Biogeosciences Discuss., 12, 14401–14440, 2015
www.biogeosciences-discuss.net/12/14401/2015/
doi:10.5194/bgd-12-14401-2015

Biogeosciences Discussions
Open Access

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

J. Gier[1], S. Sommer[1], C. R. Löscher[2], A. W. Dale[1], R. A. Schmitz[2], and T. Treude[1,a]

[1]GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany
[2]Institute for Microbiology, Christian-Albrechts-University Kiel, Kiel, Germany
[a]present address: University of California, Department of Earth, Planetary & Space Sciences and Department of Atmospheric & Oceanic Sciences, Los Angeles, USA

Received: 22 July 2015 – Accepted: 27 July 2015 – Published: 2 September 2015

Correspondence to: J. Gier (jgier@geomar.de) and T. Treude (ttreude@g.ucla.edu)

Published by Copernicus Publications on behalf of the European Geosciences Union.

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 taken at six stations (70–1025 m) along a depth transect at 12° S, covering anoxic and hypoxic bottom water conditions. Benthic N_2 fixation was detected at all sites, with high rates measured in OMZ mid-waters between the 70 and 253 m and lowest N_2 fixation rates below 253 m down to 1025 m water depth. SR rates were decreasing with increasing water depth, with highest rates at the shallow site. Benthic N_2 fixation depth profiles largely overlapped with SR depth profiles, suggesting that both processes are coupled. The potential of N_2 fixation by SR bacteria was verified by the molecular analysis of nifH genes. Detected nifH sequences clustered with SR bacteria that have been demonstrated to fix N_2 in other benthic environments. Depth-integrated rates of 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 controlled by additional various environmental factors. The organic matter availability and the presence of sulfide appear to be major drivers for benthic diazotrophy. It was further found that N_2 fixation was not inhibited by high ammonium concentrations. N_2 fixation rates in OMZ sediments were similar to rates measured in other organic-rich sediments. Overall, this work improves our knowledge on N sources in marine sediments and contributes to a better understanding of N cycling in OMZ sediments.

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 the marine productivity (Ward and Bronk, 2001; Gruber, 2008) and this limitation makes N_2 fixation the dominant source of bioavailable N

BGD
12, 14401–14440, 2015

(i.e. ammonium (NH_4^+)) in the marine environment (Falkowski et al., 1998; Strous et al., 1999; Brandes and Devol, 2002).

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

Latest investigations argue that N_2 fixation in the water column cannot be totally attributed to phototrophic cyanobacteria, but that also heterotrophic prokaryotes contribute a substantial part (Riemann et al., 2010; Farnelid et al., 2011; Dekaezemacker et al., 2013; Fernandez et al., 2015) similar to marine benthic habitats. This relation was shown for the Peruvian oxygen minimum zone (OMZ), where proteobacterial clades were dominating and 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 received more attention (Fulweiler et al., 2007; Bertics et al., 2010, 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). The nitrogenase activity was positively influenced by a variety of environ-

BGD
12, 14401–14440, 2015

mental 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., bioturbated coastal sediments showed enhanced N_2 fixation activity mediated by SR bacteria, adding new dissolved inorganic N to the system (Bertics et al., 2010; Bertics and Ziebis, 2010). Further coupling of N_2 fixation to SR was found 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 SR bacteria carry the *nifH* gene for encoding the nitrogenase enzyme (Sisler and ZoBell, 1951; Riederer-Henderson and Wilson, 1970; Zehr and Turner, 2001) and were shown to actively fix N_2 in culture experiments (Riederer-Henderson and Wilson, 1970). Therefore, we need to better understand SR bacteria and their potential to fix N in the environment.

So far, the distribution of benthic N_2 fixation and its relevance for N cycling in the Peruvian OMZ, (defined by dissolved oxygen $< 20\,\mu mol\,kg^{-1}$, 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 the benthic N cycle (Bohlen et al., 2011). This process is mediated by the filamentous sulfide-oxidizing *Thioploca* bacteria (Schulz, 1999; Schulz and 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.

The high input of labile organic carbon to the Peruvian OMZ sediments (Dale et al., 2015) should support benthic N_2 fixation. SR bacteria could considerably contribute to N_2 fixation in these organic-rich OMZ sediments, given that several SR bacteria (e.g. *Desulfovibrio* spp., Riederer-Henderson and Wilson, 1970; Muyzer and 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.,

BGD
12, 14401–14440, 2015

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 identification and quantification 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 should shed light into the diazotrophic community inhabiting these sediments. The overall knowledge gained 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 in the eastern tropical south Pacific ocean at the Central Peruvian coast (Kamykowski and Zentara, 1990). The Peruvian OMZ ranges between 50 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, Table 1) off Peru are affected by coastal trapped waves (Gutiérrez et al., 2008), trade winds (Deutsch et al., 2014) or 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). Bottom water O_2 concentrations varied greatly with water depth and were below the detection limit (5 µM) at stations from 70 to 407 m water depth. Bottom water O_2 increased from 19 µM at 770 m water depth to 53 µM at 1025 m water depth, indicating the lower boundary of the OMZ (Dale et al., 2015). Between 70 and 300 m water depth, the sediment surface was colonized by dense filamentous mats of sulfur-oxidizing bacteria, presumably of the genera *Thioploca* spp. (Gutiérrez et al., 2008;

BGD
12, 14401–14440, 2015

Mosch et al., 2012). This bacteria are able to glide up to 1 cm h^{-1} through the sediment in order to feed on hydrogen sulfide (Fossing et al., 1995; Jørgensen and Gallardo, 1999; Schulz, 1999). Sediments at the lower boundary (770 and 1025 m) of the OMZ were shown to have a variety of macrofaunal organisms e.g. ophiuroids, gastropods, and crustaceans (Mosch et al., 2012).

The 12° S region is in the center of extensive upwelling and features high primary productivity (Pennington et al., 2006). Sediments at 12° S have higher rates of particulate organic carbon (2–5 times) compared to other continental margins and a high carbon burial efficiency at deep stations, indicating high 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 sediment accumulation rates of 0.45 cm yr^{-1}, while rates between 0.07 and 0.011 cm yr^{-1} were found in OMZ mid-waters and below the OMZ. Sediment 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.

