The Functionality of Iron Minerals in Environmental Processes

Scientific Report

Short Visit Grant

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1. Purpose of visit

The purpose of visiting the Max Plank Institute (MPI) for Marine Microbiology in Bremen was to learn specialized techniques, which will be applied in the SNF (Swiss National Science Foundation) funded project 'Methane oxidation pathways at oxic-anoxic boundaries in lakes'. Within this project lead by Prof. Dr. Bernhard Wehrli (Eawag/ETH) and Dr. Carsten Schubert (Eawag), the key aim is to investigate how methane oxidation is coupled to the redox cycles of nitrogen, iron and manganese in the suboxic zones of stratified lakes. In marine systems, anaerobic methane oxidation via sulfate reduction is the dominant pathway by which the methane flux into the atmosphere is impeded. In stratified lakes the mechanism by which methane is oxidized is less well understood and often observed oxygen concentration profiles cannot account for the amount of oxidized methane. Thus, in such environments methane oxidation could be linked to microbial activity, which utilizes alternative electron acceptors, such as nitrate, nitrite or iron and manganese oxides.

In order to test this hypothesis one task will include the incubation of lake water from four different stratified lakes (Lake Rotsee, Switzerland; Lake Kivu, Africa; Lake La Cruz, Spain and Lake Powell, Canada) with different electron acceptors (nitrate, nitrite, sulfate, iron and manganese oxides) in order to evaluate which electron acceptors are responsible for anaerobic oxidation of methane (AOM) in the suboxic zones of lakes. Moreover, oxidation rates will be investigated by incubations with a labeled substrate ($^{13}CH_4$). Hence, not only will the involved constituents be discovered but the rate of AOM will be assessed. Samples from labeling experiments will be analyzed for single-cell isotopic signatures using nanoSIMS, (secondary ion mass spectrometry on a nanometer scale).

Above mentioned nanoSIMS sample preparation and measurements are planned to be carried out in collaboration with the MPI in Bremen. Thus, the main purpose of the visit was to learn how to prepare environmental samples for nanoSIMS measurement. Furthermore, in-situ incubation experiments are a firmly established method at the MPI and are applied mainly in marine systems. Within the scope of this project, these incubation techniques and subsequent analytical tools will be applied to lacustrine systems. During the visit to the MPI, obtaining information about how to setup these incubations and carry out labeling experiments encompassed another goal.

2. Description of work carried out during the visit

NanoSIMS sample preparation

A major portion of the time spent at the MPI involved learning how to prepare samples for nanoSIMS measurement. NanoSIMS is an analytical tool, which supplies information about the distribution of an element on the surface of a sample and its isotopic composition. During measurement the sample is hit with primary ions from a focused cesium beam and subsequent ions emanating from the sample are assessed in a mass analyzer. The nanoSIMS ion beam at the MPI in Bremen can be focused to a spot size of less than 50 nm, which provides high resolution. A certain group of organisms or species can be identified by halogen in-situ hybridization (HISH), where the 16S rRNA gene is hybridized with specific HRP (horseradish peroxidase)-labeled probes. These hybridized genes are then stained with a ^{19}F-labeled tyramide, which allows identification of the targeted organisms and quantification of their activity with nanoSIMS.

Usually samples for nanoSIMS would be prepared from incubation experiments with labeled isotope compounds in order to trace the assimilation of certain elements into the cells. Since environmental samples were not available from the project yet, bacterial cultures were used to learn sample preparation. A brief summary of the completed procedure is given in the following:

- **Sputter Coating** of 0.2 µm GTTP filters with gold-palladium in a sputter coating device as the nanoSIMS requires the sample to be conductive.
- **Fixation** of a sample from a bacterial culture with paraformaldehyde and filtered onto the gold-coated filter.
- **Bleaching** with hydrochloric acid (HCl) in order to remove any endogenous peroxidases from the cells.
- **Permeabilization** treatment with lysozyme to ensure permeability of the cell walls.
- **Hybridization** for 6 h in a hybridization buffer (specific for each probe) along with the probe/s.
- **Washing** of filter in a washing buffer. The buffer constituents and concentrations are specific to the type of hybridization buffer used.
- **Amplification** of the hybridized genes with a fluorescently labeled tyramide (Oregon Green), which only binds to the HRP.
- **Counterstaining** with DAPI, which binds to all DNA, in order to visualize all cells in the sample.

