We would like to thank both referees for their critical and constructive comments to our manuscript. Their comments helped to significantly improve the quality and clarity of the manuscript. We hope that our answers and revisions are sufficient to accept this work for publication in Biogeosciences. Please find our responses to each of the individual comments below.

Referee # 1 Dr. Riemann
Received and published 22 September 2015

Review of Gier et al. 2015. The paper concerns N2 fixation and sulfate reduction (SR) in sediments below OMZ waters off Peru. The work demonstrates an interesting coupling between N2 fixation and SR, as also suggested by nifH gene analyses. Moreover, the study indicates that organic matter load and sulfide are major drivers of N2 fixation. The paper contributes to the compiling data on factors regulating diazotrophy and specifically to the rather limited number of studies from sediments. The paper is generally well written, clear, and to the point. My points of criticism are overall minor, but should improve the readability and clarity of the paper.

1. The wording should be changed at several places in the abstract. The current version seems to indicate that rates were measured in water, and not just in sediments. For instance line 6: “measured in OMZ mid-waters”; line 8: “Benthic N2 fixation profiles” etc. Please, make sure the reader cannot be misled to believe that water samples were analyzed.
The wording in the abstract regarding the measurements has been changed according to the referee´s suggestions.

2. P1, l. 11. Define nifH genes
A definition regarding the *nifH* gene has been added.

3. P1, l14. Delete “various”
“Various” has been deleted.

4. P6, l1. “These bacteria…”
Changed.

5. P6, l10-14. Unclear where this information comes from
The author information (Dale et al., 2015) has been added.

6. P7, l16-22. It would be good to reduce the overall length of the manuscript. This section could be easily reduced. Most readers will know the principle of acetylene reduction.
We thank the reviewer for this suggestion. We reduced the method part regarding the description of the acetylene reduction assay.

7. p8, l5. Specify whether samples were analyzed onboard or stored somehow.
Samples were analyzed onboard and this information has been added.

8. P8, l13. OK, but why were they expressed as NA. Isn’t that just confusing? If keeping it as NA, then please explain why.
As both referees pointed out that it is confusing to have nitrogenase activity (NA) and N_2 fixation in the manuscript, values were recalculated for N_2 fixation and all figures, tables and text were changed accordingly and we now only refer to N_2 fixation.

9. P10, l2. Please, specify how many sequences were obtained per sample. Also, describe negative controls and whether they were blank.
The information regarding the sequences and the negative controls has been added.

10. P10, l14. How can you in the description of your sediments cite literature which is published before this sampling was carried out? This is your Results section – you should describe your results, not those of others.
Thanks for noticing. We agree with the referee and deleted this citation from the results part.

11. P10, l18. Redundant, described 3-4 lines higher up.
The sentence has been deleted.

12. P13. It should be evident from the text why the authors are interested in looking at C/N ratios. It is not enough to address that later in the discussion. Likewise, it should be explained why data on DIC flux are reported (Fig. 4), also how this was measured is unclear to me.
Information on why we looked at the C/N ratios and DIC values, as well as how DIC was measured has been added.

13. P14, l8. Rephrase. A novel clade cannot belong to anything. It may be related to something…
The sentence has been rephrased.

14. P15. L5-6. Again, this sounds like water samples. Please, rephrase
Rephrased.

15. P15, l6. “Sometimes both depth profiles revealed similar trends”. Clarify what is meant by depth profiles.
Clarified.

16. P15, l8. “were”
Corrected.

17. P15, l21. What does “this study” refer to?
“This study” referred to the citation in the sentence before. The sentence was changed to make this clear.

18. P. 15, l28. “SR bacteria were…”
Corrected.

19. P16, l11-15. Needs work. That samples have a “certain diversity” is not informative. Unclear what “these results” refer to (line 13). Farnelid et al. did not sample an OMZ (line 15).
The paragraph has been rephrased and the citation Farnelid et al. has been removed.

20. P17, l10-11. Weird and unclear sentence. Please, revise or remove.
The sentence has been removed.

21. P17, l20-28. I have not understood the point with the DIC fluxes. Please, make this clearer here as well as earlier in the manuscript.
As stated at comment number 12, information on the DIC fluxes has been added.

22. P20, l7-8. Sentence is out of context. Please, clarify the point or remove.
The sentence has been removed.

23. Figure 1, text. Please, define MUC.

Has been defined.

24. Figure 6, text. Delete “expressed”. Clarify whether the sizes of the triangles are proportional to the number of sequences within each triangle. Moreover, indicate on the figure how many clones the triangles etc represent.
Expressed has been deleted. The sizes of the triangles should not be used for quantification. To make it clearer, all triangles were changed to the same size and the information how many clones each triangle represent has been added inside the triangles.

Referee # 2 Dr. Ionescu
Received and published: 11 November 2015

The paper by Gier et al discusses N fixation in oxygen minimum zones in marine sediments (specifically off the coast of Peru). The study suggests a link between sulfate reduction and N fixation in these environments and supports this previously mentioned hypothesis by rates measurements and phylogenetic data. This paper adds to our understanding regarding diazotrophy in sediments as well as highlights our gap in knowledge on the matter by showing that not all patterns can be explained by the presented data. The paper is generally well written with some exceptions where the English can be improved and the wording can be phrased in a more accurate manner.
The manuscript was cross-checked by an English speaker.

I tried to highlight these places in the comments below. Additionally as stated below the figures are not suited to the page size used by the journal and hence are often not readable.
We tried to improve the readability and clarity of the figures.

Page 14408 line 4 – The definition of formalin is an aqueous solution of 37% (m:v) formaldehyde. Hence 37 % formalin would mean 13 % formaldehyde. I guess this is not what the authors meant. To avoid misunderstandings, I suggest using 37% formaldehyde solution.
We agree with the referee and changed the information according to his suggestion.

Page 14408 line 5 – The acetylene reduction assay should not be used for longer than 48 h. Some consider this to be too long as well. The reason is that the saturation of the enzyme with acetylene leads to a lack of fixed N and reduction in cell viability and accordingly N-fixation (See for examples Seitzinger and Garber, 1987 MEPS 37 and references therein).
We agree with the referee and we are aware that incubation with acetylene can lead to a potential lack of fixed N, however to the best of our knowledge this is the standard method used for the determination of N_2 fixation in sediments (15N rate determinations are not feasible in sediments as incubation times would need to be several weeks to months to achieve a signal above the natural 15N sediment background). We have added in a recent citation (Bertics et al., 2013) that describes the method in further detail and we point towards this limitation of the method in the manuscript.

Page 14408 line 14. If you have converted the NA from C2H4 reduction to N fixation, why do the graphs in Fig 3 still discuss C2H4. While the value of 3 is not fixed for all environments it is indeed widely used. If you used it you can now refer to N2 rather than C2H4.
As both referees pointed out that it is confusing to have nitrogenase activity (NA) and N_2 fixation in the manuscript, values were recalculated for N_2 fixation and all figures, tables and text were changed accordingly and we now only refer to N_2 fixation.

Page 14409 line 27: 1 µl of BSA is not very informative as we don't know the concentration of the stock solution nor the reaction volume.
The information has been added.

Page 14410 line 25: No need for "The" in "The St. 9".
Changed.

Page 14411 line 3: "The deepest St. 10" means that there are several stations named St. 10 and this is the deepest of them. I suggest "The deepest station (10; 1025 m)…" Or "St. 10 (the deepest; 1-25 m) ..."
Changed.

Page 14411 line 11: Erase “The” in “The St. 4 and 6”.
Corrected.
Page 14411 line 16: The shallowest St 1 – see my previous comment about the deepest St 10.
Corrected.

Page 14412 line 2: “Sediment depth profiles of N2 fixation activity are expressed in nitrogenase activity (NA), i.e. without the conversion factor of 3 C2H4: 1 N2” – Why convert in some cases (integrated rates) and not everywhere. Either you trust the conversion factor or you don’t – no need to confuse the reader. Providing N2 fixation rates also allows for direct comparison with other studies. Please change this.
As both referees pointed out that it is confusing to have nitrogenase activity (NA) and N_2 fixation in the manuscript, values were recalculated for N_2 fixation and all figures, tables and text were changed accordingly and we now only refer to N_2 fixation.

Page 14412 line 9: In all cases so far you used the abbreviation St. even when several stations where mentioned why here the full word stations.
Corrected.

Page 14412 line 8-10: The choice of sentence structure is not clear – Simply state: NA and SR rates where high (or highest) at the shallow St…. and lowest at deep St…
Changed.

Page 14412 line 11 – page 14413 line 13: This section is messy and hard to follow. For example, St 1 has its own paragraph while the other stations are mentioned in a single paragraph. I also find this section too detailed. I believe you should only highlight the important things from the figures and not literally describe the graphs.
The paragraph has been shortened and only highlights from the graphs are specified. We hope this improves the clarity of this section.

Page 14413 line 15: The rate conversion was done from C2H4 to N2 and not to N (same in Fig. 4). Also the units (mmol) is missing.
Corrected.

Page 14413 line 25, 27, 28: mmol N2
Corrected.

Page 14414 line 7: Instead of “three novel clades and seven novel clades…” write “three and seven novel clades were detected, respectively”.
Changed.

Page 14414 line 15: For the sake of correctness add: for a “known” Vibrio species…
Corrected.

Page 14416 line 21: The term heterotrophic N2 fixation is a bit obscure as autotrophy refers to carbon. If the authors refer to N2 fixation by heterotrophs this should be stated in such a manner.
The term heterotrophic has been clarified.

Page 14416 line 23: The integrated N2 fixation rate and the Corg concentration clearly showed similar trends. Nevertheless, the use of the word “correlated” requires a statistical measure which I believe was not provided. Either provide such data (which should be straight forward) or rephrase the sentence to address the similarity in trends.
We agree with the referee and have rephrased the sentences accordingly.

Page 14417 line 22. Fig 5 should be Fig 4.
Corrected.

Figures:
Fig 2 – The figure is probably designed to cover and entire page (A4 or Letter). However, this is not the format used by this journal. Hence he printed figure is not readable. Online viewing requires as well magnification to 250 % for clear reading. Consider splitting into two panels spanning two pages.
The final format of Biogeosciences is letter format, hence the Fig. will be printed on a full page.

Fig. 3 – A similar problem as above with the addition of long text as the axis title. This cannot be read at 100% magnification on a screen or print.
The figure, as well as the axis title has been changed and the fonts were increased.

Fig. 4. As stated before I believe the correct unit is mmol N2 and not mmol N. Fonts need to be increased.
We agree with the referee and changed the unit. Also the fonts were increased.

Fig. 5. The same comment as above. Additionally, the yellow line and text are hardly visible.
The whole figure and all fonts have been increased, the yellow line has been darkened and the unit was changed accordingly.

Fig. 6. Needless to say that this is useless in print or at standard screen viewing. The fonts need to be larger. Sequences from this study should be bold. The shaded frames should be positioned in the background of the tree and not above it as they hide the text. Consider cutting the tree into two sections on two pages.
We agree with the referee and tried our best to increase the quality of the whole figure. The sequences from this study have been increased and were made bold. The shaded frames were changed to a transparent design for a better visibility. We considered cutting the tree into two sections, however this would make a direct comparison and association of the sequences more difficult for the reader and therefore we decided to show the tree on one page.

Nitrogen fixation in sediments along a depth transect through the Peruvian oxygen minimum zone

Jessica Gier[1*], Stefan Sommer[1], Carolin R. Löscher[2], Andrew W. Dale[1], Ruth A. Schmitz[2], Tina Treude[1,3*]

[1] GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany

[2] Institute for Microbiology, Christian-Albrechts-University Kiel, Germany

[3]Present address: University of California, Los Angeles, Department of Earth, Planetary & Space Sciences and Department of Atmospheric & Oceanic Sciences, USA

Correspondence: jgier@geomar.de, ttreude@g.ucla.edu

Abstract

Benthic nitrogen (N_2) fixation and sulfate reduction (SR) were investigated in the Peruvian oxygen minimum zone (OMZ). Sediment samples, ~~retrieved by a multiple corer~~ were retrieved by a multiple corer ~~taken~~ at six stations (70 - 1025 m water depth) along a depth transect at 12°S, covering anoxic and hypoxic bottom water conditions. Benthic N_2 fixation was detected at all sites using the acetylene reduction assay, with high rates ~~measured in OMZ mid-waters~~ between ~~the~~ 70 m and 253 m and ~~lowest~~ lower ~~N_2 fixation~~ rates at greater depth~~below 253 m down to 1025 m water depth~~. SR rates ~~were decreasing~~decreased with increasing water depth~~., with highest rates at the shallow site~~. Benthic N_2 fixation and SR depth profiles in sediments showed similar qualitative trends ~~largely overlapped with SR depth profiles~~, suggesting a coupling of ~~that~~ both processes ~~are coupled~~. ~~The p~~Potential ~~of benthic~~ N_2 fixation by ~~SR~~ sulfate-reducing bacteria was verified by the molecular analysis of *nifH* genes. Detected *nifH* sequences, i.e., the key functional gene for N_2 fixation, encoding for the nitrogenase enzyme, clustered with sulfate-reducing ~~SR~~ bacteria that have been demonstrated to fix N_2 in other benthic environments. Depth-integrated rates of benthic N_2 fixation and SR showed no direct correlation along the ~~12°S~~ transect, suggesting that the benthic diazotrophs in the Peruvian OMZ ~~are being~~ is controlled by additional ~~various~~ environmental factors such as~~. The~~ organic matter and free sulfide~~availability and the presence of sulfide appear to be major drivers for benthic diazotrophy~~. It was further found that N_2 fixation in OMZ sediments was not inhibited by

Formatiert: Tiefgestellt

high ammonium concentrations. N_2 fixation rates in OMZ sediments were similar to rates measured in other organic-rich sediments. Overall, this ~~work~~ study improves our knowledge on fixed N sources and ~~in marine sediments and contributes to a better understanding of~~ N cycling in ~~OMZ sediments~~oxygen deficient environments.