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 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.

BGD
12, 14401–14440, 2015

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 particulate organic carbon and particulate organic nitrogen contents were analyzed using a Carlo–Erba element analyzer (NA 1500).

2.4 Benthic nitrogenase activity

At each of the six stations, one MUC core was sliced in a cold 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) 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 and 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.

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 Table 1). Two sets of triplicate controls

BGD
12, 14401–14440, 2015

($10\,cm^3$) were processed for every station. Sediment was collected from each core liner from 0–5, 5–10, 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 %).

The increase of C_2H_4 in each sediment slice was measured 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 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 and 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 integrated N_2 fixation were calculated from three integrated rates per station.

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

BGD
12, 14401–14440, 2015

incubated for ~ 12 h 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 for the NA. 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 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 s. 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 BSA (Fermentas). The TopoTA Cloning® Kit (Invitrogen,

BGD
12, 14401–14440, 2015

Carlsbad, USA) was used for cloning of PCR amplicons, according to the manufacturer's protocol. Sanger sequencing (120 *nifH* sequences) was performed by the Institute of Clinical Molecular Biology, Kiel, Germany. Sequences were ClustalW aligned in MEGA 6.0 (Tamura et al., 2007), and a maximum likelihood tree was constructed on a 321 bp fragment and visualized in iTOL (Letunic and Bork, 2007, 2011). Reference sequences were obtained using BlastX on the NCBI database (Sequence submission being in progress).

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 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 *Thioploca* 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 the 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), sediment appeared dark olive green between 0–17 cm and olive green with white patches between 17–20 cm. At this station, *Thioploca* 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 between 0–12 cm and appeared brown to olive green with-

BGD
12, 14401–14440, 2015

out white dots 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 (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 C/N ratio between 0–20 cm of 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 at the sediment surface to 2022 µM at 20 cm. The St. 4 and 6 showed intermediate NH_4^+ concentrations between 300 and 800 µM at 20 cm, respectively. At St. 8 (407 m) the NH_4^+ concentration increased from 0.7 µM in the surface to 107 µM at 20 cm. The two deep stations (St. 9 and 10) had the lowest NH_4^+ concentrations with 33 and 22 µM at 20 m sediment depth, respectively.

The SO_4^{2-} concentrations remained relatively constant in the surface sediments of 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. Sulfide increased from 280 µM in 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. 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 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.

BGD
12, 14401–14440, 2015

3.2 Benthic nitrogen fixation and sulfate reduction (SR)

For an easy comparison of SR rates with N_2 fixation only the sediment depths between 0–20 cm are considered. Sediment depth profiles of N_2 fixation activity are expressed in nitrogenase activity (NA), i.e. without 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).

Highest 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 NA and SR rates were high at the shallower stations 1, 4, and 6 (70, 144, 253 m), NA and SR rates were lowest at the three deeper stations 8–10 (407, 770, 1025 m).

At St. 1, NA and SR rates showed different trends in the top layer of the cores, but depth profiles aligned below. While St. 1 had the highest SR rates of all sites, reaching 248 nmol SO_4^{2-} cm^{-3} d^{-1} at 0–1 cm, NA was not highest at this station. Only St. 1 had considerably 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 NA and SR revealed peaks close to the surface. 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 decreasing towards the bottom of the core. St. 6 (253 m) had the highest NA of all stations. After decreasing from 6.6 ± 0.7 nmol C_2H_4 cm^{-3} d^{-1} in the surface 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 cm. Although 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 NA rates were measured at St. 8 (407 m) (0.77 ± 0.37 nmol C_2H_4 cm^{-3} d^{-1} in the surface), as well as very low SR rates (0–

BGD
12, 14401–14440, 2015

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 NA and SR rates showed a peak at 5 cm and at 7 cm, respectively. At St. 9 NA was low in the surface and at 20 cm sediment depth, with a peak in activity at 4–5 cm (1.2 ± 0.12 nmol C_2H_4 cm^{-3} d^{-1}). At St. 10 (1025 m), NA rates were low throughout the sediment core, ranging between 0.23 ± 0.03 nmol C_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, 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 below detection, which could point either to the absence of SR or to the complete loss of total reduced inorganic ^{35}S 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 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, SR rates ranged from ~ 4.6 mmol SO_4^{2-} m^{-2} d^{-1} at St. 1 to 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 inversely correlated between St. 1 and St. 6. 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 was not conform with the relatively lower 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 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 0.05 ± 0.005 N m^{-2} d^{-1} and 0.01 ± 0.001 N m^{-2} d^{-1}, respectively and also integrated SR rates were lowest at St. 9

BGD
12, 14401–14440, 2015

(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.

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 and 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 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 SR bacteria, such as *Desulfovibrio vulgaris* (Riederer-Henderson and Wilson, 1970; Muyzer and Stams, 2008) and *Desulfonema limicola* (Fukui et al., 1999). One cluster (OMZ 144 m) belonged to *Vibrio diazotrophicus* (Guerinot et al., 1982), which has the unique property for a *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 (TAS801) from the Pacific Ocean (AY896428.1).

BGD
12, 14401–14440, 2015

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 nitrogenase activity (NA) 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 depth profiles revealed similar trends. However, while peaks in SR where very pronounced, maximum 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 NA and SR were determined in replicate MUC cores, which had a sampling distance of up to 50 cm, depending on the location of the cores in the instrument. The observed NA is therefore not directly fuelled 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. The more surprising finding is that integrated rates of NA and SR showed opposite trends at the three shallowest stations, pointing to potential environmental control mechanisms (see Sect. 4.2).

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

BGD
12, 14401–14440, 2015

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 SR bacteria *Desulfovibrio vulgaris*, which is a confirmed diazotroph (Sisler and ZoBell, 1951; Riederer-Henderson and 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 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).

