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RESEARCH PAPER

Axial diffusion of respired CO_2 confounds stem respiration estimates during the dormant season

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Abstract

• ***Key message*** **Efflux-based estimates of stem respiration in oak trees during the dormant season were biased by axial diffusion of locally respired CO_2. Light-induced axial CO_2 diffusion along the stem due to woody tissue photosynthesis may lead to equivocal estimates of stem respiratory coefficients during the dormant season, which are generally used to estimate maintenance respiration throughout the year.**

• ***Context*** Stem CO_2 efflux (E_A) does not reflect respiratory rates of underlying tissues. Recent research has focused on the significance of CO_2 transport via the transpiration stream. However, no studies have yet addressed the potential role of light-induced axial CO_2 diffusion on E_A during the dormant season when there is no transpiration.

• ***Aims*** This study investigated to which extent woody tissue photosynthesis and axial diffusion of respired CO_2 affect E_A during the dormant season.

• ***Methods*** E_A was measured in a stem cuvette on dormant oak trees in a growth chamber at constant temperature. Different rates of axial CO_2 diffusion were induced by woody tissue photosynthesis by means of illuminating stem sections at varying distances from the stem cuvette, while light was excluded from the remainder of the tree.

• ***Results*** Axial diffusion of respired CO_2 led to reductions in E_A of up to 22% when the stem section closest to the cuvette was exposed to light.

• ***Conclusion*** Dormant-season efflux-based estimates of stem respiration might be biased by axial diffusion of respired CO_2, particularly in open forest stands with sufficient light penetration. Consequently, this may lead to ambiguous estimates of dormant season E_A coefficients (Q_{10} and E_{A_0}) generally used to estimate maintenance respiration throughout the year.

Keywords *Quercus robur* L. · Woody tissue photosynthesis · Stem CO_2 efflux · Internal CO_2 transport · Maintenance respiration

1 Introduction

Within forest ecosystems, CO_2 efflux from woody tissue to the atmosphere represents a substantial component of ecosystem respiration, accounting for 5–35% (Salomón et al. 2017b; Yang et al. 2016). The wide range in these estimates partially reflects differences observed between different forest ecosystems (Chambers et al. 2004; Ryan et al. 1995; Yang et al. 2012), but is additionally resulting from our limited ability to accurately measure and model this respiratory flux at the tree level and to upscale it to larger spatial scales (Meir et al. 2017; Ryan et al. 2009).

However, advances have been made on using efflux-based measurements to accurately quantify woody tissue respiration during the growing season. While past studies

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mainly focused on the exponential relationship between stem temperature and stem CO_2 efflux (E_A) (Maier et al. 1998; Ryan et al. 1995; Stockfors 2000), most recent studies highlight temperature-independent factors interfering with E_A (Etzold et al. 2013; Rodríguez-Calcerrada et al. 2014; Salomón et al. 2017a; Salomón et al. 2015; Saveyn et al. 2007a; Saveyn et al. 2008; Tarvainen et al. 2017; Teskey et al. 2017; Yang et al. 2012). In particular, CO_2 originating from woody tissue respiration can diffuse to the atmosphere remote from the site of respiration, as dissolved CO_2 is transported away from the site of respiration via the transpiration stream (Teskey and Mcguire 2002). Teskey and McGuire (2007) found that internal transport of respired CO_2 might account for up to 70% of the CO_2 derived from stem respiration, which may explain E_A reductions during periods of high transpiration (Bowman et al. 2005; Martin et al. 1994; McGuire et al. 2007; McGuire and Teskey 2004; Negisi 1974; Salomón et al. 2017a; Salomón et al. 2016; Saveyn et al. 2007b). Moreover, Bloemen et al. (2013) showed that a considerable fraction of E_A might be derived from belowground respired CO_2 transported with the transpiration stream, indicating the need to continuously measure E_A as well as transport of respired CO_2 via the transpiration stream to accurately quantify and interpret stem respiratory fluxes (Steppe et al. 2015; Teskey et al. 2017; Teskey et al. 2008; Trumbore et al. 2013).

When no sap flow occurs, other factors that may influence efflux-based estimates of stem respiration are often neglected. For example, anaplerotic fixation (Berveiller and Damesin 2008; Gessler et al. 2008; Hilman et al. 2017) and woody tissue photosynthesis in chlorophyll containing bark and xylem tissues can assimilate part of the locally respired CO_2 (Ávila et al. 2014; Pfanz et al. 2002; Tarvainen et al. 2017; Wittmann and Pfanz 2018), present in tree stems at CO_2 concentrations ($[CO_2]$, %) ranging from < 1 to over 26% (Teskey et al. 2008). For instance, a reduction in E_A was observed in young birch trees under illumination because up to 97% of the respired CO_2 was locally assimilated by woody tissue photosynthesis (Wittmann et al. 2006). Variable refixation rates were found along the stem of 90-year old Scots pine trees, depending on light availability and bark chlorophyll content, with a reduction in E_A of 28% in the upper stem section (Tarvainen et al. 2017). While an opaque stem cuvette is generally used to measure E_A, preventing local assimilation of respired CO_2, woody tissue photosynthesis in stem or branch sections remote from the site of measurement might account for observed non-temperature-related variations in E_A. Saveyn et al. (2008) initially suggested that woody tissue photosynthesis in stem sections above or below the stem cuvette might reduce internal $[CO_2]$ within these sections, inducing axial diffusion of CO_2 away from the site of respiration. Isotope labelling techniques coupled with isotope ratio laser spectroscopy have recently confirmed the potential of light-induced axial CO_2 diffusion to alter sub-daily CO_2 diffusion patterns in tree stems (Salomón et al. 2019).