1. Introduction

Only 6 % of nitrogen (N) in seawater is bioavailable (Gruber, 2008). This bioavailable N is mainly present in the form of nitrate (NO_3^-), whereas the large pool of ~~available~~ atmospheric dinitrogen gas (N_2) is only available for N_2 fixing microorganisms (diazotrophs). ~~Therefore,~~ N ~~is~~ often ~~controlling~~ limits ~~the~~ marine productivity (Ward & Bronk, 2001; Gruber, 2008) and the largest ~~this limitation makes N_2 fixation the dominant~~ source of bioavailable N (i.e. ammonium (NH_4^+)) in the marine environment is N_2 fixation (Falkowski et al., 1998; Strous et al., 1999; Brandes & Devol, 2002).

Formatiert: Tiefgestellt

To date, the quantitative contribution of diazotrophs in the marine N cycle remains unclear and numerous estimates of global sources and sinks of global N have ~~exist, lead~~ ing to an unbalanced budget with deficits of around 200 Tg N yr^{-1} (~~Gruber, 2004; Brandes et al., 2007; Capone & Knapp, 2007;~~ Codispoti, 2007). ~~In most studies, oceanic N sinks are either estimated to be higher than oceanic N sources, suggesting that~~This suggests that either previous ~~determination of~~ N_2 fixation rate~~s~~ determinations have been underestimated ~~(Montoya et al., 1996; Codispoti, 2007)~~ (Großkopf et al., 2012) or that N loss processes are overestimated (Codispoti, 2007). ~~But also almost b~~However, also balanced budgets such as ~~exist that calculated~~ ~265 Tg N yr^{-1} for N sources and ~275 Tg N yr^{-1} for N sinks exist (Gruber, 2004). These ~~B~~budget discrepancies illustrate that the current knowledge on diazotroph~~ys~~ and the marine N cycle is still limited.

~~Latest~~ Recent investigations argue that N_2 fixation in the water column cannot be totally attributed to phototrophic cyanobacteria, but that also heterotrophic prokaryotes contribute ~~a~~ substantially ~~part~~ (Riemann et al., 2010; Farnelid et al., 2011; Dekaezemacker et al., 2013; Löscher et al., 2014; Fernandez et al., 2015) ~~similar to marine benthic habitats~~. This ~~relation~~ was shown for the Peruvian oxygen minimum zone (OMZ), where proteobacterial clades ~~were~~ dominated ~~and~~ with heterotrophic diazotrophs ~~mainly~~

~~occurred~~, indicating that cyanobacterial diazotrophs are of minor importance in this area (Löscher et al., 2014).

Pelagic N_2 fixation has been studied mostly in the oligotrophic surface oceans, but it was not until the past decade that ~~also~~ benthic habitats began to receive~~d~~ more attention (Fulweiler et al., 2007; Bertics et al., 2010; Bertics et al. 2013). Most studies on benthic N_2 fixation focused on coastal environments (Capone et al., 2008 and references therein). For example, subtidal sediments in Narragansett Bay (Rhode Island) were found to switch from being a net sink in the form of denitrification to being a net source of bioavailable N by N_2 fixation, caused by a decrease of organic matter deposition to the sediments (Fulweiler et al., 2007). Shallow brackish-water sediments off the Swedish coast revealed benthic N_2 fixation along with a diverse diazotrophic community (Andersson et al., 2014). N_2 fixation~~The nitrogenase activity~~ was positively influenced by a variety of environmental factors, such as salinity and dissolved inorganic nitrogen, while wave exposure had a negative influence. Recent work revealed that benthic N_2 fixation is often linked to sulfate-reducing ~~(SR)~~ bacteria~~,~~ ~~e.g.,~~ For instance, bioturbated coastal sediments showed enhanced N_2 fixation activity mediated by sulfate-reducing ~~SR~~ bacteria, adding new dissolved inorganic N to the system (Bertics et al., 2010; Bertics & Ziebis, 2010). Further coupling of N_2 fixation to SR was ~~found~~ observed in organic-rich sediments of the seasonal hypoxic Eckernförde Bay (Baltic Sea) (Bertics et al., 2013), as well as in the sub-tidal, heterotrophic sediments of Narragansett Bay (Rhode Island, USA) (Fulweiler et al., 2013). Several sulfate-reducing ~~SR~~ bacteria carry the functional gene marker for N_2 fixation, the *nifH* gene ~~for encoding the nitrogenase enzyme~~ (Sisler & ZoBell, 1951; Riederer-Henderson & Wilson, 1970; Zehr & Turner, 2001) and were shown to actively fix N_2 in culture experiments (Riederer-Henderson & Wilson, 1970). ~~Therefore, we need to better understand SR bacteria and their potential to fix N in the environment.~~ However, information on sulfate-reducing bacteria and their contribution to N2 fixation in the environment is rather sparse and makes this one of the remaining questions to be solved.

So far, the distribution of benthic N_2 fixation and its relevance for N cycling in the Peruvian (OMZ), defined by dissolved oxygen < 20 µmol kg^{-1} (Fuenzalida et al., 2009)~~,~~ are unknown. The shelf and the upper slope in the Peruvian OMZ represent recycling sites of dissolved inorganic N with dissimilatory NO_3^- reduction to NH_4^+ being the dominant process ~~driving~~ in

Formatiert: Schriftart: 12 Pt., Englisch (USA)

Formatiert: Tiefgestellt

the benthic N cycle (Bohlen et al., 2011). This process is mediated by the filamentous sulfide-oxidizing *Thioploca* bacteria (Schulz, 1999; Schulz & Jørgensen, 2001). Along with dissimilatory NO_3^- reduction to NH_4^+, ~~also~~ benthic denitrification by foraminifera between 80 and 250 m water depth occurs in the Peruvian OMZ (Glock et al., 2013). These authors calculated a potential NO_3^- flux rate of 0.01 to 1.3 mmol m^{-2} d^{-1} via this pathway and suggested that foraminifera could be responsible for most of the benthic denitrification.

The high input of labile organic carbon to ~~the~~ Peruvian OMZ sediments (Dale et al., 2015) and subsequent SR should ~~support~~ favor benthic N_2 fixation. Sulfate-reducing ~~SR~~ bacteria could considerably contribute to N_2 fixation in these organic-rich OMZ sediments, given that several sulfate-reducing ~~SR~~ bacteria (e.g. *Desulfovibrio spp*. (Riederer-Henderson & Wilson, 1970; Muyzer & Stams, 2008)) carry the genetic ability to fix N_2, and provide an important bioavailable N source for non-diazotrophic organisms (Bertics et al., 2010; Sohm et al., 2011; Fulweiler et al., 2013). We therefore hypothesize a possible coupling of N_2 fixation and SR in sediments off Peru. The aim of the present study was ~~the~~ to identify~~ication~~ and quantify~~ication of~~ benthic N_2 fixation along a depth transect through the Peruvian OMZ, together with potentially coupled SR. Additionally, the identification of bacteria facilitating these processes will help to understand ~~should shed light into~~ the diazotrophic community structure of ~~inhabiting~~ these sediments. The overall knowledge gained is useful ~~will be used~~ to better constrain benthic N cycling in OMZs and to improve our knowledge on sources and sinks of fixed N.

2. Materials and Methods

2.1 Study area

The most extensive OMZ worldwide ~~developed~~ is found in the eastern tropical south Pacific ocean at the Central Peruvian coast (Kamykowski & Zentara, 1990). The Peruvian OMZ ranges between 50 m and 700 m water depth with oxygen (O_2) concentrations below the detection limit in the mid-waters (Stramma et al., 2008). The mean water depth of the upper OMZ boundary deepens during intense El Niño Southern Oscillation years and can reach a depth of 200 m (Levin et al., 2002) with oxygenation episodes reaching concentrations of up to 100 µM O_2 (Gutiérrez et al., 2008). O_2 concentrations (Fig. 1, Tab. 1) off Peru are ~~affected~~ modulated by coastal trapped waves (Gutiérrez et al., 2008), trade

winds (Deutsch et al., 2014) ~~or~~ and subtropical-tropical cells (Duteil et al., 2014), and can vary on monthly to interannual time-scales (Gutiérrez et al., 2008).

At 12°S, the OMZ extends from water depths between 50 and 550 m (Dale et al., 2015) (Fig. 1). During our field work, ~~B~~bottom water O_2 concentrations varied greatly with water depth and were below the detection limit (5 µM) at stations from 70 m to 407 m water depth. Bottom water O_2 increased ~~from~~ to 19 µM at 770 m water depth ~~to~~ and 53 µM at 1025 m water depth, indicating the lower boundary of the OMZ (Dale et al. 2015). Between 70 m and 300 m water depth, the sediment surface was colonized by dense filamentous mats of sulfur-oxidizing bacteria, presumably of the genera Mari~~T~~t*hioploca spp* ~~(Gutiérrez et al., 2008; Mosch et al., 2012)~~. ~~This~~ These bacteria are able to glide up to 1 cm h^{-1} through the sediment in order to ~~feed~~access ~~on~~ hydrogen sulfide (Fossing et al., 1995; Jørgensen & Gallardo, 1999; Schulz, 1999). Sediments at the lower boundary (770 m and 1025 m) of the OMZ ~~were shown to have~~host a variety of macrofaunal organisms e.g. ophiuroids, gastropods, and crustaceans (Mosch et al., 2012).

The 12°S region is in the center of an extensive upwelling zone and features high primary productivity (Pennington et al., 2006). Sediments at 12°S have higher rates of particulate organic carbon accumulation (2-5 times) compared to other continental margins and a high carbon burial efficiency ~~at deep stations~~, indicating ~~high~~ preferential preservation of organic matter in ~~sediments below~~ the Peruvian OMZ (Dale et al., 2015). The shelf (74 m) of the Peruvian OMZ is characterized by high sedimentation ~~accumulation~~ rates of 0.45 cm yr^{-1}, while mid-waters and below the OMZ show rates between 0.07 and 0.011 cm yr^{-1} ~~were found in OMZ mid-waters and below the OMZ, additionally. sSediment porosity was high at the shelf stations and in OMZ mid-waters (0.96 – 0.9) and was lowest (0.74) at the deepest 1024 m station (Dale et al., 2015).~~

2.2 Sampling

Sediment samples were taken in January 2013~~,~~ at six stations (70, 144, 253, 407, 770, and 1025 m) ~~at 12°S~~ along a depth transect at 12°S in the OMZ off Peru (Fig. 1) during an expedition on RV Meteor (M92). January represents austral summer, i.e. the low upwelling season in this area (Kessler, 2006). Samples were retrieved using a TV-guided multiple corer (MUC) equipped with seven core liners. The core liners had a length of 60 cm and an inner diameter of 10 cm. Location, water depth, temperature, and O_2 concentration (from

Dale et al. 2015) at the six sampling stations are listed in Table 1. Retrieved cores for microbial rate measurements were immediately transferred to cold rooms (4-9 °C) for further processing.

2.3 Geochemical analyses

Porewater analysis and the determination of sediment properties and geochemical data have been previously described in detail by Dale et al. (2015). In short, the first core was subsampled under anoxic conditions using an argon-filled glove bag, to preserve redox sensitive constituents. NH_4^+ and sulfide concentrations were analyzed on a Hitachi U2800 UV/VIS spectrophotometer using standard photometric procedures (Grasshoff et al., 1999), while sulfate (SO_4^{2-}) concentrations were determined by ion chromatography (Methrom 761).

The second replicate core was sampled to determine porosity by the weight difference of the fresh sediment subsamples before and after freeze-drying. ~~The p~~Particulate organic carbon and particulate organic nitrogen contents were analyzed using a Carlo-Erba element analyzer (NA 1500).

2.4 Benthic ~~nitrogenase activity~~ nitrogen fixation

At each of the six stations, one MUC core was sliced in a ~~cold~~refrigerated container (9°C) in 1-cm intervals from 0 – 6 cm, in 2-cm intervals from 6 – 10 cm, and in 5-cm intervals from 10 – 20 cm. The acetylene reduction assay (Capone, 1993; Bertics et al. 2013) was applied, to quantify nitrogenase activity ~~(NA)~~. ~~This application is an *ex situ* method, based on the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) by the nitrogenase enzyme, which reduces other small triple bond molecules, like acetylene (Lockshin & Burris 1965; Dilworth, 1966). The temporal increase of C_2H_4 in samples can be measured by flame ionization gas chromatography (Hardy et al. 1968; Stewart et al. 1967). Thereby, the amount of C_2H_2 reduced to C_2H_4 serves as an indication for N_2 fixation rates.~~ To convert from nitrogenase activity to N_2 fixation, a conversion factor of 3 C_2H_4:1 N_2 was applied (Patriquin & Knowles, 1972; Donohue et al., 1991; Orcutt et al., 2001; Capone et al., 2005) ~~was applied~~, which was previously used to measure N_2 fixation in sediments (Welsh et al., 1996; Bertics et al., 2013).