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 controlling 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 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 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 sed-

BGD
12, 14401–14440, 2015

iments in the Atlantic Ocean (Hartwig and 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 and 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 at the same stations by Dale et al. (2015) during the expedition, did not correlate with integrated N_2 fixation rates, but instead roughly followed the pattern of SR rates along water depth (Fig. 5). The highest integrated SR rate and DIC flux was found at St. 1 (70 m), whereas the lowest integrated SR rate and DIC flux was found 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 (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 long, unfrozen storage of the samples (see methods).

BGD
12, 14401–14440, 2015

4.2.2 Ammonium

Interestingly, the highest N_2 fixation was measured in sediments colonized by the sulfur-oxidizing and nitrate-reducing filamentous bacteria *Thioploca* spp. (Schulz, 1999; Schulz and Jørgensen, 2001). *Thioploca* 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* 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 increasing to 2022 µM at 20 cm (Fig. 2), while no inhibition of N_2 fixation was found. Though, 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. N_2 fixation was measured in the Carmens River estuary (New York) and was still abundant at 2800 µM NH_4^+ (Capone, 1988). In general, these studies suggested that the impact of NH_4^+ on N_2 fixation is more complex than previously thought and 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 and 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 and

BGD
12, 14401–14440, 2015

Whiting, 1986; McGlathery et al., 1998). In the study of Yoch and Whiting (1986) it was shown that enrichment cultures of *Spartina alterniflora* salt marsh sediment reacted with different N_2 fixation inhibition stages on 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 were found in the form of localized organic matter hot spots (Brandes and Devol, 2002; Bertics and Ziebis, 2010), and could also occur for NH_4^+.

4.2.3 Sulfide

Sulfide is a known inhibitor for many biological processes (Reis et al., 1992; Joye and 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 and Oleszkiewicz, 1991). Interactions of sulfide with benthic N_2 fixation have so far not been investigated, and 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 the O_2 sensitivity of the key enzyme nitrogenase (Postgate, 1998; Dixon and 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

BGD
12, 14401–14440, 2015

bottom water O_2 concentrations in the Peruvian OMZ were below the detection limit at the St. 1 to 8 (70 to 407 m), thereby mainly excluding benthic macrofauna, O_2 concentrations increased to levels above 40 µM at St. 10 (1025 m), supporting a diverse bioturbating and bioirrigating benthic macrofauna community (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 (Table 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).

BGD
12, 14401–14440, 2015

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

Acknowledgements. 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

BGD
12, 14401–14440, 2015

discussions. We further thank the authorities of Peru for the permission to work in their territorial waters. 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.

The article processing charges for this open-access publication were covered by a Research Centre of the Helmholtz Association.

References

Andersson, B., Sundbäck, K., Hellman, M., Hallin, S., and Alsterberg, C.: Nitrogen fixation in shallow-water sediments: spatial distribution and controlling factors, Limnol. Oceanogr., 59, 1932–1944, 2014.

Bertics, V. J. and Ziebis, W.: Bioturbation and the role of microniches for sulfate reduction in coastal marine sediments, Environ. Microbiol., 12, 3022–3034, 2010.

Bertics, V. J., Sohm, J., Treude, T., Chow, C., Capone, D., Fuhrman, J., and Ziebis, W.: Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulfate reduction, Mar. Ecol.-Prog. Ser., 409, 1–15, 2010.

Bertics, V. J., Löscher, C. R., Salonen, I., Dale, A. W., Gier, J., Schmitz, R. A., and Treude, T.: Occurrence of benthic microbial nitrogen fixation coupled to sulfate reduction in the seasonally hypoxic Eckernförde Bay, Baltic Sea, Biogeosciences, 10, 1243–1258, doi:10.5194/bg-10-1243-2013, 2013.

Bohlen, L., Dale, A. W., Sommer, S., Mosch, T., Hensen, C., Noffke, A., Scholz, F., and Wallmann, K.: Benthic nitrogen cycling traversing the Peruvian oxygen minimum zone, Geochim. Cosmochim. Ac., 75, 6094–6111, 2011.

Brandes, A., Devol, A. H., and Deutsch, C.: New developments in the marine nitrogen cycle, Chem. Rev., 107, 577–89, 2007.

Brandes, J. A. and Devol, A. H.: A global marine-fixed nitrogen isotopic budget: implications for Holocene nitrogen cycling, Global Biogeochem. Cy., 16, 1–14, 2002.

Capone, D. G.: Benthic nitrogen fixation, in: Nitrogen Cycling in Coastal Marine Environments, edited by: Blackburn, T. H. and Sorensen, J., John Wiley & Sons Ltd, 85–123, 1988.

BGD
12, 14401–14440, 2015

Capone, D. G.: Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure, in: Handbook of Methods in Aquatic Microbial Ecology, edited by: Kemp, P. F., Sherr, B. F., Sherr, E. B., and Coles, J. J., CRC Press LLC, Boca Raton, 621–631, 1993.

Capone, D. G. and Knapp, A. N.: A marine nitrogen cycle fix?, Nature, 16, 159–160, 2007.

Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A. F., and Carpenter, E. J.: Nitrogen fixation by *Trichodesmium* spp.: an important source of new nitrogen to the tropical and subtropical North Atlantic Ocean, Global Biogeochem. Cy., 19, 1–17, 2005.

Capone, D. G., Bronk, A. A., Mulholland, M. R., and Carpenter, E. J.: Nitrogen in the Marine Environment, 2nd edn., Elsevier, 2008.

Chen, J.-S.: Nitrogen fixation in the clostridia, in: Genetics and Regulation of Nitrogen Fixation in Free-Living Bacteria, Nitrogen Fixation: Origins, Applications, and Research Progress, edited by: Klipp, W., Masepohl, B., Gallon, J. R., and Newton, W. E., Kluwer Academic Publishers, Dordrecht, 53–64, 2005.

Codispoti, L. A.: An oceanic fixed nitrogen sink exceeding 400 Tg N a^{-1} vs. the concept of homeostasis in the fixed-nitrogen inventory, Biogeosciences, 4, 233–253, doi:10.5194/bg-4-233-2007, 2007.

