What's the flux? Unraveling how CO_2 fluxes from trees reflect underlying physiological processes

Tree stems and branches emit carbon dioxide (CO_2) at rates that per unit area can rival emissions from leaves or the soil surface and summed over a forest stand can comprise 14–30% of the total CO_2 efflux (Chambers *et al.*, 2004; Ryan *et al.*, 2009). Stem CO_2 fluxes have predictable patterns of variation with growth rate, stand age, and elevation (Chambers *et al.*, 2004; Ryan *et al.*, 2009; Robertson *et al.*, 2010). Over the past decade observations of diel covariation of CO_2 efflux with sapflux rates measured in tree stems have led to the conclusion that internal transport of CO_2 within the stem strongly influences the measured CO_2 efflux at the surface (Teskey *et al.*, 2008). In this issue of *New Phytologist*, Bloemen *et al.* (pp. 555–565) report on a tracer experiment that demonstrates not only upward transport of $^{13}CO_2$ added to the transpiration stream, and emission of this label along the stem, but also fixation of a significant fraction of the added CO_2 in canopy branches, petioles and, to a minor extent, leaves. The study of Bloemen *et al.* adds to the growing literature that demonstrates the utility of isotope labeling studies to understand allocation and carbon (C) cycling in trees (Powers & Marshall, 2011; Epron *et al.*, 2012).

'*Dynamic approaches for measuring continuous diurnal CO_2 fluxes and transport in the transpiration stream need to be more widely applied.*'

Processes influencing stem CO_2 efflux

A number of factors can influence the efflux of CO_2 measured by a flux chamber covering a segment of tree stem (Fig. 1). The cambium is the site of formation of new tissue, that is, of growth, while maintenance respiration produces CO_2 in all living tissues. The C being respired may derive from recent photosynthetic products transported in the phloem (e.g. Powers & Marshall, 2011) and from storage reserves. The pathways for respiration may vary with time or tree species: recently $^{18}O/^{16}O$ measurements in oxygen (O_2) provided the first evidence for the alternative oxidase pathway contributing to respiration in some tree stems (Angert *et al.*, 2012a). CO_2 may also be locally fixed by photosynthetic tissues found under the bark before it is lost to the atmosphere.

Low rates of diffusion, especially across the cambium, can cause high CO_2 concentrations in stems, and internal O_2 concentrations can drop to very low levels (Spicer & Holbrook, 2005; Teskey *et al.*, 2008). CO_2 is highly soluble, and will dissolve in (or exsolve from) stem water, depending on local saturation conditions, which in turn are controlled by factors such as temperature and pH. Uptake of CO_2 directly from the soil atmosphere, once thought potentially important, has largely been shown to be minor (see summary in Bloemen *et al.*). Hence the source of CO_2 emitted to the atmosphere from the bark surface can reflect a combination of local growth and maintenance respiration, other local processes producing CO_2 (including potentially decomposition in heartwood) or CO_2 from respiration in other tissues (e.g. roots) that has been transported into the volume beneath a chamber in solution. However, there can also be net export in the xylem water stream, as indicated by the fate of the tracer added by Bloemen *et al.* The measured chamber flux at any given time is thus the complex result of transport in, transport out and respiration minus photosynthesis in local tissues. Use of a dark chamber will exclude local photosynthesis.

Observations of a relationship between sapflux and CO_2 efflux provide a clue as to whether CO_2 is net imported or exported from the volume of stem under a chamber attached to the stem surface (see Fig. 1, modified from Teskey *et al.*, 2008). Other evidence for net CO_2 transport away from the region of efflux measurement comes from lower-than-expected efflux rates compared with what is expected given the construction costs of wood (Ryan *et al.*, 2009), and potentially from higher efflux rates in canopy branches (Teskey *et al.*, 2008). Changes in local temperature and/or pH can change respiration rates and also cause changes in CO_2 solubility (Kunert & Mercado Cárdenas, 2012).

Stem anatomy, including bark thickness and tree hydraulics, likely influences the importance of the mechanisms and can help explain observations such as changes in CO_2 efflux with stand age or tree size, or differences between similar trees growing in different environments (Ryan *et al.*, 2009). Bloemen *et al.* report results from labeling *Populus deltoides*, the eastern cottonwood tree, which has very high transpiration rates and generally is found in riparian zones. As noted by Ubierna *et al.* (2009) most studies that have reported relationships between sapflux and CO_2 efflux have been made in tree species with high sapflux rates and small conducting area. By contrast, the large conifer trees investigated by Ubierna *et al.* (2009), with lower overall sapflux, did not demonstrate such relationships, and even crown removal did not change the rates of CO_2 efflux from stems they studied.

