimated based on the calculations of Moore et al. (2008).

Species	Soil [CO_2] (%)	Extra E_{stem} (µmol m^{-2} s^{-1})	Contribution (%)
Pine	3.85	1.07	11.92
Oak	4.12	1.14	67.18
Mean	3.99	1.11	19.30

Soil solution enrichment with $^{13}CO_2$ proved the uptake of soil CO_2 by tree roots for high soil [CO_2], but no prove was obtained for lower (natural) concentrations. Therefore, it is possible that the increased E_{stem} rates rather are an indirect effect of increased soil [CO_2] than actual uptake, especially for lower (natural) conditions. In a field study of De Bel (2014) internal root [CO_2] and [CO_2] of the surrounding soil were measured during several days whereby the root [CO_2] was generally higher than the soil [CO_2]. Because the natural net root CO_2 efflux will therefore be from the roots into the soil, it is highly unlikeable that tree roots take up CO_2 from the soil solution against the natural gradient. Therefore, it is possible that under natural concentrations another effect is observable. Assume a tree growing in natural conditions. Within its roots, respired CO_2 can either diffuse to the soil environment or be transported with the transpiration stream (Aubrey & Teskey, 2009) and theoretically, when all conditions remain constant, an equilibrium between both carbon fluxes will exist at root level. In the stem, CO_2 from local woody tissue respiration (R_{wt}) and CO_2 transported upward with the transpiration stream can efflux to the atmosphere, where again an equilibrium exists between

diffusion into the xylem sap and diffusion to the atmosphere (McGuire et al., 2007). An increase in the soil [CO_2] relative to the observed concentrations under natural conditions can alter the CO_2 equilibria at root level by altering the balance between respired CO_2 efflux into the soil and internal transport. As a result, less CO_2 will efflux to the soil environment and more will be transported upward in the tree, where an increased internal [CO_2] will further affect the equilibrium with E_{stem} and more specifically increase measured E_{stem} rates. Of course, eventually some soil CO_2 can be taken up by tree roots, but this effect will be small as suggested by several studies (Ford et al., 2007; Teskey & McGuire, 2007). For a higher soil [CO_2], there is a point where the soil [CO_2] becomes higher than the internal root [CO_2] and therefore E_{stem} rates will become higher than under natural conditions. This way, soil CO_2 uptake will increase the internal [CO_2] and therefore increase E_{stem}.

De Bel (2014) checked if there was any correlation between the soil [CO_2] and the [CO_2] inside the roots, but no relevant trends could be detected from their data. In the same trees, Wittocx (2014) measured E_{stem} rates and also here no correlation with the soil [CO_2] was detected. Although, soil [CO_2] ranged between 0.4 and 4.8%, on average the concentration remained stable around 1% and outliers were scarce. The stability of the natural soil [CO_2] is probably the reason why no correlation with E_{stem} was observed. However, it is also possible that the equilibria hypothesis only holds for small trees and that for bigger trees the relative role of the equilibria decreases. For small trees R_{root} could still have been low (Teskey & McGuire, 2007), resulting in lower internal [CO_2]. In addition small roots lack a periderm (see Section 3.3.1), whereby CO_2 diffusion between the roots and the soil environment will occur easier than in older, more suberized roots (Teskey et al., 2008). For the data of this thesis, E_{stem_c} contributions (Table 5-1) were compared with tree dimensions, but no correlation was found. Most studies investigating the uptake of soil CO_2 seem to have used small plants and rather low CO_2 concentrations. For example, Ford et al. (2007) used *Pinus taeda* seedlings of approximately five months old and soil solutions with a mean [CO_2*] of 1.8 mM. It is possible that, if the equilibria hypothesis holds, they would have observed an increased E_{stem}, although actual CO_2 uptake was small.

Finally, to our knowledge, most $^{13}CO_2$ experiments focused on assimilated CO_2 (Hibberd & Quick, 2002; Ford et al., 2007; McGuire et al., 2009; Bloemen et al., 2013). Therefore, $^{13}CO_2$ enriched solutions were infused into the base of tree stems or roots were exposed to $^{13}CO_2$ enriched soil solutions. Either way, tissues samples were used to determine the CO_2 fixed by photosynthetic active cells. Bloemen et al. (2013) infused a ^{13}C labeled solution in the base of a tree stem and found that up to 17% of the label was assimilated, while the remainder diffused to the atmosphere via E_{stem} and E_{branch}, indicating that most of the internal CO_2 effluxes to the atmosphere. It is possible that soil $^{13}CO_2$ enrichment studies focusing on the assimilated $^{13}CO_2$ slightly underestimate the uptake of soil