Feldfunktion geändert

Serum vials (60 mL) were flushed with N_2 and filled with 10 cm^3 sediment from each sampling depth (triplicates). The samples were flushed again with N_2, crimp sealed with

butyl stoppers and injected with 5 mL of C_2H_2 to saturate the nitrogenase enzyme. Serum vials were stored in the dark ~~and~~ at 9 °C, which reflected the average *in situ* temperature along the transect (compare with Tab. 1). Two sets of triplicate controls (10 cm^3) were processed for every station. Sediment was collected from each core liner from 0 – 5 cm, 5 – 10 cm, and from 10 – 20 cm and placed in 60 mL serum vials. One set of controls was used to identify natural C_2H_4 production~~,~~ without the injection of acetylene, and the second control set was fixed with 1 mL ~~formalin (~~37.5%~~).~~ formaldehyde solution.

The increase of C_2H_4 in each sediment slice was measured onboard over one week (in total 5 time points, including time zero) using gas chromatography (Hewlett Packard 6890 Series II). From each serum vial, a 100 µl headspace sample was injected into the gas chromatograph and the results were analyzed with the HP ChemStation gas chromatograph software. The gas chromatograph was equipped with a packed column (Haye SepT, 6 ft, 3.1 mm ID, Resteck) and a flame ionization detector. The carrier gas was helium and the combustion gases were synthetic air (20 % O_2 in N_2) and hydrogen. The column had a temperature of 75°C and the detector temperature was 160°C.

~~Sediment depth profiles were expressed in NA. To convert from NA to N_2 fixation, a conversion factor of 3 C_2H_4:1 N_2 for the integrated rates was applied. This conversion factor is based on comparisons between the C_2H_2 reduction assay and ^{15}N incubations (Patriquin & Knowles, 1972; Donohue et al., 1991; Orcutt et al., 2001; Capone et al., 2005) and was previously used to measure N_2 fixation in sediments (Welsh et al., 1996; Bertics et al., 2013).~~ Standard deviation for depth profiles was calculated from three replicates per sediment depth and error bars for standard deviation of ~~for~~ integrated N_2 fixation were calculated from three integrated rates per station.

Feldfunktion geändert

Feldfunktion geändert

2.5 Sulfate reduction rates

One MUC core per station was used for determination of SR activity. First, two replicate push cores (length 30 cm, inner diameter 2.6 cm) were subsampled from one MUC core. The actual push core length varied from 21 - 25 cm total length. Then, 6 µl of the carrier-free $^{35}SO_4^{2-}$ radio tracer (dissolved in water, 150 kBq, specific activity 37 TBq $mmol^{-1}$) was injected into the replicate push cores in 1-cm depth intervals according to the whole-core injection method (Jørgensen, 1978). The push cores were incubated for ~12h at 9°C. After incubation, bacterial activity was stopped by slicing the push core into 1-cm intervals and

transferring each sediment layer into 50 mL plastic centrifuge tubes filled with 20 mL zinc acetate (20% w/w). Controls were done in triplicates from different depths and first fixed with zinc acetate before adding the tracer. Rates for SR were determined using the cold chromium distillation procedure according to Kallmeyer et al. (2004).

It should be mentioned that the yielded SR rates have to be treated with caution due to long (up to 3 half-life times of ^{35}S) and unfrozen storage. Storage of SR samples without freezing has recently been shown to result in the re-oxidation of ^{35}S-sulfides (Røy et al., 2014). In this reaction, FeS is converted to ZnS. The released Fe^{2+} reacts with O_2 and forms reactive Fe(III). The Fe(III) oxidizes ZnS and FeS, which are the major components of the total reduced inorganic sulfur species, resulting in the generation of SO_4^{2-} and hence an underestimation of SR rates. However, because all SR samples in the present study were treated the same way, we trust the relative distribution of activity along sediment depth profiles and recognize potential underestimation of absolute rates.

2.6 *nifH* gene analysis

Core samples for DNA analysis were retrieved from the six stations and were sliced in the same sampling scheme as described for ~~the N~~Abenthic N_2 fixation. Approximately 5 mL sediment from each depth horizon was transferred to plastic whirl-paks® (*Nasco,* Fort Atkinson, USA), frozen at -20 °C and transported back to the home laboratory. To check for the presence of the *nifH* gene, DNA was extracted using the FastDNA® SPIN Kit for Soil (MP Biomedicals, CA, USA) following the manufacturer's instructions with a small modification. Sample homogenization was done in a Mini-Beadbeater™ (Biospec Products, Bartlesville, USA) for 15 seconds. PCR amplification, including primers and PCR conditions, was done as described by Zehr et al. (1998), using the GoTaq kit (Promega, Fitchburg, USA) and additionally 1 µL bovine serum albumin ~~BSA~~ (20 mg mL^{-1} (Fermentas)). The TopoTA Cloning® Kit (Invitrogen, Carlsbad, USA) was used for cloning of PCR amplicons, according to the manufacturer's protocol. Sanger sequencing (122 *nifH* sequences) was performed by the Institute of Clinical Molecular Biology, Kiel, Germany~~.~~ For the sampling sites 70 m, 144 m, 253 m, 407 m, 770 m, and 1025 m water depth the number of obtained sequences was 22, 24, 24, 13, 18, and 21, respectively. Negative controls were performed using the PCR mixture as described without template DNA; no amplification was detected. Sequences were ClustalW aligned in MEGA 6.0 (Tamura et al., 2007), and a maximum likelihood tree

Formatiert: Tiefgestellt

Formatiert: Hochgestellt

Formatiert: Schriftart: 12 Pt., Englisch (USA)

was constructed on a 321 ~~bp~~ base pair fragment and visualized in iTOL (Letunic & Bork, 2007, 2011). Reference sequences were obtained using BlastX on the NCBI database. ~~(Sequence submission being in Progress).~~ Sequences were submitted to Genbank (Accession numbers: KU302519 - KU302594).

3. Results

3.1 Sediment properties

Although sediment description and porewater sampling was done down to the bottom of the core, the focus here is on sediments from 0 – 20 cm where ~~NA~~ benthic N_2 fixation was investigated.

Sediments at the shelf station (St.) 1 (70 m) were black between 0 – 1 cm and then olive green until 20 cm. Only a few metazoans (polychaetes) were observed in the surface sediment. The sediment surface was colonized by dense filamentous mats of sulfur-oxidizing Mari~~T~~t*hioploca spp.* ~~(Gutiérrez et al., 2008; Mosch et al., 2012)~~. These bacteria reached down to a sediment depth of 36 cm in the sediment cores. The sediment ~~at~~ on the outer shelf St. 4 (144 m) was dark olive green from 0 – 13 cm and dark grey until 20 cm. ~~At the sediment surface and in MUC cores, *Thioploca spp.* was visible.~~ At St. 6 (253 m), which was within the OMZ core, sediment appeared dark olive green between 0 – 17 cm and olive green with white patches between 17 – 20 cm. At this station, Mari~~T~~t*hioploca spp*. was abundant. Uniquely, surface sediments (0 – 3 cm) at St. 8 (407 m), consisted of a fluffy, dark olive-green layer mixed with white foraminiferal ooze. This layer also contained cm-sized phosphorite nodules with several perforations (ca. 1 - 3 mm in diameter). Below 2 cm, the sediment consisted of a dark olive green, sticky clay layer. No *Thioploca* mats were found at St. 8. ~~The~~ St. 9 (770 m) was below the OMZ. Sediments were brown to dark olive green with white ~~dots~~ particles between 0 – 12 cm and appeared brown to olive green without white ~~dots~~ particles below this depth. Organisms such as anemones, copepods, shrimps and various mussels were visible with the TV-guided MUC and in sediment cores. The deepest St. ~~10~~ (10; 1025 m) had dark olive green sediment from 0 – 20 cm and black patches from 17 – 20 cm. The sediment was slightly sandy and was colonized with polychaete tubes at the surface and organisms that were also present at St. 9. For further sediment core descriptions see also Dale et al. (2015).

Geochemical porewater profiles of NH_4^+, SO_4^2, sulfide, organic carbon content, and organic C/N ratio between 0 – 20 cm ~~of~~ at the six stations are shown in Fig 2. In all cores, NH_4^+ concentrations increased with sediment depth. The highest NH_4^+ concentration was reached at St. 1 (70 m), increasing from 316 µM in the upper cm~~at the sediment surface~~ to 2022 µM at 20 cm. ~~The~~ St. 4 and 6 showed intermediate NH_4^+ concentrations between 300 µM and 800 µM at 20 cm, respectively. At St. 8 (407 m) the NH_4^+ concentration increased from 0.7 µM ~~in~~ at the surface to 107 µM at 20 cm. The two deep stations (St. 9 and 10) had the lowest NH_4^+ concentrations with 33 µM and 22 µM at 20 m sediment depth, respectively.

The SO_4^{2-} concentrations remained relatively constant in the surface sediments ~~of~~ along the transect. Only at ~~the shallowest~~ St. 1, a decrease from 28.7 µM in the surface layer to 19.4 µM at 20 cm was observed. Along with the decrease in SO_4^{2-}, only St. 1 revealed considerable porewater sulfide ~~buildup~~accumulation. Sulfide increased from 280 µM ~~in~~ at the surface sediment to 1229 µM at 20 cm.

Organic carbon content decreased with increasing sediment depth at St. 1 (70 m), 9 (770 m), and 10 (1025 m). The highest surface organic carbon content (~15 wt%) was found at St. 6~~.~~, whereas ~~T~~the lowest ~~surface organic carbon content~~ (~2.6 wt%) was detected at the deep St. 10. The average (0 - 20 cm) organic carbon value (Fig. 5) increased from St. 1 to St. 6 (15 ± 1.7 wt%) and decreased from St. 6 to the lowest value at St. 10 (2.4 ± 0.4 wt%). C/N ratios, as a proxy for the freshness of the organic matter, increased with increasing sediment depth (Fig. 5). The lowest ~~benthic~~ surface C/N ratio (6.2) was measured at the shallow St. 1, while the highest surface C/N ratio (11) was found at St. 10.

3.2 Benthic nitrogen fixation and sulfate reduction (SR)

For a~~n~~ straighforward~~easy~~ comparison of SR rates with benthic N_2 fixation ~~NA~~ only the sediment depths between 0 – 20 cm are considered. Sediment depth profiles are expressed as ~~in nitrogenase activity (NA)~~N_2 fixation, ~~i.e.~~ that is, with~~out~~ the conversion factor of 3 C_2H_4:1 N_2 ~~to achieve actual N_2 fixation rates. The conversion to N_2 fixation was applied only for the estimation of integrated rates (0 – 20 cm).~~

Formatiert: Tiefgestellt

Highest N_2 fixation ~~NA~~ and SR rates were detected in the surface sediments (0 – 5 cm) and both rates tended to decrease with increasing sediment depth (Fig. 3). ~~While~~ N_2 fixation ~~NA~~ and SR rates were high at the shallow~~er stations~~ St. 1, 4, and 6 (70 m, 144 m, 253 m) ~~and,~~

~~NA and SR rates were lowest~~and lowest at the ~~three~~ deep~~er stations~~ St. 8 – 10 (407 m, 770 m, 1025m).

At St. 1, N_2 fixation ~~NA~~ and SR rates showed different trends in the top layer of the cores, but depth profiles were more aligned below. ~~While~~ Although St. 1 had the highest SR rates of all sites, reaching 248 nmol SO_4^{2-} cm^{-3} d^{-1} at 0 – 1 cm, N_2 fixation ~~NA~~ was not highest at this station. Only St. 1 had considerable~~ly~~ porewater sulfide concentrations and a decrease of SO_4^{2-} concentration with increasing sediment depth, as well as the highest NH_4^+ concentrations throughout the core.

At St. 4 (144 m), both N_2 fixation ~~NA~~ and SR revealed peaks close to the surface. N_2 fixation ~~NA~~ decreased ~~from 3.5 ± 0.6 nmol C_2H_4 cm^{-3} d^{-1} to 0.9 ± 0.08 nmol C_2H_4 cm^{-3} d^{-1}~~ between 0 – 8 cm and increased below 8 cm.~~, reaching 2.2 ± 1.2 nmol C_2H_4 cm^{-3} d^{-1} at 20 cm~~. This increase was not observed in SR rates, which were highest in the surface (181 nmol SO_4^{2-} cm^{-3} d^{-1}) and decreased~~ing towards the bottom of the core. St. 6 (253 m) had the highest N_2 fixation ~~NA~~ of all stations~~. After decreasing from 6.6 ± 0.~~, with~~7 nmol C_2H_4~~ rates of 4.0 ± 0.5 nmol N_2 cm^{-3} d^{-1} in the surface cm ~~to 1.7 ± 0.2 nmol C_2H_4 cm^{-3} d^{-1} in 6 – 8 cm, NA increased to 2.5 ± 2.2 nmol C_2H_4 cm^{-3} d^{-1} with a peak at 10 – 15 c~~m. Although N_2 fixation ~~NA~~ and SR had corresponding depth profiles, the highest SR rate of all stations was not detected at St. 6 ~~(18 nmol SO_4^{2-} cm^{-3} d^{-1})~~. Very low N_2 fixation ~~NA~~ rates were measured at St. 8 (407 m) (0.~~77~~ 5 ± 0.~~37~~ 25 nmol ~~C~~N_2H_4 cm^{-3} d^{-1} in the surface), as well as very low SR rates (0 – 4.3 nmol SO_4^{2-} cm^{-3} d^{-1}). This station was unique due to the presence of foraminiferal ooze, phosphorite nodules and a sticky clay layer below 2 cm. ~~Here, NA was extremely low below 2 cm, not exceeding 0.09 ± 0.04 nmol C_2H_4 cm^{-3} d^{-1}. The~~ N_2 fixation ~~NA~~ and SR rates showed a peak at 5 cm and at 7 cm, respectively. At St. 9 (770 m) N_2 fixation ~~NA~~ was low in the surface and at 20 cm sediment depth, with a peak in activity at 4 – 5 cm (~~1.2~~0.8 ± 0.08~~12~~ nmol ~~C~~N_2H_4 cm^{-3} d^{-1}). At St. 10 (1025 m), N_2 fixation ~~NA~~ rates were low throughout the sediment core, not exceeding ~~ranging between~~ 0.~~23~~ 16 ± 0.02~~3~~ nmol ~~C~~N_2H_4 cm^{-3} d^{-1}~~. in surface sediments and 0.06 ± 0.01 nmol C_2H_4 cm^{-3} d^{-1} in 10 – 15 cm. In accordance with this observation, t~~This site had the lowest organic carbon content throughout the core (between 2.6 wt% at the surface and 1.9 wt% at 20 cm), as well as low NH_4^+ concentrations. At St. 9 (below 9 cm depth) and St. 10 (entire core) SR rates were

Formatiert: Abstand Nach: 10 Pt.