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

Dale, A. W., Sommer, S., Lomnitz, U., Montes, I., Treude, T., Liebetrau, V., Gier, J., Hensen, C., Dengler, M., Stolpovsky, K., Bryant, L. D., and Wallmann, K.: Organic carbon production, mineralisation and preservation on the Peruvian margin, Biogeosciences, 12, 1537–1559, doi:10.5194/bg-12-1537-2015, 2015.

Dekaezemacker, J. and Bonnet, S.: Sensitivity of N_2 fixation to combined nitrogen forms (NO_3^- and NH_4^+) in two strains of the marine diazotroph *Crocosphaera watsonii* (Cyanobacteria), Mar. Ecol.-Prog. Ser., 438, 33–46, 2011.

Dekaezemacker, J., Bonnet, S., Grosso, O., Moutin, T., Bressac, M., and Capone, D. G.: 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 Biogeochem. Cy., 27, 768–779, 2013.

BGD
12, 14401–14440, 2015

Deutsch, C., Berelson, W., Thunell, R., Weber, T., Tems, C., McManus, J., Crusius, J., Ito, T., Baumgartner, T., Ferreira, V., Mey, J., and van Geen, A.: Centennial changes in North Pacific anoxia linked to tropical trade winds, Science, 345, 665–668, 2014.
Dixon, R. and Kahn, D.: Genetic regulation of biological nitrogen fixation, Nat. Rev. Microbiol., 2, 621–631, 2004.
Donohue, M. J. O., Moriarty, D. J. W., and MacRae, I. C.: Nitrogen fixation in sediments and the rhizosphere of the seagrass *Zostera capricorni*, Microbiol. Ecol., 22, 53–64, 1991.
Duteil, O., Böning, C. W., and Oschlies, A.: Variability in subtropical-tropical cells drives oxygen levels in the tropical Pacific Ocean, Geophys. Res. Lett., 41, 1–9, 2014.
Falkowski, P. G., Barber, R. T., and Smetacek, V.: Biogeochemical controls and feedbacks on ocean primary production, Science, 281, 200–207, 1998.
Farnelid, H., Andersson, A. F., Bertilsson, S., Al-Soud, W. A., Hansen, L. H., Sørensen, S., Steward, G. F., Hagström, Å., and Riemann, L.: Nitrogenase gene amplicons from global marine surface waters are dominated by genes of non-cyanobacteria, PloS One, 6, e19223, 1–9, 2011.
Fernandez, C., Farías, L., and Ulloa, O.: Nitrogen fixation in denitrified marine waters, PloS One, 6, e20539, 1–9, 2011.
Fernandez, C., González, M. L., Muñoz, C., Molina, V., and Farias, L.: Temporal and spatial variability of biological nitrogen fixation off the upwelling system of central Chile (35–38.5° S), J. Geophys. Res.-Oceans, 120, 3330–3349, 2015.
Fossing, H., Gallardo, V. A., Jørgensen, B. B., Hüttel, M., Nielsen, L. P., Schulz, H., Canfield, D. E., Forster, S., Glud, R. N., Gundersen, J. K., Küver, J., Ramsing, N. B., Teske, A., Thamdrup, B., and Ulloa, O.: Concentration and transport of nitrate by the mat-forming sulphur bacterium Thioploca, Nature, 374, 713–715, 1995.
Fuenzalida, R., Schneider, W., Garces-Vargas, J., Bravo, L., and Lange, C.: Vertical and horizontal extension of the oxygen minimum zone in the eastern South Pacific Ocean, Deep-Sea Res. Pt. II, 56, 992–1008, 2009.
Fukui, M., Teske, A., Assmus, B., Muyzer, G., and Widdel, F.: Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus desulfonema), Arch. Microbiol., 172, 193–203, 1999.
Fulweiler, R. W., Nixon, S. W., Buckley, B. A., and Granger, S. L.: Reversal of the net dinitrogen gas flux in coastal marine sediments, Nature, 448, 180–182, 2007.

BGD
12, 14401–14440, 2015

Fulweiler, R., Brown, S., Nixon, S., and Jenkins, B.: Evidence and a conceptual model for the cooccurrence of nitrogen fixation and denitrification in heterotrophic marine sediments, Mar. Ecol.-Prog. Ser., 482, 57–68, 2013.

Fulweiler, R. W., Heiss, E. M., Rogener, M. K., Newell, S. E., LeCleir, G. R., Kortebein, S. M., and Wilhelm, S. W.: Examining the impact of acetylene on N-fixation and the active sediment microbial community, Front. Microbiol., 6, 1–9, 2015.

Glock, N., Schönfeld, J., Eisenhauer, A., Hensen, C., Mallon, J., and Sommer, S.: The role of benthic foraminifera in the benthic nitrogen cycle of the Peruvian oxygen minimum zone, Biogeosciences, 10, 4767–4783, doi:10.5194/bg-10-4767-2013, 2013.

Grasshoff, K., Kremlingl, K., and Ehrhardt, M.: Methods of Seawater Analysis, 3rd edn., Wiley-VCH, Weinheim, 1999.

Gruber, N.: The dynamics of the marine nitrogen cycle and its influence on atmospheric CO_2 variations, in: Carbon Climate Interactions, edited by: Oguz, T. and Follows, M., 97–148, 2004.

Gruber, N.: The marine nitrogen cycle? Overview and challenges, in: Nitrogen in the Marine Environment, edited by: Capone, D. G., Bronk, D. A., Mulholland, M. R., and Carpenter, E. J., Elsevier, Amsterdam, 1–50, 2008.

Guerinot, M. L., West, P. A., Lee, J. V., and Colwell, R. R.: Vibrio diazotrophicus sp. nov., a Marine Nitrogen-Fixing Bacterium, Int. J. Syst. Bacteriol., 32, 350–357, 1982.

Gutiérrez, D., Enríquez, E., Purca, S., Quipúzcoa, L., Marquina, R., Flores, G., and Graco, M.: Oxygenation episodes on the continental shelf of central Peru: remote forcing and benthic ecosystem response, Prog. Oceanogr., 79, 177–189, 2008.

