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REVIEW

Root xylem CO_2 flux: an important but unaccounted-for component of root respiration

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Abstract

Key message **In tree roots, a large fraction of root-respired CO_2 remains within the root system rather than diffusing into the soil. This CO_2 is transported in xylem sap into the shoot, and because respiration is almost always measured as the flux of CO_2 into the atmosphere from plant tissues, it represents an unaccounted-for component of tree root metabolism.**

Abstract Root respiration has been considered a large component of forest soil CO_2 efflux, but recent findings indicate that it may be even more important than previous measurements have shown because a substantial fraction of root-respired CO_2 remains within the tree root system and moves internally with the transpiration stream. The high concentration of CO_2 in roots appears to originate mainly within the root. It has been suggested that plants can take up dissolved inorganic carbon (DIC) from soil, but under most conditions uptake from soil is minimal due to the root-to-soil diffusion gradient, which suggests that most of the CO_2 in root xylem is derived from root respiration. Estimates of the internal flux of CO_2 through root xylem are based on combined measurements of sap flow and internal [CO_2]. Results quantifying root xylem CO_2 flux, obtained for a limited number of species, have raised important concerns regarding our understanding of tree respiration. Taken together, the results of these studies call into question the partitioning of ecosystem respiration into its above- and belowground components, and redefine the energetic costs of tree root metabolism and hence estimates of belowground carbon allocation. Expanding our observations of root xylem CO_2 flux to more species and at longer time scales, as well as improving the techniques used to study this process, could be fruitful avenues for future research, with the potential to substantially revise our understanding of root respiration and forest carbon cycles.

Keywords Tree roots · Soil respiration · Xylem CO_2 transport · Tree carbon cycle · Carbon allocation

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Introduction

A common belief among scientists working on belowground respiration in forests is that root-respired CO_2 diffuses into the soil environment and thereby contributes to CO_2 efflux from soils. Recent results (Aubrey and Teskey 2009; Grossiord et al. 2012; Bloemen et al. 2014a) indicate that a fraction of belowground respired CO_2 may remain within the root system rather than diffusing into the soil. For instance, Aubrey and Teskey (2009) estimated that twice the amount of CO_2 derived from root respiration was transported via the transpiration stream as diffused into the soil environment. As respiring cells in roots lie in close

proximity to xylem tissue, respired CO_2 can dissolve in xylem water within the roots and be transported above ground via the transpiration stream rather than diffusing into the soil, implying that we are substantially underestimating the autotrophic component of belowground respiration.

The root xylem CO_2 flux have been estimated only for a few species over short time periods of up to 1 week (Aubrey and Teskey 2009; Grossiord et al. 2012; Bloemen et al. 2014a). However, the results from these studies all indicate that root xylem CO_2 is a very important, but unaccounted-for, component of root and tree respiration. The ramifications of these new results are that the energetic cost of tree root metabolism is much larger than previously assumed and because the largest portion of root-derived CO_2 diffuses into the atmosphere aboveground from tree stems and branches (up to 94 %, Bloemen et al. 2013b), the current understanding of the magnitude of autotrophic respiration in above- and belowground components might be incorrect. Hence, accounting for root xylem CO_2 flux is essential for accurate quantification and partitioning of the autotrophic component of forest ecosystem respiration into its subcomponents. Therefore, there is a need to measure the magnitude of root xylem CO_2 flux in more species and on longer time scales to allow us to accurately account for below- and above-ground tree respiration.

The movement of CO_2 in roots and soil

Respiration occurs in living cells throughout the root, in the epidermis, phloem, and xylem parenchyma in the center of the root (Raven et al. 1999). As the gaseous CO_2 is released by respiration in living cells, a portion dissolves in water and moves into and through the xylem rather than diffusing outwards into the surrounding soil and contributing to soil CO_2 efflux (Fig. 1).

Dissolved CO_2 from belowground respiration can be transported upwards with the transpiration stream (Aubrey and Teskey 2009; Grossiord et al. 2012; Bloemen et al. 2013b, 2014a), which contributes to the high internal [CO_2] observed in tree tissues (<1 to over 23 %, Teskey et al. 2008). The internal [CO_2] in the xylem is generally higher than that of forest soil, where [CO_2] is often in the range of 1–2 % (Pumpanen et al. 2003). For example, Teskey and McGuire (2007) simultaneously measured soil [CO_2] at 15 cm depth and internal [CO_2] at the stem base of *Platanus occidentalis* trees and observed substantially higher [CO_2] in the stem (mean 7.6 %) compared with the soil (mean 1.2 %). Similarly, Ubierna et al. (2009) measured higher [CO_2] in the xylem at the bottom of conifer tree stems compared with [CO_2] in neighboring soil and concluded that the xylem CO_2 flux from belowground had likely resulted from the upward transport of root-respired CO_2, rather than transport of CO_2 derived from the soil. So far, only one study has simultaneously measured [CO_2] directly in roots (as opposed to the stem base) and in soil. In those recently concluded measurements, substantially higher [CO_2] was observed in large roots of *Liriodendron tulipifera* and *Fagus grandifolia* compared with the neighboring soil environment (De Bel 2014). From these studies we conclude that under most conditions it should be expected that a decreasing [CO_2] gradient exist from the root to the soil, which explains why almost all of the upward flux of CO_2 in root xylem must be derived from root respiration.

