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## Hydrogen isotope offsets between palmitic acid and phytol increase during cyanobacterial blooms

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Hydrogen isotope fractionation between source water and lipids is highly variable among different taxonomic groups, and also among different compound classes within individual organisms. This variability results in lipid  $\delta^2\text{H}$  values that often span as much as two orders of magnitude more variability than that of environmental waters within typical ecosystems, indicating that lipid  $\delta^2\text{H}$  values may provide valuable biochemical and ecological information. These applications of lipid  $\delta^2\text{H}$  values remain underexplored.

Recent results from algal batch cultures indicate that hydrogen isotope fractionation by cyanobacteria differs significantly compared to eukaryotic algae. In particular, in single species cultures with constant water  $\delta^2\text{H}$  values, cyanobacteria tend to produce fatty acids that are slightly  $^2\text{H}$ -enriched compared to those from most eukaryotic algae, while phytol from cyanobacteria is very  $^2\text{H}$ -depleted compared to phytol from eukaryotes. This results in larger offsets between the  $\delta^2\text{H}$  values of phytol and fatty acids for cyanobacteria than those observed in eukaryotic algae. In order to determine if  $\delta^2\text{H}$  offsets between fatty acids and phytol change in freshwater lakes with variable abundance of cyanobacteria, we collected algal biomass from two depths in the water column of Rotsee, a small lake in central Switzerland, every second week from January 2019 to February 2020. During this time the percentage of algal biovolume from cyanobacteria ranged from 0 to 82 %, with two distinct cyanobacterial blooms occurring in July and October.

Water isotopes in the lake were relatively stable throughout the year, with water  $\delta^2\text{H}$  values varying by  $< 10$  ‰. Lipid  $\delta^2\text{H}$  values, on the other hand, displayed extreme variability throughout the year. Palmitic acid (C 16:0)  $\delta^2\text{H}$  values varied by nearly 100 ‰ ( $-282$  to  $-192$  ‰), while those of phytol varied by more than 200 ‰ ( $-417$  to  $-168$  ‰). Consistent with expectations based on the results of cultures of single algal species, cyanobacterial blooms were characterized by larger offsets between the  $\delta^2\text{H}$  values of palmitic acid and phytol, and these offsets were positively correlated with the percentage of total algal biovolume attributable to cyanobacteria ( $R^2 = 0.29$ ;

$p < 0.01$ ). These results suggest that hydrogen isotope offsets between palmitic acid and phytol in sediments have the potential to be developed as proxies for past cyanobacterial blooms, and demonstrate that hydrogen isotopes of lipids in the geologic record that are produced by many different types of aquatic organisms are more likely to be driven by ecological changes rather than changes in water isotopes.