Formatiert: Tiefgestellt

below detection, which could point either to the absence of SR or to the complete loss of total reduced inorganic sulfur due to the long, unfrozen storage (see methods).

Integrated N_2 fixation (0 – 20 cm) increased from St. 1 to St. 6, with the highest rate (0.4 ± 0.06 N_2 m^{-2} d^{-1}) at St. 6 (253 m), and decreased from St. 6 (407 m) to St. 10 (1025 m) (Fig. 4). ~~Integrating SR rates over 0 to 20 cm sediment depth,~~ Integrated SR rates (0 to 20 cm) ranged from ~4.6 mmol SO_4^{2-} m^{-2} d^{-1} at St. 1 to below detection~~0 mmol SO_4^{2-} m^{-2} d^{-1}~~ at St. 9 (Fig. 4). Overall, integrated SR rates decreased with increasing water depth. Integrated N_2 fixation rates and SR were ~~almost~~ in general inversely correlated between St. 1 and St. 6, and ~~. Overall, N_2 fixation rates~~ followed the organic carbon content from St. 1 to St. 6 (70 – 253 m) (Fig. 5). Both parameters had the highest value at St. 6. This pattern did not hold ~~was not conform with~~ for the relatively low~~er~~ integrated SR rate at St. 6. The C/N ratio, averaged over 20 cm, increased with increasing water depth (Fig. 5). Regarding the three deep stations, the lowest integrated N_2 fixation rate (0.008 ± 0.002 N_2 m^{-2} d^{-1}) was detected at St. 8 (407 m). Also the integrated SR rate was low at this site (~0.46 mmol SO_4^{2-} m^{-2} d^{-1}). At St. 9 and 10 (770 and 1025 m), integrated N_2 fixation ~~had low rates of~~was low at 0.05 ± 0.005 N_2 m^{-2} d^{-1} and 0.01 ± 0.001 N_2 m^{-2} d^{-1}, respectively and ~~also~~ integrated SR rates were also lowest at St. 9 (770 m). From St. 8 to 10 a decrease of integrated N_2 fixation and SR together with the average organic carbon content was detected.

No activity was detected ~~In~~in controls for N_2 fixation and SR ~~no activity was detected~~.

3.3 Molecular analysis of the *nifH* gene

NifH gene sequences were detected at all six sampling sites and clustered with Cluster I proteobacterial sequences and Cluster III sequences as defined by Zehr & Turner (2001) (Fig. 6). In Cluster I and Cluster III, three ~~novel clades~~ and seven novel clades were detected, respectively. In general, most of the ~~novel~~ previously unidentified clades belong to uncultured bacteria. One distinct novel clade was found for ~~the~~ St. 1 – 6~~. Furthermore, several clades consisting of different stations were found~~. No Cluster I cyanobacterial *nifH* sequences were detected and no potential PCR contaminants were present (Turk et al., 2011). In this study, detected sequences clustered with sulfate-reducing ~~SR~~ bacteria, such as *Desulfovibrio vulgaris* (Riederer-Henderson & Wilson, 1970; Muyzer & Stams, 2008) and *Desulfonema limicola* (Fukui et al., 1999). One cluster (OMZ 144 m) ~~belonged~~ was closely related to *Vibrio diazotrophicus* (Guerinot et al., 1982), which has the unique property for

Formatiert: Tiefgestellt

a known *Vibrio* species to perform N_2 fixation and ~~-~~which was found previously in the water column of the OMZ off Peru ~~(P7-M773-28)~~ (Löscher et al., 2014). The other organisms with which OMZ sequences clustered belonged to the genera of bacteria using fermentation, namely *Clostridium beijerincki* (Chen, 2005), and to the genera of iron-reducing bacteria, namely *Geobacter bemidjiensis* (Nevin et al., 2005). In addition, several sequences were phylogenetically related to ~~an uncultured bacterium from the Eastern Tropical South Pacific (KF151591.1) and~~ a gamma proteobacterium (Zehr & Turner, 2001) ~~(TAS801)~~ from the Pacific Ocean ~~(AY896428.1)~~.

4. Discussion

4.1 Coupling of benthic nitrogen fixation and sulfate reduction

Based on the high organic matter input to Peruvian sediments underneath the OMZ we hypothesized a presence of N_2 fixation and it's coupling to sulfate reduction (SR). We confirmed the presence of N_2 fixation ~~NA~~ in sediments at all sampled stations along the depth transect ~~between 70 and 1025 m water depth~~. This activity was generally enhanced~~,~~ where SR peaked and sometimes both activity depth profiles revealed similar trends. However, while peaks in SR w~~h~~ere very pronounced, maximum N_2 fixation ~~NA~~ showed a much broader distribution over depth. This discrepancy indicates that N_2 fixation might be partly coupled to processes other than SR (see *nifH* discussion below). But it should be kept in mind that the N_2 fixation ~~NA~~ and SR were determined in replicate MUC cores, which had a sampling distance of up to 50 cm, depending on ~~the location~~where ~~of~~ the core~~s~~ liners were situated in the ~~instrument~~multiple corer. Nonetheless, it appears that ~~T~~the observed N_2 fixation ~~NA~~ is ~~therefore~~ not directly ~~fuelled~~fueled by ~~the observed~~ SR activity. ~~Trends might vary naturally.~~ We are ~~also~~ aware of potential microbial community shifts driven by the addition of C_2H_2 (Fulweiler et al., 2015). However, a community shift would be expected to cause rather an underestimation of absolute N_2 fixation rates. Further, incubation with acetylene can lead to a potential lack of fixed N; however, to the best of our knowledge this is the standard method used for the determination of N_2 fixation in sediments (Bertics et al., 2013).

The more surprising finding is that integrated rates of N_2 fixation ~~NA~~ and SR showed opposite trends at the three shallowest stations, pointing to potential environmental control mechanisms (see 5.2).

The coupling between N_2 fixation and SR has been previously suggested for coastal sediments off California, where ~~(Bertics & Ziebis, 2010). In this study~~ N_2 fixation significantly decreased when SR was inhibited (Bertics & Ziebis, 2010). Different studies confirmed that sulfate-reducing ~~SR~~ bacteria, such as *D.esulfovibrio vulgaris* can supply organic-rich marine sediments with bioavailable N through N_2 fixation (Welsh et al., 1996; Nielsen et al., 2001; Steppe & Paerl, 2002; Fulweiler et al., 2007; Bertics et al., 2013; Fulweiler et al., 2013). ~~–~~Fulweiler et al. (2013) conducted a study in sediments of the Narrangaset Bay and found several *nifH* genes related to sulfate-reducing ~~SR~~ bacteria, such as *Desulfovibrio spp.*, *Desulfobacter spp*. and *Desulfonema spp.*, suggesting that sulfate-reducing ~~SR~~ bacteria ~~are~~ were the dominant diazotrophs.

The *nifH* gene sequences obtained in our study strongly indicated the genetic capability of sulfate reducers in the Peruvian sediments to conduct N_2 fixation. They clustered with the sulfate-reducing ~~SR~~ bacteria *Desulfovibrio vulgaris,* which is a confirmed diazotroph (Sisler & ZoBell 1951; Riederer-Henderson & Wilson 1970)**,** as well as *Vibrio diazotrophicus*, which recently clustered with sequences from the Peruvian OMZ water column (Fernandez et al., 2011; Löscher et al., 2014). Sequences taken from the seasonally hypoxic Eckernförde Bay in the Baltic Sea also clustered with *Desulfovibrio~~.~~ vulgaris* (Bertics et al., 2013), suggesting a major involvement of ~~SR~~ sulfate-reducing bacteria in N_2 fixation in organic-rich sediments underlying OMZs. Interestingly, we detected several new *nifH* gene clusters in the Peruvian OMZ that have not been identified yet (Fig. 6). ~~These findings suggest certain diversity among the benthic diazotrophic community and a possible coupling of N_2 fixation also to processes other than SR, which might explain some of the discrepancies between the two activities (see above). These results add to the growing evidence that "heterotrophic" N_2 fixation is dominant in the Peruvian OMZ (Farnelid et al., 2011; Fernandez et al., 2011; Löscher et al., 2014).~~

The molecular analysis further indicates that not all of the benthic diazotrophs are known sulfate-reducing ~~SR~~ organisms. Therefore, a ~~possible~~ coupling of N_2 fixation also to processes other than SR is ~~likely~~possible, which might explain some of the discrepancies

Formatiert: Schriftart: 12 Pt., Englisch (USA)

Formatiert: Standard, Abstand Nach: 0 Pt.

Formatiert: Schriftart: 12 Pt., Englisch (USA)

Formatiert: Schriftart: 12 Pt., Englisch (USA), Tiefgestellt

Formatiert: Schriftart: 12 Pt., Englisch (USA)

Formatiert: Schriftart: 12 Pt., Englisch (USA)

between N_2 fixation and SR ~~the two~~ activity~~ies~~ (see above). Other relevant processes may include the usage of reduced carbon compounds as previously suggested for diazotrophic organisms in the water column of the Peruvian OMZ (Dekaezemacker et al., 2013; Löscher et al., 2014).

Formatiert: Schriftart: 12 Pt., Tiefgestellt

Formatiert: Schriftart: 12 Pt., Englisch (USA)

Formatiert: Schriftart: 12 Pt., Englisch (USA)

Formatiert: Schriftart: 12 Pt., Schriftartfarbe: Dunkelrot, Englisch (USA)

Feldfunktion geändert

4.2 Environmental factors potentially controlling benthic N_2 fixation

The observed differences between integrated N_2 fixation and SR along the depth transect indicate potential environmental factors that ~~are~~ control~~ling~~ the extent of benthic N_2 fixation, which will be discussed in the following section.

4.2.1 Organic matter quantity and quality

A major driver for microbial processes such as SR and ~~"heterotrophic"~~ N_2 fixation by potentially heterotrophic organism is the availability of the organic material (Jørgensen, 1983; Howarth et al., 1988; Fulweiler et al., 2007). Integrated N_2 fixation and average organic carbon content ~~correlated~~ showed similar trends along the Peruvian OMZ depth transect (Fig. 5). Thus, organic matter availability appears to be a major factor controlling N_2 fixation at this study site. Low N_2 fixation rates were previously shown to be related to low organic matter content in slope sediments in the Atlantic Ocean (Hartwig & Stanley, 1978). This pattern is supported by the study of Bertics et al. (2010), which showed that burrow systems of the bioturbating ghost shrimp *Neotrypaea californiensis* can lead to enhanced organic matter availability in deeper sediment layers, resulting in high rates of N_2 fixation. However, high organic matter availability does not always result in enhanced N_2 fixation rates. Subtidal sediments in the Narragansett Bay were found to switch from being a net sink via denitrification to being a net source of bioavailable N via N_2 fixation (Fulweiler et al., 2007). This switch from N sink to N source was caused by a decrease of organic matter deposition to the sediments, which was in turn triggered by low primary production in the surface waters. ~~Especially this switch is an interesting feature, showing us that there are still major gaps in our understanding of benthic N_2 fixation.~~

Besides quantity also the quality of organic matter in sediments is a major factor influencing microbial degradation processes (Westrich & Berner, 1984). In the Peruvian OMZ sediments, the average C/N ratio increased with water depth indicating that the shallow stations received a higher input of fresh, labile organic material compared to the deeper stations. Similar trends were reported for a different depth transect off Peru (Levin

et al., 2002). However, an increase of the C/N ratio with depth would suggest highest integrated N_2 fixation rate at the shallowest St. 1 (70 m), which however is not in line with our observation that shows an increase in rate from St. 1 (70) to St. 6 (253 m) (Fig. 5). Similarly, DIC fluxes measured using benthic chambers at the same stations can be used as an indicator for organic matter degradation rates ~~were at the same stations during the expedition by (~~Dale et al., ~~(~~2015). The DIC flux did not correlate with integrated N_2 fixation rates, but instead roughly followed the pattern of SR rates along water depth (Fig. 4~~5~~). The highest integrated SR rate and DIC flux ~~was~~ were found at St. 1 (70 m), whereas the lowest ~~integrated SR rate and DIC flux was found~~ occurred at St. 10 (1025 m). Assuming that SR is largely responsible for organic matter remineralization ~~, i.e. DIC fluxes,~~ in the sediments below the OMZ (Bohlen et al., 2011; Dale et al. 2015), the difference between integrated SR and DIC flux is expected to mainly represent ~~the underestimated fraction, which likely resulted from the~~ the long duration of~~,~~ unfrozen storage of the samples (see methods).

