LETTERS

Detoxification of sulphidic African shelf waters by blooming chemolithotrophs

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Coastal waters support ~90 per cent of global fisheries and are therefore an important food reserve for our planet[1]. Eutrophication of these waters, due to human activity, leads to severe oxygen depletion and the episodic occurrence of hydrogen sulphide—toxic to multi-cellular life—with disastrous consequences for coastal ecosytems[2–5]. Here we show that an area of ~7,000 km^2 of African shelf, covered by sulphidic water, was detoxified by blooming bacteria that oxidized the biologically harmful sulphide to environmentally harmless colloidal sulphur and sulphate. Combined chemical analyses, stoichiometric modelling, isotopic incubations, comparative 16S ribosomal RNA, functional gene sequence analyses and fluorescence *in situ* hybridization indicate that the detoxification proceeded by chemolithotrophic oxidation of sulphide with nitrate and was mainly catalysed by two discrete populations of γ- and ε-proteobacteria. Chemolithotrophic bacteria, accounting for ~20 per cent of the bacterioplankton in sulphidic waters, created a buffer zone between the toxic sulphidic subsurface waters and the oxic surface waters, where fish and other nekton live. This is the first time that large-scale detoxification of sulphidic waters by chemolithotrophs has been observed in an open-ocean system. The data suggest that sulphide can be completely consumed by bacteria in the subsurface waters and, thus, can be overlooked by remote sensing or monitoring of shallow coastal waters. Consequently, sulphidic bottom waters on continental shelves may be more common than previously believed, and could therefore have an important but as yet neglected effect on benthic communities.

The subtropical eastern boundary current regions (for example Peru-Humboldt, California, Canary and Benguela) are naturally eutrophic, and although they comprise only 0.1% of the global ocean volume, 17% of the global fish catch occurs there[6]. In terms of primary productivity, the most productive of these systems is the Benguela system off Namibia[6]. However, the Benguela system is the least productive with respect to fish catch, yielding a catch of only 0.5×10^6 tons of fish per year (in comparison with $\sim 11 \times 10^6$ tons per year in the Peru-Humboldt system)[6]. This apparent anomaly has been attributed to the episodic occurrence of hydrogen sulphide gas, which is toxic to economically important fish in the southwest African shelf waters. In fact, scientific reports of massive fish mortality in the Namibian coastal waters associated with sulphidic shelf waters date back more than half a century[7,8]. The regulation of these sulphidic events is poorly understood; however, their initiation has been attributed to either the eruptive release of methane and sulphide from gas-loaded shelf sediments[9,10] or the diffusive flux of sulphide from the sulphidic sediments[11]. Even less is known about the termination of these events, and it is unclear by which route the detoxification of hydrogen sulphide proceeds.

During an RV *Alexander von Humboldt* cruise (AHAB1) in January 2004, we recorded the spatial extent and the temporal development of a sulphidic event in the southwest African shelf waters (Fig. 1; see also Supplementary Information). The shelf waters were generally depleted in oxygen below 60-m water depth north of 27° S, and towards the end of the cruise, on 24 January 2004, sulphide-containing waters were recorded to cover approximately 7,000 km^2 of the shelf (Fig. 1a). At the start of the cruise, on 8 January, the shelf waters off Walvis Bay (23° S) were sulphide free, but 120 km farther south (24° S) we found sulphidic bottom waters. A sampling site at 24° S (18 km off shore; Fig. 1), located at the centre of this sulphidic water mass, was visited three times during a period of nine days. At this site, sulphide concentrations 3 m above the sediment increased from 5 μM on 10 January to 19 μM on 15 January and to 25 μM on 18 January (Supplementary Fig. 1). A similar increase in sulphide concentrations was observed in the originally sulphide-free bottom waters at 23° S from 17 to 23 January. Assuming that the concentration profile of sulphide at 24° S represents a steady state (Fig. 2b; see also Supplementary Discussion), we calculated a flux of ~8 mmol m^{-2} d^{-1} from the sediment for 24° S by using a reaction diffusion model[12], which is comparable to the range (12–22 mmol m^{-2} d^{-1}) of sedimentary sulphide fluxes previously reported for this region[11]. On the basis of the benthic sulphide flux, it would require 8 to 15 days to accumulate the observed sulphide concentration in the shelf waters observed on 15 January (Fig. 2b), without taking sulphide oxidation in the water column into account.