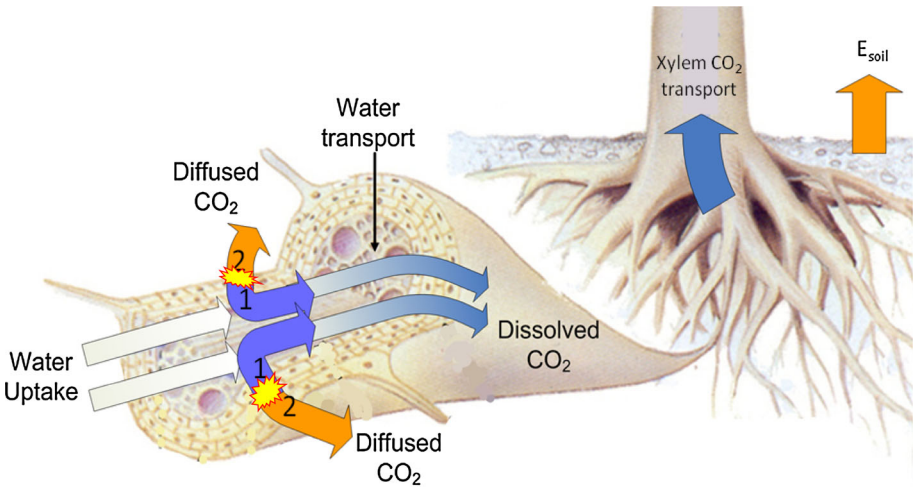

Fig. 1 Conceptual model of root-respired CO_2 transport in the transpiration stream. *Stars* indicate points of respiration in living root tissues, where root-respired CO_2 remains within the root, dissolves in the water taken up by the root, and is transported with the transpiration stream in the xylem vessels (*1*) or where root-respired CO_2 diffuses from inside the root outward into the soil (*2*). As a result there are two aboveground fluxes of root-respired CO_2, one that contributes to soil CO_2 efflux and one transported internally via the transpiration stream that contributes to efflux from aboveground tissues (drawing adapted from Cruiziat and Tyree 1990)

Nevertheless, there is a common misunderstanding regarding the source of CO_2 transported through the xylem from belowground. This probably developed because, until recently, scientists were largely unaware of the high [CO_2] in stems and roots. In the 1960s, crop growers believed that plants could take up and assimilate soil-dissolved inorganic carbon (DIC). In various experiments, plants were irrigated with water enriched with CO_2, thereby increasing the amount of soil DIC available for plant carbon gain (see references in Enoch and Olesen 1993). However, in these experiments the contribution of soil DIC uptake to plant carbon gain was very small, generally less than 1 % (Enoch and Olesen 1993). However, whether soil DIC enters a root depends on the concentration gradient between root and soil. So if soil [CO_2] is higher than that of the root, soil DIC will enter the root. This explains why some studies have shown a substantial contribution of soil DIC to the total [CO2] in the xylem sap, e.g., in willow (Vapaavuori and Pelkonen 1985; Vuorinen et al. 1989; Vuorinen and Kaiser 1997), tobacco (Hibberd and Quick 2002), barley (Stolwijk and Thimann 1957; Vuorinen and Kaiser 1997), peas (Stolwijk and Thimann 1957), summer wheat (Schäfer 1988), tomato (Stemmet et al. 1962), and beans (Amiro and Ewing 1992) (Table 1). Those studies were performed in hydroponic culture and the DIC concentrations were higher than normally found in soil, which reversed the [CO_2] gradient from root to soil, allowing the uptake of a substantial amount of DIC from the hydroponic solution.