Hartwig, E. O. and Stanley, S. O.: Nitrogen fixation in Atlantic deep-sea and coastal sediments, Deep-Sea Res., 25, 411–417, 1978.

Howarth, R. W., Marino, R., Lane, J., and Cole, J. J.: Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance, Limnol. Oceanogr., 33, 669–687, 1988.

Jørgensen, B. B.: The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark), Limnol. Oceanogr., 22, 814–832, 1977.

Jørgensen, B. B.: A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments, Geomicrobiol. J., 1, 11–27, 1978.

Jørgensen, B. B.: SCOPE 21 – the major biogeochemical cycles and their interactions, in: Processes at the Sediment–Water Interface, 1983.

BGD
12, 14401–14440, 2015

Jørgensen, B. B. and Gallardo, V. A.: *Thioploca* spp.: filamentous sulfur bacteria with nitrate vacuoles, FEMS Microbiol. Ecol., 28, 301–313, 1999.
Joye, S. B. and Hollibaugh, J. T.: Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments, Sciences, 270, 623–625, 1995.
Kallmeyer, J., Ferdelman, T. G., Weber, A., Fossing, H., and Jørgensen, B. B.: Evaluation of a cold chromium distillation procedure for recovering very small amounts of radiolabeled sulfide related to sulfate reduction measurements, Limnol. Oceanogr.-Meth., 2, 171–180, 2004.
Kamykowski, D. and Zentara, S.-J.: Hypoxia in the world ocean as recorded in the historical data set, Deep-Sea Res., 37, 1861–1874, 1990.
Kartal, B., Kuypers, M. M. M., Lavik, G., Schalk, J., Op den Camp, H. J. M., Jetten, M. S. M., and Strous, M.: Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium, Environ. Microbiol., 9, 635–642, 2007.
Kessler, W. S.: The circulation of the eastern tropical Pacific: a review, Prog. Oceanogr., 69, 181–217, 2006.
Knapp, A. N.: The sensitivity of marine N_2 fixation to dissolved inorganic nitrogen, Front. Microbiol., 3, 1–14, 2012.
Letunic, I. and Bork, P.: Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation, Bioinformatics, 23, 127–128, 2007.
Letunic, I. and Bork, P.: Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy, Nucleic Acids Res., 39, 1–4, 2011.
Levin, L., Gutierrez, D., Rathburn, A., Neira, C., Sellanes, J., Munoz, P., Gallardo, V., and Salamanca, M.: Benthic processes on the Peru margin: a transect across the oxygen minimum zone during the 1997–98 El Nino, Prog. Oceanogr., 53, 1–27, 2002.
Löscher, C. R., Großkopf, T., Desai, F. D., Gill, D., Schunck, H., Croot, P. L., Schlosser, C., Neulinger, S. C., Pinnow, N., Lavik, G., Kuypers, M. M. M., Laroche, J., and Schmitz, R. A.: Facets of diazotrophy in the oxygen minimum zone waters off Peru, ISME J., 8, 1–13, 2014.
McCartney, D. M. and Oleszkiewicz, J. A.: Sulfide inhibition of anaerobic degradation of lactate and acetate, Water Res., 25, 203–209, available at: http://www.sciencedirect.com/science/article/pii/004313549190030T (last access: 6 February 2015), 1991.
McGlathery, K. J., Risgaard-Petersen, N., and Christensen, B. P.: Temporal and spatial variation in nitrogen fixation activity in the eelgrass Zostera marina rhizosphere, Mar. Ecol.-Prog. Ser., 168, 245–258, 1998.

BGD
12, 14401–14440, 2015

Montoya, J. P., Voss, M., Kahler, P., and Capone, D. G.: A simple, high-precision, high-sensitivity tracer assay for N_2 fixation, Appl. Environ. Microb., 62, 986–993, 1996.
Mosch, T., Sommer, S., Dengler, M., Noffke, A., Bohlen, L., Pfannkuche, O., Liebetrau, V., and Wallmann, K.: Factors influencing the distribution of epibenthic megafauna across the Peruvian oxygen minimum zone, Deep-Sea Res. Pt. I, 68, 123–135, 2012.
Muyzer, G. and Stams, A. J. M.: The ecology and biotechnology of sulphate-reducing bacteria, Nat. Rev. Microbiol., 6, 441–454, 2008.
Nevin, K. P., Holmes, D. E., Woodard, T. L., Hinlein, E. S., Ostendorf, D. W., and Lovley, D. R.: *Geobacter bemidjiensis* sp. nov., and *Geobacter psychrophilus* sp. nov., two novel Fe(III)-reducing subsurface isolates, Int. J. Syst. Evol. Micr., 55, 1667–1674, 2005.
Nielsen, L. B., Finster, K., Welsh, D. T., Donelly, A., Herbert, R. A., de Wit, R., and Lomstein, B. A.: Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments from *Zostera noltii* and *Spartina maritima* meadows, Environ. Microbiol., 3, 63–71, 2001.
Orcutt, K. M., Lipschultz, F., Gundersen, K., Arimoto, R., Michaels, A. F., Knap, A. H., and Gallon, J. R.: A seasonal study of the significance of N_2 fixation by *Trichodesmium* spp. at the Bermuda Atlantic Time-series Study (BATS) site, Deep-Sea Res. Pt. II, 48, 1583–1608, 2001.
Orsi, T. H., Werner, F., Milkert, D., Anderson, A. L., and Bryant, W. R.: Environmental overview of Eckernförde Bay, northern Germany, Geo-Mar. Lett., 16, 140–147, 1996.
Patriquin, D. and Knowles, R.: Nitrogen fixation in the rhizosphere of marine angiosperms, Mar. Biol., 16, 49–58, 1972.
Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Calienes, R., and Chavez, F. P.: Primary production in the eastern tropical Pacific: a review, Prog. Oceanogr., 69, 285–317, 2006.
Postgate, J. R.: The Sulphate-Reducing Bacteria, Cambridge University Press, 1979.
Postgate, J. R.: The Fundamentals of Nitrogen Fixation, Cambridge University Press, 1982.
Postgate, J. R.: Nitrogen Fixation, 3rd edn., Cambridge University Press, Cambridge, 1998.
Reis, M. A., Almeida, J. S., Lemos, P. C., and Carrondo, M. J.: Effect of hydrogen sulfide on growth of sulfate reducing bacteria, Biotechnol. Bioeng., 40, 593–600, 1992.
Riederer-Henderson, M.-A. and Wilson, P. W.: Nitrogen fixation by sulphate-reducing bacteria, J. Gen. Microbiol., 61, 27–31, 1970.