At all stations with sulphidic bottom waters, sulphide disappeared well below the oxic zone (Figs 1d–f, 2a, b), and at 24° S the sulphide front descended ~15 m in the suboxic water column between 15 and 18 January despite increasing sulphide concentration in the lower bottom waters (Supplementary Fig. 3). This apparent sulphide sink in the suboxic zone strongly suggests that sulphide was oxidized anaerobically in the water column. In the absence of oxygen, nitrate is the best electron acceptor for sulphide oxidation. Nitrate and sulphide profiles overlapped in the suboxic zone between depths of ~86 and 90 m at 24° S (Fig. 2b). The sulphide flux into the suboxic zone at 24° S was ~2.3 mmol m^{-2} d^{-1}, whereas the nitrate flux was 3.3 mmol m^{-2} d^{-1}.

In addition to dissolved sulphide, ammonium diffused from the sediments into the suboxic zone (Fig. 2a). Similar concentrations of ammonium and sulphide were detected in the bottom water, but ammonium disappeared just above dissolved sulphide at 80 m, indicating that ammonium was also oxidized anaerobically. Recently, it was shown that anaerobic ammonium oxidation with nitrate and/or

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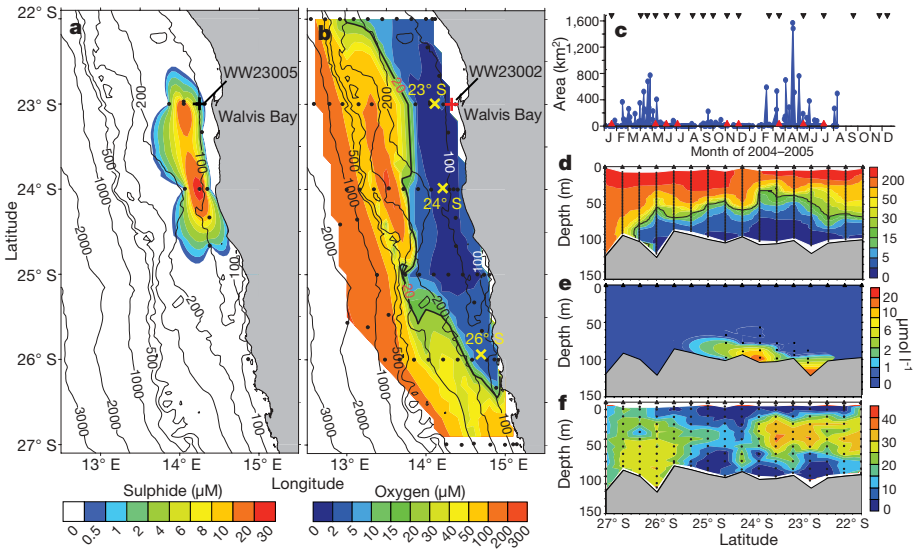

Figure 1 | Spatial and temporal distribution of sulphidic shelf waters off Namibia during 2004–2005. **a**, **b**, Bottom-water concentrations (~2 m above the sediment) of sulphide (**a**) and oxygen (**b**). **c**, Occurrence of, and surface water area with turquoise discolouration specific to the presence of, colloidal sulphur (S^0; blue trace)[28]; black triangles indicate the monitoring (and major) cruises, red triangles indicate the detection of free sulphide in shelf waters at 23° S during these cruises. **d**–**f**, North–south transects (100–130-m water depth) parallel to the Namibian coast for the concentrations of oxygen (**d**), sulphide (**e**) and nitrate (**f**). All data incorporated in the contour plots were retrieved from 124 CTD-rossette and pump-CTD casts between 4 and 20 January 2004, during the cruise AHAB1. The main sampling sites at 23° S, 24° S and 26° S are marked with crosses in **b**. Our monitoring stations at positions WW23005 and WW23002 are indicated with pluses in **a** and **b**, respectively. The black numbering on the thin contours indicates water depth in metres. The red numbering on the thick contour in **b** indicates oxygen concentration in μM.

