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# On-line monitoring with isotope-specific laser spectroscopy reveals temporal variability of the oxygen isotope exchange between leaf water and carbon dioxide

Nicolas Brüggemann (1) and Carola Blessing (2)

(1) Forschungszentrum Jülich GmbH, Agrosphere (IBG-3), Jülich, Germany (n.brueggemann@fz-juelich.de, +49 2461 61-2518), (2) ETH Zürich, Institute of Agricultural Sciences, Universitätstrasse 2, 8092 Zürich, Switzerland

The oxygen isotope composition of atmospheric CO2 carries the information of the role of the terrestrial biosphere in the global carbon cycle. However, its proper interpretation requires understanding of the underlying processes, also in response to short-term changes of environmental conditions. The most important process determining the 18O-signature of atmospheric CO2 and its spatial and temporal variation is the interaction with plants due to oxygen exchange of CO2 with 18O-enriched leaf water – a process catalyzed by the enzyme carbonic anhydrase, which is commonly assumed as quantitative. Understanding the mechanisms and temporal variability of oxygen isotope exchange between CO2 and leaf water is key to reliable quantification of the contribution of the terrestrial biosphere to the 18O-signature of atmospheric CO2.

As not only leaf-respired CO2 is subject to oxygen exchange, but also CO2 involved in retroflux – i.e. the proportion of CO2 that is not fixed in photosynthesis and diffuses back to the atmosphere –, the oxygen isotope signal of CO2 depends on its gross (one-way) fluxes into and out of plant leaves. Accounting for one-way fluxes could explain the high 18O enrichment of CO2 during darkness, observed in several studies. However, up to now the mechanism of the even higher 18O-enrichment of CO2 just after darkening is unclear. This study aimed at elucidating the processes involved in oxygen isotope exchange between atmospheric CO2 and leaf water by means of cuvette experiments with potted Norway spruce (Picea abies) and grey poplar (Populus x canescens) plants under controlled environmental conditions and varying light intensities. Beside the determination of gas exchange and associated parameters, like stomatal conductance or intercellular CO2 concentration, CO2 and H2O concentration and isotopic composition were measured with high time resolution with tunable diode laser absorption spectroscopy (TDLAS) for CO2 and wavelength-scanned cavity ring-down spectroscopy (WS-CRDS) for H2O.

The isotopic exchange between CO2 and leaf water was higher during illumination than during darkness for untreated spruce and for poplar at the beginning of the measurements. In contrast, this relation changed over the course of the experiments with grey poplar, revealing the same pattern as in Norway spruce treated with pesticide with a higher degree of equilibration during darkness. One possible reason could be due to stress, induced by drought in poplar and by pesticide in spruce. Pronounced post-illumination peaks of $\delta$18O-CO2 were found for Norway spruce, especially after illumination with high light intensities, whereas they were less pronounced in grey poplar. Additionally, "post-darkness" depressions of $\delta$18O-CO2 were found right after the onset of the light period for both Norway spruce and grey poplar. Application of pesticide to Norway spruce as treatment against aphids artificially decreased stomatal conductance and hence gas exchange compared to the pre-treatment phase. Post-illumination peaks of $\delta$18O-CO2 in pesticide-treated Norway spruce were much less pronounced, if not absent. Both increased stomatal conductance and light-enhanced dark respiration could be identified as most likely causes of these post-illumination peaks, albeit not unambiguously in all cases. Changes in oxygen isotopic composition of leaf water after darkening or changes in CO2 concentration gradients could be ruled out as significant for post-illumination peaks of $\delta$18O-CO2. In contrast, post-darkness depressions of $\delta$18O-CO2 could not be explained with the parameters measured or calculated in this study. The proportion of CO2 that isotopically equilibrated with chloroplast water was at most times clearly below 1 for both Norway spruce and grey poplar, running counter to the common assumption of quantitative isotopic equilibration between CO2 and water within the leaf. This study emphasizes the need for including the mechanisms responsible for the temporal variability of oxygen exchange between atmospheric CO2 and leaf water in regional and global modeling of biosphere-atmosphere exchange on the basis of oxygen isotopic signatures.