CO_2 because Rubisco is known to discriminates between carbon isotopes with a slightly preference for the lighter isotope ^{12}C (Roeske & O'Leary, 1984; Whitton, 2012). This isotopic discrimination is only achieved when the $[CO_2]$ is high (Whitton, 2012), as is usually the case for internal CO_2 in trees. It is possible that slightly more $^{13}CO_2$ is taken up from the soil solution than is indicated by the assimilation in photosynthetic tissues. This could also explain the difference in the conclusions of Ford et al. (2007) and Moore et al. (2008). Finally, it should be noted that in an experiment of Ubierna et al. (2009) ^{13}C labeling of soil solution CO_2 did not result in an enrichment of the stem xylem sap, despite clear evidence that the water itself had been taken up. However, it should be kept in mind that the study of Ubierna et al. (2009) was performed on large conifer trees, and as discussed in the previous paragraph the question is whether the effects are the same for small trees as compared to mature trees.

6.3.2. Impact of sap velocity on stem CO_2 efflux

In literature, E_{stem} is assumed to be negatively correlated with sap flow (F_s) (Teskey & McGuire, 2002; McGuire et al., 2007). However, others have reported no influence of F_s on E_{stem} (Edwards & Wullschleger, 2000; Maier & Clinton, 2006) and Levy et al. (1999) even found a positive relationship, that could have been a result of upward transported water with high $[CO_2]$ from the root system. In general, it is difficult to establish relations between E_{CO2} and the influencing factors because they vary in complex ways. In addition, tissue size and time of the year may affect the relative contributions of CO_2 diffusing from the xylem and CO_2 released by R_{wt} (Teskey et al., 2008). McGuire et al. (2007) suggested that the relationship between E_{CO2} and F_s is driven by variations of the xylem $[CO_2]$ caused by the diluting effect of F_s. The $[CO_2]$ of water entering the roots is substantially lower than the xylem sap $[CO_2]$. Therefore, as F_s rates increase the sap $[CO_2]$ becomes more diluted and less CO_2 fluxes to the atmosphere (McGuire et al., 2007). However, as commented by McGuire et al. (2009), when the water supplied to the root system contains a high $[CO_2]$ this dilution effect will probably be limited or even be eliminated.

For this thesis, F_s rates were measured during the uptake of enriched soil solutions. F_s of the last three treatments (5, 10 and 20%) seem to increase approximately linearly (Figure 5-2; a linear regression of this last three datapoints in time results in an R^2 of 0.99 and 0.97 for pines and oaks, respectively), probably in relation to growth. Especially for oaks, mean F_s for the 1.5% treatment was higher than expected. A possible reason for this extreme value could be that oak trees were still adapting to the conditions in the growth chamber after they were removed from the greenhouse. However, there was a sufficient time period of ten days between the first day in the growth chamber and the first experimental E_{stem} measurements during which the trees were already regularly irrigated and probably adapted to the growth chamber conditions. Another explanation for the

higher F_s could be the adapted experimental conditions to prevent leaf scorching of the oaks. After the 1.5% treatment, lights were switched to a slightly higher position and the relative humidity was altered to 75% instead of 50%. Therefore, F_s rates could be decreased for the following treatments due to changed experimental conditions. As described in the previous paragraph, the effect of F_s on E_{stem} is not entirely clear. Because later treatments (5, 10 and 20%) used water with a high [CO_2] it is possible that higher F_s rates increased the internal [CO_2] of the trees, rather than diluting it. This could mean that when F_s would been have higher, E_{stem} rates also would have been slightly higher.

