Microsocsm Incubation Series II

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Project
» Coupling of carbonate cycling and redox reactions in the bioturbated zone of marine sediments (Carbonate cycling bioturbated zone)

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Abstract
In this study, we examine cable bacteria metabolic activity and growth dynamics using the functional expression of net electrogenic activity revealed by pH patterns, inferred reaction rate distributions, and filamentous cell abundance. We demonstrate in experimental microcosms, the potential for substantial cable bacteria activity in Mn- and Fe-depleted carbonate muds from Florida Bay, USA, and document their biogeochemical impacts in the near absence of Mn and Fe.

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Dataset Description

The detailed data plot figures are given in Yin, H., Aller, J.Y., Furman, B.T., Aller, R.C., and Zhu, Q. (2022). Cable bacteria activity and impacts in Fe and Mn depleted carbonate sediments. Marine Chemistry 246, 104176. doi.org/10.1016/j.marchem.2022.104176

Methods & Sampling

Florida Bay sediment was hand-collected from the upper ~ 10 cm of carbonate mudbank deposits in January 2019 (25.116367°N, 80.817970°W, 21°C) packed and sealed into plastic bags, and transported to Stony Brook University

A set of microcosm incubations in January 2019, termed series II, time series sampling and resolution of sulfide distributions. In addition, we followed the responses of electrogenic activity to deoxygenation of overlying water at the end of series II. (See Table 1; Yin et al. 2022).

Time series chemical compositions in individual microcosms were monitored using planar optodes, extracted porewater, and visible imaging. Bulk sediment was initially sieved through a 1-mm pore size nylon screen mesh with no added water followed by manual homogenization with a plastic spatula.

Experiment series II sediment was stored in sealed bottles without aeration in the dark at 4 °C for 1 month before use. Sediment was introduced into six cylindrical microcosms and two rectangular microcosms fitted with optical sensors. Microcosms were monitored and sampled serially as described subsequently.

Microcosms were overlain with continuously aerated seawater at 22° C. The overlying seawater was replaced regularly (~ 3-d intervals) with filtered seawater (10 µm pore size filter; salinity ~25) collected from off West Meadow Beach (Long Island Sound, New York, USA). The final sampling and termination of series II after 106 days followed a 6 hour period of deoxygenation of overlying water that was designed to examine responses of possible electrogenic activity to lack of oxygen.

Data Processing Description

Microcosms were monitored as a function of depth for pH, O2, H2S, and cable bacteria abundance throughout the 3-month incubation period in order to determine the time points of core sectioning for bulk pore water solute compositions (Ca2+, Na+, Mg2+, SPO43- and total alkalinity). When sampled, the whole rectangular microcosms and cylindrical cores were vertically sectioned (in air; each core processing took less than 15 min; resolution: 0.25 cm above 2 cm depth; 0.5 cm downwards), centrifuged (3000 rpm, 10 min), and the porewater was filtered (VWR polyethersulfone syringe filters; 0.2 µm pore size) for analyses of Ca2+, Na+, Mg2+, SPO43- and total alkalinity. Series II incubation series were sectioned on day 3, 26, 43 (2 cylindrical microcosms each time; A & B), and 106 (duplicate rectangular microcosms, A & B). On day 106, overlying water anoxia was generated and maintained by continuously purging with a mixture of N2 and CO2 gases (CO2, 0.04%), while keeping the overlying water at a pH of 8 (monitored by a pH electrode). Anoxia was confirmed using an oxygen electrode. 2D pH and H2S distribution patterns in the box microcosms were imaged using planar optical sensors before and after the anoxic incubation. The concentrations of other solutes (Ca2+, Na+, Mg2+, SPO43- and total alkalinity) were measured in porewater extracted immediately after the anoxic incubation.

Porewater alkalinity was measured using the bromophenol blue colorimetric method (Sarazin et al., 1999). Reactive inorganic phosphate (H2PO4-, HPO42-, PO43-; abbreviated as SPO43-) was determined colorimetrically using molybdenum blue (Murphy and Riley, 1962). NO2- and NO3- were measured based on the acidic Griess reaction method (LOD<1 µM) (Miranda, et al.,2001). Ca2+, Na+, and Mg2+ concentrations were determined using a Horiba JY Ultima 2C ICP-OES Spectrometer.

Samples for examination of the microbial community were collected on day 1, 20, 26, 43, and 106 from the rectangular microcosms in series II. In series II experiments, 3-ml cutoff plastic syringes were inserted 5 cm vertically at random positions into microcosms. The subcores were subsequently sliced and preserved in 2% formaldehyde (resolution: 0.25 cm until 1 cm depth; 0.5 cm from 1 to 3 cm depth; 1 cm downwards). Aliquots of homogenized sediment samples were dried and used to normalize abundances to dry weight (Aller, et al., 2019). Cable bacteria filament identity was confirmed by FISH with a *Desulfobulbaceae*-specific oligonucleotide probe (DSB706) (Manz et al, 1992). Cable bacteria filaments were morphologically distinctive (Schauer, et al., 2014), and we could also readily identify them using acridine orange (AO) using epifluorescence microscopy. Compared with the FISH method, AO stained samples generally showed longer filament lengths due to less handling, and better precision of cell counts. Additionally, we used AO rather than 4′, 6-diamidino-2-phenylindole (DAPI) for identifying and counting bacteria because it gave better contrast in our natural sediments.