4.2.2 Ammonium

Interestingly, the highest N_2 fixation was measured in sediments colonized by the sulfur-oxidizing and nitrate-reducing filamentous bacteria Mari~~T~~t*hioploca spp*. (Schulz, 1999; Schulz & Jørgensen, 2001; Gutiérrez et al., 2008; Salman et al., 2011; Mosch et al., 2012). Mari~~T~~t*hioploca* facilitates dissimilatory NO_3^- reduction to NH_4^+, which preserves fixed N in the form of NH_4^+ in the environment (Kartal et al., 2007). OMZ sediments off Peru are generally rich in NH_4^+ (Bohlen et al., 2011). This co-occurrence of ~~*Thioploca*~~ *Marithioploca* and N_2 fixation was puzzling since high concentrations of NH_4^+, could inhibit N_2 fixation (Postgate, 1982; Capone, 1988; Knapp, 2012). It remains questionable why microorganisms should fix N_2 in marine sediments, when reduced N species are abundant. Some doubt remains as to the critical NH_4^+ concentration that inhibits N_2 fixation and whether the inhibitory effect is the same for all environments (Knapp, 2012). For example, NH_4^+ concentrations up to 1000 µM did not fully suppress benthic N_2 fixation in a hypoxic basin in the Baltic Sea (Bertics et al., 2013), indicating that additional environmental factors must control the distribution and performance of benthic diazotrophs (Knapp, 2012). We observed high porewater NH_4^+ concentrations at the shallow St. 1 with 316 µM at the sediment surface (0 – 1 cm) increasing to 2022 µM at 20 cm (Fig. 2), while no inhibition of N_2 fixation was found. ~~Though~~However, we cannot exclude that a partial suppression

occurred. Inhibition experiments of N_2 fixation with NH_4^+ have been conducted in several environments with different ~~findings~~results. For example, benthic N_2 fixation was measured in the Carmens River estuary (New York) with ambient ~~and was still abundant at 2800 µM~~ NH_4^+ concentrations of 2800 µM (Capone, 1988). In general, these studies suggested that the impact of NH_4^+ on N_2 fixation is more complex than previously thought and poorly understood~~hitherto hardly known~~.

One explanation for why diazotrophs still fix N under high NH_4^+ concentrations could be that bacteria try to preserve the intracellular redox state by N_2 fixation functioning as an excess for electrons, particularly with a deficient Calvin–Benson–Bassham pathway, as it was shown for photoheterotrophic nonsulfur purple bacteria (Tichi & Tabita, 2000). Previous studies on benthic environments propose that the organic carbon availability can reduce an inhibition of N_2 fixation by abundant NH_4^+ (Yoch & Whiting, 1986; McGlathery et al., 1998). In the study of Yoch & Whiting (1986), ~~it was shown that~~ enrichment cultures of *Spartina alterniflora* salt marsh sediment showed~~reacted with~~ different N_2 fixation inhibition stages ~~on~~ for different organic matter species. Another explanation could be that microniches, depleted in NH_4^+ exist between the sediment grains, which we were unable to track with the applied porewater extraction techniques (Bertics et al., 2013). Such microniches ~~wehre~~ are found in the form of localized organic matter hot spots (Brandes & Devol, 2002; Bertics & Ziebis, 2010), and could also ~~occur for~~supply NH_4^+.

4.2.3 Sulfide

Sulfide is a known inhibitor for many biological processes (Reis, et al., 1992; Joye & Hollibaugh, 1995) and could potentially affect N_2 fixation (Tam et al., 1982). The shallow St. 1 was the only station with sulfide in the porewater, reaching 280 µM in surface sediments and 1229 µM in 20 cm (Fig. 2). The presence of relatively high concentrations of sulfide might explain why N_2 fixation was lower at St. 1 compared to St. 6, despite the higher quality, i.e. lower C/N ratio, of organic matter at this station. Because SR rates were highest at St. 1 (Fig. 4), we exclude direct inhibition on SR, although the effect has generally been reported (Postgate, 1979; McCartney & Oleszkiewicz, 1991). Interactions of sulfide with benthic N_2 fixation have so far not been investigated, and hence we can ~~therefore~~ not rule out a partial inhibition of N_2 fixation by sulfide.

4.2.4 Oxygen

Dissolved O_2 can have a considerable influence on N_2 fixation~~, because of~~ due to the O_2 sensitivity of the key enzyme nitrogenase (Postgate, 1998; Dixon & Kahn, 2004). Bioturbating and bioirrigating organisms can transport O_2 much deeper into sediments than molecular diffusion (Orsi et al., 1996; Dale et al., 2011). In coastal waters, the bioturbation and bioirrigation activity of ghost shrimps was found to reduce N_2 fixation~~,~~ when sediments were highly colonized by these animals (Bertics et al., 2010). While bottom water O_2 concentrations in the Peruvian OMZ were below the detection limit at ~~the~~ St. 1 to 8 (70 m to 407 m), thereby mainly excluding benthic macrofauna, O_2 concentrations increased to ~~levels~~ above 40 µM at St. 10 (1025 m) where~~, supporting~~ a diverse bioturbating and bioirrigating benthic macrofauna community was observed (Mosch et al. 2012). Accordingly, this station revealed some of the lowest N_2 fixation activity. We are, however, unable to decipher whether O_2, low organic matter content, and/or the low C/N ratio was responsible for this low activity. ~~Furthermore, several marine diazotrophs have developed strategies to protect the nitrogenase from O_2 (Jørgensen, 1977).~~

4.3 Comparison of benthic N_2 fixation in different environments

We compiled a list of N_2 fixation rates from different marine environments to gain an overview of the magnitude of N_2 fixation rates measured in the Peruvian OMZ sediments (Tab. 2). We found that N_2 fixation rates from the Peruvian sediments exceed those reported for open ocean sediments (2800 m) (Howarth et al., 1988), bioturbated coastal lagoon sediment (Bertics et al., 2010) and sediments >200 m water depth (Capone, 1988). The highest integrated N_2 fixation rate determined in our study (0.4 mmol N m^{-2} d^{-1}, St. 6) closely resembles highest rates found in salt marsh surface sediments (0.38 mmol N m^{-2} d^{-1}) and Zostera estuarine sediments (0.39 mmol N m^{-2} d^{-1}) (Capone, 1988). Further, our rates were characterized by a similar range of N_2 fixation rates that were previously measured in an organic-rich hypoxic basin in the Baltic Sea (0.08 - 0.22 mmol N m^{-2} d^{-1}, Bertics et al., 2013). Different to the above examples, our N_2 fixation rates were 8.5 times lower compared to shallow (< 1 m) soft-bottom sediment off the Swedish coast (Andersson et al., 2014) and 17 times lower than coral reef sediments (Capone, 1988). However, in these environments, phototrophic cyanobacterial mats contributed to benthic N_2 fixation. Given the dark incubation, N_2 fixation of the present study seems to be attributed to

heterotrophic diazotrophs, which is additionally confirmed by the *nifH* gene analysis, where none of the sequences clustered with cyanobacteria (Fig. 6).

5. Summary

To the best of our knowledge, this is the first study combining N_2 fixation and SR rate measurements together with molecular analysis in OMZ sediments. We have shown that N_2 fixation occurred throughout the sediment and that elevated activity often overlapped with peaks of SR. The molecular analysis of the *nifH* gene confirmed the presence of heterotrophic diazotrophs at all sampling sites. Sequences clustered with sulfate-reducing ~~SR~~ bacteria, such as *Desulfovibrio vulgaris*, which is a known diazotroph in sediments. In combination, our results suggest that N_2 fixation and SR were coupled to a large extend, but ~~that~~ additional coupling to other metabolic pathways cannot be ruled out completely. The major environmental factor controlling benthic diazotrophs in the OMZ appears to be the organic matter content. ~~-~~Sulfide was identified as a potential inhibitor for N_2 fixation. We further found no inhibition of N_2 fixation by high NH_4^+ concentrations, highlighting gaps in our understanding of the relationship between NH_4^+ availability and the stimulation of N_2 fixation. N_2 fixation rates determined in the Peruvian OMZ sediments were in the same range of other organic-rich benthic environments, underlining the relation between organic matter, heterotrophic activity, and N_2 fixation.

Author contribution

J. G. and T. T. collected samples and designed experiments. J. G. performed nitrogen fixation experiments and T. T. conducted sulfate reduction experiments. S. S. and A. W. D. measured porosity, DIC, organic carbon content and C/N. J. G., T. T., C. R. L. and S. S. analyzed the data. J. G. and C. R. L. performed ~~PCR assay and sequence~~molecular analysis. J. G. prepared the manuscript with contributions from all co-authors and T. T. supervised the work.

Acknowledgments

We would like to thank the captain and the crew of the RV Meteor cruise M92, as well as S. Kriwanek, A. Petersen and S. Cherednichenko of the GEOMAR Technology and Logistics Center, for all of their assistance in field sampling. We also thank B. Domeyer, A. Bleyer, U. Lomnitz, R.

Suhrberg, S. Trinkler and V. Thoenissen for geochemical analyses. Additional thanks goes to the members of the Treude and Schmitz-Streit working groups, especially V. Bertics for her methological guidance, G. Schuessler, P. Wefers, N. Pinnow, and B. Mensch for their laboratory assistance and to J. Maltby and S. Krause for scientific discussions. We further thank the authorities of Peru for the permission to work in their territorial waters. We thank the editor and two reviewers for their valuable comments. This study is a contribution of the Sonderforschungsbereich 754 "Climate – Biogeochemistry Interactions in the Tropical Ocean" (www.sfb754.de), which is supported by the German Research Foundation.

References

Andersson, B., Sundbäck, K., Hellman, M., Hallin, S. & Alsterberg, C. (2014). Nitrogen fixation in shallow-water sediments: Spatial distribution and controlling factors. *Limnology and Oceanography*. 59 (6). p.pp. 1932–1944.

Bertics, V.J., Löscher, C.R., Salonen, I., Dale, A.W., Gier, J., Schmitz, R.A. & Treude, T. (2013). Occurrence of benthic microbial nitrogen fixation coupled to sulfate reduction in the seasonally hypoxic Eckernförde Bay, Baltic Sea. *Biogeosciences*. 10 (3). p.pp. 1243–1258.

Bertics, V.J., Sohm, J., Treude, T., Chow, C., Capone, D., Fuhrman, J. & Ziebis, W. (2010). Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulfate reduction. *Marine Ecology Progress Series*. 409. p.pp. 1–15.

Bertics, V.J. & Ziebis, W. (2010). Bioturbation and the role of microniches for sulfate reduction in coastal marine sediments. *Environmental Microbiology*. 12. p.pp. 3022–3034.

Bohlen, L., Dale, A.W., Sommer, S., Mosch, T., Hensen, C., Noffke, A., Scholz, F. & Wallmann, K. (2011). Benthic nitrogen cycling traversing the Peruvian oxygen minimum zone. *Geochimica et Cosmochimica Acta*. 75 (20). p.pp. 6094–6111.

Brandes, A., Devol, A.H. & Deutsch, C. (2007). New developments in the marine nitrogen cycle. *Chemical reviews*. 107 (2). p.pp. 577–89.

Brandes, J.A. & Devol, A.H. (2002). A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling. *Global Biogeochemical Cycles*. 16 (4). p.pp. 1–14.

Capone, D.G. (1983). Benthic nitrogen fixation. In: E. J. Carpenter & D. G. Capone (eds.). *Nitrogen in the Marine Environment*. New York: John Wiley & Sons Ltd, pp. 85–123.

Capone, D.G. (1988). Benthic Nitrogen Fixation. In: T. H. Blackburn & J. Sorensen (eds.). *Nitrogen cycling in coastal marine environments*. John Wiley & Sons Ltd, pp. 85–123.

Capone, D.G. (1993). Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In: P. F. Kemp, B. F. Sherr, E. B. Sherr, & J. J. Coles (eds.). *Handbook of methods in aquatic microbial ecology*. Boca Raton: CRC Press LLC, pp. 621–631.

Capone, D.G., Bronk, A.A., Mulholland, M.R. & Carpenter, E.J. (2008). *Nitrogen in the marine environment*. 2nd Ed. Elsevier.

Capone, D.G., Burns, J. a., Montoya, J.P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A.F. & Carpenter, E.J. (2005). Nitrogen fixation by Trichodesmium spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Global Biogeochemical Cycles*. 19. p.pp. 1–17.

Capone, D.G. & Knapp, A.N. (2007). A marine nitrogen cycle fix? *Nature*. 16 (445). p.pp. 159–160.

Chen, J.-S. (2005). Nitrogen Fixation in the Clostridia. In: W. Klipp, B. Masepohl, J. R. Gallon, & W. E. Newton (eds.). *Genetics and Regulation of Nitrogen Fixation in Free-Living Bacteria*. Nitrogen Fixation: Origins, Applications, and Research Progress. Dordrecht: Kluwer Academic Publishers, pp. 53–64.

