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Insight

The quandary of sources and sinks of CO_2 efflux in tree stems—new insights and future directions

Roberto L. Salomón[1,2,†*], Juliane Helm[3,4,†], Arthur Gessler[5,6], Thorsten E. E. Grams[7], Boaz Hilman[3], Jan Muhr[8], Kathy Steppe[2], Christiane Wittmann[9] and Henrik Hartmann[3,10]

[1]Universidad Politécnica de Madrid (UPM), Departamento de Sistemas y Recursos Naturales, Research Group FORESCENT, Antonio Novais 10, 28040, Madrid, Spain
[2]Department of Plants and Crops, Laboratory of Plant Ecology, Ghent University, Faculty of Bioscience Engineering, Coupure Links 653, 9000 Ghent, Belgium
[3]Max-Planck-Institute for Biogeochemistry, Biogeochemical Processes, Hans-Knöll-Str. 10, 07743 Jena, Germany
[4]Department of Environmental Sciences – Botany, Basel University, Schönbeinstr. 6, Basel CH-4056, Switzerland
[5]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zurcherstrasse 111, 8903 Birmensdorf, Switzerland
[6]Institute of Terrestrial Ecosystems, ETH Zürich, Rämistrasse 101, 8902 Zurich, Switzerland
[7]Technical University of Munich, Ecophysiology of Plants, Land Surface – Atmosphere Interactions, Von-Carlowitz-Platz 2, 85354 Freising, Germany
[8]Department of Forest Botany and Tree Physiology, Laboratory for Radioisotopes, Georg-August Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[9]Faculty of Biology, Botanical Garden, University of Duisburg-Essen, Universitätsstrasse 5, 45117 Essen, Germany
[10]Institute for Forest Protection, Julius Kühn Institute Federal Research Centre for Cultivated Plants, Erwin-Baur-Straße 27, 06484 Quedlinburg, Germany

*Corresponding author (roberto.salomon@upm.es)

†Shared first authorship.

Stem respiration (R_S) substantially contributes to the return of photo assimilated carbon to the atmosphere and, thus, to the tree and ecosystem carbon balance. Stem CO_2 efflux (E_{CO_2}) is often used as a proxy for R_S. However, this metric has often been challenged because of the uncertain origin of CO_2 emitted from the stem due to post-respiratory processes. In this *Insight*, we (i) describe processes affecting the quantification of R_S, (ii) review common methodological approaches to quantify and model R_S and (iii) develop a research agenda to fill the most relevant knowledge gaps that we identified. Dissolution, transport and accumulation of respired CO_2 away from its production site, reassimilation of respired CO_2 via stem photosynthesis and the enzyme phosphoenolpyruvate carboxylase, axial CO_2 diffusion in the gas phase, shifts in the respiratory substrate and non-respiratory oxygen (O_2) consumption are the most relevant processes causing divergence between R_S and measured stem gas exchange (E_{CO_2} or O_2 influx, I_{O_2}). Two common methodological approaches to estimate R_S, namely the CO_2 mass balance approach and the O_2 consumption technique, circumvent some of these processes but have yielded inconsistent results regarding the fate of respired CO_2. Stem respiration modelling has recently progressed at the organ and tree levels. However, its implementation in large-scale models, commonly operated from a source-driven perspective, is unlikely to reflect adequate mechanisms. Finally, we propose hypotheses and approaches to advance the knowledge of the stem carbon balance, the role of sap pH on R_S, the reassimilation of respired CO_2, R_S upscaling procedures, large-scale R_S modelling and shifts in respiratory metabolism during environmental stress.

Keywords: apparent respiratory quotient, cortical photosynthesis, $O_2$2 influx, PEPC, stem respiration modelling, xylem CO_2 transport.

Introduction

Plant autotrophic respiration substantially contributes to the ecosystem's carbon (C) balance (Luyssaert et al. 2007). During the last decade, plant autotrophic respiration released approximately six times the amount of CO_2 emitted from fossil fuel burning, ca 60 and 10 Pg C $year^{-1}$, respectively (Friedlingstein et al. 2022). As the tree stem comprises most of the biomass in plant woody species (Poorter et al. 2012), a large fraction of plant autotrophic respiration occurs in the living tissues of the stem, with stem respiration (R_S) estimates accounting for 5–35% of ecosystem respiration (Campioli et al. 2016, Salomón et al. 2017). Globally, R_S has been estimated to be 6.7 ± 1.1 Pg C $year^{-1}$ (Yang et al. 2016), in the same order of magnitude as CO_2 fossil fuel emissions. Despite the importance of R_S in the ecosystem and global C budgets, we can still not accurately estimate R_S, given the uncertainties related to the origin and fate of gases (CO_2 and O_2) involved in stem respiration (Teskey et al. 2008, 2017, Trumbore et al. 2013). Therefore, a more comprehensive understanding of the CO_2 and O_2 fluxes and processes involved in stem respiratory physiology would help establish a universal measurement protocol, which could eventually facilitate comparison among studies and refine plant respiration estimates in large-scale models.

Aerobic respiration requires O_2 and respiratory substrates, while CO_2 and water are released as by-products of this catabolic reaction (Kader and Saltveit 2002). Therefore, CO_2 efflux (E_{CO_2}) and O_2 influx (I_{O_2}) can potentially be used as metrics of respiration rates. Respiration can be easily determined from the CO_2 release or the O_2 consumption in isolated mitochondria and cells, but it becomes more complicated when measuring respiration rates of plant organs in situ.

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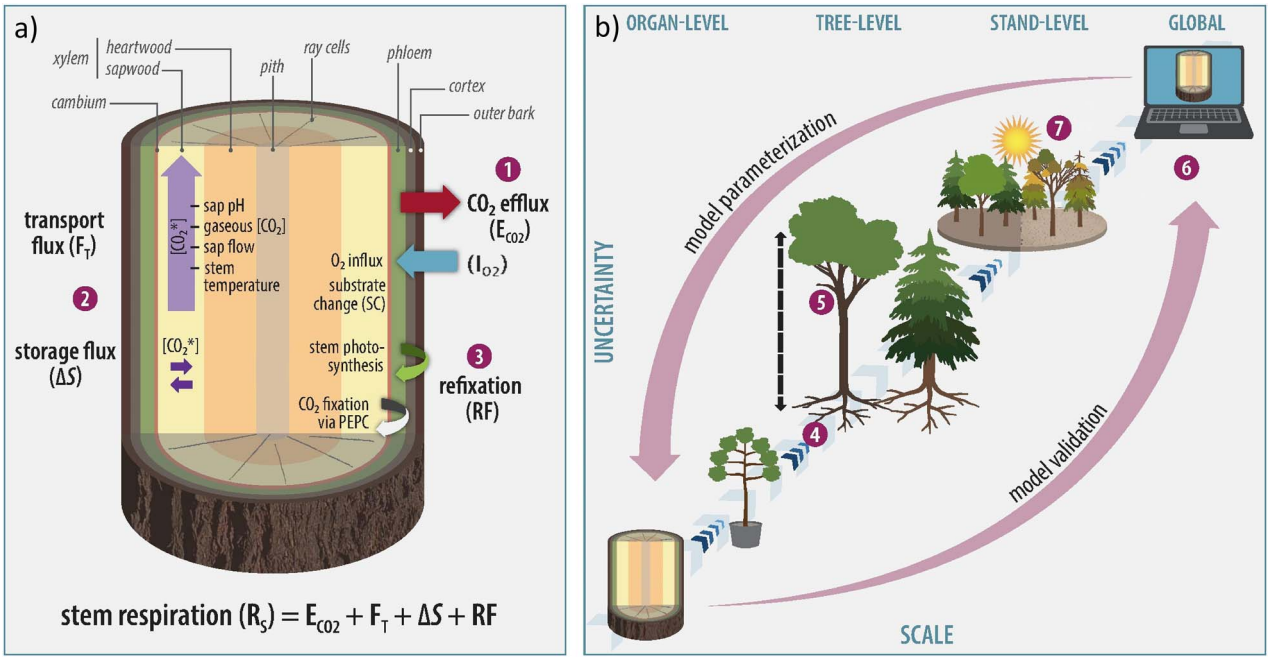