BGD
12, 14401–14440, 2015

Riemann, L., Farnelid, H., and Steward, G. F.: Nitrogenase genes in non-cyanobacterial plankton: prevalence, diversity and regulation in marine waters, Aquat. Microb. Ecol., 61, 235–247, 2010.
Røy, H., Weber, H. S., Tarpgaard, I. H., Ferdelman, T. G., and Jørgensen, B. B.: Determination of dissimilatory sulfate reduction rates in marine sediment via radioactive 35 S tracer, Limnol. Oceanogr.-Meth., 12, 196–211, 2014.
Schulz, H. N.: Dense populations of a giant sulfur bacterium in Namibian shelf sediments, Science, 284, 493–495, 1999.
Schulz, H. N. and Jørgensen, B. B.: Big bacteria, Ann. Rev. Microbiol., 55, 105–137, 2001.
Sisler, F. D. and ZoBell, C. E.: Nitrogen fixation by sulfate-reducing bacteria indicated by nitrogen/argon ratios, Science, 113, 511–512, 1951.
Sohm, J. A., Webb, E. A., and Capone, D. G.: Emerging patterns of marine nitrogen fixation, Nat. Rev. Microbiol., 9, 499–508, 2011.
Steppe, T. F. and Paerl, H. W.: Potential N2 fixation by sulfate-reducing bacteria in a marine intertidal microbial mat, Aquat. Microb. Ecol., 28, 1–12, 2002.
Stramma, L., Johnson, G. C., Sprintall, J., and Mohrholz, V.: Expanding oxygen-minimum zones in the tropical oceans, Science, 320, 655–658, 2008.
Strous, M., Kuenen, J. G., and Jetten, M. S.: Key physiology of anaerobic ammonium oxidation, Appl. Environ. Microb., 65, 3248–3250, 1999.
Tam, T.-Y., Mayfield, C. I., Inniss, W. E., and Knowles, R.: Effect of sulfide on nitrogen fixation in a stream sediment–water system, Appl. Environ. Microb., 43, 1076–1079, 1982.
Tamura, K., Dudley, J., Nei, M., and Kumar, S.: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0, Mol. Biol. Evol., 24, 1596–1599, 2007.
Tichi, M. A. and Tabita, F. R.: Maintenance and control of redox poise in Rhodobacter capsulatus strains deficient in the Calvin–Benson–Bassham pathway, Arch. Microbiol., 174, 322–333, 2000.
Turk, K. A., Rees, A. P., Zehr, J. P., Pereira, N., Swift, P., Shelley, R., Lohan, M., Woodward, E. M. S., and Gilbert, J.: Nitrogen fixation and nitrogenase (nifH) expression in tropical waters of the eastern North Atlantic, ISME J., 5, 1201–1212, 2011.
Ward, B. B. and Bronk, D. A.: Net nitrogen uptake and DON release in surface waters: importance of trophic interactions implied from size fractionation experiments, Mar. Ecol.-Prog. Ser., 219, 11–24, 2001.

BGD
12, 14401–14440, 2015

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

Westrich, J. T. and Berner, R. A.: The role of sedimentary organic matter in bacterial sulfate reduction?: the G model tested, Limnol. Oceanogr., 29, 236–249, 1984.

Yoch, D. C. and Whiting, G. J.: Evidence for NH_4^+ switch-off regulation of nitrogenase activity by bacteria in salt marsh sediments and roots of the grass *Spartina alterniflora*, Appl. Environ. Microb., 51, 143–149, 1986.

Zehr, J. P. and Turner, P. J.: Nitrogen fixation? Nitrogenase genes and gene expression, in: Methods in Microbiology, vol. 30, edited by: Paul, J. H., Academic Press, San Diego, CA, 271–286, 2001.

Zehr, J. P., Mellon, M., Braun, S., Litaker, W., Steppe, T., and Paerl, H. W.: Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat, Appl. Environ. Microb., 61, 2527–2532, 1995.

Zehr, J. P., Mellon, M. T., and Zani, S.: New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (nifH) genes, Appl. Environ. Microb., 64, 3444–3450, 1998.

BGD
12, 14401–14440, 2015

Table 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, 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	11 Jan	12°13.492′	77°10.511′	70	14	bdl
4	MUC 11	9 Jan	12°18.704′	77°17.790′	144	13.4	bdl
6	MUC 6	7 Jan	12°23.322′	77°24.181′	253	12	bdl
8	MUC 23	15 Jan	12°27.198′	77°29.497′	407	10.6	bdl
9	MUC 17	13 Jan	12°31.374′	77°35.183′	770	5.5	19
10	MUC 28	19 Jan	12°35.377′	77°40.975′	1025	4.4	53

BGD
12, 14401–14440, 2015

Table 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 sediments	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