Codispoti, L.A. (2007). An oceanic fixed nitrogen sink exceeding 400 Tg N a−1 vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences*. [Online]. 4 (2). p.pp. 233–253. Available from: http://www.biogeosciences.net/4/233/2007/.

Dale, A.W., Sommer, S., Bohlen, L., Treude, T., Bertics, V.J., Bange, H.W., Pfannkuche, O., Schorp, T., Mattsdotter, M. & Wallmann, K. (2011). Rates and regulation of nitrogen cycling in seasonally hypoxic sediments during winter (Boknis Eck, SW Baltic Sea): Sensitivity to environmental variables. *Estuarine, Coastal and Shelf Science*. 95 (1). p.pp. 14–28.

Dale, A.W., Sommer, S., Lomnitz, U., Montes, I., Treude, T., Liebetrau, V., Gier, J., Hensen, C., Dengler, M., Stolpovsky, K., Bryant, L.D. & Wallmann, K. (2015). Organic carbon production, mineralisation and preservation on the Peruvian margin. *Biogeosciences*. 12. p.pp. 1537–1559.

Dekaezemacker, J. & Bonnet, S. (2011). Sensitivity of N2 fixation to combined nitrogen forms (NO3 − and NH4+) in two strains of the marine diazotroph Crocosphaera watsonii (Cyanobacteria). *Marine Ecology Progress Series*. 438. p.pp. 33–46.

Dekaezemacker, J., Bonnet, S., Grosso, O., Moutin, T., Bressac, M. & Capone, D.G. (2013). Evidence of active dinitrogen fixation in surface waters of the eastern tropical South Pacific during El Niño and La Niña events and evaluation of its potential nutrient controls. *Global Biogeochemical Cycles*. 27 (3). p.pp. 768–779.

Deutsch, C., Berelson, W., Thunell, R., Weber, T., Tems, C., McManus, J., Crusius, J., Ito, T., Baumgartner, T., Ferreira, V., Mey, J. & van Geen, A. (2014). Centennial changes in North Pacific anoxia linked to tropical trade winds. *Science*. 345 (6197). p.pp. 665–8.

Dixon, R. & Kahn, D. (2004). Genetic regulation of biological nitrogen fixation. *Nature reviews. Microbiology*. 2 (8). p.pp. 621–631.

Donohue, M.J.O., Moriarty, D.J.W. & Rae, I.C. Mac (1991). Nitrogen Fixation in Sediments and the Rhizosphere of the Seagrass Zostera capricorni. *Microbiology Ecology*. 22. p.pp. 53–64.

Duteil, O., Böning, C.W. & Oschlies, A. (2014). Variability in subtropical-tropical cells drives oxygen levels in the tropical Pacific Ocean. *Geophysical Research Letters*. 41. p.pp. 1–9.

Falkowski, P.G., Barber, R.T. & Smetacek, V. (1998). Biogeochemical Controls and Feedbacks on Ocean Primary Production. *Science*. 281 (5374). p.pp. 200–7.

Farnelid, H., Andersson, A.F., Bertilsson, S., Al-Soud, W.A., Hansen, L.H., Sørensen, S., Steward, G.F., Hagström, Å. & Riemann, L. (2011). Nitrogenase gene amplicons from global marine surface waters are dominated by genes of non-cyanobacteria. *PloS one*. 6 (4). p.pp. 1–9.

Fernandez, C., González, M.L., Muñoz, C., Molina, V. & Farias, L. (2015). Temporal and spatial variability of biological nitrogen fixation off the upwelling system of central Chile (35-38.5°S). *Journal of Geophysical Research: Oceans*. 120 (5). p.pp. 3330–3349.

1005 Fossing, H., Gallardo, V.A., Jørgensen, B.B., Hüttel, M., Nielsen, L.P., Schulz, H., Canfield,
1006 D.E., Forster, S., Glud, R.N., Gundersen, J.K., Küver, J., Ramsing, N.B., Teske, A.,
1007 Thamdrup, B. & Ulloa, O. (1995). Concentration and transport of nitrate by the mat-
1008 forming sulphur bacterium Thioploca. *Nature*. 374. p.pp. 713–715.

1009 Fuenzalida, R., Schneider, W., Garces-Vargas, J., Bravo, L. & Lange, C. (2009). Vertical
1010 and horizontal extension of the oxygen minimum zone in the eastern South Pacific
1011 Ocean. *Deep-Sea Research Part II*. 56 (16). p.pp. 992–1008.

1012 Fukui, M., Teske, A., Assmus, B., Muyzer, G. & Widdel, F. (1999). Physiology,
1013 phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus
1014 desulfonema). *Archives of microbiology*. 172 (4). p.pp. 193–203.

1015 Fulweiler, R., Brown, S., Nixon, S. & Jenkins, B. (2013). Evidence and a conceptual model
1016 for the co‐occurrence of nitrogen fixation and denitrification in heterotrophic marine
1017 sediments. *Marine Ecology Progress Series*. 482. p.pp. 57–68.

1018 Fulweiler, R.W., Heiss, E.M., Rogener, M.K., Newell, S.E., LeCleir, G.R., Kortebein, S.M.
1019 & Wilhelm, S.W. (2015). Examining the impact of acetylene on N-fixation and the
1020 active sediment microbial community. *Frontiers in Microbiology*. 6 (418). p.pp. 1–9.

1021 Fulweiler, R.W., Nixon, S.W., Buckley, B. a & Granger, S.L. (2007). Reversal of the net
1022 dinitrogen gas flux in coastal marine sediments. *Nature*. 448 (7150). p.pp. 180–2.

1023 Glock, N., Schönfeld, J., Eisenhauer, A., Hensen, C., Mallon, J. & Sommer, S. (2013). The
1024 role of benthic foraminifera in the benthic nitrogen cycle of the Peruvian oxygen
1025 minimum zone. *Biogeosciences*. 10. p.pp. 4767–4783.

1026 Grasshoff, K., Kremlingl, K. & Ehrhardt, M. (1999). *Methods of Seawater Analysis*. 3rd Ed.
1027 Weinheim: Wiley–VCH.

1028 Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M.M.M., Lavik, G.,
1029 Schmitz, R. a., Wallace, D.W.R. & LaRoche, J. (2012). Doubling of marine dinitrogen-
1030 fixation rates based on direct measurements. *Nature*. 000. p.pp. 1–4.

1031 Gruber, N. (2004). The dynamics of the marine nitrogen cycle and its influence on
1032 atmospheric CO2 variations. In: T. Oguz & M. Follows (eds.). *Carbon Climate*
1033 *interactions*. pp. 97–148.

1034 Gruber, N. (2008). The Marine Nitrogen Cycle : Overview and Challenges. In: D. G.
1035 Capone, D. A. Bronk, M. R. Mulholland, & E. J. Carpenter (eds.). *Nitrogen in the*
1036 *Marine Environment*. Amsterdam: Elsevier, pp. 1–50.

1037 Guerinot, M.L., West, P. a., Lee, J. V. & Colwell, R.R. (1982). Vibrio diazotrophicus sp.
1038 nov., a Marine Nitrogen-Fixing Bacterium. *International Journal of Systematic*
1039 *Bacteriology*. 32 (3). p.pp. 350–357.

1040 Gutiérrez, D., Enríquez, E., Purca, S., Quipúzcoa, L., Marquina, R., Flores, G. & Graco, M.
1041 (2008). Oxygenation episodes on the continental shelf of central Peru: Remote forcing
1042 and benthic ecosystem response. *Progress in Oceanography*. 79 (2-4). p.pp. 177–189.

1043 Hartwig, E.O. & Stanley, S.O. (1978). Nitrogen fixation in Atlantic deep-sea and coastal
1044 sediments. *Deep Sea Research*. 25 (4). p.pp. 411–417.

1045 Howarth, R.W., Marino, R., Lane, J. & Cole, J.J. (1988). Nitrogen fixation in freshwater,
1046 estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and*
1047 *Oceanography*. 33. p.pp. 669–687.

1048 Jørgensen, B.B. (1978). A comparison of methods for the quantification of bacterial sulfate
1049 reduction in coastal marine sediments. *Geomicrobiology Journal*. 1. p.pp. 11–27.

1050 Jørgensen, B.B. (1983). *SCOPE 21 -The Major Biogeochemical Cycles and Their*
1051 *Interactions. Processes at the Sediment-Water Interface*. In: 1983.

1052 Jørgensen, B.B. & Gallardo, V.A. (1999). Thioploca spp .: filamentous sulfur bacteria with
1053 nitrate vacuoles. *FEMS Microbiology Ecology*. 28. p.pp. 301–313.

1054 Joye, S.B. & Hollibaugh, J.T. (1995). Influence of sulfide inhibition of nitrification on
1055 nitrogen regeneration in sediments. *Sciences*. 270. p.pp. 623–625.

1056 Kallmeyer, J., Ferdelman, T.G., Weber, A., Fossing, H. & Jørgensen, B.B. (2004).
1057 Evaluation of a cold chromium distillation procedure for recovering very small
1058 amounts of radiolabeled sulfide related to sulfate reduction measurements. *Limnology*
1059 *and Oceanography: Methods*. 2. p.pp. 171–180.

1060 Kamykowski, D. & Zentara, S.-J. (1990). Hypoxia in the world ocean as recorded in the
1061 historical data set. *Deep Sea Research Part A. Oceanographic Research Papers*. 37
1062 (12). p.pp. 1861–1874.

1063 Kartal, B., Kuypers, M.M.M., Lavik, G., Schalk, J., Op den Camp, H.J.M., Jetten, M.S.M. &
1064 Strous, M. (2007). Anammox bacteria disguised as denitrifiers: nitrate reduction to
1065 dinitrogen gas via nitrite and ammonium. *Environmental microbiology*. 9 (3). p.pp.
1066 635–42.

1067 Kessler, W.S. (2006). The circulation of the eastern tropical Pacific: A review. *Progress in*
1068 *Oceanography*. 69. p.pp. 181–217.

1069 Knapp, A.N. (2012). The sensitivity of marine N2 fixation to dissolved inorganic nitrogen.
1070 *Frontiers in microbiology*. 3. p.pp. 1–14.

1071 Letunic, I. & Bork, P. (2007). Interactive Tree Of Life (iTOL): An online tool for
1072 phylogenetic tree display and annotation. *Bioinformatics*. 23. p.pp. 127–128.

1073 Letunic, I. & Bork, P. (2011). Interactive Tree of Life v2: Online annotation and display of
1074 phylogenetic trees made easy. *Nucleic Acids Research*. 39. p.pp. 1–4.

1075 Levin, L., Gutierrez, D., Rathburn, A., Neira, C., Sellanes, J., Munoz, P., Gallardo, V. &
1076 Salamanca, M. (2002). Benthic processes on the Peru margin: a transect across the
1077 oxygen minimum zone during the 1997–98 El Nino. *Progress In Oceanography*. 53.
1078 p.pp. 1–27.

1079 Löscher, C.R., Großkopf, T., Desai, F.D., Gill, D., Schunck, H., Croot, P.L., Schlosser, C.,
1080 Neulinger, S.C., Pinnow, N., Lavik, G., Kuypers, M.M.M., Laroche, J. & Schmitz,
1081 R.A. (2014). Facets of diazotrophy in the oxygen minimum zone waters off Peru. *The*
1082 *ISME journal*. p.pp. 1–13.

1083 McCartney, D.M. & Oleszkiewicz, J.A. (1991). Sulfide inhibition of anaerobic degradation
1084 of lactate and acetate. *Water Research*. 25 (2). p.pp. 203–209.

1085 McGlathery, K.J., Risgaard-Petersen, N. & Christensen, B.P. (1998). Temporal and spatial
1086 variation in nitrogen fixation activity in the eelgrass Zostera marina rhizosphere.
1087 *Marine Ecology Progress Series*. 168. p.pp. 245–258.

1088 Mosch, T., Sommer, S., Dengler, M., Noffke, A., Bohlen, L., Pfannkuche, O., Liebetrau, V.
1089 & Wallmann, K. (2012). Factors influencing the distribution of epibenthic megafauna
1090 across the Peruvian oxygen minimum zone. *Deep Sea Research Part I: Oceanographic*
1091 *Research Papers*. 68. p.pp. 123–135.

1092 Muyzer, G. & Stams, A.J.M. (2008). The ecology and biotechnology of sulphate-reducing
1093 bacteria. *Nature reviews. Microbiology*. 6. p.pp. 441–54.

1094 Nevin, K.P., Holmes, D.E., Woodard, T.L., Hinlein, E.S., W, O.D. & R, L.D. (2005).

1095 Geobacter bemidjiensis sp. nov. and Geobacter psychrophilus sp. nov., two novel
1096 Fe(III)-reducing subsurface isolates. *International Journal of Systematic and*
1097 *Evolutionary Microbiology*. 55. p.pp. 1667–1674.

1098 Nielsen, L.B., Finster, K., Welsh, D.T., Donelly, A., Herbert, R. a, de Wit, R. & Lomstein,
1099 B. a (2001). Sulphate reduction and nitrogen fixation rates associated with roots,
1100 rhizomes and sediments from Zostera noltii and Spartina maritima meadows.
1101 *Environmental microbiology*. 3 (1). p.pp. 63–71.