Using data from their field study, Aubrey and Teskey (2009) calculated the potential contribution of soil CO_2 to the quantity of CO_2 in the xylem at the base of the stem. They made the assumption that all the CO_2 in soil solution could enter the root and contribute to the CO_2 present in the xylem. Under that unlikely scenario, they estimated that for *Populus deltoides* trees growing in the field only 8 % of the total xylem CO_2 flux could have been derived from the uptake of soil DIC. However, since the CO_2 gradient would almost always be in the reverse direction, because xylem [CO_2] was generally in the range of 10–20 % and soil [CO_2] was <2 %, it is highly likely that 8 % is a large overestimation of the actual contribution of soil DIC to the [CO_2] measured in the xylem. Supporting this idea, ^{13}C label dissolved in water applied to soil around either *Pinus* seedlings (Ford et al. 2007) or large conifer trees (Ubierna et al. 2009) showed that soil DIC uptake had only a small effect on aboveground plant carbon gain and CO_2 efflux,

Table 1 Studies on the uptake and transport of soil-dissolved inorganic carbon (DIC) in plants classified by type of experiment, method, type of plant/setup, and species

Method	Type of plant/setup, species	Main finding	References
Pot and field experiments			
^{13}C soil DIC labeling	Potted seedlings, *Pinus taeda*	Soil DIC uptake contributed 0.8 % to plant carbon gain	Ford et al. (2007)
	Field-grown, conifers	Soil DIC uptake did not affect stem CO_2 efflux	Ubierna et al. (2009)
ARQ determination	Field-grown, tropical trees	Soil ARQ ~1 implied no soil DIC uptake	Angert et al. (2012)
Belowground xylem CO_2 flux measurements	Field-grown, *P. deltoides*	8 % of xylem CO_2 flux was from soil DIC uptake	Aubrey and Teskey (2009)
	Field-grown, *Q. robur*	Belowground xylem CO_2 flux was derived from root respiration	Bloemen et al. (2014a)
Hydroponic experiments			
^{14}C soil DIC labeling	Hydroponics, *Salix*	Soil ^{14}C label was found in the leaves and shoots	Vapaavuori and Pelkonen (1985)
	Hydroponics, *Salix*	Soil ^{14}C label was found in the leaves and shoots	Vuorinen et al. (1989)
	Hydroponics, *Salix*	Soil CO_2 enrichment led to higher PEPc activity	Vuorinen and Kaiser (1997)
	Hydroponics, tobacco	^{14}C label was fixed in photosynthetic cells	Hibberd and Quick (2002)
	Hydroponics, peas and barley	Little CO_2 was taken up by roots	Stolwijk and Thimann (1957)
	Hydroponics, summer wheat	Root-absorbed DIC was 1.21 % of total C assimilation	Schäfer (1988)
	Hydroponics, tomato	^{14}C label was found in the leaves and shoots	Stemmet et al. (1962)
	Hydroponics, bean	Uptake of DIC was related to transpiration	Amiro and Ewing (1992)

ARQ apparent respiratory quotient (ratio of CO_2 emission/O_2 uptake ratio), *PEPc* phosphoenolpyruvate-carboxylase

respectively. Further, uptake of CO_2 from soil by roots should result in an apparent respiratory quotient (ARQ, ratio of CO_2 emission/O_2 uptake ratio) of <1. Angert et al. (2012) observed an average ARQ of approximately 1 in air sampled from the soil near the roots of different tropical trees, suggesting a lack of uptake of soil DIC by the root system.

High root [CO_2] indicates the presence of barriers to diffusion

The cause of the high [CO_2] in tree roots, relative to soil, may be analogous to a previously observed oxygen concentrating mechanism in roots of wetland species (Colmer 2003b; De Simone et al. 2003; Aubrey and Teskey 2009). In these species, roots form substantial barriers to gas exchange in the outer cell layers, leading to both increased [CO_2] and low radial O_2 loss (Colmer 2003a; Colmer et al. 2006; Soukup et al. 2007; Abiko et al. 2012). Highly porous aerenchyma tissue in root and shoots of wetland species provides a low resistance pathway for gas movement, enabling internal aeration (Colmer 2003b). Moreover, aquatic plants have mechanisms by which CO_2 derived from sediments and/or from respiration in submerged rhizomes and root tissues moves internally from belowground to the shoot (Brix 1990; Colmer 2003b). Upland trees lack such aerenchyma tissue, making the transpiration stream the principal internal pathway of upward movement of carbon from belowground.

As root systems age, an increasing proportion of the tissue becomes suberized (Kramer and Kozlowski 1979), which, along with inner and outer bark development in secondary roots, may form a substantial barrier for radial diffusion of root-respired CO_2 into the soil. Most research on the effect of suberization on gas diffusion at the root soil interface has been performed on upland crops (Abiko et al. 2012) and grasses (Soukup et al. 2007). Root suberization has been studied in this context on only two tree species, and it was associated with decreased radial oxygen loss and hence increased root [CO_2] (De Simone et al. 2003).

