Supplementary Material

Differences between tree stem CO_2 efflux and O_2 influx rates cannot be explained by internal CO_2 transport or storage in large beech trees

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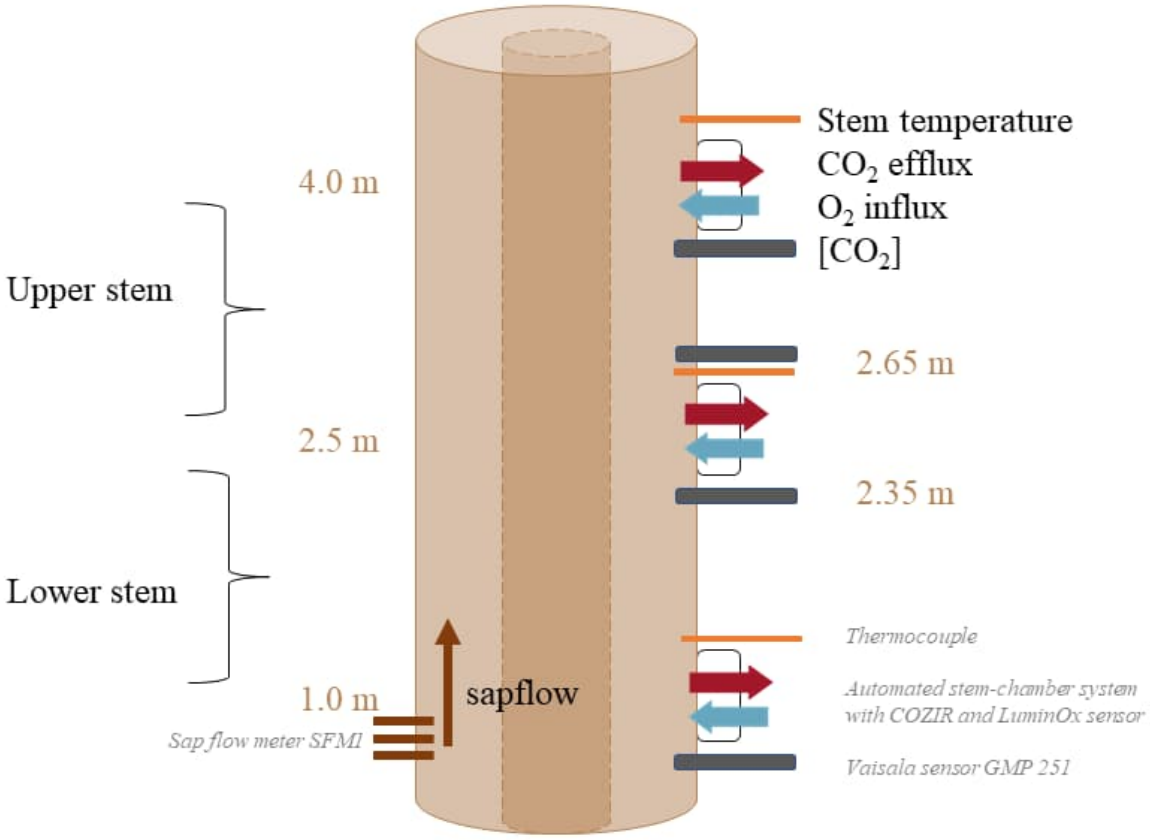
The following Supporting Information is available for this article:

Figure S1 Schematic overview of the experimental set-up and measurement devices installed on the stem at different stem levels.

Figure S2 (a) Beech tree with stem chambers (CO_2 efflux, O_2 influx) and Vaisala sensors (internal [CO_2]) at different stem heights. (b) Close-up of the equipment with butty butyl sealant for isolation from the atmosphere.