Figure 1. Schematic of stem post-respiratory processes that affect stem respiration (R_S) estimates (a), and research needs and upscaling procedures to improve estimates of R_S at different spatial scales (a, b). (a) A tree stem section highlighting respiratory fluxes, namely stem CO_2 efflux (E_{CO_2}), stem O_2 influx (I_{O_2}), CO_2 flux through the xylem (F_T), CO_2 storage (ΔS) and CO_2 refixation (RF). Numbers in circles refer to specific items in the research agenda: ❶ combination of the extended mass balance approach ($R_S = E_{CO_2} + F_T + \Delta S + RF$) with I_{O_2} measurements helping to elucidate the fate of respired CO_2.❷ Accurate measurements of sap pH are key for quantifying the DIC in the sap solution ([CO_2*]). ❸ Refixation (RF) of respired CO_2 via SP or PEPC enzyme causes differences between stem surface measurements (E_{CO_2} and I_{O_2}) and R_S. (b) Upscaling from organ-level measurements to forest stands and above. The upscaling procedure involves ❹ comparing processes in saplings and mature trees and ❺ scaling up to the whole-tree level. ❻ The upscaling ladder of model parameterization and validation can be implemented in stem to stand level respiration models, progressively improved by 'ground-truthing' with observational and experimental data at larger spatial scales (red arrows). ❼ Environmental factors should be accounted for in R_S studies. Uncertainty in R_S estimates increases with observational scale and knowledge gaps from the organ to the global level.

Tree stems consist of a superposition of several tissues, namely the bark, cortex, phloem, cambium and xylem, all containing living cells that consume O_2 and release CO_2 during respiration (Figure 1a). However, CO_2 and O_2 fluxes measured at the stem surface may not accurately reflect the respiration rates of tissues underneath (Teskey et al. 2008, 2017, Trumbore et al. 2013). Yet, E_{CO_2} is widely used as a proxy of R_S for methodological simplicity, which can lead to confounding net gas fluxes with stem respiration.

Several processes preclude respired CO_2 from being locally emitted and atmospheric O_2 from being locally consumed. For instance, respired CO_2 can dissolve in the xylem sap solution and be transported and stored, impeding its radial diffusion to the atmosphere (Teskey et al. 2008, 2017). This is less of a problem when I_{O_2} is used as a proxy of R_S because of the lower O_2 solubility in water. Another process causing further divergences between R_S and both E_{CO_2} and I_{O_2} is the reassimilation of respired CO_2 through stem photosynthesis (SP; Avila et al. 2014, Steppe et al. 2015, Berry et al. 2021). By contrast, reassimilation of CO_2 via the phosphoenolpyruvate carboxylase (PEPC) enzyme only decouples R_S from E_{CO_2} but not from I_{O_2}, as O_2 is not involved in the PEPC-mediated fixation of inorganic carbon (Angert et al. 2012*b*, Hilman et al. 2022). Less explored processes that can decouple E_{CO_2} and I_{O_2} measurements from actual R_S will be addressed in detail in a later section.

The complexity of processes simultaneously occurring in tree stems confounds the interpretation of CO_2 and O_2 fluxes measured at the stem surface. No methodological approach can disentangle all these processes simultaneously, allowing a straightforward measurement of R_S in situ, defined here from a biochemical perspective as the O_2 consumption and CO_2 production through oxidative catabolic pathways. Therefore, there is no scientific consensus on measuring (and even defining) R_S (see Box 1 in O'Leary et al. 2019), which greatly hinders progress in this research field. Uncertainties and technical challenges in R_S estimation at the site of measurement propagate all the way through to quantification of woody tissue respiration at the whole-tree level, including branch and coarse roots respiration, and to modelling of tree respiration at the ecosystem and larger spatial scales (Meir et al. 2017). In addition, despite recent advances in the mechanistic modelling of R_S at the organ and tree level (Hölttä and Kolari 2009, Schiestl-Aalto et al. 2015, Salomón et al. 2020), terrestrial biosphere models (TBMs) are still lacking realistic implementations of woody tissue respiration (Atkin et al. 2017, Fatichi et al. 2019), probably due to pertaining paradigm of source-driven tree C cycling (Fatichi et al. 2014, Zuidema et al. 2018, Cabon et al. 2022).

In this *Insight*, we aim to provide an updated overview of processes that confound the interpretation of stem CO_2 and O_2 fluxes as proxies of R_S (Section 1). We compile results from methodological approaches that circumvent confounding processes, including mechanistic modelling, as a means to integrate theory and observational data (Section 2). Finally, we propose a detailed research agenda with observational and experimental studies, technical and analytical tools, and modelling approaches to overcome sources of uncertainty in R_S estimates and eventually improve R_S upscaling procedures (Section 3).

Table 1. Summary of processes affecting CO_2 efflux and O_2 influx measurements at the stem surface (E_{CO_2} and I_{O_2}) as stem respiration (R_S) proxies.

Process	R_S vs E_{CO_2}	R_S vs I_{O_2}
CO_2 (and O_2) dissolution and transport and storage in the xylem	(+) Local net import (−) Local net export	(±) (1/30 of the effect on CO_2)
Refixation of respired CO_2 via stem photosynthesis	(−) Consumption	(−) Production
Refixation of respired CO_2 via PEPC enzyme	(−) Consumption	
Light-induced axial CO_2 diffusion	(+) Local net import (−) Local net export	
Shifts in the respiratory substrate		(+) Lipid-dominated R_S
Non-respiratory O_2 consumption		(+) Consumption

The difference between actual R_S and the gas (CO_2 or O_2) flux measurement at the stem surface in the second and third columns, respectively, denotes R_S overestimation (+), underestimation (−), limited effect (±) and no effect (empty spaces).

Section 1: Processes and fluxes affecting stem respiration estimates

Table 1 summarizes processes that hinder the straightforward measurement of the respiratory activity of living tissues underneath the stem surface. The following subsections describe these processes individually, some illustrated in Figure 1a.

CO_2 transport and storage in the xylem

Tree stems link the canopy to the root system, transporting mainly water and nutrients upwards through the xylem while distributing carbohydrates and other solutes downwards through the phloem. The vertical acropetal transport pathway in the xylem is essential for understanding the origin and fate of respired CO_2. For more than 40 years, we have known that the dissolution of respired CO_2 in the xylem sap and its subsequent transport or storage impede its radial diffusion to the atmosphere (Negisi 1979, Hari et al. 1991). According to Henry's law, the partial pressure of gaseous CO_2 in the xylem gas spaces is in equilibrium with the CO_2 concentration dissolved in the xylem sap, with the degree of dissolution being strongly dependent on sap temperature and pH. The dissolved inorganic carbon (DIC) includes carbonic acid (H_2CO_3) and two deprotonated forms, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (McGuire and Teskey 2002).