Although diffusion in the axial direction is higher than in the radial direction (Sorz and Hietz 2006), it is still considered insignificant relative to internal transport of CO_2 via the transpiration stream (Hölttä and Kolari 2009). The aim of this study was to quantify the extent to which axial diffusion of respired CO_2 affect E_A during the dormant season. We measured under controlled condition stem CO_2 efflux with a stem cuvette on dormant oak (*Quercus robur* L.) trees and induced axial CO_2 gradients by means of facilitating woody tissue photosynthesis in stem sections at varying heights, while the remainder of the tree was excluded from light. We hypothesised that a fraction of locally respired CO_2 would be transported axially instead of radially diffusing into the atmosphere, and therefore, the effect of axial CO_2 diffusion on E_A would be more pronounced when stem sections closer to the cuvette were illuminated. If true, previous efflux-based estimates of stem respiration during the dormant season, generally used to partition respiration into growth and maintenance components on a seasonal basis, may need reconsideration.

2 Materials and methods

2.1 Plant material and measurement conditions

Experiments were conducted under controlled conditions in a growth chamber (2 m × 1.5 m × 2 m, height × width × length) during winter. Measurements were performed on two 4-year-old oak (*Quercus robur* L.) trees, hereafter referred to as oak_1 and oak_2, with both an approximate height of 2 m and a diameter at stem base of 3.22 and 3.19 cm, respectively. Both trees were previously grown outdoors in 50-l containers containing potting mixture (LP502D, Peltracom nv, Gent, Belgium) and fertiliser (Basacot Plus 6M, Compo Benelux nv, Deinze, Belgium). Each tree was placed into the growth chamber 1 week prior to measurements, allowing adaptation to indoor conditions. Air temperature (T_{air}) and relative humidity (RH) were kept constant during the entire experiment and were measured with a type-T thermocouple (Omega, Amstelveen, the Netherlands), and a capacitive RH-sensor (Model HIH-3605-A, Honeywell, Morristown, NJ, USA), respectively. Densely packed fluorescent lamps (TL'D 36 W/85, Philips, Eindhoven, the Netherlands) at the ceiling of the growth chambers produced a constant background photosynthetic active radiation (PAR) of 140 μmol m^{-2} s^{-1} during the

entire experiment, which was measured with a quantum sensor (model Li-190, Li-COR, Lincoln, TE, USA) just above the canopy. All data was recorded with a data logger (HP 34970A, Hewlett-Packard, Palo Alto, CA, USA) at a 1-min interval and averaged over 5-min intervals.

2.2 Stem CO_2 efflux

E_A was measured on a stem section located 30 cm above the soil surface, with a stem diameter of 3.06 and 3.02 cm for oak_1 and oak_2, respectively. Stem cuvettes were 13-cm long, constructed of Polycarbonate film (Roscolab Ltd., London, UK) and sealed with adhesive closed-cell foam gasket material and non-caustic silicone (RS components Benelux, Anderlecht, Belgium). Outside air was mixed in a 50-l buffer barrel to obtain stable inlet air [CO_2] and was pumped to the stem cuvette with a membrane pump (model 2-Wisa, Hartmann and Braun, Frankfurt am Main, Germany) at an average flow rate of 1.1 l min^{-1}, which was measured with a flow meter (model 5860S, Brooks Instruments, Ede, the Netherlands). The [CO_2] of air leaving the stem cuvette was measured with an infrared gas analyser (IRGA, LI-7000, Li-COR, Lincoln, TE, USA) and was compared to the [CO_2] of air leaving a reference cuvette. The reference cuvette had same dimensions as measurement cuvettes and enclosed a PVC tube of 3.2-cm outer diameter. The IRGA was zeroed every hour to correct for possible drift during measurements. For this, an automatic multiplexer switched the reference flow through the measuring flow of the IRGA. E_A was calculated according to Long and Hallgren (1985) and was expressed per unit of surface area. Stem cuvettes were leak-tested prior to the start of the experiment and sealed where needed. Stem temperature (T_{stem}) was measured 2 cm below and 2 cm above the stem cuvette with 1-cm-long home-made thermocouple needle (type T, Omega Engineering Omega, Amstelveen, the Netherlands) to verify constant T_{stem} during the experiment.