An illustration of the effect of the barriers to diffusion of CO_2 from roots can be seen in studies of root respiration that have measured excised roots (see review by Kuzyakov 2006). In these studies, after excision, root CO_2 efflux rate first increase and subsequently decrease over time (Rakonczay et al. 1997). By excising the roots, barriers to radial diffusion are removed, and a large amount of root-respired CO_2 that was concentrated in the roots can diffuse into the incubation chamber, causing a high apparent respiration rate at the start of the measurement, followed by a rapid decrease (Chapin and Tryon 1982; Rakonczay et al. 1997; Kuzyakov 2006; Marsden et al. 2008b). In particular, in coarse roots there can be a strong flush of CO_2 efflux directly after root excision, as observed in *Eucalyptus* (Marsden et al. 2008a), because these roots possess more substantial barriers to radial diffusion. Diffusion of root xylem CO_2 can contribute to significantly higher apparent respiration rates after excision than rates observed in intact living roots (Makita et al. 2012).

Current methods to measure root xylem CO_2flux

As first described by Aubrey and Teskey (2009), root xylem CO_2 flux (F_t, mg C h^{-1}) can be estimated from measurements of sap flow (F_s, g h^{-1}) and gaseous [CO_2] (vol %), along with tissue temperature (°C), and xylem pH which allow the calculation of the total quantity of DIC in xylem sap ([CO_2^*], mM)(Fig. 2):

$$F_t = F_s \times [CO_2^*] \times a \quad (1)$$

With a the atomic weight of carbon. [CO_2^*] is the sum of [CO_2]$_{aq}$, [H_2CO_3], [HCO_3^-], and [CO_3^{2-}], and cannot be measured in situ (Aubrey et al. 2011). Instead, it is calculated based on Henry's law, which states that the partial

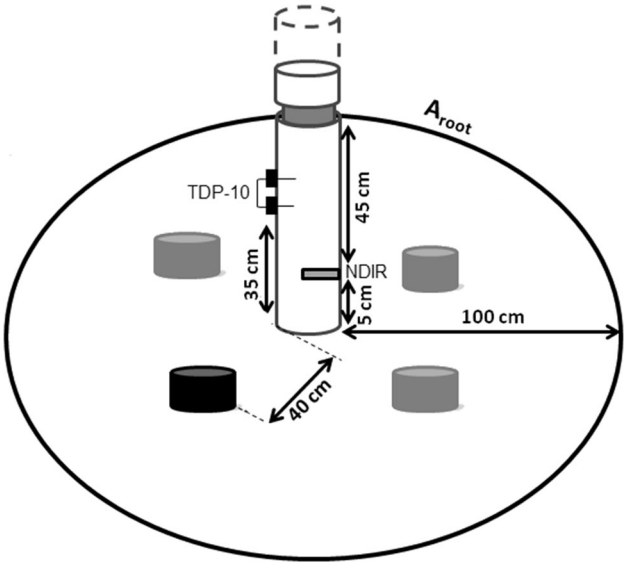

Fig. 2 Schematic of the experimental setup used by Bloemen et al. (2014a) to estimate root xylem CO_2 flux and soil CO_2 efflux, indicating the distances and positions of the stem girdle and equipment installed on the stem and within the soil area occupied by the roots (A_{root}) to measure the flux of root-respired CO_2 transported in the transpiration stream and soil CO_2 efflux. The black and grey chamber(s) represent the measurement of E_{soil} with automated and manual chambers, respectively. Chambers were located at 40 cm from the stem. *NDIR* non-dispersive infrared CO_2 sensor, *TDP-10* thermal dissipation probe. The stem thermocouple installed 3 cm above the NDIR sensor and the manual chambers installed 70 cm from the stem for E_{soil} measurements are not shown (from Bloemen et al. 2014a)

pressure of a gas over a solution is proportional to the concentration of that gas in the solution (Stumm and Morgan 1996):

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

where K_1 and K_2 are the first and second acidity constants, respectively, K_H is the Henry constant (all of the constants are temperature dependent) and pCO_2 is the partial pressure of CO_2 over the solution, which is equal to measured xylem $[CO_2]$.

Gaseous xylem $[CO_2]$ can be measured continuously in situ. Non-dispersive infrared (NDIR) sensors have become small enough that they can be inserted directly into a hole drilled into the xylem of medium- and large-diameter trees (an example is the GMM 221 sensor manufactured by Vaisala Inc., Helsinki, Finland). The NDIR sensors have recently been favored over the micro-electrodes used in previous studies of internal $[CO_2]$ in tree stems (McGuire and Teskey 2002, 2004) because they have shown greater stability, less temperature sensitivity, and better reliability under field conditions (Teskey and McGuire 2007). However, the NDIR sensors that are currently available are larger than the CO_2 micro-electrodes, so their application is limited to larger diameter roots or stems. Further, insertion of an NDIR sensor requires creation of a relatively large hole in the tissue, which may have unknown effects on $[CO_2]$ and sap flow in the xylem.