After dissolution in the xylem sap, CO_2 can be transported upwards through the transpiration stream (see ❷ in Figure 1a), as evidenced by isotopic labeling (e.g., McGuire et al. 2009, Powers and Marshall 2011, Bloemen et al. 2013, Salomón et al. 2021). Therefore, locally emitted CO_2 at a certain stem level may originate from respiration below the measurement point, such as the roots or lower parts of the stem. In this scenario of a net import of CO_2 into the monitored stem segment, E_{CO_2} measurements will overestimate actual R_S. Alternatively, locally respired CO_2 can be transported upwards to the upper part of the stem and the canopy, diffusing into leaf-internal air spaces and the atmosphere away from the CO_2 production site (Hanson et al. 2016). In this scenario of net export of respired CO_2, E_{CO_2} measurements would underestimate R_S. Import and export of respired CO_2 in and out of the monitored stem segment likely co-occur under natural conditions according to dynamic vertical and radial gradients of [CO_2] along the stem and across the stem-atmosphere continuum. Accumulation and depletion of dissolved CO_2 over time can also occur, again causing R_S under- and over-estimation when E_{CO_2} measurements are used as proxies of respiratory activity (McGuire and Teskey 2004). However, the storage flux has been consistently evaluated to be of limited relevance (Section 2). Because the solubility of O_2 in water is approximately 30 times lower than that of CO_2 (Dejours 1981), I_{O_2} largely circumvents the issue of O_2 import or export at the site of measurement (discussed in detail in Section 2) and would be more appropriate to reflect actual R_S in the absence of any other process listed below.

Refixation of respired CO_2 via stem photosynthesis

Photosynthesis in twigs is considered near-ubiquitous in angiosperm species (Rosell et al. 2015), and more than 300 species spanning different biomes are considered capable of conducting SP (see ❸ in Figure 1a) (Berry et al. 2021). The process consumes endogenous CO_2 (Sprugel 1991, Pfanz et al. 2002, Saveyn et al. 2010, Avila et al. 2014, Steppe et al. 2015) and exogenous CO_2 in cases of stems with the presence of stomata, lenticels or cracks, which can facilitate the diffusion of atmospheric CO_2 through epidermal or peridermal tissues (Avila et al. 2014). Endogenous CO_2 originates from respiring cells in the cortex, phloem, cambium and xylem parenchyma. When illuminated, chloroplast-containing cells assimilate part of the xylem CO_2 (Strain and Johnson 1963, Pfanz et al. 2002, Wittmann et al. 2006), which has much higher concentrations than in the atmosphere, commonly one to two orders of magnitude (see Stutz and Anderson 2021 for a recent data compilation), due to the high diffusion barriers of stem tissues (Sorz and Hietz 2006, Steppe et al. 2007, 2015, Salomón et al. 2021).

Stem photosynthesis is often quantified as the difference between E_{CO_2} under light and dark conditions (e.g., Cernusak and Marshall 2000, Saveyn et al. 2010, Bloemen et al. 2016, Tarvainen et al. 2017, De Roo et al. 2020*a*, 2020*b*). Refixation of respired CO_2 via SP can range between 7% and 123% of E_{CO_2} (reviewed by Avila et al. 2014), with the latter value implying a net C gain, and varies with species, stem age (Aschan et al. 2001, Damesin 2003, Wittmann and Pfanz 2008) and vertical position in the stem (Tarvainen et al. 2017). Nevertheless, most studies have been performed in young, green twigs, while data from mature trees with thick bark are relatively scarce (but see Strain and Johnson 1963, Tarvainen et al. 2017), for which refixation rates are substantially lower (Wittmann and Pfanz 2008, Vick and Young 2009). The decline in SP with stem ageing is attributed to changes in structural and functional traits, such as bark optical properties, chlorophyll and nitrogen content, and the area-to-mass ratio of the stem cortex (Cernusak and Marshall 2000,

Wittmann and Pfanz 2008). In addition to contributing to the tree C economy, SP increases the cortical O_2 concentration under illumination and counteracts temporal/spatial hypoxia within woody tissues (Wittmann and Pfanz 2014, 2018) and constitutes an alternative C source under drought conditions when leaf photosynthetic activity is limited, thereby reducing the risk of C starvation and hydraulic failure (Vandegehuchte et al. 2015, Cernusak and Cheesman 2015, De Baerdemaeker et al. 2017, Ávila-Lovera et al. 2018, De Roo et al. 2020*a*).

Stem photosynthesis affects both E_{CO_2} and I_{O_2} due to the CO_2 consumption and O_2 production occurring during the Calvin cycle and the light reactions, respectively. It can completely offset the CO_2 respiratory outflow in young twigs (Avila et al. 2014), reduce it by 50% in 4-m height trees (De Roo et al. 2020*b*), by 25% in the upper part of the stem of mature pines, or have a negligible effect at breast height (Tarvainen et al. 2017). Given this enormous variability, SP is critical when interpreting the net gas (CO_2 and O_2) exchange measured at the stem surface. Nevertheless, most studies assessing stem E_{CO_2} and I_{O_2} intentionally avoid local SP by using opaque chambers or cuvettes (see, e.g., references in Table 2) covered with aluminium foil or any other solar reflective material.

Light-induced axial CO_2 diffusion

More commonly ignored is the effect that SP can have above and below the opaque chamber, indirectly affecting the CO_2 emission at the measurement point. Assuming that the intercellular air spaces in the cortex are interconnected, internal [CO_2] gradients may develop along the light/shade boundaries above and below the stem opaque chamber. Internal CO_2 may accumulate in shaded stem sections, while CO_2 may deplete in illuminated areas due to SP. Shading or illumination of a stem outside a clamp-on chamber could then affect internal [CO_2] gradients in the axial direction, altering the radial E_{CO_2} of the stem section enclosed in the opaque chamber. This process was first suggested by Saveyn et al. (2008*a*, 2008*b*) when observing light-induced reductions in temperature-normalized E_{CO_2} during the dormant season in deciduous oaks. Under these conditions, xylem CO_2 transport and reduced cell turgor could not explain such E_{CO_2} decreases. To directly provide evidence of this effect, De Roo et al. (2019) covered in one experiment the whole stem of 4-year-old oak trees with aluminium foil to inhibit SP. Then, stem segments above the opaque chamber were temporarily uncovered and illuminated to enable SP. Illumination immediately above the measurement chamber induced E_{CO_2} reductions of up to 22%, progressively decreasing with the distance from the chamber (De Roo et al. 2019). Similar results were achieved in 4-year-old aspen trees, with E_{CO_2} reductions of 10–25% associated with SP of stem segments above and below the opaque chamber (De Roo et al. 2020*b*). These experiments provided evidence for the non-negligible effect of artifactual, light-induced vertical [CO_2] gradients in E_{CO_2} measurements. We could expect a similar mechanism to hold for O_2, with lower internal [O_2] underneath the opaque chamber altering vertical gradients and I_{O_2} measurements. However, we are unaware of any studies that have addressed this specifically. Here, it is worth noting that while light-induced axial CO_2 diffusion in the gas phase can be quantitatively relevant in young trees with thin bark that allows light to penetrate, this might be less of an issue in mature trees with thick bark and presumably negligible SP rates.

Refixation of respired CO_2 via PEPC enzyme

Phosphoenolpyruvate carboxylase is a ubiquitous cytosolic enzyme (Chollet et al. 1996) that is present in plants, green algae and cyanobacteria (O'Leary et al. 2011). It is the main substrate provision mechanism for photosynthetic C assimilation in C4 and CAM plants (Nimmo 2006, Gowik and Westhoff 2011, O'Leary et al. 2011). Phosphoenolpyruvate carboxylase also plays a central role in CO_2 fixation in anaplerotic metabolic pathways to replenish the tricarboxylic acid (TCA) cycle intermediates in all plant tissues, independently of their photosynthesis type (Chollet et al. 1996, Berveiller et al. 2007, Werner and Gessler 2011, Abadie and Tcherkez 2019). Biochemically, bicarbonate (HCO_3^-) binds to phosphoenolpyruvate (PEP), and the resulting oxaloacetate (OAA) is transformed into organic acids, e.g., malate and aspartate (Chollet et al. 1996). As it occurs with respired CO_2, the fate of the PEPC-mediated malate production is uncertain (Hilman et al. 2019). Malate can be locally processed in the TCA cycle of respiring cells, releasing back CO_2. It can also be transported through the xylem via the transpiration stream (Schill et al. 1996, Patonnier et al. 1999) and increase the malate pool in leaves (Gessler et al. 2009), causing light-enhanced dark respiration during day–night transitions (Werner and Gessler 2011). Alternatively, malate can be loaded into the phloem sieve tubes and exported downwards (Hoffland et al. 1992). In fact, malate contributed up to 2% of the phloem C pool in several tree species (Gessler et al. 2013), and phloem-transported malate can reach the root system (Touraine et al. 1992), releasing it into the soil solution as an exudate (Shane et al. 2004).