1102 Orcutt, K.M., Lipschultz, F., Gundersen, K., Arimoto, R., Michaels, A.F., Knap, A.H. &
1103 Gallon, J.R. (2001). A seasonal study of the significance of N2 fixation by
1104 Trichodesmium spp. at the Bermuda Atlantic Time-series Study (BATS) site. *Deep*
1105 *Sea Research Part II: Topical Studies in Oceanography*. 48 (8-9). p.pp. 1583–1608.

1106 Orsi, T.H., Werner, F., Milkert, D., Anderson, A.L. & Bryant, W.R. (1996). Environmental
1107 overview of Eckernförde Bay , northern Germany. *Geo-Marine Letters*. 16. p.pp. 140–
1108 147.

1109 Patriquin, D. & Knowles, R. (1972). Nitrogen fixation in the rhizosphere of marine
1110 angiosperms. *Marine Biology*. 16 (1). p.pp. 49–58.

1111 Pennington, J.T., Mahoney, K.L., Kuwahara, V.S., Kolber, D.D., Calienes, R. & Chavez,
1112 F.P. (2006). Primary production in the eastern tropical Pacific: A review. *Progress in*
1113 *Oceanography*. 69. p.pp. 285–317.

1114 Postgate, J.R. (1998). *Nitrogen fixation*. 3rd Ed. Cambridge: Cambridge University Press.

1115 Postgate, J.R. (1982). *The Fundamentals of Nitrogen Fixation*. Cambridge University Press.

1116 Postgate, J.R. (1979). *The Sulphate-Reducing Bacteria*. Cambridge University Press.

1117 Reis, M.A., Almeida, J.S., Lemos, P.C. & Carrondo, M.J. (1992). Effect of hydrogen sulfide
1118 on growth of sulfate reducing bacteria. *Biotechnology and bioengineering*. 40 (5). p.pp.
1119 593–600.

1120 Riederer-Henderson, M.-A. & Wilson, P.W. (1970). Nitrogen Fixation by Sulphate-reducing
1121 Bacteria. *Journal of General Microbiology*. 61. p.pp. 27–31.

1122 Riemann, L., Farnelid, H. & Steward, G.F. (2010). Nitrogenase genes in non-cyanobacterial
1123 plankton: Prevalence, diversity and regulation in marine waters. *Aquatic Microbial*
1124 *Ecology*. 61 (3). p.pp. 235–247.

1125 Røy, H., Weber, H.S., Tarpgaard, I.H., Ferdelman, T.G. & Jørgensen, B.B. (2014).
1126 Determination of dissimilatory sulfate reduction rates in marine sediment via
1127 radioactive 35 S tracer. *Limnology and Oceanography: Methods*. 12. p.pp. 196–211.

1128 Schulz, H.N. (1999). Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf
1129 Sediments. *Science*. 284 (5413). p.pp. 493–495.

1130 Schulz, H.N. & Jørgensen, B.B. (2001). Big bacteria. *Annual review of microbiology*. 55.
1131 p.pp. 105–137.

1132 Sisler, F.D. & ZoBell, C.E. (1951). Nitrogen Fixation by Sulfate-reducing Bacteria Indicated
1133 by Nitrogen/Argon Ratios. *Science*. 113. p.pp. 511–512.

1134 Sohm, J.A., Webb, E.A. & Capone, D.G. (2011). Emerging patterns of marine nitrogen
1135 fixation. *Nature reviews. Microbiology*. 9 (7). p.pp. 499–508.

1136 Steppe, T. & Paerl, H. (2002). Potential N2 fixation by sulfate-reducing bacteria in a marine
1137 intertidal microbial mat. *Aquatic Microbial Ecology*. [Online]. 28. p.pp. 1–12.
1138 Available from: http://www.int-res.com/abstracts/ame/v28/n1/p1-12/.

1139 Stramma, L., Johnson, G.C., Sprintall, J. & Mohrholz, V. (2008). Expanding oxygen-

minimum zones in the tropical oceans. *Science (New York, N.Y.)*. 320 (5876). p.pp. 655–8.

Strous, M., Kuenen, J.G. & Jetten, M.S. (1999). Key physiology of anaerobic ammonium oxidation. *Applied and environmental microbiology*. 65 (7). p.pp. 3248–50.

Tam, T.-Y., Mayfield, C.I., Inniss, W.E. & Knowles, R. (1982). Effect of Sulfide on Nitrogen Fixation in a Stream Sediment- Water System. *Appl. Environm. Microbiol.* 43 (5). p.pp. 1076–1079.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*. 24. p.pp. 1596–1599.

Tichi, M.A. & Tabita, F.R. (2000). Maintenance and control of redox poise in Rhodobacter capsulatus strains deficient in the Calvin-Benson-Bassham pathway. *Archives of microbiology*. 174 (5). p.pp. 322–33.

Turk, K.A., Rees, A.P., Zehr, J.P., Pereira, N., Swift, P., Shelley, R., Lohan, M., Woodward, E.M.S. & Gilbert, J. (2011). Nitrogen fixation and nitrogenase (nifH) expression in tropical waters of the eastern North Atlantic. *The ISME journal*. 5 (7). p.pp. 1201–1212.

Ward, B.B. & Bronk, D.A. (2001). Net nitrogen uptake and DON release in surface waters: importance of trophic interactions implied from size fractionation experiments. *Marine Ecology Progress Series*. 219. p.pp. 11–24.

Welsh, D.T., Bourgues, S., de Wit, R. & Herbert, R.A. (1996). Seasonal variations in nitrogen-fixation (acetylene reduction) and sulphate-reduction rates in the rhizosphere of Zostera noltii: nitrogen fixation by sulphate-reducing bacteria. *Marine Biology*. 125. p.pp. 619–628.

Westrich, J.T. & Berner, R.A. (1984). The role of sedimentary organic matter in bacterial sulfate reduction : The G model tested ’. *Limnol. Oceanography*. 29 (2). p.pp. 236–249.

Yoch, D.C. & Whiting, G.J. (1986). Evidence for NH4+ switch-off regulation of nitrogenase activity by bacteria in salt marsh sediments and roots of the grass Spartina alterniflora. *Applied and environmental microbiology*. 51 (1). p.pp. 143–149.

Zehr, J.P., Mellon, M., Braun, S., Litaker, W., Steppe, T. & Paerl, H.W. (1995). Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. *Applied and environmental microbiology*. 61 (7). p.pp. 2527–32.

Zehr, J.P., Mellon, M.T. & Zani, S. (1998). New Nitrogen-Fixing Microorganisms Detected in Oligotrophic Oceans by Amplification of Nitrogenase (nifH) Genes. *Appl. Environ. Microbiol.* 64 (9). p.pp. 3444–3450.

Zehr, J.P. & Turner, P.J. (2001). Nitrogen Fixation : Nitrogenase Genes and Gene Expression. In: J. H. Paul (ed.). *METHODS IN MICROBIOLOGY, Volume 30*. San Diego, CA: Academic Press, pp. 271–286.

Figure captions

Fig. 1. Cross-section of dissolved O_2 concentrations (µM) along the continental margin of the Peruvian OMZ at 12°S. The vertical lines represent CTD cast for O_2 measurement during the cruise M92. Stations 1 to 10 for ~~MUC~~ multicorer (MUC) sampling are indicated by station numbers according to Dale et al. (2015).

Fig. 2: Biogeochemical porewater profiles in MUC cores from sampling stations along the 12°S depth transect. Graphs show NH_4^+ (µM), SO_4^{2-} (mM), sulfide (µM), organic carbon content (C_{org}, wt%) and the C/N ratio (molar). Information about bottom water O_2 concentrations (BW O_2, µM) is provided at the right margin.

Fig. 3: Sediment profiles of N_2 fixation ~~nitrogenase activity~~ (~~NA,~~ nmol ~~C_2H_4~~ N_2 cm^{-3} d^{-1}, average of three replicates) and sulfate reduction rates (SR, nmol SO_4^{2-} cm^{-3} d^{-1}, two replicates (R1 and R2)) from 0 - 20 cm at the six stations. The upper x-axis represents the N_2 fixation~~NA~~, while the lower x-axis represents the SR. Error bars indicate standard deviation of N_2 fixation~~NA~~.

Fig. 4: Integrated nitrogen fixation (mmol N m^{-2} d^{-1}, grey bars, average of three replicates) and integrated sulfate reduction (mmol SO_4^{2-} m^{-2} d^{-1}, green bars, two replicates) from 0 - 20 cm, including dissolved inorganic carbon (DIC, mmol m^{-2} d^{-1}, red curve from Dale et al., (2015)) and bottom water O_2 (µM, blue curve) along the depth transect (m). Error bars indicate standard deviation of N_2 fixation.

Fig. 5: Integrated nitrogen fixation (mmol N_2 m^{-2} d^{-1}, grey bars, average of three replicates), average organic carbon content (C_{org}, wt%, orange curve) and the average C/N ratio (molar, yellow curve) from 0-20 cm along the depth transect (m). Error bars indicate standard deviation.

Formatiert: Tiefgestellt

Fig. 6: Phylogenetic tree of ~~expressed~~ *nifH* genes based on the analysis of 120 sequences from the six sampling stations between 70 and 1025 m water depth. Novel detected clusters consisting of several sequences from the same sampling depth are indicated by grey triangles. Reference sequences consist of the alternative nitrogenase anfD, anfG, anfK. Cluster III sequences as defined by Zehr and Turner (2001) are highlighted in blue, Cluster I cyanobacterial sequences are highlighted in green and Cluster I proteobacterial sequences are highlighted in orange. The scale bar indicates the 10% sequences divergence. Sequences marked with an asterisk represent potential PCR contaminated products, with novel clusters distant from those clusters. Sequences determined in this study are termed OMZ plus the corresponding water depth.

Tables

Tab. 1: Sampling deployments, including station number according to Dale et al. (2015), core ID, sampling date and coordinates. Water depth (m) recorded by the ship´s winch and bottom water temperature (°C) and bottom water O_2 concentration (µM; bdl=below detection limit (5 µM)) measured by the CTD.

Station	Core ID	Date (2013)	Latitude (S)	Longitude (W)	Depth (m)	Temp. (°C)	O_2 (µM)
1	MUC 13	January 11	12°13.492’	77°10.511’	70	14	bdl
4	MUC 11	January 09	12°18.704’	77°17.790’	144	13.4	bdl
6	MUC 6	January 07	12°23.322‘	77°24.181’	253	12	bdl
8	MUC 23	January 15	12°27.198’	77°29.497’	407	10.6	bdl
9	MUC 17	January 13	12°31.374’	77°35.183’	770	5.5	19
10	MUC 28	January 19	12°35.377’	77°40.975’	1025	4.4	53

Tab. 2: Integrated rates of nitrogen fixation (mmol m^{-2} d^{-1}) in the Peruvian OMZ sediments from this study compared to other marine benthic environments. Only the highest and lowest integrated rates are shown, as well as the integrated sediment depth (cm) and the method used (ARA=acetylene reduction assay, MIMS=membrane inlet mass spectrometry).

Benthic Environment	N-fixation (mmol N m^{-2} d^{-1})	Depth of integration (cm)	Method	Reference
PERU OMZ	0.08 – 0.4	0 – 20	ARA	This study
COASTAL REGION				
Baltic Sea, hypoxic basin	0.08 – 0.22	0 – 18	ARA	Bertics et al., 2013
Bioturbated coastal lagoon	0.8 – 8.5	0 – 10	ARA	Bertics et al., 2010
Brackish-water sediment	0.03 – 3.4	0 – 1	ARA	Andersson et al., 2014
Coral reef sediment	6.09 (± 5.62)	-	-	Capone 1983
Eelgrass meadow sediment	0.15 – 0.39	0 – 5	ARA	Cole and McGlathery, 2012
Eutrophic estuary	0 – 18	0 – 20	MIMS	Rao and Charette, 2012
Mangrove sediment	0 – 1.21	0 – 1	ARA	Lee and Joye, 2006
Salt marsh surface sediment	0.38 (± 0.41)	-	-	Capone 1983
Subtidal sediment	0.6 – 15.6	0 - 30	MIMS	Fulweiler et al., 2007
Zostera estuarine sediment	0.39	-	-	Capone 1983
OPEN OCEAN				
Atlantic ocean (2800 m)	0.00008	-	-	Howarth et al., 1988
< 200 m sediments	0.02 (± 0.01)	-	-	Capone 1983
Mauritania OMZ	0.05 – 0.24	0 – 20	ARA	Bertics and Treude, unpubl