To calculate $[CO_2^*]$, xylem sap pH must also be measured, because pH has a substantial effect on the quantity of inorganic carbon that can dissolve in xylem sap (see Fig. 1 in Teskey et al. 2008; Erda et al. 2014). Below pH 5.6, most inorganic carbon in xylem sap is in the form of CO_2, but at higher pH significant quantities of CO_2 can dissolve as HCO_3^- and CO_3^{2-}. Therefore, higher root xylem CO_2 fluxes are expected for species with higher xylem sap pH, which have been reported in a range of 4.5–7.4 for several species (see Table 2 in Teskey et al. 2008). In contrast to temperature and xylem $[CO_2]$, pH is generally measured non-continuously and destructively (Aubrey et al. 2011) on sap expressed under pressure from detached twigs or stem cores. Due to the low temporal resolution of these measurements, temporal dynamics of pH in plants remain poorly understood (Aubrey et al. 2011; Erda et al. 2014). There appear to be seasonal changes in xylem sap pH, which are important to measure for accurate estimates of $[CO_2^*]$ over time, especially in species with high xylem sap pH (i.e., pH > 6.5) (Erda et al. 2014). The accuracy of currently used destructive techniques has not been directly verified, and could represent an important source of error. A new approach for measuring xylem sap pH, particularly if it was continuous, could be a useful improvement in the accuracy of $[CO_2^*]$ estimates.

Although the operational principles of sap flow measurement techniques are well-established (Smith and Allen 1996), the accuracy of these measurements is also of concern for estimating root xylem CO_2 flux. Sensors measuring sap flow rate and sap flux density for woody species are based on heat transport within the stem (Smith and Allen 1996; Vandegehuchte and Steppe 2013) and can continuously measure tree F_s. Species- and site-specific correction factors might be necessary to obtain accurate estimates of sap flow (Steppe et al. 2010; Sun et al. 2011), which is crucial to ensure that accurate estimates of root xylem CO_2 flux are obtained.

To compare soil CO_2 efflux and root xylem CO_2 flux at the same spatial scale ($F_{t,scaled}$, mg C m^{-2} h^{-1}), the latter needs to be scaled to the soil area occupied by the root system (A_{root}, m^2; Fig. 2):

Table 2 Fraction of root-respired CO_2 transported via the transpiration stream estimated for different species and for different time periods

Species	Fraction of root-respired CO_2 transported internally (%)	Period considered	Method	Reference
Populus deltoides Bartr.	50.0	Day and night	E_s and F_t flux analysis	Aubrey and Teskey (2009)
Eucalyptus PF1 clone	17.0	Daytime	Isotope analysis	Grossiord et al. (2012)
	24.0	11–15 h (high sap flow)	Isotope analysis	Grossiord et al. (2012)
Quercus robur L.	6	Day and night	Tree girdling and E_s and F_t flux analysis	Bloemen et al. (2014a)
	18.9	12–16 h (high sap flow)	Tree girdling and E_s and F_t flux analysis	Bloemen et al. (2014a)

Estimates based either on measurements of internal CO_2 transport and soil CO_2 efflux (E_s and F_t flux analysis), where the autotrophic contribution was estimated according to literature or tree girdling, or on measurements of the isotope composition of soil CO_2 efflux at natural abundance after C_4–C_3 conversion (isotope analysis)

$$F_{t,scaled} = F_t / A_{root} \quad (3)$$

Aubrey and Teskey (2009) assumed an A_{root} from the consistent stocking of trees in the plantation used in their study. Bloemen et al. (2014a) used data from root excavation measurements on additional trees to estimate the root-occupied soil surface area in their study on young 9-year-old *Quercus robur* trees. While root excavation potentially provides the most accurate determination of A_{root} of younger trees, excavation of the root system of older trees is a difficult task. Therefore, this estimate represents a large source of uncertainty for the comparison of root xylem CO_2 flux relative to soil CO_2 efflux.

Importance of root xylem CO_2 flux in trees

The transport of CO_2 from roots into stems and branches has the potential to affect many processes involved in carbon gain and loss. First, the transport of CO_2 out of roots through the xylem directly affects soil-efflux-based estimates of root respiration and therefore total belowground respiration. Three studies have addressed this issue (Aubrey and Teskey 2009; Grossiord et al. 2012; Bloemen et al. 2014a). Each study used different methodology, but all three reported that the autotrophic component of root respiration (R_a) or soil respiration (R_s) was greater when root xylem CO_2 flux was included compared with what would have been estimated by the efflux of CO_2 from the soil surface alone (Table 2).