nitrite by anammox (anaerobic ammonium oxidation) bacteria is responsible for massive nitrogen loss in the suboxic waters of the Benguela upwelling zone[13]. The calculated ammonium flux into the suboxic zone was ~2.3 mmol m^{-2} d^{-1}. Ammonium diffused 5 m farther into the suboxic zone than dissolved sulphide, and would thus have been oxidized first by nitrate/nitrite. The stoichiometry of the anammox reaction (1 mol NO_3^- or NO_2^- per mole NH_4^+) leaves 1 mmol m^{-2} d^{-1} of the nitrate flux to oxidize sulphide. Hence, 1 mol nitrate was consumed per ~2.3 mol sulphide, which is very close to the 1:2.5 stoichiometry for chemolithotrophic oxidation of sulphide with nitrate according to the following equation:

$$2NO_3^- + 5HS^- + 7H^+ \rightarrow N_2 + 5S^0 + 6H_2O$$

The detection of elemental sulphur (S^0) at a concentration of 22 μM and a water depth of 87 m (Fig. 2b) in the centre of the nitrate and sulphide mixing zone is evidence for this reaction pathway. This incomplete oxidation of sulphide to colloidal sulphur with nitrate occurs under nitrate-limiting conditions[14], which applies to the bottom waters at 24° S. Complete sulphide oxidation to sulphate may have occurred where the nitrate flux exceeded the sulphide flux, such as at 23° S on 18 January (Supplementary Fig. 2b). Additional evidence for the anaerobic oxidation of sulphide with nitrate was obtained from incubation experiments with $^{15}NO_3^-$, which indicated that denitrification was the main process of fixed inorganic nitrogen removal at 90 m at 24° S (Fig. 2c). The nitrate reduction rate of ~0.6 μM d^{-1} determined from ^{15}N experiments was also in good agreement with the rate (~0.7 μM d^{-1}) calculated for the zone of overlapping nitrate and sulphide profiles by fitting the profiles to a one-dimensional reaction-transport model[12].

Comparative 16S rRNA gene- and functional gene-sequence analyses and fluorescence *in situ* hybridization (FISH) revealed that two discrete populations of γ- and ε-proteobacteria were involved in the chemolithotrophic oxidation of sulphide with nitrate (Fig. 3; see also Supplementary Discussion). A 16S rRNA gene library retrieved from the sulphidic Namibian shelf waters was dominated by a tight cluster of γ-proteobacterial clones (121 of 271 clones, γ-proteobacterial sulphur oxidizer (GSO) cluster) displaying 95–96% sequence identity to the 16S rRNA gene sequence of '*Candidatus* Ruthia magnifica' (Fig. 3a), the sulphide-oxidizing gill endosymbiont of the mussel *Calyptogena magnifica* inhabiting sulphide-rich vent and seep environments[15,16]. 16S rRNA gene sequences of the GSO cluster were previously retrieved from sulphidic shelf waters off Namibia on 22 May 1998 (B.M.F., previously unpublished data; 80-m water depth, 24° 45.0′ S, 14° 19.5′ E, AMT6 cruise) and other sulphidic environments[17,18].

In accordance with the findings from 16S rRNA phylogeny, we also identified gene sequences encoding the alpha subunits of two enzymes in the sulphur oxidation pathway, the dissimilatory adenosine 5′-phosphosulphate reductase (AprBA) and the reverse dissimilatory sulphite reductase (rDsrAB) most closely related to '*Candidatus* Ruthia magnifica' (Supplementary Fig. 4). These endosymbionts are known to oxidize sulphide to elemental sulphur[17,19] and, subsequently, to sulphate using rDsrAB and AprBA (ref. 16). The amino-acid sequence identities were 81% (rDsrA clone Nam73_rDsr_23) and 92% (AprA clone Nam73_aprA_14), respectively (Supplementary Fig. 4). In summary, the congruent phylogenies of genes encoding 16S rRNA, rDsrA and AprA indicate the sulphur oxidation capability of the GSO bacteria (Supplementary Discussion). In the 16S rRNA gene library, we also detected a tight cluster of seven ε-proteobacterial sequences that had 94%, 92% and 83% sequence identities to *Arcobacter nitrofigilis*[20], '*Candidatus* Arcobacter sulfidicus'[21] and *Sulfurimonas denitrificans*[22], respectively (Fig. 3a). The capacity of this ε-proteobacterial group to oxidize sulphide to elemental sulphur has been demonstrated for pure cultures[23].