Figures

Fig. 1

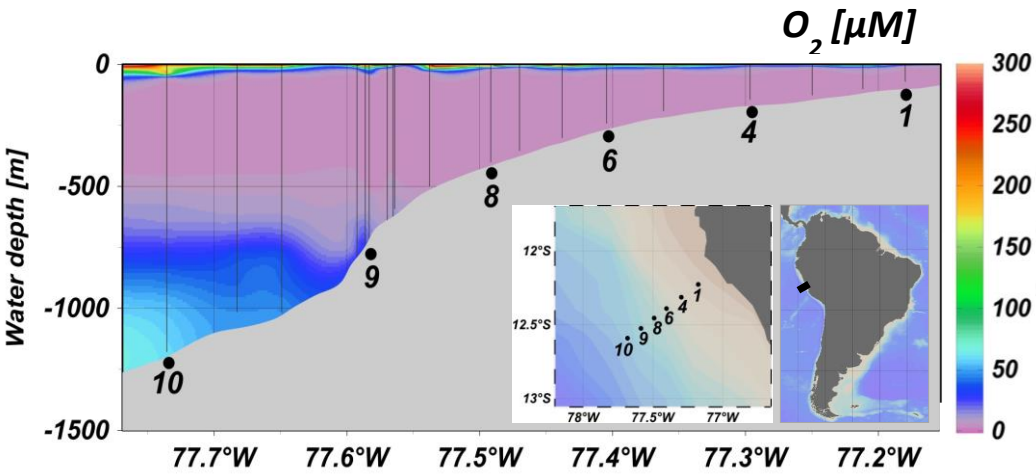


Fig. 2

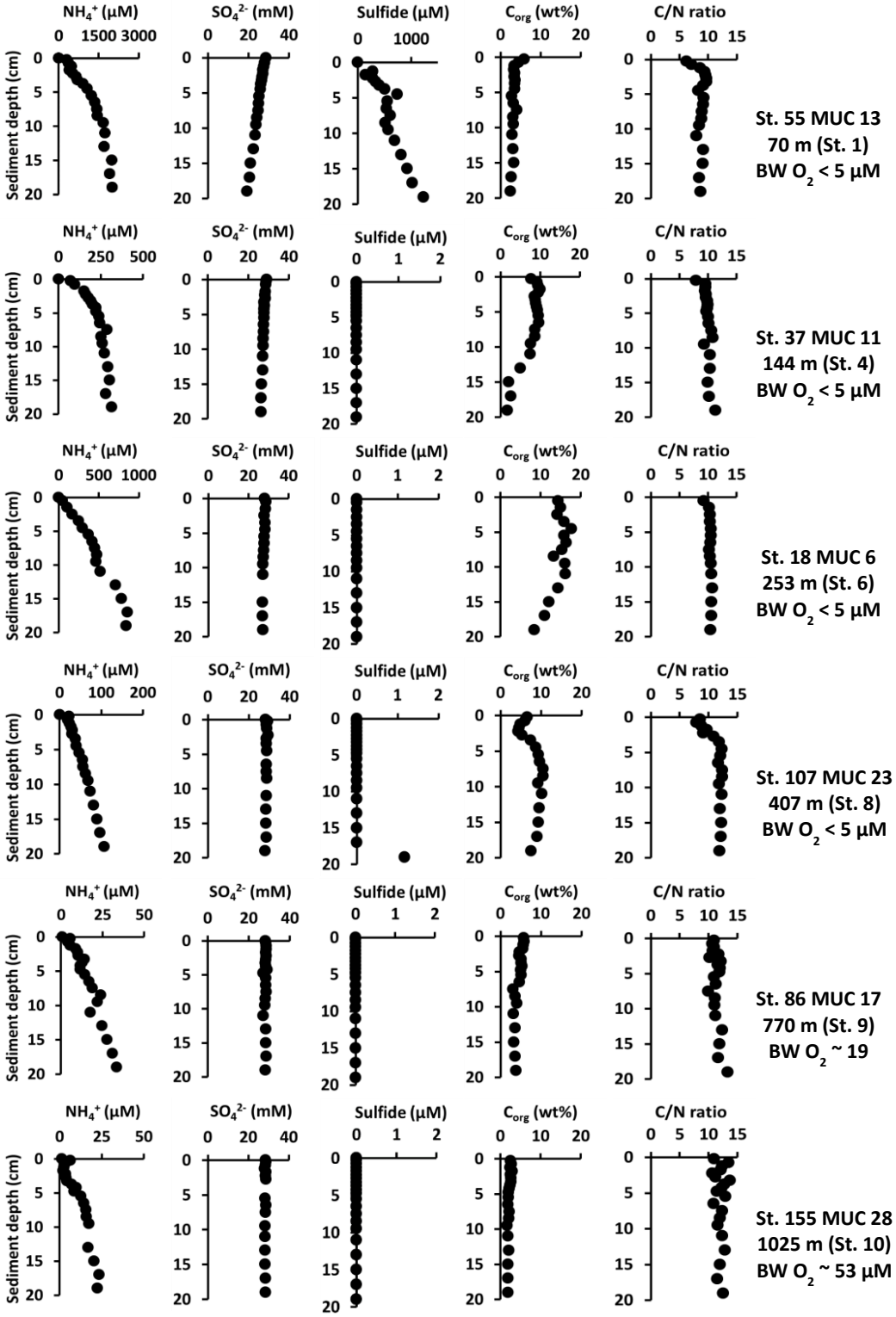

NH4+ (µM)
SO4 2- (mM)
Sulfide (µM)
Corg (wt%)
C/N ratio
Sediment depth (cm)
St. 55 MUC 13
70 m (St. 1)
BW O2 < 5 µM
St. 37 MUC 11
144 m (St. 4)
BW O2 < 5 µM
St. 18 MUC 6
253 m (St. 6)
BW O2 < 5 µM
St. 107 MUC 23
407 m (St. 8)
BW O2 < 5 µM
St. 86 MUC 17
770 m (St. 9)
BW O2 ~ 19
St. 155 MUC 28
1025 m (St. 10)
BW O2 ~ 53 µM

Fig. 3

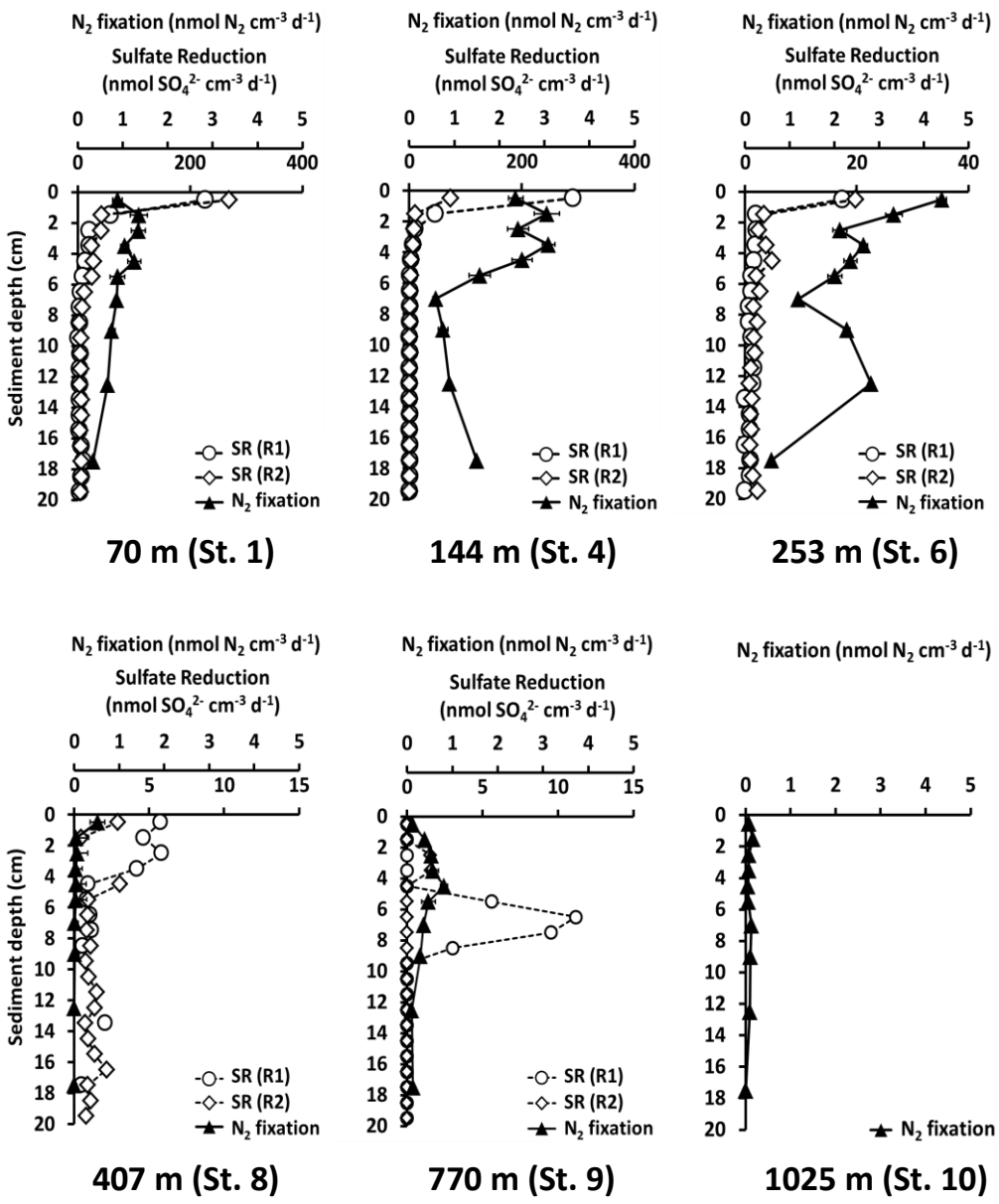

N2 fixation (nmol N2 cm-3 d-1)
Sulfate Reduction (nmol SO4 2- cm-3 d-1)
Sediment depth (cm)
SR (R1)
SR (R2)
N2 fixation
70 m (St. 1)
144 m (St. 4)
253 m (St. 6)
407 m (St. 8)
770 m (St. 9)
1025 m (St. 10)

Fig. 4

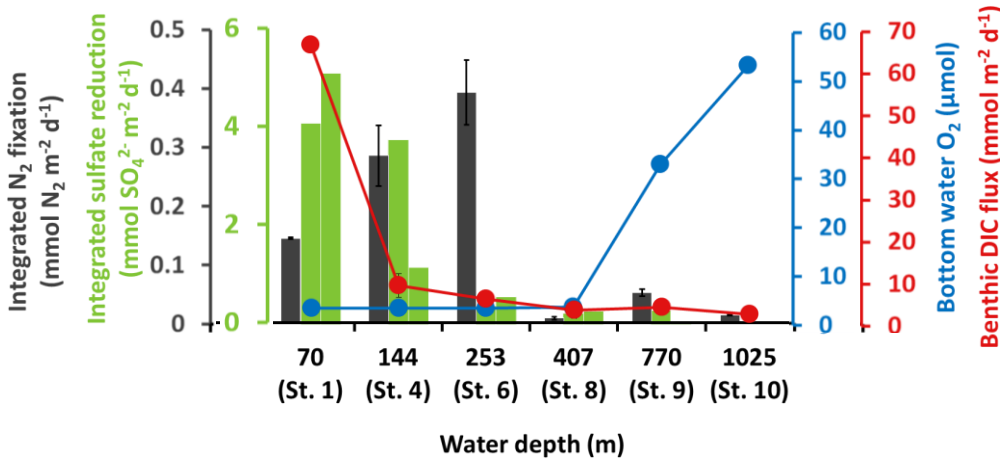
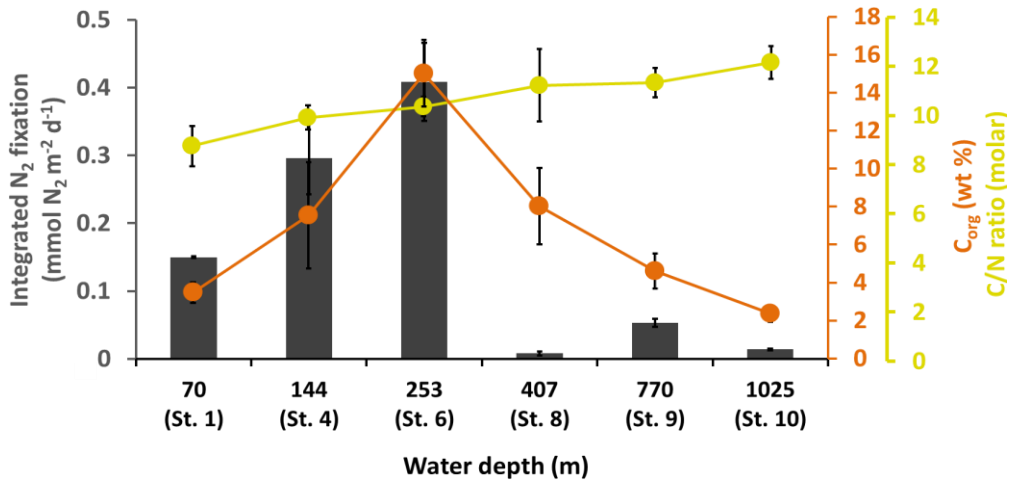

Integrated N_2 fixation (mmol N_2 m^{-2} d^{-1})
Integrated sulfate reduction (mmol SO_4^{2-} m^{-2} d^{-1})
Bottom water O_2 (µmol)
Benthic DIC flux (mmol m^{-2} d^{-1})
0
0.1
0.2
0.3
0.4
0.5
0
2
4
6
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
70 (St. 1)
144 (St. 4)
253 (St. 6)
407 (St. 8)
770 (St. 9)
1025 (St. 10)
Water depth (m)

Fig. 5


Integrated N_2 fixation (mmol N_2 m^{-2} d^{-1})
0.5
0.4
0.3
0.2
0.1
0
18
16
14
12
10
8
6
4
2
0
14
12
10
8
6
4
2
0
C_{org} (wt %)
C/N ratio (molar)
70 (St. 1)
144 (St. 4)
253 (St. 6)
407 (St. 8)
770 (St. 9)
1025 (St. 10)
Water depth (m)

1386 **Fig. 6**

1387