Method S1: Measurement of PEPC capacity



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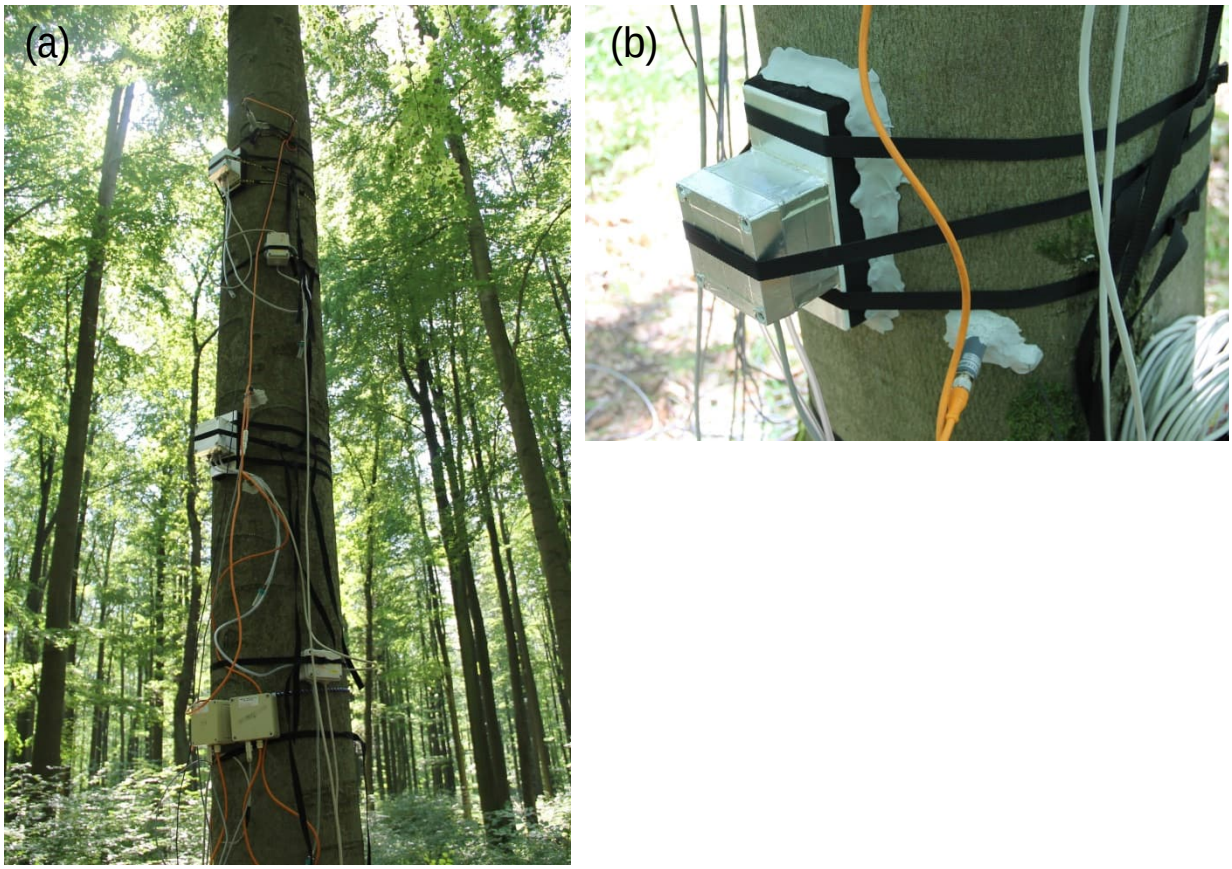

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Methods S1: Measurement of PEPC capacity

Aliquots were extracted by vigorous shaking with 500 µL of extraction buffer (20% v/v glycerol, 0.25% w/v bovine serum albumin, 1% v/v Triton X-100, 50 mM Hepes-KOH pH 7.5, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM aminocaproic acid, 1 mM benzamidine, 20 µM leupeptin, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride, 10% w/v polyvinylpolypyrrolidone and 1% w/v polyvinylpyrrolidone). After centrifugation (7 min, 3000 g, 4 °C), extracts were diluted by a factor of 2000 (w/v). Diluted extracts and NAD^+ standards (prepared in the extraction buffer and ranging from 0 to 1 nmol per well) were incubated for 20 min in 20 µl of a medium containing 100 mM Tricine-KOH pH 8.0, 20 mM $MgCl_2$, 1 unit ml^{-1} malate dehydrogenase, 10 mM $NaHCO_3$, 0.1 mM NADH, 1% w/v polyvinylpyrrolidone and phosphoenolpyruvate 0 (blanks) or 2 mM (maximal activity). The reaction was stopped with 20 µl of 0.5 M HCl. The sealed microplate was then incubated at 95 °C for 10 min to destroy NADH. After cooling down, each well was neutralised with 20 µl of NaOH 0.5 M and 0.2 M Tricine-KOH pH 9.0 to adjust the pH to 9.0. NAD^+ was then measured with 6 units ml^{-1} alcohol dehydrogenase, 100 mM Tricine-KOH pH 9.0, 4 mM EDTA, 0.1 mM PES, 0.6 mM MTT, and 500 mM ethanol. The absorbance was read at 570 nm and 30 °C until rates were stabilised. Reaction rates expressed in $mOD.min^{-1}$ were used to calculate the amount of NAD^+ formed during the first step of the assay. All pipetting steps were performed using a 96-head robot (Hamilton Star), and absorbances were measured in a filter-based microplate reader (SAFAS MP96).