6.4. Difference between species

Both experiments revealed clear differences between the two species. For the $^{12}CO_2$ experiment, both E_{stem_b} and E_{stem_t} values were substantially higher for pines compared to oaks. Although this difference is probably due to natural differences between species, pines also grew much faster than oaks, resulting in a sharper increase of E_{stem_b} in time. It is known that barriers to diffusion can differ substantially between species (see Section 2.3.2), even between individuals within a single species (Steppe et al., 2007), resulting in different E_{CO2} values. Teskey & McGuire (2002) directly manipulated xylem [CO_2*] in branches of *Quercus alba* and *Liriodendron tulipifera* and found that a 10% treatment resulted in a higher [CO_2*] increase for *Quercus alba*, but a bigger E_{branch} increase in *Liriodendron tulipifera*. They assumed that the differences in the responses of the two species were a result of differences in the effectiveness of the water infusion treatment in ring porous and diffuse porous xylem, as well as differences in the ability of CO_2 to diffuse through the cambium and bark layers into the air. Gartner et al. (2004) gave an overview of the volumetric proportions of cell wall, water and gas for commercial timber of North America. Assuming that for young trees almost all wood is sapwood, the volume proportion of cell wall, water and gas is 31, 16 and 54% for pines and 37, 39 and 25% for oaks. Because of the higher diffusion resistance in water (see Section 2.3.2) and the relative higher cell wall proportion in oaks, barriers to diffusion could have been higher for oaks. Although oaks effluxed less CO_2 to the atmosphere, the mean contribution of E_{stem_c} to E_{stem_t} was higher for oaks than for pines. The $^{13}CO_2$ experiment also revealed higher A_{13C_c} values for oaks, whereby it is possible that oaks generally took up more CO_2 from the soil solution and therefore E_{stem_c} relative to E_{stem_t} is higher for oaks. As discussed in previous paragraphs it is also possible that there are morphological or anatomical differences between species that influenced the amount of soil CO_2 that is taken up from the soil solution, or that in combination with the internal root [CO_2] the equilibria hypothesis differed between species. However, more measurements are needed to underpin this conclusion

In general, trees with ring porous xylem (e.g. *Quercus rubra*) have the highest proportion of large diameter xylem elements, smallest conducting area and fastest sap velocity. Trees with tracheid xylem (e.g. *Pinus taeda*) are characterized by the smallest diameter xylem elements, largest conducting area and slowest sap velocity (Kramer, 1979). For this study, F_s rates were opposite to what can be expected from literature data, with a mean F_s over the experimental period of 4.3 and 1.2 mL h^{-1} for pines and oaks, respectively. Although F_s rates were based on water uptake rates and therefore only an estimation, it is possible that the relative size of both tree species affected F_s rates. Mean leaf areas also differed substantially between both species (1437.9 cm^2 for pines compared to 435.1 cm^2 for oaks), possibly explaining differences in F_s rates. As pines have higher F_s rates, it could be expected that E_{stem} rates were lower (see Section 2.3.2) compared to oaks and for E_{stem_c} this actually is the case. However, studies investigating the relation between E_{CO2} and F_s usually considered one specie on a specific time for different F_s rates (McGuire et al., 2007; Saveyn et al., 2008). Because several factors affect E_{CO2} rates in a complex way, a comparison between two different species is rather difficult. For example, Teskey & McGuire (2007) concluded that the slope of the relationship between E_{CO2} and the xylem [CO_2] can change with species and therefore also the relation with F_s rates will differ.

6.5. Directions to further research

Some directions can be made for further research. First, this experiment could be repeated on a longer time scale to further investigate the results, also for bigger trees. Second, it would be interesting to combine E_{stem} measurements with an isotopic experiment, where for different soil [CO_2], E_{stem} and the isotopic composition of E_{stem} is measured. Moreover, the use of a $^{13}CO_2$ enriched solution on a lower [CO_2] would have been helpful to check if there was actual CO_2 uptake in the lower (natural) range of the soil [CO_2]. However, Ford et al. (2007) used an enriched ^{13}C soil [CO_2] to investigate the uptake of CO_2 by young *Pinus taeda* trees. The average treatment solution [CO_2*] was 1.78 mM, a concentration that fits in the lower range of the concentrations used in this experiment. Based on the observations in this experiment a [CO_2*] of 1.8 mM results in a soil [CO_2] of <1%. Therefore, the results of Ford et al. (2007) can be used as a estimation of the result that could have been obtained by repeating this $^{13}CO_2$ experiment for a lower [CO_2]. Ford et al. (2007) observed a stem tissue enrichment of approximately (estimated from their graph) 50‰. In comparison, mean stem tissue enrichment in this experiment was 168‰. This substantial difference supports the equilibria hypothesis.