A sulphide oxidation rate of ~7 fmol per cell per day was determined for these pure cultures of ε-proteobacteria oxidizing sulphide with nitrate[23]. Assuming a similar cell-specific activity for the Namibian shelf waters, 2.5×10^5 cell ml^{-1} would be required in order to account for the observed sulphide oxidation rates in the suboxic zone. Quantification of the ε-proteobacterial genus *Arcobacter* and members of the GSO cluster by FISH revealed a spike in cell densities of $2.3 \times 10^5 \pm 3{,}000$ cell ml^{-1} (8.7% of 4,6-diamidino-2-phenylindole (DAPI)) and $3.1 \times 10^5 \pm 2{,}000$ cell ml^{-1} (11.1% of DAPI), respectively, at the top of the sulphidic zone, where nitrate disappears

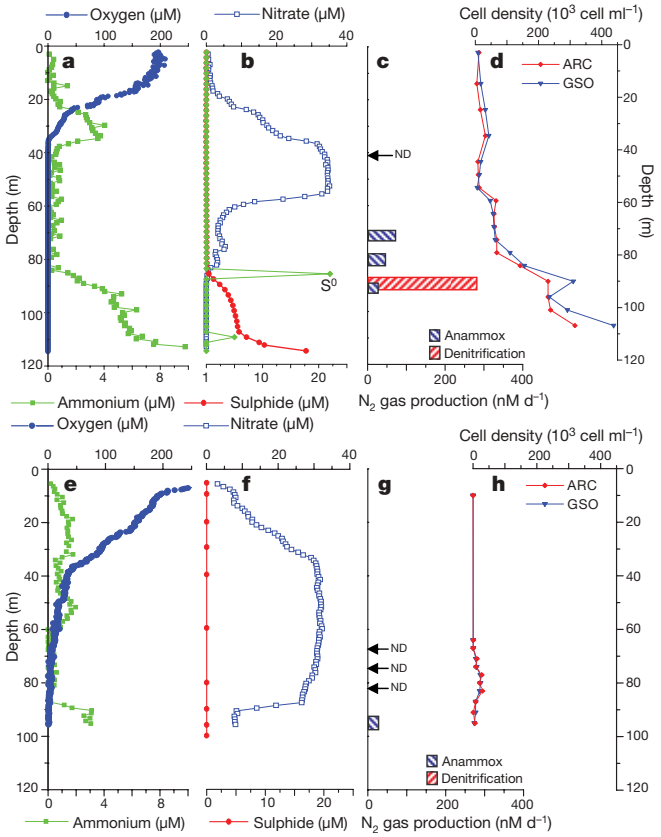

Figure 2 | Chemical zonation and distribution of indicators for bacterial sulphide oxidation. **a**–**d**, Data from 24° S, 15 January 2004; **e**–**h**, data from 23° S, 8 January 2004. **a**, **e**, Concentrations of oxygen and ammonium; **b**, **f**, concentrations of nitrate, sulphide (continuously measured, red line) and colloidal sulphur (S^0, green diamonds, same scale as sulphide). Note that colloidal sulphur that forms upon incomplete anaerobic oxidation of sulphide maximizes exactly in the zone of overlapping nitrate and sulphide profiles at 24° S, and that there is a second colloidal sulphur peak at ~110 m, where there is a change in sulphide gradient, which may reflect an earlier oxidation front. **c**, **g**, Denitrification and anammox rates determined from *in situ* $^{15}NO_3^-$ incubations. ND, not detected. **d**, **h**, Depth profile of ε-proteobacteria (*Arcobacter*) as determined by *in situ* hybridization with the specific ARC94 oligonucleotide probe and γ-proteobacteria hybridizing with the specific GSO477 oligonucleotide probe. The location of the sampling sites is shown in Fig. 1.