Following the method development with the FISH technique, the lengths of cable bacteria filaments were enumerated primarily from AO stained samples. Abundances were calculated from the summed filament lengths and an average cell size of 2 µm. Sediment subsamples were first diluted 100-fold with 3% saline water and homogenized. AO stained samples were deposited on black GVS polycarbonate track-etched filters (0.2 µm pore size). Cable bacteria filaments were counted within 10 randomly selected fields (1000× magnification; defined by a Whipple reticle) including any that extended beyond the grid field (the same filament only counted once). The inclusion of parts of filaments outside the chosen grid field had the potential to overestimate the cable cell abundance as they sometimes expanded across multiple grid fields. Other filamentous bacteria like *Beggiatoa* can be easily excluded from erroneous enumeration based on the distinctive cell morphology. No non-filament cells were counted as cable bacteria, although they can apparently occur as isolated, single cells (Muller, et al., 2000).

The pH imaging planar optical sensors were made by immobilizing 8-hydroxy-1,3,6- pyrenetrisulfonic acid trisodium salt (HPTS) into a transparent polyester film (Zhu, et al., 2006). The responses of the sensor films were captured using a commercially available digital camera (EOS Rebel T7i), following ratiometric calibration (emission wavelength: 545 nm; excitation wavelengths: 510 nm and 430 nm). pH data reported here are on

the NBS scale. Oxygen sensing films were made with platinum tetrakis (pentafluorophenyl) porphyrin (Lee and Okura, 1997). The same camera system was used with an excitation wavelength of 395 nm and an emission wavelength of 650 nm. Oxygen concentrations were calibrated based on the Stern-Volmer relationship (Lee and Okura, 1997) using a two-point calibration: in air saturated seawater (100% air saturation) and in anoxic sediment (0% air saturation). Gas permeable H2S sensors were made using the diphenylcarbazone-Zn complex (Yin, et al., 2017). Both the sample and calibration strip responses were recorded using a flatbed scanner (Canoscan 8400F). Standard solutions were made by adjusting the pH of Na2S standards below 4 to convert sulfide into dissolved H2S (> 99.9% conversion). 2D pH O2, and H2S sensor image data were converted to 1D-vertical profiles by horizontally averaging vertically oriented membranes (widths ~ 0.5 cm). In addition to the optical sensors described above, microelectrodes for pH, O2, and H2S (pH, 100 µm tip diameter; O2 and H2S, 50 µm tip diameter; Unisense A.S., Denmark) with a manual micromanipulator and a multimeter (PA2000; Unisense A.S., Denmark) were used to resolve vertical profiles of the series II rectangular microcosms on day 1, 6, 16 and 25 for O2 and H2S, and on day 2, 19 and 27 for pH. The same standards as optical sensor calibrations were used for microelectrode calibrations.

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

Coupling of carbonate cycling and redox reactions in the bioturbated zone of marine sediments (Carbonate cycling bioturbated zone)

Coverage: Great Peconic Bay, New York and Florida Bay, Florida

NSF Award Abstract:
The top meter of sediment, called the bioturbation zone, is impacted by the variety of biological activities that occur on the seafloor. Marine animals such as worms or plant-like creatures often burrow in and out of, or grow roots into sediment, respectively. Because of this, the bioturbation zone is an area of constant sediment exchange between deep water and surface sediment. Every centimeter of sediment on the ocean floor can have unique chemical properties from the centimeter of sediment above and below it, but in the bioturbation zone, the activity of marine animals mixes these layers. Usually, oceanographers hoping to study what the past chemistry of the ocean was use the chemistry of sediment because, if one knows how fast it takes a centimeter of sediment to settle on part of the seafloor, then a piece of sediment at a defined depth corresponds to a time in the past; however, in the bioturbation zone this is impossible because very old (deep) sediment may have been transferred by a marine animal upward to be mixed in with newly settled (shallow) sediment. Because of this, the bioturbation zone is less studied and generally disregarded in studies of past ocean chemistry, which means there is little, if any, information on ocean processes from the last few decades. The researchers of this project plan to change that by making field measurements, performing laboratory experiments, and developing new sensor technology to advance our understanding of bioturbation zone effects on sediment chemistry. This will be a significant advancement for chemical oceanographers and geologists who hope to understand the ocean chemistry of approximately the last 50 years. Also, the sensors developed by these investigators will be a fantastic new tool for oceanographers, and these researchers are also dedicated to supporting broadening participation in ocean sciences by mentoring students from a wide variety of diverse backgrounds.

Scientists from the State University of New York, Stony Brook propose to advance oceanographers' technical capabilities and abilities to model redox and acid-base reactions within the bioturbation zone. They plan to determine the impact of bioturbation on the dissolution, precipitation, and burial of carbonate minerals, as well as determine net alkalinity fluxes at the sediment-water interface. In past research, this team has developed optical sensors to get qualitative, 2-D images of surface sediment properties. With the progression of their work, the investigators plan to develop sensors that build on their past work to create 3-D images. By developing this 3-D image capability and making extensive field measurements and conducting laboratory experiments, they will be able to use their data to analyze the images created by the sensors quantitatively.

This will advance oceanographers' ability to model the effects of bioturbation on carbon cycling in surface sediment, which will, in turn, significantly constrain a missing component of the oceanic flux of inorganic carbon.

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