2.3 Axial CO_2 diffusion in stems

To account for the potential effect of axial CO_2 diffusion on E_A, an axial [CO_2] gradient was induced within the tree stem (Fig. 1). Light was excluded from the whole tree and the stem cuvette by loosely wrapping the tree with aluminium foil, while a 10-cm-long stem section was exposed to a movable fibre optic light source (Model FL-4000, Walz Mess und Regeltechnik, Effeltrich, Germany) producing an average PAR of 856 ± 86 μmol m^{-2} s^{-1}, which was measured with a quantum sensor (model Li-190, Li-COR, Lincoln, TE, USA) next to the stem surface during the entire experiment. A fibre optic light source was selected because it produces homogenous light distribution and does not emit heat in contrast to other standard light sources. A 10-cm-long PVC tube cut in half and covered with reflective foil at the inside was used to illuminate the opposite stem section at an average PAR of 55 ± 6 μmol m^{-2} s^{-1}. As a result, woody tissue photosynthesis occurred at this particular stem section lowering stem [CO_2] relative to the site of E_A measurement, and resulting in axial diffusion of CO_2 inside the stem (Fig. 1). The axial CO_2 gradient was altered by illuminating different stem sections along the tree: 5–15 cm, 15–25 cm, 25–35 cm and 35–45 cm above the stem cuvette, hereafter referred to as S10, S20, S30 and S40, respectively (see Fig. 4). At every position, the stem section was illuminated for 24 h while background PAR in the growth chamber was fixed at 140 μmol m^{-2} s^{-1}. Stem CO_2 efflux was recorded during three periods in each stem section: (i) 6 h before light exposure, (ii) 24 h during light exposure and (iii) 12 h after light exposure to compare light and dark conditions. Intermediate periods between stem sections were also monitored for at least 24 h until stable E_A reference readings were obtained.

2.4 Sap flow and stem diameter

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

2.5 Chlorophyll concentration

After E_A measurements in each oak tree, bark of stem sections S10 to S40 was collected for determination of bark chlorophyll concentration. Per stem section, four samples were randomly collected, immediately frozen in liquid nitrogen and stored at − 80 °C. Samples were grinded (A11 basic analytic mill, IKA-Werke GmbH & Co. KG, Staufen, Germany) and chlorophyll was extracted by adding 7.5 ml acetone (80%) to 150 mg of sample. After 24-h extraction in the dark, samples were centrifuged and the supernatant was transferred to a glass cuvette and analysed for chlorophyll concentration with a

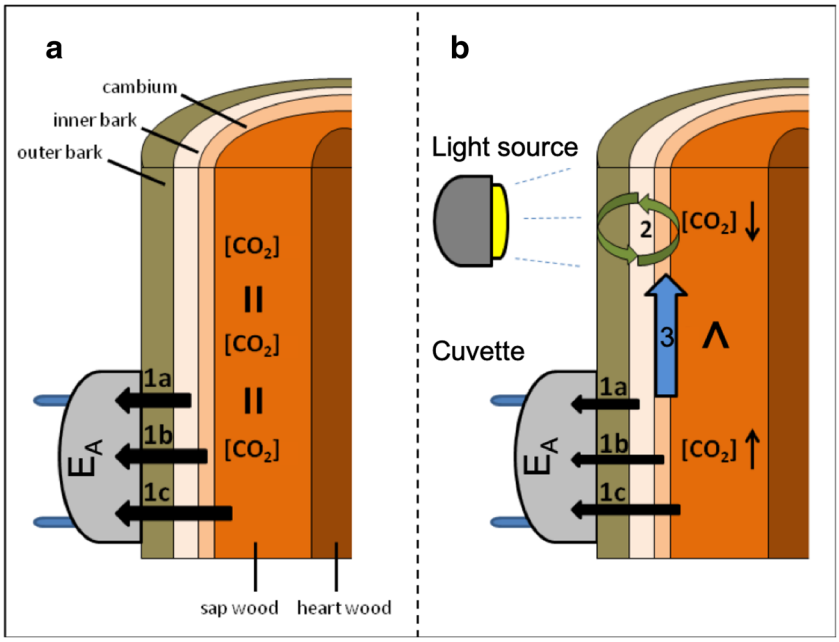

Fig. 1 A schematic overview of radial and axial CO_2 fluxes in a dormant tree stem with a stem cuvette to measure stem CO_2 efflux under different light conditions. **a** Under light exclusion, only outward radial diffusion of respired CO_2 occurs from the inner bark (1a), cambium (1b) and xylem ray cells (1c). **b** Under light exposure of a stem section remote from the stem cuvette, woody tissue photosynthesis occurs (2), while light is excluded from the remainder of the stem and the cuvette. Stem CO_2 concentration ($[CO_2]$) decreases within the illuminated stem section relative to that in the stem section enclosed in the opaque stem cuvette, resulting in upward axial CO_2 diffusion (3) to the detriment of radial CO_2 diffusion (1a, 1b and 1c). Adapted from Teskey et al. (2008)

spectrophotometer (UVIKON XL, Bio-Tek Instruments, Winooski, VT, USA) at wavelengths of 663.6 and 646.6 nm. Chlorophyll concentrations were calculated according to Porra et al. (1989) and expressed per unit of bark fresh weight (mg chl g^{-1} FW).

2.6 Temperature sensitivity of stem CO_2 efflux

To study the impact of axial CO_2 diffusion on the temperature response of E_A, trees were subjected to a temperature gradient under dark conditions and when stem section S10 was illuminated. Four temperature steps were programmed: 20, 23, 26 and 19 °C, each lasting 2 h. From the relationship between T_{stem} and E_A, new values of Q_{10} and E_{A_20} at a reference temperature of 20 °C were determined and compared to those obtained before light exposure.