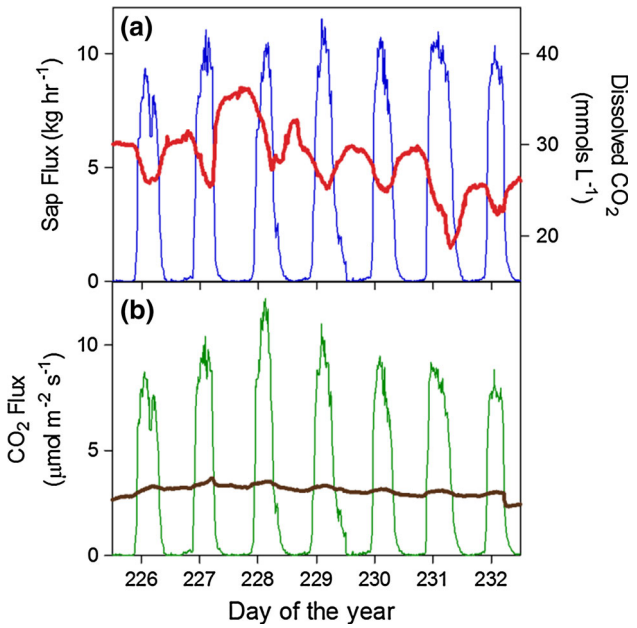

Fig. 3 **a** Pattern of mean tree sap flow (*blue line*) and dissolved CO_2 ($[CO_2^*]$) in xylem sap (*red line*) measured over a 7-day period in four 9-year-old *Populus deltoides* trees growing in a plantation. **b** The mean flux of CO_2 from the root system into the base of the stem of the same trees (*green line*) in comparison to the mean flux of CO_2 from the soil surface (*brown line*) over the same time period (from Aubrey and Teskey 2009)

The first report compared soil CO_2 efflux with xylem CO_2 flux in *Populus deltoides* trees growing in a plantation (Aubrey and Teskey 2009). During most of the daylight period more CO_2 moved internally through the xylem than fluxed from the soil surface (Fig. 3). Over a 7-day period in mid-summer the flux of CO_2 in xylem sap averaged 0.26 mol CO_2 m^{-2} day^{-1}, which was statistically equivalent to the flux of CO_2 from the soil surface, 0.27 mol CO_2 m^{-2} day^{-1}. Heterotrophic (R_h) and autotrophic (R_a) contributions to CO_2 efflux from the soil surface were not separated. Assuming that R_h and R_a contributed equally to the total CO_2 efflux from the soil, R_a greatly exceeded R_h (72 versus 28 %) when xylem CO_2 flux was included in the calculation of total belowground respiration.

The second report investigated the carbon isotope ratio of soil CO_2 efflux in a *Eucalyptus* stand planted on a site previously dominated by a C_4 grass, *Loudetia simplex* (Grossiord et al. 2012). The authors assumed that the heterotrophic ^{13}C signal of soil CO_2 efflux reflected the contribution of the C_4 grass roots to older soil carbon. Since the ^{13}C signal of C_4 plants is enriched in comparison to C_3 plants, the observed increase in ^{13}C enrichment of the soil CO_2 efflux signal during the day, compared with the signal at night, indicated that some of the $^{12}CO_2$ released from respiration of the *Eucalyptus* roots was diverted upward in the xylem during periods of active transpiration. Thus, on average over a 4-day period, 17 % of the total daily CO_2 released by root respiration was not accounted for in the measurements of soil CO_2 efflux during the daytime, with a maximum of 24 % at high rates of transpiration.

The third report examined soil CO_2 efflux and F_T in *Quercus robur* trees subjected to stem girdling compared with non-girdled control trees (Bloemen et al. 2014a). The difference in soil CO_2 efflux between the treatments was used as an estimate of the contribution of R_a. The internal flux of CO_2 in xylem sap was also calculated based on measurements of sap flow and xylem $[CO_2]$. R_a was substantially higher during daylight hours when CO_2 transported through the xylem was also included (Fig. 4). Averaged over a 5-day period in August, R_a accounted for 27 % of total soil CO_2 efflux. When xylem-transported CO_2 was added, R_a increased to 32 % of total belowground respiration. During periods of peak sap flow, 25 % of total soil CO_2 efflux was attributed to R_a, but when root xylem CO_2 flux was included, the R_a estimate increased to 45 % of total belowground respiration.