Phosphoenolpyruvate carboxylase can fix bicarbonate in stem tissues without chloroplasts-containing cells and under dark conditions (see ❸ in Figure 1a). Yet, little attention has been paid to its role as a post-respiratory sink of respired CO_2. Anaplerotic fixation in woody tissues was first evidenced in an 18-year-old *Robinia pseudoacacia* tree trunk with ^{14}C-labelled CO_2 incorporated into PEPC downstream metabolites (Höll 1974). Similarly, Hibberd and Quick (2002) demonstrated a C4-like recycling mechanism mediated by PEPC in petioles and stems of C3 tobacco plants, which may be common in other C3 species also, as high activity of PEPC has been shown in 25- to 50-year-old stems of nine woody species, including angiosperms and gymnosperms (Berveiller and Damesin 2008). As for SP, most studies assessing PEPC activity in non-foliar tissues have been performed in young stems and petioles, which are likely metabolically more active than the tree trunks of mature trees. Therefore, quantitative assessments of the amount of respired CO_2 fixed by PEPC remain highly uncertain at the whole-tree level. Recently, it was observed that PEPC capacity in stems of 130-year-old beech trees was of the same order of magnitude as that previously reported in current-year twigs (Helm et al. 2023), which calls for further research in this line. Two factors can limit the relevance of PEPC fixation in stem C budgets: (i) the limited pH buffer capacity in the cell cytoplasm may constrain the cell capacity to produce and store organic acids (Spicer and Holbrook 2007), eventually downregulating PEPC-mediated CO_2 fixation, and (ii) in species with relatively acidic sap pH (<6), not uncommon in woody species (Teskey et al. 2008),

Table 2. Summary of published studies on the relative contribution of CO_2 efflux (E_{CO_2}), xylem CO_2 transport (F_T) and CO_2 storage (ΔS) to stem respiration (R_S) applying the mass balance approach, including tree features (species, age and size) and methodological aspects to measure E_{CO_2} (open or closed configuration), F_T (xylem [CO_2], sap flow and pH measurement) and ΔS.

E_{CO_2}/R_S	F_T/R_S	$\Delta S/R_S$	Tree species/age (year)/diameter (cm)	Treatment/DOY	Method E_{CO_2}	Method F_T	Reference (Note)
0.77	0.15	0.08	*Fagus grandiflora*, 15.1 cm		Open configuration	CO_2 microelectrode TDP	(McGuire and Teskey 2004)
0.45	0.55	0.00	*Platanus occidentalis*, 10.2 cm			Sap pH from twigs	
0.83	0.14	0.02	*Liquidambar styraciflua*, 14.5 cm				
0.88	0.11	0.01	*Dacrydium cupressinum*, 18–67 cm		Open configuration	F_T predicted from sap flux density (No xylem [CO_2] readings)	(Bowman et al. 2005)
0.71	0.29		*Platanus occidentalis*	Low sap velocity	Open configuration	CO_2 microelectrode	(McGuire et al. 2007)
0.30	0.70		1–3 cm branches	High sap velocity		Sap pH from branch downstream end	
0.72	0.19	0.02	*Platanus occidentalis* mature, 19.5–24.8 cm		Open configuration	NDIR sensor TDP at opposite sides Sap pH from stem cores	(Teskey and McGuire 2007)
0.82	0.18	0.00	*Populus deltoides*	DOY 287	Open configuration	NDIR sensor	(Saveyn et al. 2008*b*)
0.93	0.09	0.00	3-year-old	DOY 296		TDP	Rainy days excluded
0.86	0.13	0.00		DOY 299		Sap pH from twigs and stem cores	
1.00	0.00	0.00	*Quercus pyrenaica*	DOY 143–144	Closed configuration	NDIR sensor,	(Salomón et al. 2016*a*)
0.95	0.05	−0.01	45-year-old	DOY 183–184		TDP at opposites sides	Results from 1.5 m height
0.97	0.04	−0.02		DOY 218–219		Sap pH from twigs	
0.97	0.03	−0.02		DOY 267–268			
0.86	0.15	−0.01	*Liriodendron tulipifera*	16.3 cm	Open configuration	NDIR,	(Fan et al. 2017)
0.89	0.12	−0.01		25.2 cm		TDP,	
0.73	0.26	0.01		31.4 cm		Sap pH from twigs	
0.54	0.41	0.05		46.2 cm			
0.46	0.55	−0.01		60.6 cm			
0.97	0.02	0.00	*Populus Canadensis* 3-yr-old		Open configuration	NDIR sensor HRM Sap pH from twigs	(Salomón et al. 2018) Control period
0.82	0.19	0	*Eucalyptus tereticornis*,	Ambient CO_2	Closed configuration	NDIR sensor,	(Salomón et al. 2019*b*)
0.96	0.04	−0.01	18.8 cm			TDP at two random azimuths	(EucFACE)
1.11	−0.10	0		Elevated CO_2		Sap pH from twigs	
0.97	0.03	0					
1.09	−0.10	0.01	*Fraxinus mandshurica* 13.1–16.1 cm		Open configuration	NDIR sensor TDP at opposites sides	(Wang et al. 2019)
0.80	0.20	0	*Betula platyphylla* 11.5–13.5 cm			Sap pH from twigs	
0.97	<0.06	–	*Pinus sylvestris* 90-year-old		Open configuration	NDIR TDP Sap pH from branches	(Tarvainen et al. 2020)
1.01	−0.01	0	*Fagus sylvatica*	Upper segment	Closed configuration	NDIR	(Helm et al. 2023)
0.65	0.36	−0.01	130-year-old	Lower segment		HRM Sap pH from twigs	

Abbreviations: DOY, day of year; HRM, Heat Ratio Method; TDP, thermal dissipation probe; NDIR, non-dispersive infrared CO_2 sensor.

most of the DIC is in the form of carbonic acid (H_2CO_3), therefore limiting the availability of bicarbonate for PEPC fixation.

Shifts in the respiratory substrate and non-respiratory oxygen consumption

Also relevant for interpreting I_{O_2} measurements as a proxy of R_S is that the amount of O_2 consumed for R_S depends on the oxidative state of the respiratory substrate (Masiello et al. 2008). For example, carbohydrates (e.g., glucose: $C_6H_{12}O_6$) are defined as having neutral C-oxidation; i.e., one mole O_2 is consumed to produce one mole CO_2 during respiratory metabolism. For more reduced substrates like lipids and fatty acids (e.g., oleic acid: $C_{18}H_{34}O_2$), more O_2 molecules are required for a complete breakdown of the molecule, resulting in more than one mole of O_2 consumed per mole of CO_2 produced. By contrast, organic acids, which are highly oxidized (e.g., oxalic acid: $C_2H_2O_4$), require less than one mole of O_2 per mole of CO_2 produced (Hilman et al. 2022). Therefore, stem O_2 influx (I_{O_2}) measurements deviate from R_S when carbohydrates do not fuel respiration. Carbohydrates constitute the largest substrate pool in woody species, and respiratory processes are assumed to be carbohydrate-dominated (Hoch et al. 2003, Plaxton and Podestá 2006). However, some tree species store substantial amounts of lipids (e.g., conifers and *Tilia* genus; Sinnott 1918, Höll 1998, Hoch et al. 2002, 2003), which are potentially consumed for respiration. For instance, under impeded photosynthetic uptake upon shading, two conifer species switched from carbohydrate-dominated respiration to a mixture of carbohydrates and lipids (Hanf et al. 2015). Similarly, pine seedlings exposed to shading were observed to shift from carbohydrate- to lipid-dominated respiration as carbohydrates progressively depleted, while under drought conditions, respiration was downregulated without an apparent shift in the respiratory substrate (Fischer et al. 2015).