2.7 Data and statistical analysis

A multi-factorial analysis of variance (ANOVA) was applied to compare chlorophyll concentration of different stem sections by considering stem section ($n = 4$, S10 to S40) as fixed factor and individual tree ($n = 2$) as random factor. To evaluate E_A, data was averaged over 1-h intervals and a repeated measures ANOVA was performed considering different experimental stages ($n = 5$, reference and S10 to S40) and time ($n = 42$, 24 h of light exposure and 18 h of reference) as fixed factors and individual tree as random factor ($n = 2$). Small-sample-size-adapted Akaikes information criterion (AIC_C) was used to determine the covariance structure that best estimated the correlation among individual trees over time. ANOVA analyses were performed using the mixed model procedure (PROC MIXED) of SAS (Version 9.1.3, SAS inc., Cary, NC, USA) with a statistical confidence of $\alpha = 0.05$.

3 Results

3.1 Microclimate, sap flow and stem diameter

Both oak_1 and oak_2 were subjected to a constant T_{air} and RH regime during the entire experiment in the growth chamber (with mean values of 19.86 ± 0.34 °C and 42.53 ± 5.08% for oak_1 and 20.37 ± 0.08 °C and 40.81 ± 2.84% for oak_2). Stem temperature was constant during the entire experiment, irrespective of the light treatment. For oak_1, averaged T_{stem} below and above the stem cuvette during the reference period was 20.52 ± 0.05 °C, which is within the range of measured average T_{stem} during the light exposure period (20.42 ± 0.18 °C to 20.75 ± 0.20 °C). Likewise, average T_{stem} of oak_2 during the reference period (20.08 ± 0.02 °C) was similar to the average T_{stem} observed during illumination (from 20.06 ± 0.05 °C to 20.16 ± 0.15 °C). Variation in E_A was

therefore independent of stem temperature. Measurements of sap flow and stem diameter variation confirmed that both trees were dormant. Heat balance data indicated that there was no heat transfer by convection within the stem, so that sap flow within the xylem did not occur. Stem diameter variations on sub-daily and daily basis were not observed (data not shown).

3.2 Stem CO_2 efflux and axial diffusion of CO_2

Average E_A during reference dark periods were 0.76 ± 0.02 and 1.04 ± 0.02 μmol m^{-2} s^{-1} for oak_1 and oak_2, respectively. Illumination of stem segments induced axial diffusion of CO_2 in the stem, which decreased E_A relative to reference dark rates (Fig. 2). The vertical distance between the illuminated stem section and the stem cuvette had a significant effect on the decrease in E_A ($P = 0.0115$). The largest decrease in E_A was observed when S10 was exposed to light. In oak_1, a reduction of 0.17 μmol m^{-2} s^{-1} was observed 24 h after light exposure (Fig. 2), i.e. 22% relative to the reference E_A (Fig. 3). In oak_2, a decrease of 0.14 μmol m^{-2} s^{-1} was detected, i.e. 13% relative to the reference E_A. In both trees, the response in E_A on S10 illumination was fast, with a time lag of approximately 1–2 h (Fig. 2).

The more remote the light-exposed stem sections were, the smaller the impact of axial CO_2 diffusion on E_A was. Illuminating S20 reduced E_A by 0.10 μmol m^{-2} s^{-1} and 0.12 μmol m^{-2} s^{-1} in oak_1 and oak_2, respectively, while a reduction of 0.06 μmol m^{-2} s^{-1} in E_A was found when exposing S30 and S40 in both trees. Due to the long distance between stem cuvette and S30 and S40, the transient decrease in E_A due to axial CO_2 diffusion was slow, with reductions in E_A of 5–10% compared to that under dark reference conditions (Fig. 3).

Axial CO_2 diffusion affected dormant season estimates of Q_{10} and E_{A_20}; Q_{10} under dark conditions was 1.75 and 2.05 for oak_1 and oak_2, respectively, and E_{A_20} was 0.98 and 1.31 μmol m^{-2} s^{-1}. When illuminating stem section S10, Q_{10} values were 1.57 and 2.25 for oak_1 and oak_2, respectively, and E_{A_20} values were 0.93 and 1.29 μmol m^{-2} s^{-1}.

3.3 Bark chlorophyll concentration

Chlorophyll concentrations ranged from 0.37 ± 0.05 to 0.45 ± 0.02 mg chl g fresh $weight^{-1}$ in oak_1 and from 0.35 ± 0.04 to 0.41 ± 0.04 mg chl g fresh $weight^{-1}$ in oak_2. No significant differences in bark chlorophyll concentration among S10, S20, S30 and S40 were observed ($P = 0.31$) (Table 1). Similar levels in chlorophyll concentration indicate similar potential of woody tissue photosynthesis along light-exposed stem sections.

