

Interactive
Comment

***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.**

V. J. Bertics et al.

vbertics@geomar.de

Received and published: 27 November 2012

We would like to thank both referees for their thoughtful and constructive reviews of our manuscript. Their suggestions and comments have greatly helped to improve our manuscript and allow for the reader to better understand our message. Below we offer our responses to each of the individual comments made by each referee.

Referee #1

1) 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 bio-energetic resources to obtain high nitrogen fixation rates. This paper does not bring us

C6028

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



any closer to the resolution of this enigma.

We agree with the referee that this remains puzzling, however it is not our intention to determine why SRB fix nitrogen, but merely to provide more evidence that N₂ fixation, possibly by SRB, exists in benthic systems. We have added in a citation (Knapp, 2012) that goes into great detail about the possible reasons for this occurrence and summarizes the many studies that have also documented benthic N₂ fixation in the presence of ammonium (and nitrate).

2) 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.

To address this comment, we have now added supplemental material that details an experiment looking at the impact of phytoplankton material on Eckernförde Bay benthic sulfate reduction rates. We therefore have experimental evidence that both temperature and organic carbon concentrations can affect these biogeochemical rates. In regards to the remaining two environmental factors (oxygen and bioturbation), we agree that these arguments remain rather speculative. However, we have shown that these two factors vary over the course of the year, along with our biogeochemical rates. We thought it better to discuss the possibility that these factors may also play a role rather than ignoring the possibility, as Eckernförde Bay is an extremely complex environment and so it is unlikely that just one or two factors dictate benthic microbial processes. In light of this referee's comment, we have restructured this section, placing those factors with more experimental support at the beginning, followed by those that remain speculative at the end.

3) The exceptional high sulfate reduction rates in November and February (Fig 4) are

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

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)

We have clarified this section to discuss how storm-induced burial, rather than mixing, may lead to pockets of organic material at depth, including a citation to a study that showed increased chlorophyll a burial at depth during winter months. We have also included other explanations for the occurrence of localized organic matter (e.g. bioturbation in February). Our best explanation for these high activities despite colder temperatures remains that there most likely are hot spots of activity surrounding localized pockets of fresh organic matter, but how those pockets got there remains speculative.

4) 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 N₂ fixation occurs in situ.

We agree that it is possible that these rates may be different from true in situ rates, and so try to use “nitrogenase activity” instead of N₂ fixation when talking about our specific data. We reserve the use of N₂ fixation for when integrating and comparing our results with previous studies. We have corrected those instances where we incorrectly used N₂ fixation instead of NA. For this study, it was our intention to look for peaks and trends in NA and correlate those instances with peaks and trends in sulfate reduction for this matter the NA is sufficient even if not completely reflect N-fixing rates.

5) Denitrification is measured via the C₂H₂ inhibition method, which is not the most state-of-the-art method to measure this biogeochemical rate (compared isotope pairing or N₂/Ar methods). This is a again a bottle method, and moreover, a lot of NO₃ has been added in this study to overcome nitrate limitation. This makes the denitrification

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

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 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 N₂-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 N₂-fixation and sulfate reduction.” This conclusion cannot be drawn from the present dataset –only two measurements were made in July and August!

As both referees had reservations concerning the denitrification measurements, all denitrification data has been removed from this paper as suggested by Referee #2. Instead, we now simply have a small section in the discussion dedicated to the possible implications of our N₂-fixation data and the N cycle in Eckernförde Bay.

6) Neither the introduction or discussion provides a perspective of the previous work on benthic N₂-fixation (and specifically, how the present rates compare to these previous studies).

This information has been added to the discussion in the section titled “Impact on Benthic N Cycling,” the section that has now replaced the previous discussion section on denitrification vs N₂ fixation in our system.

7) 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 H₂S conc < 250 µM (the porewater have presumably much higher H₂S conc where the method

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

of Cline is preferable, given the NH₄ conc up to 1200 microM)

We decided not to include sulfide measurements as there were some issues regarding the analysis. Therefore, information regarding sulfide methods/concentrations has been removed.

8) 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).

Both the Dale et al. 2012 paper and our manuscript are to be part of the same BG special issue and therefore reference each other. The graph that we include here is different from that included in the Dale et al. paper, despite it being based on the same data set, as we have differing emphases.

9) In October and November, NH₄ concentrations are low at depth, while irrigation rates are also low. This is strange as low irrigation rates would lead to accumulation of NH₄ at depth.