During this procedure, instruction on how to make the involved buffers and solutions and how to prepare the probes and tyramides was also given. Once the HISH procedure was completed, the filter was viewed under the fluorescent microscope to see if hybridization and fluorescent labeling was successful and how many cells were amplified and subsequently stained.

If the procedure worked, the areas of interest are then marked on the filter with Laser MicroDissection (LMD), which is a microscope coupled to a laser. Laser markings are necessary in order to find the cells,

which should be measured with the nanoSIMS, as the images seen with the nanoSIMS are just black and white. I received detailed training on how to select areas for nanoSIMS and how to operate the LMD and make laser markings.

In-situ incubation experiments

Learning how to setup in-situ incubation experiments encompassed another main goal of the visit to the MPI in Bremen. The goal of these experiments is to assess methane oxidation rates in the to be studied lakes and to constrain what electron acceptors are responsible for the oxidation of methane in the absence of oxygen. As the MPI has much experience in conducting such experiments in marine environments, learning how this is done was fundamental. These types of experiments are done in the field, however in this case I practiced some of the involved techniques in the lab.

In preparation for such a field campaign, I obtained information about the material and equipment needed, how to prepare a ^{13}C-labeled methane stock solution and how to select sampling depths. Once a sampling depth has been determined, I learned how to transfer water samples to serum bottles anoxically, how to amend the samples with labeled methane and stock solutions containing sulfate, nitrate, nitrite, Fe-oxides and Mn-oxides. After amendment, the larger sample volume was distributed into smaller containers (12 ml exetainers). At each time point during the incubation, an exetainer is fixed to stop microbial activity and stored until further analysis or nanoSIMS filter preparation.

Mn-oxide synthesis

For the incubation experiments, it is planned to amend with Mn-oxide among others. Mn-oxide was synthesized by two different methods. In the first procedure the oxide was made by combining potassium permanganate ($KMnO_4$) and sodium hydroxide (NaOH). After this was mixed well, manganese chloride ($MnCl_2$) was added slowly. After the solution had stirred for some time, the pH was adjusted to neutral and the precipitate washed four times in MilliQ water and finally suspended in MilliQ water.

In the second method, Mn-oxide was synthesized by preparing a $MnCl_2$ solution and allowing it to undergo oxidation with oxygen (O_2). Since the reaction is much faster at higher pH, the pH was first raised and the solution was bubbled with O_2 until a brown precipitate was visible. As before, the solid phase was washed four times and finally resuspended MilliQ water.

Analytical techniques

$^{13}CH_4$ incubation experiments are planned with amendments of sulfate (SO_4^{2-}), 15-labeled nitrate ($^{15}NO_3^-$), 15-labeled nitrite ($^{15}NO_2^-$), Fe(III)-oxide (ferrihydrite) and Mn(IV/III)-oxide (birnessite). The methane oxidation rate will be assessed by measuring the production of $^{13}CO_2$ with a gas chromatograph (GC). For this a sample at each time point is acidified with phosphoric acid, which promotes CO_2 to degas from solution. After equilibration, a headspace sample is measured with the GC. Furthermore, sample preparation to measure SO_4^{2-}, HS^-, $^{15}NO_3^-$ and $^{15}NO_2^-$ were explained and practiced.

3. Description of main results obtained

Since the visit was aimed to learn different techniques that would be necessary to carry out the proposed project, the main results comprised learning the above explained techniques. Furthermore, experimental setups were discussed and an outline of preparatory work required before incubations and nanoSIMS measurements was made.

4. Future collaboration with host institution

Generally it is envisioned to work closely with the MPI throughout the SNF project, particularly concerning the nanoSIMS measurements. At the moment nanoSIMS measurements are planned to be carried out at the MPI. However, depending on materials available at Eawag, it is possible that the entire sample preparation would be completed at the MPI as well. Performing sample preparation at the MPI could be advantageous, as the expertise and experience of the personal there could be valuable in trouble shooting problems that may arise during preparation. Moreover, sample preparation and measurement of $^{15}NO_3^-$ and $^{15}NO_2^-$ might also be completed at the MPI if facilities are not available at Eawag or at another location in Switzerland.

5. Projected publications / articles resulting or to result from the grant

In the ongoing SNF project it is envisioned that at least three publications will evolve from my PhD work. We will take care that the received grant is well acknowledged in those publications.

Once again I would like to thank the European Science Foundation for providing me with the funding for this very interesting and essential stay at the MPI.