4 Discussion

Recent advances in the field of tree respiration research fostered important discussion regarding the use of efflux-based measurements to estimate stem respiration rates (Steppe et al. 2015; Teskey et al. 2017, 2008). Where classic studies assumed that E_A equals stem respiration, it is now acknowledged that CO_2 emitted by stems is derived from a multitude of sources affected by different factors (Salomón et al. 2017a; Saveyn et al. 2007a, 2008; Steppe et al. 2015; Tarvainen et al. 2017; Yang et al. 2012). Here, we demonstrate that axial diffusion of respired CO_2 is an additional factor that should be accounted for when estimating stem respiration. Up till now, and for methodological simplicity, this flux was considered insignificant in comparison to xylem transport of respired CO_2 (Hölttä and Kolari 2009), but its importance remains unclear for efflux-based estimates of stem respiration during the dormant season. However, given that the experiment was executed on only two replicates, conclusions from this study should be taken with caution and further research with different species and across a gradient of tree sizes should be performed to more accurately quantify the magnitude of axial CO_2 diffusion in stem carbon balances.

4.1 Effect of woody tissue photosynthesis on stem CO_2 efflux

During tree dormancy, it is generally accepted that only maintenance respiratory processes contribute to E_A and that maintenance respiration dynamics are mainly driven by temperature (Amthor 2000). Notwithstanding, we observed substantial temperature-independent variations in E_A in dormant oak trees, as similarly described by Saveyn et al. (2008) in oak and beech trees. By locally illuminating different stem sections near the stem cuvette, while excluding light from the remainder of the tree, we observed pronounced decreases in E_A rates. More interestingly, we observed largest reductions in E_A when the stem section closest to the cuvette was exposed to light, with decreases up to 22% due to axial diffusion of CO_2 in stems. In this line, it has been observed that sub-daily dynamics of radial CO_2 diffusion in stems of poplar trees were mainly driven by PAR and consequent light-induced axial CO_2 gradients when woody tissue photosynthesis was allowed in stem parts adjacent to the monitored cuvette (Salomón et al. 2019). On the contrary, when woody tissue photosynthesis was disabled by means of covering woody tissues with aluminium foil, radial CO_2 diffusion was mostly explained by different factors such as the water status of the tree (Salomón et al. 2019).

Bark chlorophyll concentrations (Table 1) in dormant oak stems were within the range reported for several species (Pfanz et al. 2002). Assimilation of respired CO_2

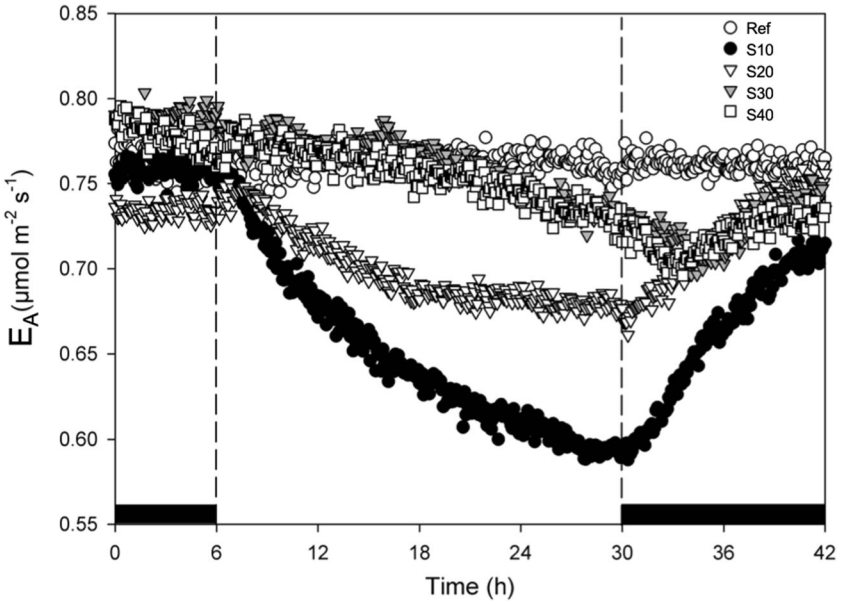

Fig. 2 Profiles of stem CO_2 efflux (E_A) measured with a stem cuvette on oak_1 when light was excluded from the entire tree (Ref) and when woody tissue photosynthesis was induced in 10-cm-long stem sections remote from the site of E_A measurement by illuminating either S10 (5–15 cm from the stem cuvette), S20 (15–25 cm from the stem cuvette), S30 (25–35 cm from the stem cuvette, grey triangles) or S40 (35–45 cm from the stem cuvette). E_A data are 5-min averages. Beginning and end of illumination periods are indicated by black boxes and dashed lines. Data of oak_2 is included as appendix (Fig. 5)

within the chlorophyll containing tissues may have locally reduced [CO_2] relative to that in stem sections where woody tissue photosynthesis was impeded. As a result, a [CO_2] gradient along the stem arose, inducing axial diffusion of respired CO_2 from the site of high to low [CO_2], according to Fick's law of diffusion (Jones 1992; Saveyn et al. 2008). As a consequence, a lower fraction of respired CO_2 diffused radially into the stem cuvette, observed as a decrease in E_A. Additionally, Fick's law of diffusion states that CO_2 diffusion along the concentration pathway is inversely proportional to its length. This explains why the largest decrease in E_A was observed during illumination of the stem section closest to the stem cuvette. In general, axial diffusion of gases within plants induced by woody tissue photosynthesis has been described to play a role in root and stem aeration in genera

