Supplemental - Occurrence of benthic microbial nitrogen fixation coupled to sulfate reduction in the seasonally hypoxic Eckernförde Bay, Baltic Sea

Experiment looking at the impact of phytoplankton organic matter addition on sulfate reduction rates in Eckernförde Bay sediments.

1 Materials and Methods

1.1 Organic carbon slurries

From two sediment cores (length ~ 60 cm, inner diameter 10 cm) collected from Eckernförde Bay on 13 April 2012, 500 ml of sediment from the top 0 - 5 cm was transferred into a 1 L glass bottle containing 500 ml of artificial seawater medium for SR bacteria from the Baltic Sea (salinity 23) (Widdel and Bak, 1992), creating a 1:1 medium:sediment slurry. The bottle was flushed with N_2, sealed with a rubber stopper and a screw cap, and kept in the dark at 13°C. From this slurry, 3 incubation slurries were created: one with no organic matter addition, one representing a "low" phytoplankton bloom, and one representing a "high" phytoplankton bloom.

Prior to organic matter addition, the energy value of freeze-dried phytoplankton material (DT´s Blend Premium, including species *Nannochloropsis oculata, Phaedactylum tricornutum* and *Chlorella)* was determined by bomb calorimetry (IKA Kalorimetersystem 200) (Linde, 2007; Pinhack, 2002). To reflect the natural phytoplankton organic carbon input (based on energy values) to the sediments, minimum and maximum values reported by Graf *et al.* (1983) served as a point of reference. These values were extrapolated over a time interval of 3 weeks (based on periods defined by Graf *et al.*, 1983) and scaled down based on the surface area of the sediment core, resulting in the amount of 0.042 g phytoplankton in the low concentration scenario and 0.294 g phytoplankton in the high-concentration scenario. Based on these amounts, the corresponding energy value for the phytoplankton concentrations was calculated to be 0.5194 kJ and 3.6361 kJ for the low- and high concentration scenarios, respectively. The proper amounts of grinded phytoplankton, representing both concentrations, were transferred into 250 ml glass bottles and combined with 200 ml of the 1:1 medium:sediment slurry. Additionally, one 250 ml bottle only contained the 200 ml of slurry and no additional organic matter. All bottles were sealed, flushed with N_2, and kept at in-situ bottom water temperature (13 °C based on CTD data) for 15 d in the dark. Every third day the slurries were shaken gently and on day 15, each of the incubations was sub-sampled for measurements of sulfate reduction rates, as well as for sulfate concentrations.

1.2 Sulfate Measurements

To determine the initial sulfate concentration in each sulfate reduction incubation slurry, 3 ml sample supernatant were extracted, filtered through a 0.2 µm PES filter and analyzed via ion chromatography (Metrohm ion-chromatograph with anion-exchange column, using carbonate-bicarbonate solution as eluent) with the IAPSO seawater standard for calibration.

1.3 Sulfate Reduction Rates

For determination of sulfate reduction rates, 20 ml from each 200-ml incubation were extracted and injected into 40 ml serum vials (triplicates). Vials were closed with a rubber stopper, crimp-sealed and flushed with N_2. Each sub-sample was injected with the radioactive tracer $^{35}SO_4^{2-}$ (6µl, 200kBq, specific activity 37 TBq $mmol^{-1}$) (Jørgensen, 1978) and incubated for 19.5 h at in-situ temperature (13°C based on CTD data) in the dark. After incubation, sub-samples were transferred to 20 ml Zinc Acetate (20% w/w) and analyzed by the cold single-step chromium distillation procedure (Kallmeyer et al. 2004). For calculation of rates, porosity was calculated based on the average sediment porosity in the upper 0 – 5 cm depth (0.89) and the proportion of medium.

2 Results

Sulfate reduction rates showed a positive correlation (R^2 = 0.69) with the energy value of added organic carbon (i.e. phytoplankton) material (Suppl. Fig. 1). The control (ØCorg +Media), containing no additional organic material, resulted in relatively low rates on the order of 23.3 ± 11.9 nmol SO_4^{2-} cm^{-3} d^{-1}. The phytoplankton "low" replicates displayed high variability with sulfate reduction rates ranging between 7.4 - 65.3 nmol SO_4^{2-} cm^{-3} d^{-1}. The phytoplankton "high" sample had higher rates compared to its analogue, ranging between 94.2 - 111.8 nmol SO_4^{2-} cm^{-3} d^{-1}.

References

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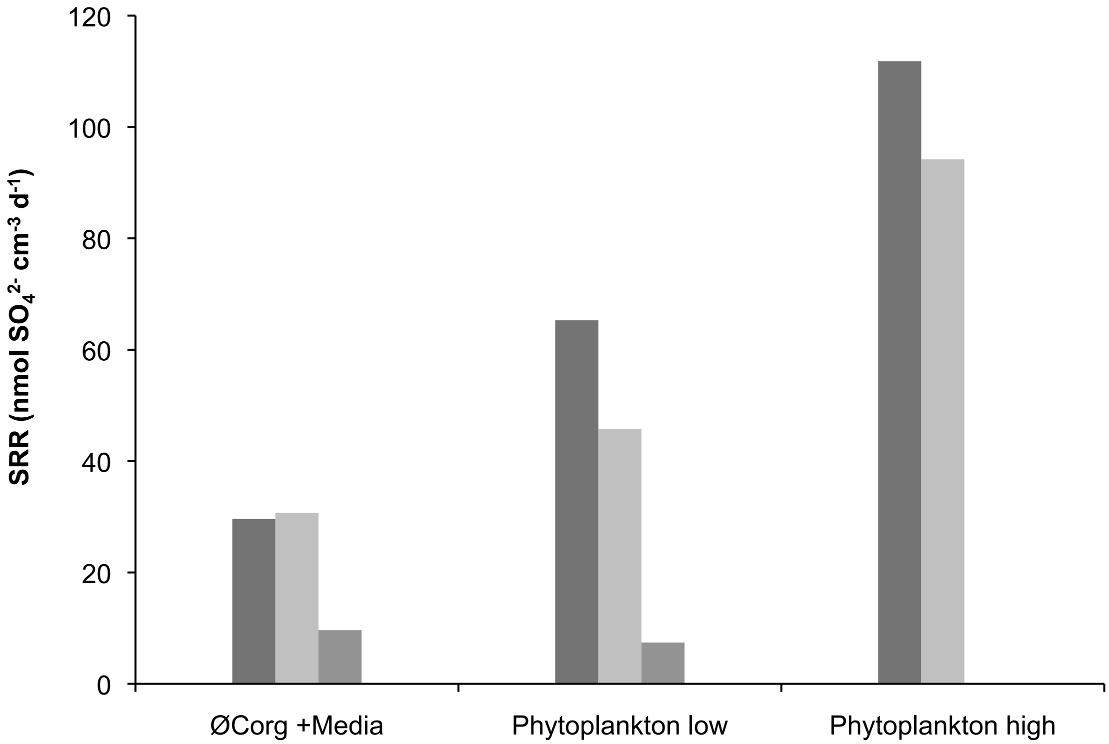
Figure Legends

Supplemental Figure 1: Sulfate reduction rates measured in the experiments with phytoplankton addition: control slurries without phytoplankton addition (ØCorg +Media) as well as slurries with low (energy value C_{org} added: 0.5194 kJ per 100 cm^3 undiluted sediment) and high phytoplankton addition (energy value C_{org} added: 3.6361 kJ per 100 cm^3 undiluted sediment). Note that for the “high” setup only two replicates were available.



Supplemental Figure 1