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Analysis of oxygen isotope ratios in phosphate for assessing the microbial metabolism in the aquatic and soil environment

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Phosphorus in the form of phosphate is one of the fundamental elements for life and is involved in numerous metabolic pathways in any living organism. The metabolic phosphoryl transfer reactions entail a change in the oxygen isotope ratios in phosphate, either by the exchange of at least one out of the four oxygen atoms with oxygen from water, or by the preferential use of lighter phosphate isotopologue. Variations of natural abundance oxygen isotope ratios in phosphate have therefore been used in the past to identify and track biological processes involved in the phosphorus cycle, in marine and terrestrial aquatic and soil environments [1].

In most of these studies, the temperature-dependent isotopic equilibrium [2] between the oxygen atoms in phosphate and in water is interpreted as the evidence that intracellular (cytosolic) phosphate has been turned over by the enzyme pyrophosphatase, which is considered to overwrite other isotope effects of enzyme-catalyzed phosphoryl transfer reactions. However, one often observes oxygen isotope ratios which are inconsistent with this equilibrium paradigm. Currently, the interpretation is limited due to the incomplete knowledge on the effect of the metabolism on the oxygen isotope ratios in phosphate and the limited number of experimentally derived phosphoryl transfer isotope effects.

Here, we evaluate the hypothesis that not only pyrophosphatase, but also other enzymes, whose presence and activity depend on the metabolic status of the organism, can determine the oxygen isotope ratios of cytosolic phosphate. Specifically, we posit that under suboptimal conditions, oxygen isotope ratios of cytosolic phosphate might differ from values according to the pyrophosphatase equilibrium assumption.

To that end, we tested our hypothesis by cultivating *E. coli* under optimal and suboptimal temperature and pH conditions while monitoring oxygen stable isotope ratios in water and cytosolic phosphate and by tracing changes in metabolism using metabolomics approaches. The results from this project have the potential to impact how oxygen isotope ratios of phosphate are interpreted in future environmental studies.

[1] Bauke (2021), *Journal of Plant Nutrition and Soil Sciences* 184, 12-19.

[2] Chang & Blake (2015), *Geochimica et Cosmochimica Acta* 150, 314-329.