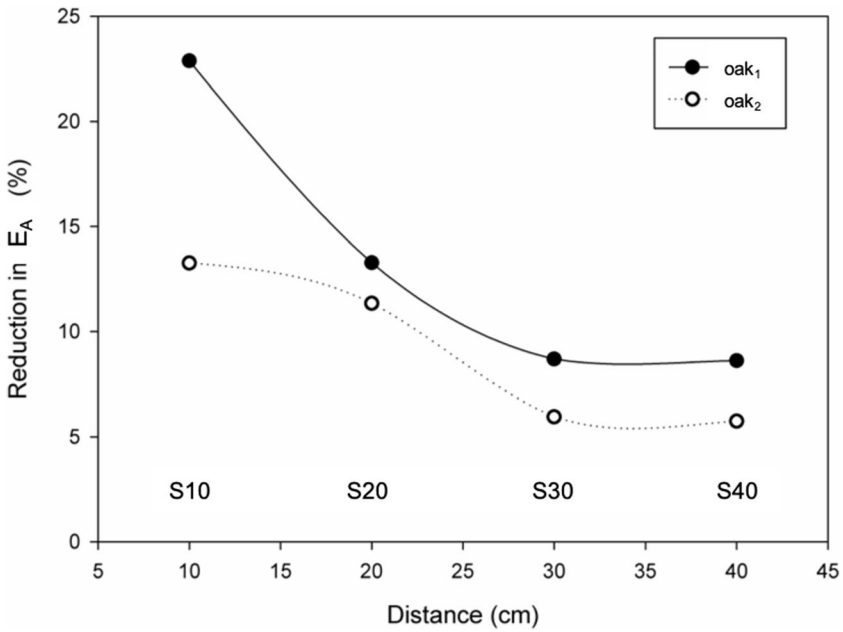

Fig. 3 Relative reduction in stem CO_2 efflux (%) measured with a stem cuvette for two dormant oak trees (oak_1 and oak_2), when illuminating 10-cm-long stem sections at different distances from the site of E_A measurement, while light is excluded from the remainder of the tree and stem cuvette. Reductions are expressed relative to E_A measurements performed when light was excluded from the entire tree

Table 1 Bark chlorophyll concentrations in two oak trees (oak_1 and oak_2) for different 10-cm-long stem sections (S10, S20, S30 and S40) along the stem exposed to light. Data are averages (± SD) of four samples per stem section per tree

Bark chlorophyll concentration		
Stem segment	oak_1 mg chl g^{-1} FW	oak_2 mg chl g^{-1} FW
S10	0.45 ± 0.08	0.37 ± 0.08
S20	0.44 ± 0.04	0.41 ± 0.04
S30	0.45 ± 0.04	0.35 ± 0.04
S40	0.37 ± 0.02	0.38 ± 0.02

of *Alnus*, *Salix*, *Betula* and *Populus* (Armstrong and Armstrong 2005; Grosse et al. 1996; Wittmann and Pfanz 2018). During illumination of aboveground plant parts, photosynthesis in chlorophyll-containing woody tissues increases the internal O_2 concentration, inducing a diffusive transfer of O_2 to parts of the tree remote from the site of O_2 production. Moreover, axial diffusion of O_2 is facilitated relative to its radial diffusion due to the anatomy of the tree stem. Sorz and Hietz (2006) reported that for *Q. robur* diffusion of O_2 in the axial direction was more than 20 times higher than in the radial direction, due to the fact that the diffusing gas encounters less cell walls when travelling along the stem axis. A similar high resistance to radial CO_2 diffusion exerted by the xylem, cambium and bark layers has been described, and this radial resistance should be considered when using efflux-based measurements to predict stem respiration rates (Steppe et al. 2007). The fact that CO_2 might diffuse rapidly in axial direction due to woody tissue photosynthesis was first suggested by Saveyn et al. (2008). Based on the specific coefficients for oxygen diffusion for *Q. robur* (6.9×10^{-8} m^2 s^{-1} at 15% moisture content; Sorz and Hietz (2006)), it is possible to estimate the time for CO_2 to diffuse axially within stems. For a 10-cm distance, oxygen axial diffusion would take around 4.6 h. Axial diffusion of CO_2 will probably take longer, given that molecules with higher mass tend to have lower diffusion coefficients (Nobel 1999), but the transfer time would be in the same order of hours. This estimate is in strong contrast with observations by Gansert (2003), who stated that gas diffusion in stems is much slower (1 m would take several years) depending on the CO_2 gradient. However, Gansert (2003) assumed that movement of gases in stems mainly will occur via the aqueous phase despite an important fraction of the stem consists of gas voids (25% gas by volume in stems of *Quercus* sp.; (MacDougal et al. 1929; Teskey et al. 2008) where diffusion occurs much faster than in water (Nobel 1999).

