**Final Summary\_BioCTrack\_300064\_Researcher Nicole R. Posth**

This study focused on the influence of carbon fixation, transport and diagenesis on the C-isotope composition of anoxygenic phototrophic bacteria communities that oxidize iron or sulfide. These bacteria fix carbon out of CO2 via enzymatic pathways which break C-O bonds. The lighter of the two stable isotopes, 12C and 13C, forms a bond that is more susceptible to cleavage and leads to a kinetic isotope effect. Different enzymatic pathways for C-fixation are associated with different C-isotope fractionation. This relationship may make it possible to use C-isotope fractionation to track a particular phototrophic metabolism in sediment by evaluating the C-isotope composition of the CO2 source and the particulate organic carbon (POC). C-isotope composition of sediment and rocks are used to understand and reconstruct life on modern and ancient Earth. The goal was to establish whether the carbon isotope signal formed by bacterial communities is derived from a particular population and whether that signal persists in settling material and sediment. Ultimately, this should aid the interpretation of bacterial carbon cycling in modern and ancient sediments.

**The microbial carbon isotope signature was followed in stratified lakes high in dissolved iron and sulfide from bacterial carbon fixation in the photic zone to diagenesis in the sediment. Parallel, laboratory-based studies determined C-isotope fractionation during carbon fixation by Fe- and S-oxidizing anoxygenic phototrophic bacteria cultured from the lakes.** Three work packages (WP) address:

**WP1:** What are the systematics and extent of C-isotope fractionation due to carbon fixation by anoxygenic phototrophic bacteria in the presence of dissolved iron and sulfur, as well as conditions of carbon surplus and limitation?

**WP2:** In mixed bacterial communities, in which multiple C-fixation pathways are used, is the final C-isotope composition measured *in situ* determined by a dominant species or is it an integration of all?

**WP3:** How do biomineralization and diagenetic processes influence the final isotopic composition of an aquatic microbial system, and the preservation potential of individual isotopic signatures?

To fulfill these tasks, the C-isotope fractionation between the DIC (CO2 source) and the POC pools were quantified both in laboratory cultures and in lake water, settling material, and sediment (∆13C = δ13CDIC - δ13CPOC, where DIC is dissolved inorganic carbon and POC is particulate organic carbon). The bulk C-isotope fractionation in meromictic lakes harboring S-oxidizing and Fe-oxidizing photoautotrophs were analyzed in light of fractionation by pure strains taking an isotope mass balance approach.

***Main results and conclusions***

1. ***Anoxygenic sulfide-oxidizing phototrophic bacteria in Lake Cadagno***

Meromictic and sulfur-rich Lake Cadagno is a model study site for modern and ancient biogeochemical processes (recent review, Tonolla et al., 2017). Light penetrates the chemocline, where oxygen-rich top waters meet sulfidic, oxygen-poor bottom waters, supporting a community of large purple sulfur bacteria (PSB) (*Chromatium okenii*), various small PSB and brown-colored, green sulfur bacteria (GSB) (*Chlorobium clathratiforme*). Recent studies determined the C-isotope fractionation (∆13C) in the sediment (where ∆13C = δ13CDIC - δ13CPOC) (Schubert et al., 2011), but ∆13C was never determined in the water column where bacterial populations actively cycle sulfur. In this study, an unusually high C-isotope fractionation of ∆13C~32‰ was determined between the δ13CDIC and δ13CPOC in Lake Cadagno´s chemocline in 2013. The 2013 study corroborated preliminary and less resolved results carried out by the grantee in a pre-study during the 2012 season. Importantly, for the study of diagenesis, this high fractionation determined at the chemocline was detected in the sediment traps as well, although the size of the fractionation was slightly decreased due to microbial degradation processes (∆13C~31‰ in October 2013).

To better interpret these high fractionations, determine their source, and understand these values in light of recent work on methane cycling in the Cadagno chemocline (Milucka et al., 2015), a carbon isotope mass balance approach was taken using C-isotope fractionation determined in pure cultures of PSB and GSB in the lab and bulk C-isotope fractionation determined in the lake. We could determine the relative contributions of the main bacterial players in Cadagno. Specifically, we found that C-isotope fractionation was driven by anoxygenic phototrophic bacteria and the influence of the PSB and GSB changed over the season. As Cadagno functions as a model environment for early euxinic oceans, our results imply that we underestimate their contribution to the C-isotope record in ancient sediments. The contribution of PSB and GSB to the bulk C-isotope fractionation in the chemocline could be traced in the settling fraction and in the sediment of Lake Cadagno. Taken together with supporting data, such as lipid biomarker studies, these results offer a firmer understanding of diagenetic influences on bacterial biomass over long geological timeframes.

1. ***Anoxygenic Fe(II)-oxidizing phototrophic bacteria and the C-isotope record of Lake La Cruz***

Anoxygenic Fe(II)-oxidizing phototrophic bacteria oxidize Fe(II), fix CO2 and produce cell-Fe(III) (hydr)oxide aggregates. These biogenic Fe(III) minerals may be important vehicles for carbon transport in the water column, in carbon preservation (Lalonde et al., 2012), but also in how biomass and Fe minerals are altered through diagenetic processes (for a review, Posth et al., 2014). To study anoxygenic Fe(II)-oxidizing phototrophic bacteria and the microbial fractionation of C-isotopes *in situ*, the grantee led a field campaign to Lake La Cruz, Spain in September 2014. Lake La Cruz is a ferruginous, methane-rich, meromictic lake harboring a population of anoxygenic phototrophs, but has not been as extensively studied as its sulfurous counterpart, Lake Cadagno. The research team included scientists from NordCEE (SDU), University of Leeds, and University of Valencia. Geochemical samples were taken from the water column, sediment traps and sediment cores.