Also important as a potential mechanism causing differences between I_{O_2} measurements and R_S is the non-respiratory O_2 consumption mediated by oxidase and dehydrogenase enzymes (Sweetlove et al. 2013, O'Leary et al. 2019) involved in cell redox balancing and documented at the leaf level (Tcherkez et al. 2012, O'Leary et al. 2019). Greater consumption of O_2 compared with CO_2 production may also occur under fast growth rates, as observed in pine needles by enthalpic growth rates derived from calorimetry-based approaches (Kruse and Adams 2008). However, little is known about this relation at the stem level, and we are unaware of literature that relates stem growth rates with simultaneous I_{O_2} and E_{CO_2} measurements.

Section 2: Methodological approaches to estimate and model stem respiration

Two approaches that allow avoidance of some (not all) of the sources of uncertainty described above can provide more accurate R_S estimates. The mass balance approach (MBA) accounts for internal and external stem CO_2 fluxes, while O_2 measurement techniques are promising as they bypass issues related to CO_2 dissolution and PEPC-mediated consumption due to the low solubility of O_2 in water and the lack of affinity of PEPC for O_2 (Section 1). The calorimetric approach briefly mentioned above is still in its infancy and technically very challenging (O'Leary et al. 2019), so we focus here on MBA and O_2 measurements.

The mass balance approach

The CO_2 mass balance approach estimates R_S by summing E_{CO_2}, CO_2 transport through the xylem (F_T) and CO_2 storage (ΔS) of a stem segment on a volume basis (mol CO_2 m^{-3} s^{-1}) (see detailed equations in McGuire and Teskey 2004):

$$R_S = E_{CO_2} + F_T + \Delta S \quad (1)$$

Stem CO_2 efflux into the atmosphere (E_{CO_2}) is calculated as the ratio of the amount of CO_2 emitted by a stem segment (mol CO_2 s^{-1}) divided by the sapwood volume (m^3) enclosed by an opaque stem chamber to avoid local SP in either open or closed configuration (Table 2). The CO_2 transport through the xylem accounts for the net CO_2 export ($F_T > 0$) and import ($F_T < 0$) from and into the stem segment, and it is estimated as the product of the sap flow rate and the vertical gradient of dissolved [CO_2] in the sap solution (sap [CO_2*] hereafter) divided by the sapwood volume. Sap [CO_2*] is estimated as the sum of DIC forms ([CO_2*] = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]) according to Henry's law (see Notes S1 available as Supplementary data at *Tree Physiology* Online). Xylem [CO_2] in the gas phase, sap temperature and sap pH should be known in order to estimate sap [CO_2*]. To measure xylem [CO_2] above and below the stem segment, microelectrodes or non-dispersive infrared (NDIR) sensors are often used (Table 2). Stem temperature is measured with thermocouples inserted 1–2 cm into the stem, and sap pH is commonly obtained from twigs, given the difficulty of collecting sap directly from the stem. The CO_2 storage flux (ΔS) accounts for the build-up ($\Delta S > 0$) and depletion ($\Delta S < 0$) of sap [CO_2*], and it is estimated as the product of its variation over time and the volumetric water content of sapwood, which can be measured in situ or, for simplicity, assumed to be 50%.

A compilation of studies applying the MBA (Table 2) shows a high variability of mean daily values in the contribution of E_{CO_2} to R_S among species (E_{CO_2}/R_S = 0.30–1.11) and a relatively limited temporal variation within trees under similar experimental conditions (Saveyn et al. 2008*b*, Salomón et al. 2016*a*, 2019*b*). According to Fick's law of diffusion, three factors determine the diffusion of xylem CO_2 into the atmosphere, hence E_{CO_2}/R_S ratios: the radial xylem CO_2 diffusivity, the radial [CO_2] concentration gradient between the stem and the atmosphere, and the length of the diffusive pathway. Large differences in radial diffusivity, up to 6-fold among tree stems of the same species (Steppe et al. 2007), may partially explain the variability in E_{CO_2}/R_S ratios among individuals. Among taxonomic clades, lower radial CO_2 diffusivity and lower xylem [CO_2] have been found in conifers with tracheid wood anatomy compared with angiosperm species with ring- or diffuse-porous anatomy (Sorz and Hietz 2006, Salomón et al. 2021). Differences in the tissue fraction of living parenchyma, higher in angiosperms (26.3%) than in conifers (7.6%) (Morris et al. 2016), can also determine differences in respiration and CO_2 build-up rates among plant functional types. Moreover, E_{CO_2}/R_S ratios commonly decrease with stem size (Fan et al. 2017) due to the increased length of the diffusive pathway and the lower surface-to-volume ratio (Cavaleri et al. 2006, Hölttä and Kolari 2009). The relative

contribution of F_T to R_S (F_T/R_S) has also shown substantial variability among species in field studies, ranging from −0.1 (net import from lower locations) to 0.55 (net export to upper locations) (Table 2). Modelling approaches suggest a balance shift from net export to net import as we move up from the ground as [CO_2] accumulates along the stem and the radial diffusive pathway is reduced as the tree tapers (Hölttä and Kolari 2009). Moreover, F_T/R_S increases with sap flow velocity (McGuire et al. 2007) and stem sapwood area (Fan et al. 2017), determining the amount of CO_2 transported upwards. The storage flux has been consistently observed to be the smallest contributor to R_S, with $\Delta S/R_S$ ratios commonly ranging from −0.02 ([CO_2*] degassing) to 0.08 ([CO_2*] dissolution), as sub-daily patterns of [CO_2*] daytime degassing and nighttime dissolution, related to thermal dynamics (CO_2 solubility increases with colder temperatures), commonly offset each other on a daily basis.

The largest uncertainty in the MBA is the accurate estimation of sap pH, as it critically affects CO_2 solubility and, subsequently, F_T and ΔS. Sap [CO_2*] exponentially increases with pH values above 6–6.5 (Teskey et al. 2008, Tarvainen et al. 2023). These values are within the reported range of sap pH (4.5–7.4; Teskey et al. 2008; Table 3), although sap pH usually fluctuates between 5.4 and 6.4 (sap pH interquartile range in Table 3). Accurate pH determination is challenging for two reasons. First, while xylem [CO_2], sap temperature and sap flow can be measured continuously, sap pH requires its extraction and is discretely measured, given the technical challenges of its continuous measurement. Therefore, most studies linearly interpolate pH readings or assume a constant pH over the study period. However, seasonal sap pH acidification commonly occurs during spring and summer months (e.g., Erda et al. 2014), with reductions up to 2.1 pH units (Table 3), the neglect of which can lead to substantial misestimation of sap [CO_2*] (up to 25%; Salomón et al. 2016*b*). To a lower extent, sap pH can also vary on a sub-daily basis, with pH nighttime declines (up to 0.4 pH units) being more commonly reported (Table 3). Adding further uncertainty to pH estimates, environmental conditions (Thomas and Eamus 2002, Aubrey et al. 2011) and the tree's soil water pool (Paudel et al. 2018) can also affect seasonal and circadian trends in sap pH. Second, sap samples are commonly taken from twigs with a pressure chamber (Table 2), given the difficulty of extracting sap from the stem. However, sap pH has been observed to be higher in the stem than in twigs in *Populus deltoides* under certain conditions (Aubrey et al. 2011), with differences up to one pH unit in *Acer platanoides* (Schill et al. 1996), introducing additional uncertainty in stem [CO_2*] estimates based on twig samples. Moreover, the release of cellular constituents due to the damage of parenchyma cells during sap sampling might further bias sap pH estimates (Tarvainen et al. 2023). Finally, another source of uncertainty in the estimation of F_T and ΔS is related to potential changes in xylem [CO_2] due to wound responses after intrusive installation of NDIR sensors (Etzold et al. 2013), which could progressively reduce the amount of CO_2 diffusing into the probe headspace in the long-term.