4.2 Implications on stem respiration estimates

Our data also allow to illustrate whether it is justified to neglect axial diffusion of respired CO_2 when estimating E_A during the growing season when transpiration occurs. Respired CO_2, either derived from below- or aboveground sources, is transported upward via the transpiration stream (Aubrey and Teskey 2009; Bloemen et al. 2013; Steppe et al. 2015) and may confound efflux-based estimates of stem and branch respiration (Salomón et al. 2017a; Teskey et al. 2008). In a previous study on two 3-year-old *Q. robur* trees grown in the same growing chamber under similar conditions, the maximal rate of internal CO_2 transport with the transpiration stream per unit of stem cross sectional area was about 0.01 μmol CO_2 cm^{-2} s^{-1} (Saveyn et al. 2007b). In another study, a maximal value of 0.05 μmol CO_2 cm^{-2} s^{-1} was observed when measuring on six 9-year-old field grown *Q. robur* trees (Bloemen et al. 2014). Converting the maximal observed reduction in E_A in our dormant trees due to axial diffusion (0.17 μmol CO_2 m^{-2} stem surface area s^{-1}) to axial transport, by multiplying with stem surface area inside the cuvette and dividing by cross-sectional area of the respective stem segment, axial CO_2 diffusion would be about 0.0006 μmol CO_2 cm^{-2} s^{-1}. This diffusion rate is only 1–6% of the internal CO_2 transport rate with the transpiration stream and therefore it is reasonable to neglect the effect of axial CO_2 diffusion on E_A when sap flow occurs. On the other hand, diffusivity of gases in stems is expected to increase with increases in stem gas volume (Sorz and Hietz 2006), which occurs during the phenological shift from the dormant to the growing season as stem volumetric water content decreases (MacDougal et al. 1929; Pausch et al. 2000). Nonetheless, this potential increase in axial CO_2 diffusion in the gas phase is unlikely large enough to rival a much larger xylem CO_2 flux dissolved in the sap solution.

Accurate efflux-based estimates of stem respiration during dormancy are essential for partitioning respiration in growth and maintenance components according to the maintenance-and-growth respiration paradigm (Amthor 1989; Maier 2001) commonly used in global models (Atkin et al. 2017). The most widely used approach to estimate growth and maintenance stem respiration is the mature tissue method (e.g. Damesin 2003; Gaumont-Guay et al. 2006; Maier 2001). This method applies the temperature sensitivity of E_A (Q_{10}) and a reference E_A measured at a reference temperature t_0 (E_{A_0}) calculated during the dormant season to estimate maintenance respiration during the whole year based on temperature measurements (Lavigne and Ryan 1997). Therefore, bias in Q_{10} and E_{A_0} coefficients due to axial CO_2 diffusion could result in substantial error when upscaling stem respiration to large spatial and temporal scales (Darenova et al. 2018). We observed changes in both parameters when the stem section just above the cuvette was illuminated in comparison with dark conditions. We suggest that differences in Q_{10} and E_{A_0}

could be even higher if stem sections on both sides of the stem cuvette (above and below) would be illuminated, as observed by Saveyn et al. (2008).

Under natural conditions, bias in estimates of Q_{10} and E_{A_0} during the dormant season might largely differ depending on the species, tree size and bark properties. On one hand, species with high bark and/or xylem chlorophyll concentrations (Pfanz et al. 2002) might exhibit large axial diffusive fluxes, particularly in stands with low stem density where light is not limiting. On the other hand, in large trees with a thick dead outer bark the light that reaches the chloroplasts might be reduced limiting axial diffusion of respired CO_2. Therefore, accounting for the effect of axial diffusion of CO_2 when estimating Q_{10} and E_{A_0} might be crucial to understand and model stem respiration, at least from a mechanistic perspective.

In conclusion, the study presented here unambiguously demonstrates the importance of axial CO_2 diffusion induced by woody tissue photosynthesis on E_A in dormant trees. However, our observations were limited to two trees of the same species. The aim of this work is to highlight an overlooked mechanism altering stem CO_2 efflux rates, but the limited sample size discourages any quantitative extrapolation to larger spatial scales. Further research in larger trees, different species and larger sample sizes would be necessary to better quantify the magnitude of axial diffusion of CO_2 across species and gradients of environmental conditions. Moreover, parallel measurements of bark properties such as bark thickness and transmission of photosynthetic photon flux density would contribute to better understand potential differences among species and tree sizes. Our above findings led us to recommend additional shading in dormant trees in upper and lower stem sections adjacent to the opaque stem cuvette to avoid axial diffusion of respired CO_2 away from the site of respiration. This recommendation will lead to more accurate Q_{10} and E_{A_0} estimates, particularly in open forest stands with sufficient light penetration. Accurate estimates of Q_{10} and E_{A_0} will improve quantification of stem respiration during the of dormant season, which is in turn crucial to better understand and predict stand-level respiration dynamics throughout the year.

