



## **A new approach to quantifying internal diffusion resistances and CO<sub>2</sub> isotope exchange in leaves**

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The oxygen isotopic composition ( $\delta^{18}\text{O}$ ) of atmospheric CO<sub>2</sub> can constrain the global CO<sub>2</sub> budget at a range of scales, offering the potential to partition net CO<sub>2</sub> exchanges into their component gross fluxes and provide insights to linkages between C and water cycles. However, there are significant limitations to utilizing the  $\delta^{18}\text{O}$  of CO<sub>2</sub> to constrain C budgets because of uncertainties associated with the isotopic exchange of CO<sub>2</sub> with terrestrial water pools. Leaf water in particular represents a critical pool with ongoing debates about its enrichment in heavy isotopes during transpiration and the hydration of CO<sub>2</sub> and its oxygen isotope exchange with this pool. Isotopic heterogeneity of the leaf water, the spatial distribution and activity of carbonic anhydrase (CA) within leaves, and resistance to diffusion of CO<sub>2</sub> from the substomatal cavity to chloroplasts are all key components with important uncertainties. Better constraints on these would significantly improve our ability to understand and model the global C budget as well as yield insights to fundamental aspects of leaf physiology. We report results using a new measurement system that permits the simultaneous measurement of the <sup>13</sup>C and <sup>18</sup>O composition of CO<sub>2</sub> and the <sup>18</sup>O isotopic composition of leaf transpiration. As this new approach permits rapid alteration of the isotopic composition of gases interacting with the leaf, key model parameters can be derived directly and simultaneously. Hence, our approach does not rely on separate measurements shifted in time from the gas exchange measurements or that may not quantify the relevant scale of heterogeneity (e.g., CA enzyme assays or bulk leaf water extraction and analysis). In particular, this new method explicitly distinguishes the leaf mesophyll resistance to CO<sub>2</sub> transport relevant for photosynthesis from the resistance required for interpreting the  $\delta^{18}\text{O}$  of CO<sub>2</sub> and allows us to derive other relevant parameters directly. This new measurement system and modeling approach allows a thorough assessment of key elements of our current understanding of CO<sub>2</sub>-H<sub>2</sub>O oxygen isotope exchange in terrestrial ecosystems.