Although there are currently only three studies available to discuss, it is important to note that all of the studies

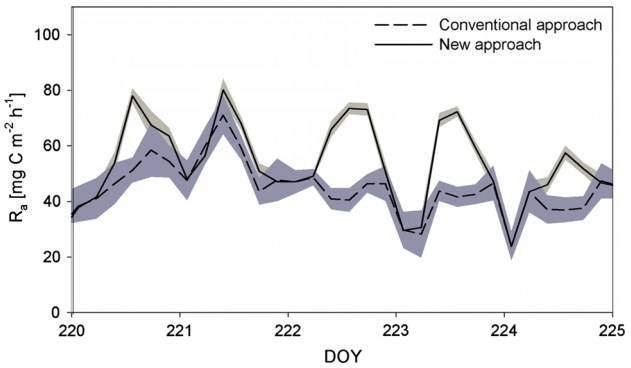

Fig. 4 Estimates of the autotrophic component of belowground respiration (R_a) calculated by the conventional approach based solely on measurements of soil CO_2 efflux (*black dashed line*), or calculated by the new approach, based on combined measurements of E_{soil} and the scaled flux of root-respired CO_2 transported in the transpiration stream (*black line*). The *shaded areas* represent standard deviation (from Bloemen et al. 2014a)

found that a significant quantity of root-respired CO_2 entered the xylem and moved upward in the transpiration stream. The results of these studies indicate that this process is an important component of our mechanistic understanding of carbon flows in trees and forests. Likewise, the variation observed among these studies suggests that there is much more to be learned about the process. There are many possible explanations for the variation in the quantity of CO_2 transported in xylem sap reported in these three studies, regardless of potential variation due to weather conditions (Saveyn et al. 2008). Root systems with large barriers to diffusion will build up high internal [CO_2] and more CO_2 will dissolve in xylem sap. Species with inherently high sap pH, such as *Populus deltoides*, will have higher sap [CO_2^*] than species with lower sap pH. The movement of CO_2 in the xylem was greatest at times when transpiration was highest, suggesting that variation in rates of transpiration among species may also be an important source of variation in the amount of CO_2 transported in sap. The different methods used in the three studies likely played a role in the variable results. The studies were also short in duration (4–7 days) which prevented the identification of seasonal differences in root xylem CO_2 flux.

Xylem-transported CO_2 also has an effect on estimates of R_a and the belowground R_a/R_h ratio. This ratio is important for understanding forest net ecosystem productivity and the contribution of autotrophs and heterotrophs to the forest carbon cycle. However, it is widely recognized that the estimate of R_h is hampered by methodological limitations (Subke et al. 2006). While R_h estimates are uncertain, the R_a estimate is also incorrect unless CO_2 transport in the xylem is included. In addition to the direct measurements described above, indirect evidence of the effects of xylem-transported CO_2 on R_s is mounting. Soil CO_2 efflux was strongly negatively correlated with evapotranspiration in a grassland ecosystem (Balogh et al. 2014) and a daytime depression in R_s was correlated with transpiration in a *Pinus sylvestris* stand, implying that xylem-transported CO_2 contributed to the reduction in R_s (Subke et al. 2009). Yang et al. (2012) found that in six temperate forest types in China, R_s was well correlated with soil temperature in the dormant season but not in the growing season, and suggested that transpiration and xylem transport of CO_2 during the growing season caused the difference.

Another effect of root-derived CO_2 in xylem sap occurs when it is transported into the shoot and increases estimates of stem and branch respiration. The upward movement of dissolved CO_2 in the xylem was first demonstrated in a *Quercus alba* tree in which a solution containing a high concentration of dissolved CO_2 was infused into the stem. The xylem sap [CO_2] above the injection point doubled compared to the initial value and there was a corresponding increase in CO_2 efflux from the stem (Teskey and McGuire 2002). Since then, there have been several studies documenting the contribution of internally transported CO_2 to the efflux of CO_2 from stems (e.g., McGuire and Teskey 2004; Gansert and Burgdorf 2005; Teskey and McGuire 2005; Steppe et al. 2007; Yang et al. 2012). One of those studies suggested that a large portion of the CO_2 that diffused from the stem over a 24-h period had been transported in sap from roots to the point of efflux (Teskey and McGuire 2007), which was first substantiated in a study on *Populus deltoides* trees in a plantation (Aubrey and Teskey 2009). The potential for long distance transport of CO_2 in the xylem was made much clearer when a large quantity of $^{13}CO_2$ was infused into the base of the stem immediately above the root collar of *Populus deltiodes* trees (Bloemen et al. 2013b). After 2 days the trees were harvested and the tissues were examined for the presence of the ^{13}C tracer. The abundance of ^{13}C had significantly increased in all tissues up to the top of the crown, including stem, branches, twigs, and leaves. However, when calculating the amount of ^{13}C recovered compared with the amount introduced into the trees, it was found that 83–94 % of the $^{13}CO_2$ had been lost, i.e., it had diffused into the atmosphere from the stem, branches, and twigs as it was transported upward. This result is a clear indication that xylem-transported CO_2 from roots and locally produced CO_2 both contribute to CO_2 efflux from the stem. Overall, xylem transport of root-respired CO_2 causes an underestimation of R_a, and it also causes an overestimation of stem, branch, and twig respiration determined from measurements of CO_2 efflux. There is some indication that xylem-transported CO_2 may also diffuse out of leaves, and therefore affect estimates of leaf respiration (Bloemen et al. 2013a, 2014b), but more evidence is needed before a conclusion about that can be reached.