BGD
12, 14401–14440, 2015

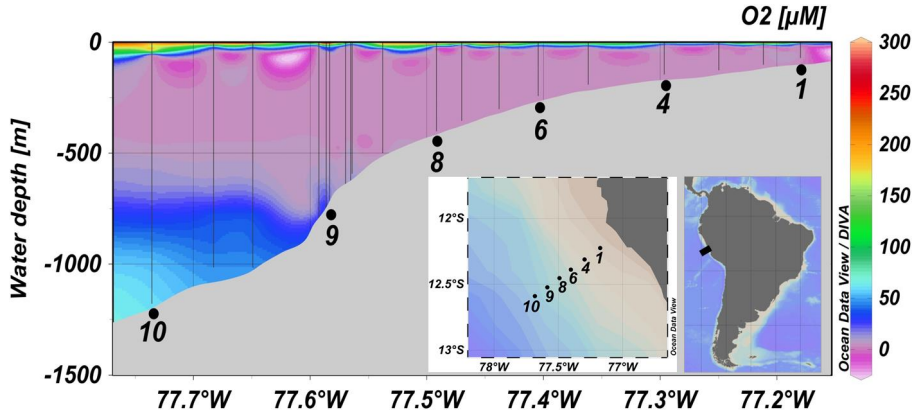
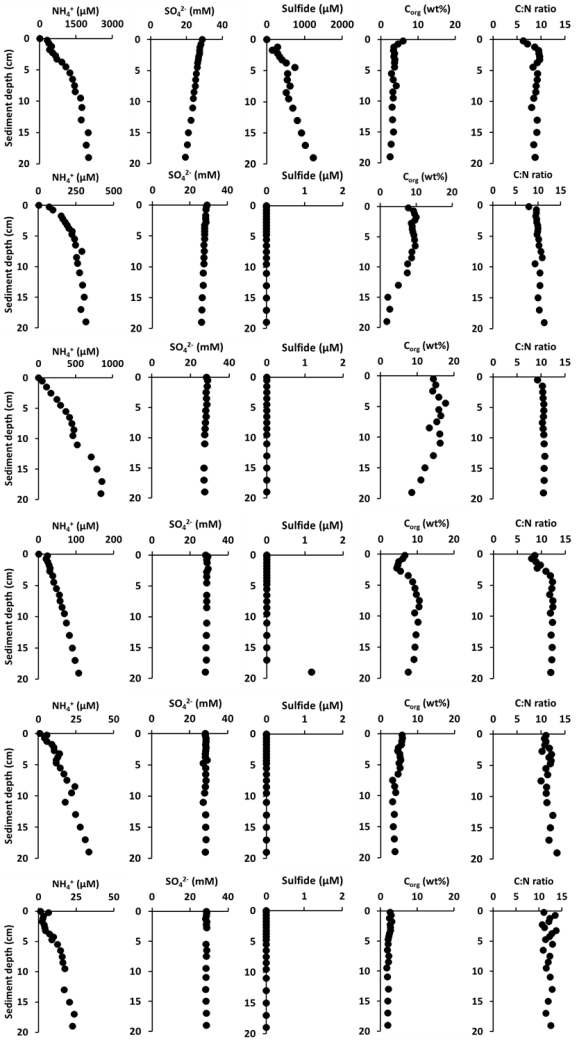


Figure 1. Cross-section of dissolved O_2 concentrations (µM) along the continental margin of the Peruvian OMZ at 12° S. The vertical lines represent CTD cast for O_2 measurement during the cruise M92. Stations 1 to 10 for MUC sampling are indicated by station numbers according to Dale et al. (2015).

BGD
12, 14401–14440, 2015



BGD
12, 14401–14440, 2015

Nitrogen fixation in sediments along a depth transect

J. Gier et al.

Figure 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.

BGD
12, 14401–14440, 2015

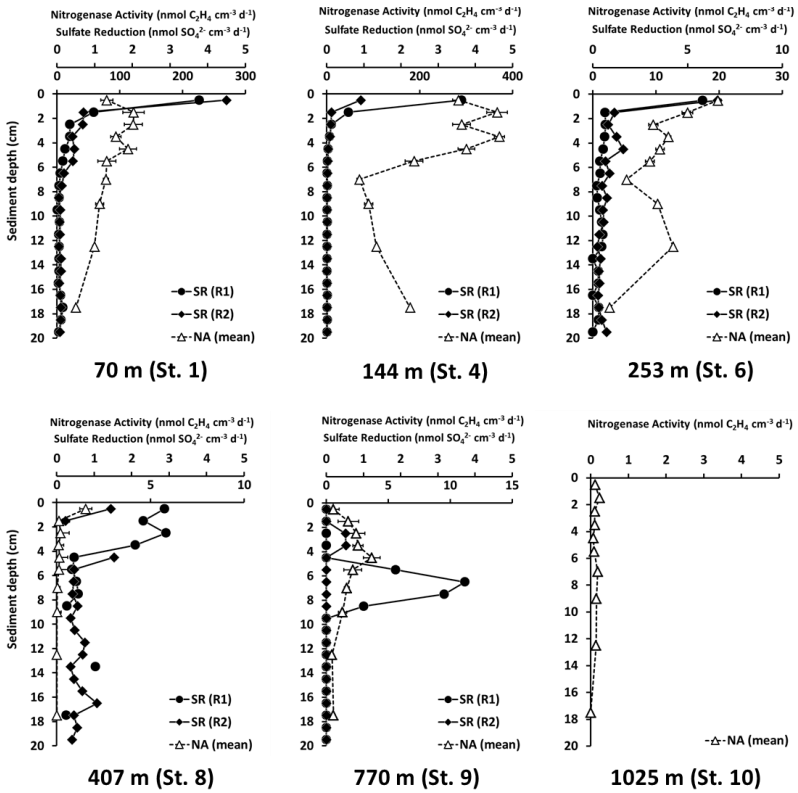


Figure 3. Sediment profiles of nitrogenase activity (NA, nmol $C_2H_4\,cm^{-3}\,d^{-1}$, average of three replicates) and sulfate reduction rates (SR, nmol $SO_4^{2-}\,cm^{-3}\,d^{-1}$, two replicates (R1 and R2)) from 0–20 cm at the six stations. The upper *x* axis represents the NA, while the lower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