O_2 measurement and the apparent respiratory quotient

Measurements of gaseous O_2 exchange in plant physiology studies are challenging due to the high background of O_2

Table 3. Compilation of studies assessing temporal (seasonal and sub-daily) variability of sap pH.

Species	pH value				Measurement location	Sub-daily trend	Seasonal trend	Reference
	Spring	Summer	Autumn	Winter				
Actinidia chinensis	5.3	6.2	6.2	6.2	Shoots	NA	Spring acidification	(Ferguson et al. 1983)
Populus × *canadensis*	5.4	6–7	7.5	7.5	Branches	NA	Spring acidification	(Sauter 1988)
Fagus sylvatica	6.0	5.6	6.0–6.5	6.0–6.5	Stem	NA	Summer acidification	(Glavac et al. 1990)
Fagus sylvatica	5.8–6.5	5.8	6.6–6.8	6.4	Stem	NA	Spring acidification	(Rennenberg et al. 1994)
Robinia pseudoacacia	5.3	5.4–5.5		5.7–6.0	Branches	NA	Spring/summer acidification	(Fromard et al. 1995)
Six savanna tree species	5–6.4 over dry-wet-dry transitions				Branches	NA	Acidification with drought for evergreen and semi-deciduous	(Thomas and Eamus 2002)
Juglans regia	5.6	NA	6.8	5.3	Twigs	NA	Winter acidification	(Alves et al. 2004)
Populus deltoides		6.9–7.2			Twigs and stem cores	Not significant under high VPD Alkalinization on cloudy days		(Aubrey et al. 2011)
Prunus domestica	5.3	5.2		5.4	Branches	Not-significant	Summer acidification	(Erda et al. 2014)
Quercus pyrenaica	6.2	6.4	6.7	NA	Twigs	Nighttime acidification	Spring acidification	(Salomón et al. 2016*b*)
Pinus cembra		6.7		5.9	Branches	NA	Summer acidification	(Losso et al. 2018)
Picea abies		7.3		6.2				
Larix decidua		6.7		5.5				
Citrus paradisi (irrigated orchard)		5.9–6.6		5.1–6.1	Branches	Nighttime acidification	Winter acidification	(Paudel et al. 2018)
Populus nigra	5.7–6.3				1-year-old stems	Nighttime acidification	NA	(Brunetti et al. 2019)

Terminology 'twigs' and 'branches' were maintained from the original studies.

in the atmosphere (21% = 210,000 p.p.m.) relative to the small changes in $[O_2]$ detectable in measurement chambers attributable to plant metabolism. In open-flow chambers, the particularly small $[O_2]$ changes can only be measured with careful gas handling that requires considerable infrastructure and labour (Stephens et al. 2007, Battle et al. 2019). Nevertheless, recent developments in high-precision techniques (see Table 4) are bringing attention to the potential of O_2 measurements to estimate R_S. Mass spectrometric analysis (Angert et al. 2012*b*), cavity-enhanced multi-gas Raman spectrometry (Fischer et al. 2015, Hanf et al. 2015) and fuel-cell-based analysers (Hilman and Angert 2016) are some of the approaches applied to measure O_2 exchange at the stem level. More recently, low-cost quenching-based O_2 sensors have proven robust enough to facilitate continuous and long-term I_{O_2} measurements in the field (Helm et al. 2021).

Aerobic respiration involves both CO_2 production and O_2 consumption. At the mitochondrial level, the CO_2-to-O_2 ratio is termed the respiratory quotient (RQ) and is mathematically related to the stoichiometry of the respiratory substrate. Therefore, respiration of lipids, carbohydrates and organic acids yields RQs below, equal and above the unit according to their oxidative status (Section 1). At the stem level, the term 'apparent' RQ (ARQ) was introduced by Angert and Sherer (2011) to underscore that the measured quotient can be affected by post-respiratory processes that consume and divert CO_2 evolved from the TCA cycle. Therefore, simultaneous I_{O_2} and E_{CO_2} measurements integrate information from the mitochondrial RQ, changes in respiratory substrate and post-respiratory processes at the stem level (Angert and Sherer 2011, Angert et al. 2012*b*, Trumbore et al. 2013, Hilman and Angert 2016, Hilman et al. 2019, 2022).

Measurements of the ARQ in tropical species ranged from 0.23 to 0.90, with mean values of 0.66 (Angert et al. 2012*b*). Similarly, mean ARQ values per species and site from different biomes ranged from 0.39 to 0.78, with mean values of 0.59 (Hilman et al. 2019). In the latter study, nine surveyed tree species had non-lipid-storing strategies, suggesting that low ARQs (<1) cannot be solely explained by lipid-dominated respiration and that post-respiratory processes might be quantitatively relevant in limiting the emission of locally respired CO_2 to the atmosphere by 30–40% (Angert and Sherer 2011, Angert et al. 2012*b*, Hilman et al. 2019, 2022). When not explained by a respiratory substrate shift, ARQ below one is expected if part of locally respired CO_2 is transported away from the measurement site. In this case, ARQ would negatively correlate with the sap flow rate. Yet, sap flow minimally affected ARQ reductions in *Quercus ilex* stems, suggesting that the contribution of F_T to the stem CO_2 mass balance might be of limited relevance while pointing to the potential role of PEPC fixation (Hilman et al. 2019). However, the fact that CO_2 easily dissolves in the sap solution does not necessarily imply a net export of locally respired CO_2, as CO_2 import from lower stem parts (Table 1) may outbalance potential F_T-induced reductions in E_{CO_2}.

By contrast, reductions in ARQ that could be attributed to shifts in the respiratory substrate were observed in conifer species. When spruce and pine seedlings were subjected to shading, respiration shifted from carbohydrate-dominated to lipid-dominated, resulting in ARQs reductions from 1.00 to 0.77–0.81 (Hanf et al. 2015). A follow-up study demonstrated that carbohydrate depletion was the main driver of such ARQ reduction (Fischer et al. 2015). Recently, the MBA

Table 4. Available O_2 techniques for stem O_2 influx measurements, highlighting the most relevant advantages and disadvantages.

Sampling procedure	Measurement/sensor/approach	Advantages	Disadvantages	References
Continuous measurement of air flowing out from a stem chamber	Simultaneous measurement of stem chamber and reference gas eliminates undesired effects of drift, pressure and temperature. This can be measured by a differential fuel-cell analyzer (Oxzilla[1])	High accuracy and temporal resolution	Costly, it requires controlled air pressure, temperature and air dryness. Setup and calibration are technically challenging	(Stephens et al. 2007, Battle et al. 2019)
A stem chamber is sampled after an incubation period during which the $[O_2]$ change is higher than in an open-flow chamber	The headspace air can be either sampled (e.g., in flasks) and measured in the laboratory or directly measured in the field. Potential analyzers are single-channel fuel cell (FC-10[2]) and quenching-based optode (Fibox 3 & 4[3])	Applicable in the field	Discrete measurements with low temporal resolution (depending on sampling intensity); limited number of measurements during incubation	(Angert and Sherer 2011, Angert et al. 2012*b*, Hilman and Angert 2016, Hilman et al. 2019)
A series of incubations are conducted by automatic ventilation of stem-chamber headspace. $[O_2]$ is measured continuously	Quenching-based sensors (Fibox 3 & 4[3], LuminOx[4])	Applicable in the field, high temporal resolution, several measurements simultaneously, low-cost	Requires in-field sensor calibrations, in situ operating of electronic equipment	(Hilman and Angert 2016, Helm et al. 2021)
	Cavity-Enhanced Raman multi-gas Spectrometry (CERS), Fibre-enhanced Raman gas spectroscopy (FERS)	High accuracy and temporal resolution	Not available commercially, costly, and requires controlled air pressure temperature, and dryness. Setup and calibration are technically challenging	(Keiner et al. 2013, Fischer et al. 2015, Knebl et al. 2018)