What do these results mean for interpretation of other ecosystem CO_2 efflux measurements?

A major conclusion of Bloemen *et al.* is that the transport of the tracer from the tree base to the canopy indicates that root respiration

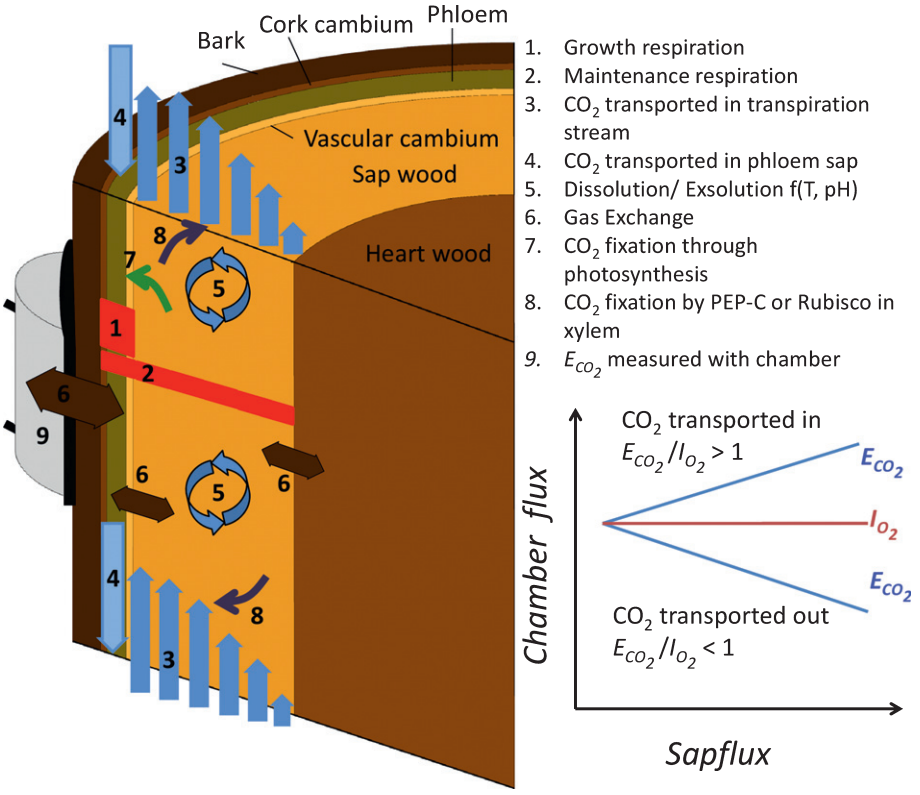

Fig. 1 Sources, sinks and transport processes in tree stems result in the net CO_2 flux observed when a chamber encloses part of the stem surface (modified after Teskey *et al.*, 2008). The degree to which the stem CO_2 efflux records the underlying process of respiration (minus any fixation) depends on the saturation state and rate of transport of the xylem water. This balance can change from day to night and with altered temperature as sun warms the outer stem. Inset: Relationship between CO_2 efflux (E_{CO_2}) and O_2 influx (I_{O_2}) in the cases of net soluble C transport into or out of the volume covered by a stem respiration chamber. At times of low transport (e.g. at night), the two fluxes should be approximately equal. Note that, if respiration is the main process, a local imbalance in E_{CO_2}: I_{O_2} means that there must be a compensating imbalance elsewhere – e.g. if CO_2 is transported to the canopy and emitted without consumption of O_2.

can be a source of at least some of the CO_2 emitted in the canopy. While the high CO_2 concentrations at the base of trees do argue for a belowground source, Bloemen *et al.* did not successfully introduce enough label via roots to demonstrate definitively the transfer of root CO_2 up the stem. Aubrey & Teskey (2009) have argued that up to 50% of root respired CO_2 may be transported upward and diffuse out higher in the tree stem or in branches. Grossiord *et al.* (2012), using isotopic differences to distinguish plant and decomposition derived soil respiration, detected a daytime reduction in autotrophic respiration from soil, albeit the 'missing' root respiration they infer is transported up the tree stem amounted to only a 17% underestimation of the autotrophic CO_2 efflux on a daily basis.