BGD
12, 14401–14440, 2015

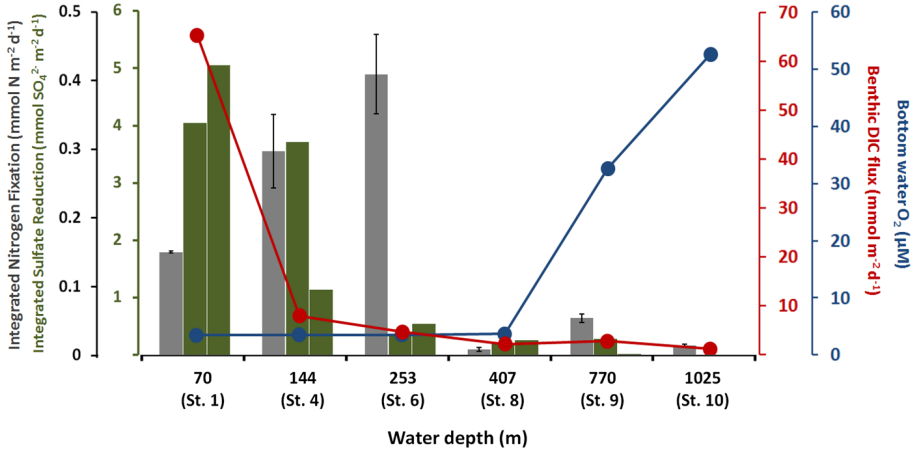


Figure 4. Integrated nitrogen fixation (mmol N m^{-2} d^{-1}, grey bars, average of three replicates) and integrated sulfate reduction (mmol SO_4^{2-} m^{-2} d^{-1}, green bars, two replicates) from 0–20 cm, including dissolved inorganic carbon (DIC, mmol m^{-2} d^{-1}, red curve) and bottom water O_2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

BGD
12, 14401–14440, 2015

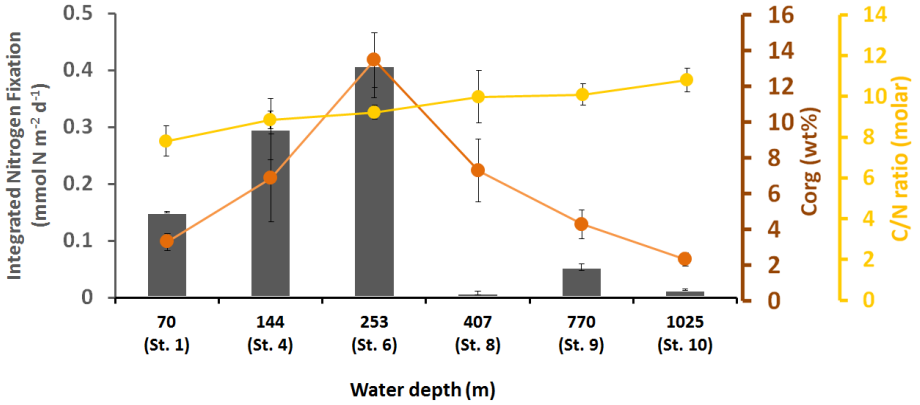


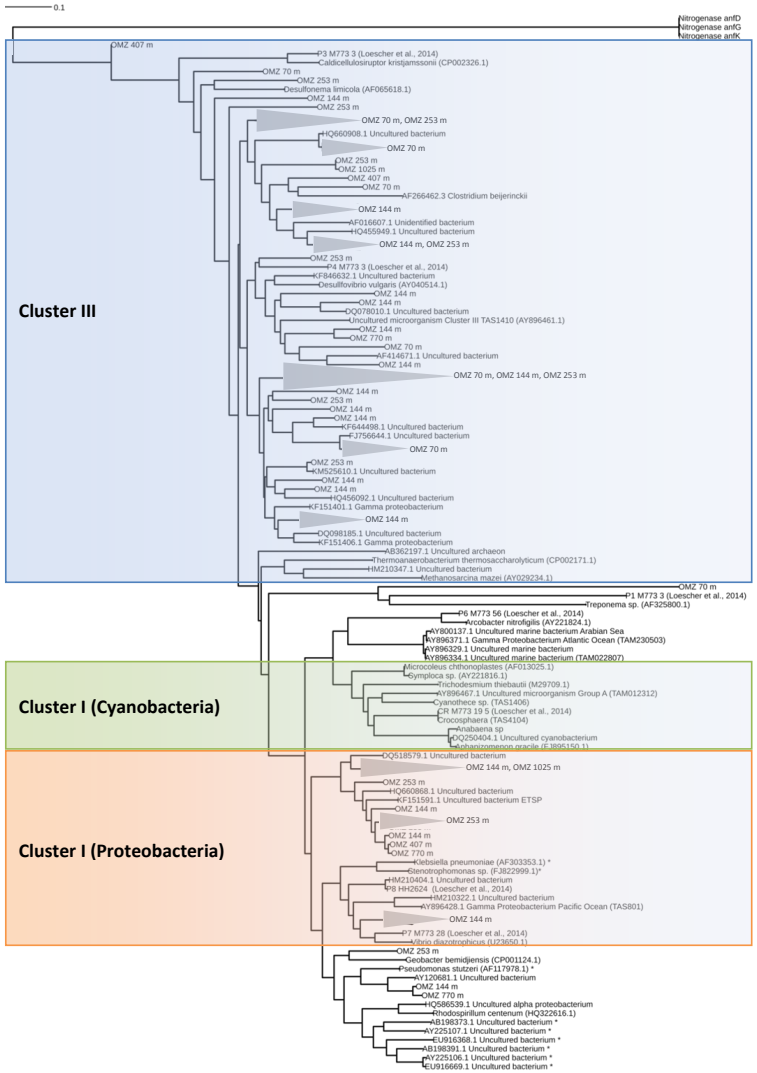
Figure 5. Integrated nitrogen fixation (mmol N m^{-2} d^{-1}, grey bars, average of three replicates), average organic carbon content (C_{org}, wt%, orange curve) and the average C/N ratio (molar, yellow curve) from 0–20 cm along the depth transect (m). Error bars indicate standard deviation.

BGD
12, 14401–14440, 2015

BGD
12, 14401–14440, 2015

Nitrogen fixation in sediments along a depth transect

J. Gier et al.



BGD
12, 14401–14440, 2015

Nitrogen fixation in sediments along a depth transect

J. Gier et al.

Figure 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.

BGD
12, 14401–14440, 2015