[1]Oxzilla; Sable Systems International, Las Vegas, NV, USA. [2]FC-10; Sable Systems International, Las Vegas, NV, USA. [3]Fibox; PreSens-Precision Sensing, Regensburg, Germany. [4]LuminOx, LOX-02-S, SST Sensing Ltd, UK.

and I_{O_2} measurements have been performed simultaneously in mature beech trees to reconcile apparent discrepancies between approaches regarding the fate of respired CO_2. The ARQ was consistently close to 0.7, while carbohydrate pools remained constant over the study period, which casts doubts on shifts in the respiratory substrate. Remarkably, F_T and ΔS did not bridge the gap between I_{O_2} and E_{CO_2} and the PEPC capacity in these mature trees was comparable to that in twigs, highlighting the need for further research on PEPC fixation to close stem C budgets (Helm et al. 2023).

Stem respiration modelling

Our limited understanding of the complex metabolic processes involved in the production and consumption of O_2 and CO_2 hinders the development of a simple biochemical respiration model (Sweetlove et al. 2013, O'Leary et al. 2019) equivalent to that of photosynthesis (Farquhar et al. 1980). Instead, the growth and maintenance respiration paradigm proposed in the early 1970s (Thornley 1970) constitutes the basis of how whole-plant respiration is currently estimated by TBMs (reviewed by Atkin et al. 2017). In most TBM, plant respiration is divided into growth (R_g) and maintenance (R_m) components. Temperature-normalized leaf R_m is commonly measured during non-growing periods and can be estimated from the empirical relationship between dark leaf respiration (R_d) and foliar nitrogen (N) content (Smith and Dukes 2013) or as a function of the maximum carboxylation capacity of the enzyme Rubisco (V_{cmax}). Once leaf R_m at a reference temperature is determined, temperature-driven variation in R_m is accounted for by the Q_{10} parameter, which reflects the relative increase of R_m for a 10 °C rise in temperature according to Arrhenius kinetics (Ryan 1991). Then, leaf R_m respiration is scaled up to the whole-plant level using tree biomass partitioning and N allocation patterns, given the well-known link between N content and protein turnover rates involved in maintenance metabolism (Reich et al. 2008). Finally, whole-tree growth respiration is commonly estimated as a fixed fraction of the difference between gross primary production (GPP) and whole-plant R_m (Atkin et al. 2017). Therefore, this source-driven perspective of plant C cycling indirectly estimates woody tissue (and stem) respiration from leaf-derived parameters (GPP, V_{cmax} and R_d). However, the coordination between photosynthetic and respiratory metabolism in leaves (Wang et al. 2020) is unlikely to regulate respiration in non-photosynthetic stems, as denoted by different thermal acclimation responses (Smith et al. 2019) and N–R_d relations (Reich et al. 2008) between organs.

Biophysical modelling of R_S has advanced in recent years. Hölttä and Kolari (2009) developed a model integrating CO_2 diffusion and solubility processes in different stem compartments (heartwood, sapwood, phloem and outer bark) to interpret E_{CO_2} measurements. The CASSIA model constituted another step forward for more mechanistic modelling of stem and plant respiration (Schiestl-Aalto et al. 2015). CASSIA considers the sink strength of growth and respiratory processes in different tree organs to reflect intra-annual and inter-annual growth variability, and it was successfully applied in a boreal conifer stand (Schiestl-Aalto et al. 2015, 2019). More recently, TReSpire was developed to determine R_S independent of leaf metabolism and thus allows decoupling from source-driven models (Salomón et al. 2020). TReSpire simulates water and carbon fluxes and estimates respiratory trait parameters commonly used in large-scale models, such as the growth yield (Y_G), the temperature-normalized R_m per unit of N (R_{m_N}), and its temperature sensitivity (Q_{10}). It has proven helpful in capturing the sink strength of growth and respiratory processes across species and, importantly, at sub-daily and seasonal temporal scales (Meir et al. 2020, Salomón et al. 2022). We encourage the reader particularly interested on R_S modelling to consult the supplementary material, where we use TReSpire to showcase the sensitivity of sap [CO_2*], E_A and R_S to variations in sap pH and stem size through two modelling exercises. These exercises aim to illustrate the potential of mechanistic modelling in providing theoretical backup to empirical observations without aiming at testing novel hypotheses.

Taken together, observations and model outcomes from R_S studies applying the MBA and O_2 measurement techniques pinpoint existing challenges to reduce and disentangle measurement uncertainties for accurate estimations of R_S. In the next section, we identify some of these challenges that we believe are most relevant for improving R_S estimation accuracy, including upscaling procedures, that should be addressed via observational studies, manipulative experiments and modelling approaches.

Section 3: Research agenda

For each research challenge identified, we describe the knowledge gap, pose the corresponding hypothesis and then suggest methodological approaches to address it. Note that the enumerated items in the research agenda refer to the numbering shown in Figure 1a and b.

Closing the stem C mass balance

We still lack a complete understanding of the fate of CO_2 in tree stems, making estimates of R_S from E_{CO_2} and I_{O_2} measurements uncertain. Complementary approaches to quantify R_S, like simultaneous measurements of I_{O_2}, E_{CO_2} and internal CO_2 fluxes, can help disentangle the different post-respiratory processes involved in CO_2 removal from the production site. We expect that the CO_2- and O_2-based methods will allow us to quantify the magnitude of the different contributors to R_S and help interpret each other, assuming that the imbalance between E_{CO_2} and I_{O_2} could be largely explained by an extended mass-balance approach that accounts for internal fluxes and (F_T and ΔS) and the refixation of respired CO_2 (RF) via both SP and PEPC-driven fixation:

$$I_{O2} - E_{CO_2} = F_T + \Delta S + RF \quad (2)$$

We propose the simultaneous measurement of variables required to apply the MBA and ARQ to the same trees under the same experimental conditions, extending recent observations (Helm et al. 2023) to species with different wood anatomy and under manipulative conditions to assess how the limitation of specific fluxes (e.g., F_T, SP, axial CO_2 diffusion) affects the remaining ones.

Uncertainty of sap pH readings and CO_2 internal fluxes

Given the high impact of sap pH on the calculation of sap [CO_2*] and internal fluxes (F_T and ΔS), uncertainties in pH

measurement can result in substantial errors in the stem mass balance. Accurate and continuous measurements of stem sap pH in situ are urgently needed, for which no suitable method is currently available due to technical constraints. Nevertheless, advanced technologies from different fields capable of registering pH spatial and temporal variability would limit uncertainties in F_T and ΔS estimates. For instance, approaches from medical disciplines like microdialysis, based on a passive diffusion principle which would not require sap extraction, could help achieve continuous readings of the xylem sap of trees in vivo. This approach has proven successful in monitoring phosphate concentration for 24 h in beech tree stem segments (Jeřábek et al. 2020). We recently attempted a similar approach in mature beech trees, but the pH probes provided reliable readings only for about 2–3 h (unpublished data), probably due to a contact loss between the probe and xylem sap. Technical difficulties in successfully using such probes in trees that must be addressed are (i) ensuring constant contact with xylem sap, being aware of embolism formation and wound responses when placing probes in the xylem, and (ii) avoiding contamination with cellular constituents by damaging living cells in the parenchymatic tissue (Tarvainen et al. 2023).