Tracer studies by Powers & Marshall (2011) as well as Bloemen *et al.* show that ^{13}C-labeled CO_2 added to the xylem stream indeed is transported upward, emitted and a fraction refixed in the canopy. In the Bloemen *et al.* study, an estimated 6–17% of the added tracer was fixed in photosynthetic tissues in branches and petioles. Hence recycling of CO_2 within the plant is potentially quite important – perhaps especially so when CO_2 concentrations in the atmosphere were lower than those of today (Teskey *et al.*, 2008).

Hibberd & Quick (2002) provide an additional mechanism for internal C transport, based on the capture of CO_2 by PEP-carboxylase, after which it can be removed from the site of respiration as malate. Their labeling experiments indicate that malate transported in the xylem enters the bundle-sheath cells, and can be used for photosynthesis, in this 'C_4 like' mechanism. Bloemen *et al.* found that most of their labeled CO_2 was fixed in branches and in leaf petioles, which agrees well with transported C being fixed in bundle-sheath cells.

How can we derive an estimate of the 'real' stem respiration flux?

The various effects of temperature and transpiration velocity can affect CO_2 efflux rates over a day–night cycle. One way to estimate fluxes might be to choose to sample at night, when transpiration flux is near zero (Teskey & McGuire, 2002). However, this is also the coolest time of day, so this might underestimate daytime respiration in tissues (such as the cambium) that may warm significantly over the daytime period (Kunert & Mercado Cárdenas, 2012). A second method is to measure CO_2 evolution or O_2 uptake (Teskey & McGuire, 2002; Spicer & Holbrook, 2005) on excised wood. Apart from damaging the tree (or the tissues with heat generated on sampling), such methods must be used with care as the degassing of high CO_2 in wood pores can initially yield too-high CO_2 fluxes (Teskey & McGuire, 2002).

Another possibility is to use *in situ* O_2 uptake as a measure of respiration (Angert & Sherer, 2011). Because O_2 is much less soluble in water than CO_2, the molar flux of O_2 into stems should roughly equal that of CO_2 out if transport is minimal, given the stoichiometry of the respiration substrate in most woody tissues. In cases where CO_2 respired elsewhere is transported and emitted in the stem and canopy, we would expect CO_2 release to exceed O_2 uptake (i.e. $E_{CO_2}/I_{O_2} > 1$). In cases where locally derived CO_2 is exported, the ratio of CO_2 release to O_2 uptake will be ≤ 1 (Fig. 1 inset).

In Amazonian tropical forest trees, Angert *et al.* (2012b) found the CO_2 efflux from the stems was on average only 0.66 ($\pm$ 0.18) of the O_2 influx, in other words about one third of the CO_2 respired in the stem section beneath the chamber is not locally emitted, but transported away. Some of this 'removed CO_2' can be carried by the xylem stream, as shown by Bloemen *et al.* However, the flux of CO_2 that can be removed in this way is constrained by the chemistry of the carbonate system, and is governed by the xylem pH which is seldom > 7 (Teskey *et al.*, 2008). Angert *et al.* (2012b) concluded, based on estimates of stem [CO_2] and xylem pH, that the rate of dissolved inorganic C export might not remove CO_2 at the rate required, and suggest that the C might be exported in organic (e.g. malate), rather than inorganic form.

Progress in understanding the sources and magnitudes of CO_2 fluxes in tree stems is being made rapidly, and linking actively respiring tissues local to, and remote from, the point of measurement. Future studies taking advantage of pulse labeling in trees with different water-use strategies would be useful for resolving conditions where stem xylem water transport in stems significantly impact soil CO_2 efflux. Radiocarbon measurements of stem CO_2 can help resolve questions about whether the C being respired (and potentially translocated) derives from storage reserves vs fresh photosynthetic products. Dynamic approaches for measuring continuous diurnal CO_2 fluxes and transport in the transpiration stream need to be more widely applied. New sensor methods for O_2 measurement can add information that allows separation of transport from local physiological processes. Future studies should also focus on quantitative measurements of the photosynthetic fraction supported by both inorganic, and organic C, transported internally in the xylem.

Meanwhile, we need to be careful about invoking the process 'respiration' when really we are measuring CO_2 flux. Ultimately the CO_2 emitted from a stem is produced by physiological processes, but the challenge remains identifying what portion is produced by local tissues, which will facilitate much-needed mechanistic understanding of factors controlling autotrophic respiration.

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