

## Detecting plant-climate interactions over decades-millennia using NMR isotopomer analysis

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Increasing CO<sub>2</sub> and climate change affect photosynthesis, which creates a critical influence on the global C cycle and on the future productivity of crops and forests. Manipulative experiments (e.g. FACE) impose step increases in [CO<sub>2</sub>], and are limited to few locations and to time spans of years, while responses over decades and centuries are critical for Earth system models.

To overcome these limitations, we have developed a new method – isotopomer analysis – that allows deducing plant C metabolism by analysis of primary plant photosynthates (Ehlers et al., PNAS 2015, 15585) or tree rings. We apply the method to material from manipulation (CO<sub>2</sub>, T) experiments, and to remnant - including subfossil - plant material. Thus, metabolic responses can be identified in FACE experiments, and it can be tested to what degree these responses are maintained during gradual environmental changes over decades-millennia. Isotopomer proxies developed using FACE experiments can then be used to reconstruct physiological and climatic changes by retrospective analysis, thus bridging a gap between experimental plant sciences and paleo research.

In experiments on annual plants, we have found that specific deuterium isotopomers in photosynthetic glucose reflect the ratio of oxygenation to carboxylation at Rubisco, a central metabolic branching that is the origin of the photorespiration flux in all C<sub>3</sub> plants. We found that increasing atmospheric [CO<sub>2</sub>] over the 20th century has reduced the photorespiration / photosynthesis ratio in all investigated C<sub>3</sub> species, with no evidence for acclimatory reactions by the plants. Results on the peat moss *Spagnum fuscum* suggest a mechanism for increasing peat accumulation rates, a major global C sink.

For 12 tree species from five continents, we observe that the CO<sub>2</sub> increase since industrialization has reduced the photorespiration / photosynthesis ratio. However, the observed reduction is ca. 50 % smaller than expected from CO<sub>2</sub> manipulation experiments. The smaller suppression of photorespiration in trees may be explained by increases in leaf temperature, suggesting that increasing temperatures may already be reducing the CO<sub>2</sub> fertilization effect on the global scale. These results may explain the discrepancy between strong CO<sub>2</sub> fertilization inferred from <sup>13</sup>C measurements yet lack of biomass increases.

Finally, we will stress advantages of isotopomers for studies of plant metabolism on millennial time scales: First, isotopomers multiply the information content, because glucose contains seven deuterium and six <sup>13</sup>C isotopomers. Second, RATIOS of isotopomers are independent of the isotope compositions of a plant's substrates H<sub>2</sub>O and CO<sub>2</sub>, which often are poorly constrained over paleo time scales. Third, because isotopomer abundances are set by specific biochemical reactions, very strong correlations between isotopomers and environmental variables are observed. Forth, parallel reconstructions of physiological and climate signals from the same samples may inform analyses of plant-climate interactions (Augusti et al., Chem. Geol 2008).