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Data availability The datasets generated and/or analysed during the current study are available in the Zenodo repository (De Roo et al. 2019), https://doi.org/10.5281/zenodo.2633214.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

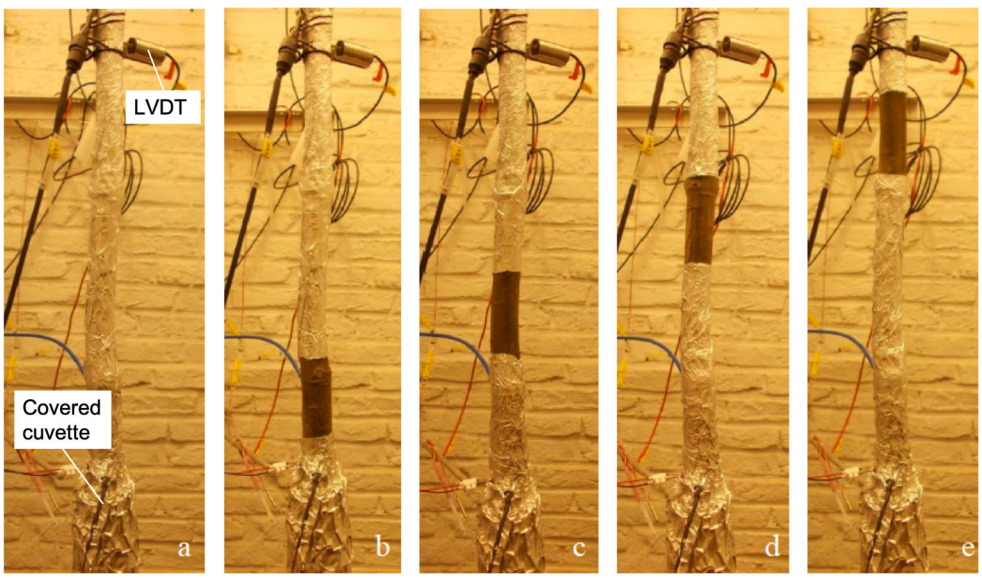

Fig. 4 Photograph of the experimental setup, showing dark conditions (**a**) and the uncovering of section S10 (**b**), section S20 (**c**), section S30 (**d**) and section S40 (**e**), respectively

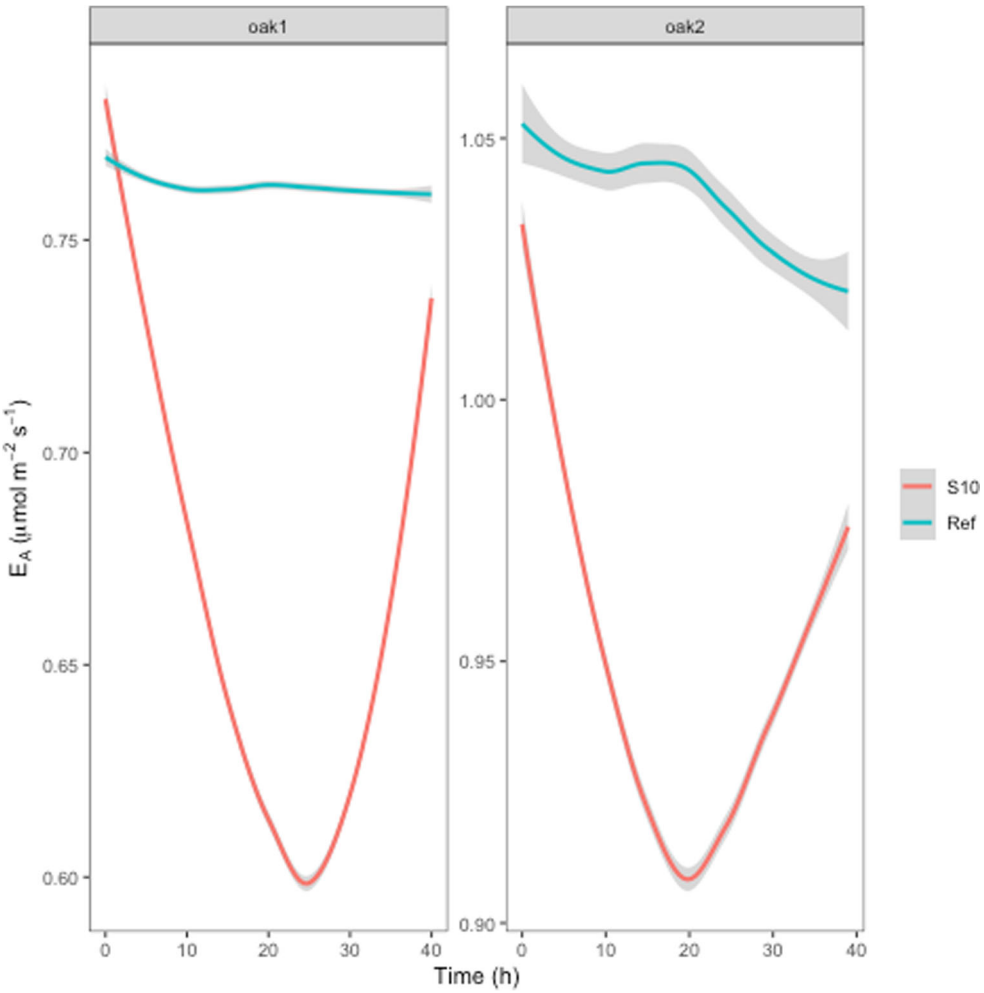

Fig. 5 Profiles of stem CO_2 efflux (E_A) measured with a stem cuvette on oak_1 and oak_2 when light was excluded from the entire tree (Ref) and when woody tissue photosynthesis was induced in 10-cm-long stem sections remote from the site of E_A measurement by illuminating S10 (5–15 cm from the stem cuvette). Loess regression was performed to visually clarify the similar pattern of both trees

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