Photosynthetic and anaplerotic refixation of respired CO_2

Stem photosynthesis (SP) is commonly measured in green twigs, branches and seedlings, but rarely in stems of mature trees. Therefore, uncertainties remain about the effect of stem age, diameter and bark optical properties on the photosynthetic potential of mature woody tissues along the stem vertical axis and its influence on R_S estimates. For instance, we expect that SP would be lower in species with dark-coloured bark (e.g., oak and pine trees) than in species with light-coloured bark (e.g., beech or poplar trees), with intraspecific variability being modulated by bark thickness. Moreover, we expect SP efficiency to be largely determined by optimal chloroplast allocation according to light transmission properties in stems of variable age, allometry and location within the canopy. We propose comprehensive measurements of SP pigment content, stem photochemical (photosystem II) activity via chlorophyll fluorescence, optical properties of the outer (periderm) and inner bark (cortex) (Wittmann and Pfanz 2016), and stem gas exchange along the stem axis of different species to evaluate potential tradeoffs between pigment allocation efficiency and stem C gain.

Although the non-photosynthetic CO_2 fixation catalyzed by PEPC in leaves is well known, its role in non-foliar tissues remains largely unexplored. Therefore, the magnitude of CO_2 PEPC-mediated refixation remains highly speculative in the stem C budget. We expect that consistent stem ARQs below the unit and ARQ sub-daily variability (see Hilman et al. 2019) might be partly explained by PEPC activity. Isotopic studies with ^{13}C- or ^{14}C-labelled CO_2 to track PEPC-mediated fixation in the malate (and derived products) pools over time, along the stem axis, and in root exudates via compound-specific isotope analysis would provide quantitative information on the significance of this process as a recycling mechanism. Parallel studies performing enzymatic assays (Bénard and Gibon 2016) would further allow comparison between PEPC capacity and activity in vivo.

Scaling carbon flux dynamics from small to large trees

A large body of studies measuring stem gas exchange has been performed in seedlings, saplings or small trees for methodological simplicity. However, the fate of respired CO_2 depends largely on stem size, and this methodological bias could distort our perspective of stem CO_2 fluxes. Xylem CO_2 diffusion is limited in large stems due to the long radial CO_2 diffusive pathway, and the relative contribution of E_{CO_2} to R_S is expected to decrease with stem size. By contrast, F_T is expected to increase with sapwood area due to more dissolved CO_2 being transported upwards. If more CO_2 is transported away from the measurement site, stem ARQs will decrease. Likewise, stem size likely reduces SP and PEPC-mediated fixation on a volume basis when comparing saplings and mature trees. To test these hypotheses, E_{CO_2}, I_{O_2}, F_T, SP and PEPC-mediated fixation measurements should be performed under comparable abiotic conditions for saplings and mature trees of contrasted size.

Scaling observations to the whole-tree level

Upscaling the gas exchange at the stem to the whole-tree level is challenging (Meir et al. 2017). Drivers of R_S and ARQs might differ among different organs (roots, stem, branches and leaves) according to their morphological, anatomical and physiological traits. Assuming ca 30% of stem-respired CO_2 is not emitted locally (Hilman et al. 2019), its emission in upper tree parts and organs will increase ARQs upwards. This hypothesis could be tested by simultaneous xylem [CO_2] and ARQ measurements along the stem vertical axis, branches and leaves. Moreover, there is evidence that root-respired CO_2 can be recycled in upper tree organs (Bloemen et al. 2013, Salomón et al. 2021) and that xylem-transported CO_2 can be assimilated in C3 and C4 leaves (Stutz and Hanson 2019*a*, 2019*b*). This recycling mechanism, critical in drier scenarios (CO_2 is assimilated with minimal water loss), is poorly understood, and we still ignore the total amount of CO_2 recycled at the whole-tree level. Isotopic approaches allow quantitative assessment of the fate of respired CO_2. Labelling could be performed via ^{13}C-CO_2 tracer into the xylem (Powers and Marshall 2011, Bloemen et al. 2013), gaseous ^{13}C-CO_2 to the canopy (Joseph et al. 2020) or phloem feeding of ^{13}C-labelled carbohydrates as a respiratory substrate (Gessler et al. 1998). Subsequently, cavity ring-down laser spectroscopy can be applied for real-time measurements of emitted ^{13}C-CO_2 (Salomón et al. 2019*a*, Salomón et al. 2021). Alternatively, online measurements of the xylem CO_2 ^{13}C (and ^{18}O) isotopologues composition could be performed via spectrometry using an adapted online system where the probe design and the laser spectrometer target CO_2 instead of water (Gessler et al. 2022). Whole-tree chambers would be ideal for these experimental approaches; however, high costs limit their broad use.

Modelling R_S to large spatial scales

Estimation of tree respiration in models is based on foliar tissue parameters, but respiratory metabolism of (non-photosynthetic) stem tissues differs from that of foliar tissues. The implementation of modelling structures and algorithms that (i) decouple leaf and woody tissue respiration (Salomón et al. 2020), (ii) account for its differential thermal acclimation (Smith et al. 2019), (iii) consider the partially sink-driven

nature of woody tissue respiration (Schiestl-Aalto et al. 2015, Salomón et al. 2020) and (iv) reflect the physical properties of sapwood and bark (Hölttä and Kolari 2009, Westerband et al. 2022) will improve the mechanistic representation of whole-plant respiration in large-scale models, which constitutes one of the largest sources of uncertainty in net primary production globally (Dietze et al. 2014). Improvements in the global modelling of leaf respiration (e.g., Heskel et al. 2016, Huntingford et al. 2017) have been achieved following the compilation of a global database of leaf respiratory traits (GlobResp; Atkin et al. 2015). We propose that a similar strategy should be adopted for R_S modelling. As a first step forward, compiling a global database of stem respiratory traits (R_{m_N}, Q_{10} and Y_G) would be helpful for hypothesis testing regarding stem respiratory regulation and acclimation along broad gradients of climatic conditions, eventually helping to refine model algorithms and estimates of whole-plant respiration in large-scale models.

Respiratory shifts under environmental stress

When investigating tree responses to ongoing global warming and climate extremes, the downregulation and acclimation of respiratory metabolism and shifts in reserve consumption are critical to understanding how trees cope with stressful conditions. Under heat and drought, leaf photosynthesis is limited following stomatal closure, and trees rely heavily on storage compounds, including soluble carbohydrates, starch and lipids. Trees can tap into older C reserve pools (Muhr et al. 2018) and switch from pure carbohydrate to lipid metabolism (Fischer et al. 2015) to buffer stress-induced C starvation. We hypothesize that the modulation in respiratory metabolism and shifts in the respiratory substrate under unfavourable conditions would be related to the species-specific water use economy. For instance, drought-avoiding species that close stomata early during stress also downregulate respiratory C losses to maintain stable storage pools. By contrast, drought-tolerant species will maintain respiratory metabolism for extended periods, likely consuming older and lipidic reserves to a larger extent. To test this hypothesis, we propose combined measurements of respiratory- and hydraulic-related traits, ARQs and bomb radiocarbon (^{14}C) dating (Muhr et al. 2013) under gradients of heat and drought stress to assess respiratory acclimation and the composition and age of the respiratory substrate.

Conclusions

Substantial progress has been made during the last two to three decades in stem respiration research. Here, we have reviewed the growing body of evidence demonstrating that stem E_{CO_2} and I_{O_2} measurements should be interpreted cautiously as several non-respiratory processes can cause divergences between net gas exchange measured at the stem surface (E_{CO_2} and I_{O_2}) and the respiratory activity of tissues underneath (R_S). Although there is no gold-standard approach to quantify R_S unequivocally, we now have the tools to disentangle all these respiratory and non-respiratory processes affecting stem C budgets. The research agenda proposed here should be helpful as a roadmap to keep advancing knowledge during the coming years on the regulation and upscaling of stem respiratory metabolism, particularly relevant yet uncertain, in climate change scenarios.

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Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

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Data availability statement

There are no new data in this *Insight* paper.

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