

## ***Interactive comment on “Occurrence of benthic microbial nitrogen fixation coupled to sulfate reduction in the seasonally hypoxic Eckernförde Bay, Baltic Sea” by V. J. Bertics et al.***

**Anonymous Referee #1**

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This paper presents a seasonal study on sulfate reduction and benthic nitrogen fixation in a seasonal hypoxic setting. The authors make a strong case that benthic nitrogen fixation activity is linked to the activity of sulfate reducing bacteria (based on potential rate measurements of nitrogen fixation). This is very convincing.

However, the linkage between sulfate reduction (or nitrogen fixation) and changing environmental factors over the season (the main part of the discussion) is far less strong, because lack of quantification of correlation.

Most of all, it remains puzzling to me why sulfate reducing bacteria, that live in a pool of high ammonium concentrations in hypoxic sediments (see fig 4), would invest costly

C3495

bio-energetic resources to obtain high nitrogen fixation rates. This paper does not bring us any closer to the resolution of this enigma.

General comments

There is no quantitative statistical or correlation analysis to support the claims made on the relation between environmental factors and benthic biogeochemical rates. Section 4.2.1 suggests a direct link between organic matter input and sulfate reduction rates. However, this is concluded from a very qualitative inspection of the data. This comment is equally applicable to the other environmental factors discussed in section 4.2.

The exceptional high sulfate reduction rates in November and February (Fig 4) are explained as hotspots of mineralization due to downmixing of macrophyte debris by storms (without given further evidence). Yet this means that this fresh macrophyte debris must be mixed down to 10 to 25 cm. This is highly unlikely in such cohesive sediments. So it remains enigmatic why there are such high rates at these times of low temperatures (when one expects lowest activity)

Nitrogen fixation is measured via the acetylene reduction assay. This is a bottle method, which renders the reported nitrogen fixation rates as potential rates. These could be quite far off from in situ rates. So the question is how much true N<sub>2</sub> fixation occurs in situ.

Denitrification is measured via the C<sub>2</sub>H<sub>2</sub> inhibition method, which is not the most state-of-the-art method to measure this biogeochemical rate (compared isotope pairing or N<sub>2</sub>/Ar methods). This is again a bottle method, and moreover, a lot of NO<sub>3</sub> has been added in this study to overcome nitrate limitation. This makes the denitrification rates reported really potential rates (which might be far off from in situ rates). This is acknowledged in the text, but not in the abstract. So persons that only read the abstract can be misled.

The comparison in table 1 of the current potential rate to the modeled values of Dale

C3496

et al is really an apple and orange comparison (despite the superscript indication in Table 1, this is highly confusing). As a consequence, one can certainly not make the conclusion as in 6512- L “In August and September, denitrification greatly outweighed N<sub>2</sub>-fixation, while in February the rates were almost equal.” as this refers to entirely different types of data in winter and summer.

6512 –L15 “Denitrification also showed a seasonal pattern similar to that of N<sub>2</sub>-fixation and sulfate reduction.” This conclusion cannot be drawn from the present dataset – only two measurements were made in July and August!

Neither the introduction or discussion provides a perspective of the previous work on benthic N<sub>2</sub>-fixation (and specifically, how the present rates compare to these previous studies).

#### Specific comments

Methods reported should pertain to the dataset provided. The methods section describes sulphide determination in the porewater, but no data are given. Moreover, the correct ref for the sulphide method is: Fonselius, S., Dyrssen, D., and Yhlen, B. 1999. Determination of hydrogen sulphide. In Methods of seawater analysis, 3rd edition. Ed. by K. Grasshoff et al. Wiley-VCH, Germany. This method is suitable for low H<sub>2</sub>S conc < 250 microM (the porewater have presumably much higher H<sub>2</sub>S conc where the method of Cline is preferable, given the NH<sub>4</sub> conc up to 1200 microM)

Fig. 2. These bio-irrigation results were already published somewhere else (Dale et al 2012). It is unclear to me why they are repeated in this paper (with a dedicated figure).

In October and November, NH<sub>4</sub> concentrations are low at depth, while irrigation rates are also low. This is strange as low irrigation rates would lead to accumulation of NH<sub>4</sub> at depth.

6510-L25 How can bubble irrigation transport particulate organic matter downwards? This is not possible.

C3497

#### Technical comments

6491-L17 Middleburg misspelled

6492-L15 Or an incorrect estimate of sources and sinks. . .

6493-L26 mean water depth

6494-L12 Bange and Treude (2012). Reference is missing in list

6494-L20 density is calculated not measured (and not mentioned in the results either)

6494-L20 oxygen concentration: be more specific, from rosette Winkler or optode sensor?

6494-L22 Manufacturer MUC?

6494-25 Immediate processing -> this is misleading. Simply state time period between coring and core processing.

6495-L18 Porosity was determined by drying a known volume of sediment. This does not make sense. The volume is not known when weighing procedure described. Was the solid phase density of the sediment determined (or a fixed number assumed, e.g. 2.65 g cm<sup>3</sup>)? Was a salt correction employed?

6495-L26 irrigation rates are estimated by modeling, not approximated

6500-L16. It is remarkable that the sediment porosity decreases during the period of hypoxia. My experience with hypoxic sediments is that they become fluffier during the period of hypoxia.

6501 Benthic microbial turnover rates and geochemistry -> strange title. Microbial turnover rates are not discussed. Change to “Biogeochemical process rates” or similar

6505-L19 macrophyte debris

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Interactive comment on Biogeosciences Discuss., 9, 6489, 2012.

C3498