To be able to estimate the concentration gradient between roots and the soil environment, it would have been helpful to know the internal root or stem [CO_2]. For 8 year old *Pinus taeda* stems, the

internal [CO_2] is previously reported in the range of 1.0-8.0% (Maier and Clinton 2006) and for 25.4 cm radius *Quercus rubra* stems in the range of 13.5-16.5% (Jensen, 1967). De Bel (2014) observed mean root [CO_2] of 8.5% in *Fagus grandifolia* and 5.2% in *Liriodendron tulipifera*, indicating that substantial differences exist between species. As Teskey & McGuire (2007) suggested that larger trees with larger root systems may accumulate more CO_2 in the xylem sap, the internal [CO_2] of the trees in this experiment could have been substantially lower, limiting the diluting effect of water taken up from the soil solution. A lower internal [CO_2] in combination with increased soil [CO_2] could be an explanation for the observed contribution of E_{stem_c} in this small trees. Finally, although trees were watered on a regular basis and it was tried to keep trees on the point of draining, there was no active regulation of the water status and F_s rates. Saveyn et al. (2007) revealed the importance of the water status on E_{stem} and therefore this should be kept in mind for further research.

7. Conclusion

Two growth room experiments were used to investigate the uptake of soil CO_2 by tree roots and more specifically the influence of the soil CO_2 concentration on the stem CO_2 efflux. The results of the $^{12}CO_2$ experiment revealed an increase in stem CO_2 efflux rates for increased soil CO_2 concentrations. The contribution of the corrected stem CO_2 efflux to the total treatment stem CO_2 efflux after irrigation with a CO_2 enriched solution was 31.0 ± 1.8% for pines and 38.6 ± 2.4% for oaks. However, a large difference in the contribution of the uptake of soil CO_2 solution to stem CO_2 efflux was observed among the different treatments. The soil CO_2 concentration after irrigation with CO_2 enriched solution ranged between 0.3-8.5%, where the natural concentration for forest soils is reported in the range of 0.5-1.5%. For both species, the $^{13}CO_2$ experiment confirmed the uptake of soil CO_2 for high soil CO_2 concentrations relative to the natural range. Because the internal CO_2 concentration of tree roots is mostly reported to be higher than the CO_2 concentration of the surrounding soil, uptake of soil CO_2 would be against the concentration gradient. Under natural conditions an equilibrium exists between root-respired CO_2 that diffuses into the soil environment or is transported internally. An increased soil CO_2 concentration alters the natural concentration gradient, whereby the internal CO_2 concentration increases throughout the tree roots and stem, resulting in an increased transport of respired CO_2 in the stem and an increased CO_2 efflux at stem level. Therefore, for natural soil CO_2 concentrations root respiration will be the largest source of internal belowground respired CO_2, although elevated soil CO_2 concentrations can indirectly affect stem CO_2 efflux. In addition, uptake of soil CO_2 at high concentrations can also decrease the diluting effect of water taken up from the soil and for high soil CO_2 concentrations even increase internal CO_2 concentrations, resulting in an increased E_{stem}. However, this experiment used 1 year old trees and it is possible that effects are different for more mature trees.

The results of this experiment are a trigger to open up the discussion about soil CO_2 uptake. However, a more complete combination of both experiments performed in this thesis would be helpful to investigate the actual soil CO_2 uptake for lower CO_2 concentrations. In general, this experiment could be repeated on a larger scale, with more species, using a larger number of both $^{12}CO_2$ and $^{13}CO_2$ treatments at different concentrations over a longer experimental period. This way, we will be able to test if our results on smaller trees can be extrapolated to larger trees and have more detailed results for a varying range of soil CO_2 concentrations. In addition, the internal root and stem CO_2 concentration could be measured to monitor whether internal CO_2 increases after $^{12}CO_2$ treatment. Finally, water status of the trees should be actively regulated or more precisely measured to make sure sap flow rates do not affect results.

In conclusion, these results once more confirmed the complex nature of internal CO_2 inside trees. Because CO_2 efflux is a combination of many different processes, related to the different sources and sinks of respired CO_2 inside trees, estimations of woody tissue respiration based on CO_2 efflux rates are not accurate. Although in forest soils under natural conditions the CO_2 concentration will be mostly stable, this experiment indicates a potential influence on stem CO_2 efflux when the soil CO_2 concentration increases substantially. Especially in light of the predicted increase in atmospheric CO_2 concentrations and the related increase in soil CO_2 concentrations (Andrews & Schlesinger, 2001; Jastrow et al., 2005), the gradient between soil and roots will be altered (Karberg et al., 2005). More research considering the different CO_2 pathways inside trees and between trees and their environment will gradually increase our understanding of the tree carbon balance and the role of forest ecosystems in the global carbon budget.

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