Carbon dioxide transported in xylem from roots also contributes to total tree carbon gain. As the CO_2 moves though the xylem and diffuses through the bark, it encounters chlorophyll-containing cells that can use it for photosynthesis. In the Bloemen et al. (2013b) study, up to 17 % of the $^{13}CO_2$ that moved through the shoot was refixed. Of that 17 %, 3 % was fixed in stems, 11 % in branches and 3 % in leaves. The amount of refixation of root-respired CO_2 is likely to be species-dependent. We speculate that species with thick bark that allows little light to reach photosynthetic cells in the stem likely recycle little transported CO_2, while species with thin bark and green stems and branches likely recycle a much larger amount.

In addition to photosynthetic refixation, there is some evidence that the phosphenolpyruvate carboxylase (PEP_C) pathway may be used to recycle xylem-transported CO_2. Recent investigations have shown the importance of PEP_C for non-photosynthetic carbon fixation in stems (e.g., Berveiller et al. 2007; Berveiller and Damesin 2008), but there are few recent studies in roots. Experiments using radioactive carbon isotopes first showed fixation of CO_2 in roots in the mid-twentieth century. Detached barley (*Hordeum vulgare*) roots were submerged in a solution containing $H^{11}CO_3^-$, took up the solution for a 92-min period, and retained almost 5 % of the ^{11}C label as reduced carbon (Overstreet et al. 1940). The exudate from the unsubmerged cut tops of the roots also contained reduced ^{11}C, suggesting that fixed carbon was transported in the xylem stream. Poel (1953) supplied excised barley roots with a solution enriched with $NaH^{14}CO_3$ and found that the bicarbonate was taken up and reduced. Products were organic acids and amines, consistent with fixation in the tricarboxylic acid cycle, which is now known to be supplied with carbon compounds catalyzed by PEP_C. Jackson and Coleman (1959a, b) provided evidence that PEP_c catalyzed the majority of CO_2 fixed in bean (*Phaseolus vulgaris*) roots. In an experiment using cell-free enzyme preparations from stem and root wood of *Robinia pseudoacacia*, Höll (1974) found that $^{14}CO_2$ was transformed into organic compounds in the presence of PEP, but not when PEP was absent, indicating that PEP_c was the carboxylating enzyme. The author concluded that in living trees, respiration was the likely source of CO_2 for the reaction, but that cells surrounding the xylem could also use CO_2 from the transpiration stream. More recently, Hibberd and Quick (2002) reported that C_3 tobacco showed characteristics of C_4 photosynthesis in cells that surround the xylem. They suggested that malate derived from PEP_c activity in the roots was transported through the xylem and decarboxylated at sites of photosynthesis in the stem and petiole, representing a more spatially separated version of the C_4 photosynthetic pathway. All of these studies have indicated the importance of PEP_c-catalyzed carbon fixation in plant tissues, but the magnitude of this fixation pathway in the carbon economy of trees has never been quantified.

Conclusion and future prospects

In trees, a large portion of respired CO_2 remains within the root system and is transported away from the site of respiration via the transpiration stream rather than diffusing into the soil. The decreasing CO_2 gradient from inner root tissues to the soil indicates that under most conditions the internal CO_2 transported through root xylem is mainly derived from root respiration and not from soil DIC uptake. Measurements of $[CO_2]$ in both tree roots and neighboring soil for different tree species will be essential to increase recognition of the importance of root-respired CO_2 to the internal CO_2 flux in trees. Although current studies are limited to a small number of species and short measurement periods, they all indicate that root xylem CO_2 flux represents an important unaccounted-for component of root respiration and call into question our contemporary understanding of tree carbon cycling. These studies highlight the need to measure root xylem CO_2 flux, as well as the amount of root-respired CO_2 diffusing through the soil, to accurately determine R_a and R_s. Since a substantial fraction of the root-derived CO_2 may diffuse from stem and branch tissues into the atmosphere, current methods for estimating autotrophic respiration in above-and below-ground tissues based on CO_2 efflux into the atmosphere are likely to be inaccurate. Laser-based $^{13}CO_2$ efflux measurements in combination with stem isotope labeling as performed by Bloemen et al. (2013b) could be used to accurately quantify aboveground efflux of xylem-transported root-respired CO_2. Moreover, studies of root xylem CO_2 flux on additional species, in varied ecosystems, and over longer time periods have the potential to redefine our understanding of the energetic costs of tree root metabolism and our assessment of the components of tree and forest carbon cycles.

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