



## The importance of internal CO<sub>2</sub> gradients in tree roots for assessing belowground carbon allocation

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In trees, it is known that allocation of recent assimilates belowground fuels metabolic processes like root respiration. Nonetheless, the fraction of carbon allocated belowground remains poorly quantified as the energetic costs of tree root metabolism remain largely unknown. Current estimates of root respiration are calculated from measurements of CO<sub>2</sub> efflux from roots or soil. However, a substantial portion of CO<sub>2</sub> released by root respiration might remain within the tree root system rather than diffusing into the soil environment, indicating that root respiration consumes substantially more carbohydrates than previously recognized.

We measured internal CO<sub>2</sub> concentration ([CO<sub>2</sub>]) and sap flow in three longitudinal sections of two large roots of American beech (*Fagus grandifolia*) and yellow poplar (*Liriodendron tulipifera*) trees (n=4 trees per species), while simultaneously measuring [CO<sub>2</sub>] in neighboring soil. We hypothesized that [CO<sub>2</sub>] would be lowest in soil and increase from the root tip to the base of the stem. We observed substantially higher [CO<sub>2</sub>] in tree roots (on average  $8.5 \pm 2.0$  and  $5.2 \pm 1.9$  Vol% for American beech and yellow poplar, respectively) compared with the soil environment ( $1.0 \pm 0.4$  and  $1.3 \pm 1.3$  Vol% around American beech and yellow poplar, respectively), indicating that root tissues exert substantial barriers to outward diffusion of respired CO<sub>2</sub>. Moreover, we observed an internal [CO<sub>2</sub>] gradient from root tip to stem base which suggests that progressively more respired CO<sub>2</sub> dissolved in flowing xylem sap as it moved from the soil through the root xylem.

These results confirm that a fraction of root-respired CO<sub>2</sub> concentrates in the xylem sap of the root system and fluxes upward within the tree. This CO<sub>2</sub> that is removed from the site of respiration cannot be accounted for with measurements of CO<sub>2</sub> efflux from roots or soil, indicating that efflux-based techniques underestimate the energetic costs of tree root metabolism and therefore the amount of carbon that is allocated belowground.