We agree, however, sulfate reduction rates (a major measure of organic matter degradation) indicate that this is possible. We can't really explain why but offer the idea that perhaps with no macrofauna present and reduced storm activity, there is less transport of fresh organic carbon to deeper layers in these months. Additionally, Dale et al. (2012) offer the idea that bubble irrigation during these months may be leading to the advection of ammonium out of the system.

10) 6510-L25 How can bubble irrigation transport particulate organic matter downwards? This is not possible.

We have deleted the last sentence of the section that discuss this transport.

11) 6491-L17 Middleburg misspelled

Corrected

BGD

9, C6028–C6036, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



12) 6492-L15 Or an incorrect estimate of sources and sinks. . .

Changed

13) 6493-L26 mean water depth

Corrected

14) 6494-L12 Bange and Treude (2012). Reference is missing in list

This citation has been replaced as the introduction to this special issue has not been submitted yet.

15) 6494-L20 density is calculated not measured (and not mentioned in the results either)

Density has been removed.

16) 6494-L20 oxygen concentration: be more specific, from rosette Winkler or optode sensor?

Corrected

17) 6494-L22 Manufacturer MUC?

This information has been added.

18) 6494-25 Immediate processing -> this is misleading. Simply state time period between coring and core processing.

Corrected

19) 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 cm³)? Was a salt correction employed?

We have clarified this in the text.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



20) 6495-L26 irrigation rates are estimated by modeling, not approximated

Corrected

21) 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.

The decrease was so minimal that we have deleted this statement.

22) 6501 Benthic microbial turnover rates and geochemistry -> strange title. Microbial turnover rates are not discussed. Change to “Biogeochemical process rates” or similar

Changed

23) 6505-L19 macrophyte debris

Corrected

Referee #2

1) I have some strong reservations about the “denitrification” data used in this paper. The authors correctly identify the measurements as potential rates in the methods, but then treat them as environmentally-relevant rates in the results and discussion. My questions about this measurement are not only semantic, but also about the entire relevance of the measurement. The acetylene block approach might be used where NO_x- concentrations are known, but in this application they only tell about the potential for enzyme activity. In highly reducing sediment, sources of oxidized NO_x are minimal because of lack of oxygen for nitrifying bacteria and the potential for sulfide-induced inhibition/death of nitrifiers. The very interesting profile of denitrification shows strong vertical structure, with the highest potential rates >2-3 cm below the sediment-water interface. For these rates to have relevance, the NO_x would need to either be formed at depth (unlikely) or diffuse/bioadvect from surface sediment layers. Moreover, there is a high potential that DNRA would strongly compete for NO_x. The authors should

BGD

9, C6028–C6036, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



either identify an environmental relevance of these data, or delete them. The paper does not require these data to be a useful and valuable contribution.

As both referees had reservations concerning the denitrification measurements, all denitrification data has been removed from this paper as suggested by Referee #2. Instead, we now simply have a small section in the discussion dedicated to the possible implications of our N₂-fixation data and the N cycle in Eckernförde Bay.

2) 6495 Would be nice to mention Br analysis technique and the fact that the coefficient is pore water derived.

This information has been added.

3) 6506 Microenvironment referene: Brandes, J.A., Devol, A.H., 1995. Simultaneous nitrate and oxygen respiration in coastal sediments: Evidence for discrete diagenesis. *Journal of Marine Research* 53, 771-797.

This reference has been added.

4) 6507 Convert the integral of SR to equivalent oxygen units and predict O₂ penetration (Cai, W.-J., Sayles, F.L., 1996. Oxygen penetration depths and fluxes in marine sediments. *Marine Chemistry* 52, 123-131). This whole argument seems poorly set up. High oxygen will not result in deep oxygen penetration in these sulfidic sediments.

We agree that oxygen penetration will not necessary increase and therefore conclude: "Overall, fluctuations in Eckernförde Bay water column oxygen concentrations probably serve as more of an indication of organic matter availability to the benthos (i.e. bacterial degradation of organic matter in the water column leads to consumption of oxygen, with the remaining organic matter settling on the seafloor), rather than directly influencing sulfate reduction and N₂-fixation in the sediment." We have added in a reference that measured O₂ penetration into these sediments.

5) 6510 The idea of bubble irrigation also in: Roden, E.E., Tuttle, J.H., 1992. Sulfide Release from Estuarine Sediments Underlying Anoxic Bottom Water. *Limnology and*

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

Oceanography 37, 725-738.

This reference has been added.

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

BGD

9, C6028–C6036, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

C6036