In parallel, C-isotope fractionation was tested in cultures of anoxygenic Fe(II)-oxidizing phototrophic bacteria, i.e., *Rhodobacter ferrooxidans* sp. SW2 and *Chlorobium ferrooxidans* sp. KoFox. A larger fractionation was observed in the PSB cultures than in the GSB cultures, where ∆13C~24‰ was determined for the PSB and ∆13C~10‰ for the GSB. The C-isotopic composition of organic carbon in cell-Fe(III) mineral aggregates formed by PSB *Rhodobacter ferrooxidans* sp. SW2 was δ13CPOC ~ -38‰ in comparison to values of δ13CPOC ~ -20‰ for GSB *Chlorobium ferrooxidans* sp. KoFox. Cycling and diagenesis experiments with the biogenic Fe(III) minerals formed by these strains suggest a ~3-5‰ shift in the C-isotope composition of the organic carbon in the cell-Fe(III) mineral aggregates due to temperature and pressure diagenesis. This shift was not observed in similar experiments carried out with sulfide-oxidizing photoautotrophs and suggests Fe(III) mineral transformation influences C-isotopic composition of biogenic minerals after temperature and pressure diagenesis (Posth et al., 2013; 2014). Further analysis, isotopic mass balance calculations and pending metagenome and mineralogical analysis of Lake La Cruz will help constrain the processes behind this observation.

1. ***C-isotope fractionation in Spathi Bay and implications for microbial Fe, As, and C cycling***

The grantee joined Swedish collaborators in Milos Island, Greece (6/2014) to investigate microbial carbon fixation and the As, Fe and Mn cycles in the water column, microbial mats, and sediment of this shallow, hydrothermally active bay using isotope analysis and microbial community profiling. Based on the findings, it is proposed that hydrothermal temperature gradients off Milos Island control the dissolution of CO2 into the sediment pore water. The hydrothermally active sediments are marked by a low genetic abundance compared to reference sediments not affected by direct hydrothermal activity. These shallow submarine hydrothermal vents differ from hydrothermal environments at seafloor spreading centers as in the light-penetrated shallow vent environments, photosynthetic as well as chemosynthetic processes plays an important role. Indeed, the quantification of the specific genes involved in autotrophic carbon fixation in Spathi Bay indicates that the RuBisCo form II (Calvin cycle) is the predominant CO2 fixation pathway in the arsenic-CO2-rich shallow submarine hydrothermal sediments as supported by the isotopic data. This differs from modern deep-sea hydrothermal settings in which the reverse tricarboxylic acid cycle (rTCA cycle) appears to be the main route to carbon fixation (Campbell& Cary, 2004; Nakagawa & Takai, 2008).

***Study Impact:*** Results of BioCTrack will help interpretation of the rock record to understand the nature and evolution of early life on our planet. By extension, we can understand how life and environment continues to evolve in relation to one another on a changing planet. The project public website can be found at: <http://www.sdu.dk/om_sdu/institutter_centre/i_biologi/forskning/forskningsprojekter/bioctrack>

**References cited:**

Campbell BJ, Cary SC: Abundance of Reverse Tricarboxylic Acid Cycle Genes in Free-Living Microorganisms at Deep-Sea Hydrothermal Vents. *Appl Environ Microbiol* 2004, 70(10):6282-6289.

Lalonde K, Mucci A, Ouellet A, Gelinas Y (2012) Preservation of organic matter in sediments promoted by iron. *Nature,* 483, 198-200.

Milucka, J., Kirf, M., Lu, L., Krupke, A., Lam, P., Littmann, S., Kuypers, M.M.M., Schubert, C.J. (2015) Methane oxidation coupled to oxygenic photosynthesis in anoxic waters. *ISME J.* 1-12.

Musat N, Halm H, Winterholler B, Hoppe P, Peduzzi S, Hillion F, Horreard F, Amann R, Jørgensen BB, Kuypers MMM (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *PNAS,* 105, 17861-17866.

Nakagawa S, Takai K: Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance. *FEMS Microbiology Ecology* 2008, 65(1):1-14.

**Posth NR**, Koehler I, Swanner ED, Schroeder C, Wellmann E, Binder B, Konhauser KO, Neumann U, Berthold C, Nowak M, Kappler A (2013) Simulating Precambrian banded iron formation diagenesis. *Chem. Geol.,* 362, 66-73.

**Posth NR**, Canfield DE, Kappler A (2014) Biogenic Fe(III) minerals: From formation to diagenesis and preservation in the rock record. *Earth-Science Reviews,* 135, 103-121.

Schubert CJ, Vazquez F, Losekann-Behrens T, Knittel K, Tonolla M, Boetius A (2011) Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di Cadagno). *FEMS Microbiology Ecology,* 76, 26-38.

Tonolla M, Storelli N, Danza F, Ravasi D, Peduzzi S[, **Posth NR**](http://findresearcher.sdu.dk:8080/portal/en/persons/nicole-posth(c784f3ed-3549-4b45-9c64-f560c54e2935).html), Cox R, Jørgensen M, Gregersen L, Daugbjerg N & Frigaard N-U. 2017, '[Special Study: Lake Cadagno](http://findresearcher.sdu.dk:8080/portal/en/publications/ecology-of-meromictic-lakes(53e892a7-7a21-4a22-814e-58763973d25f).html)'. in R Gulati, A Degermendzhy & E Zadereev (eds), *Ecology of Meromictic Lakes.* Springer Verlag. Ecological Studies.