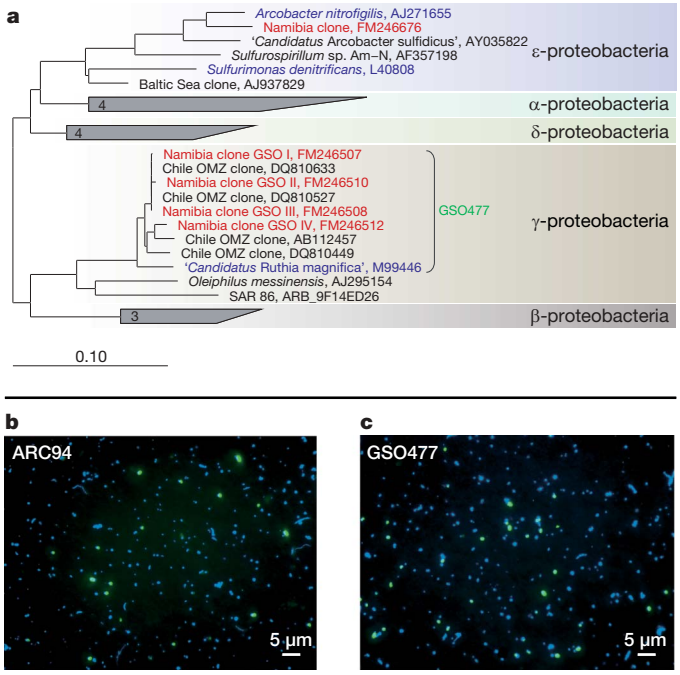

Figure 3 | Affiliation and morphology of sulphide-oxidizing bacteria from the Namibian shelf waters. **a**, Phylogenetic tree of the identified γ- and ε-proteobacterial phylotypes based on comparative 16S rRNA sequence analysis. Sequences written in red were obtained from the Namibian upwelling system and sequences written in blue are of special interest to this study. The phylogenetic tree was calculated by maximum-likelihood analysis using the ARB software package[29] Scale bar shown in units of sequence divergence. **b**, **c**, Micrographs of Namibian bacterioplankton from the sulphidic layer (24° S, 95 m) stained with DAPI (blue) and specific probes (green). Probe ARC94 (**b**) targets the 16S rRNA of the ε-proteobacterial genus *Arcobacter*. Probe GSO477 (**c**) targets the γ-proteobacterial GSO cluster and hybridized to a morphologically homogenous population of coccoidal cells.

(~90 m; Figs 2b, d). Below this upper spike, cell abundances on 15 January increased with depth, indicating that their activity optimum (that is, the sulphide–nitrate transition) was previously located closer to the sediment. Substantial changes in the position of the sulphide–nitrate transition at 24° S were also observed during the second week of our cruise, with the sulphide front descending between 15 and 18 January as described above (Supplementary Fig. 3). The second colloidal sulphur peak at ~110 m at 24° S supports our hypothesis that the sulphide oxidation front was previously located closer to the sediment (Fig. 2b).

Although the cell abundance of the described γ- and ε-proteobacterial populations were sufficient to account for the observed sulphide oxidation rates, our rDsrAB functional gene library indicates that other microbes might also be involved in the detoxification of the sulphidic Namibian waters (Supplementary Discussion). Further north, at 23° S, γ- and ε-proteobacterial cells were present only in low abundance ($<0.2 \times 10^5$ cell ml^{-1}) on 8 January, when no sulphide was detected (Fig. 2e–h), but were three to four times more abundant (each $>0.6 \times 10^5$ cell ml^{-1}) in the sulphidic waters ten days later (Supplementary Fig. 2b, d). This increase in cell abundance was accompanied by an increase in abundance of the gene encoding the GSO-related enzyme rDsrA (Supplementary Discussion). Although there was no detectable denitrification at 23° S on 8 January, substantial denitrification occurred in the zone of overlapping nitrate and sulphide profiles on 18 January (Supplementary Fig. 2b, c). The cell-specific denitrification rates (~8 fmol N_2 per cell per day) at 23° S on 18 January were similar to those detected at 24° S on 15 January (~6 fmol N_2 per cell per day), providing additional evidence for the involvement of these organisms in the anaerobic sulphide oxidation. These chemolithotrophs were not detected at nearby stations before or after our cruise, or in the suboxic waters to the south of the sulphidic water mass (at 26° S; Supplementary Fig 2e–h). In addition to the rapid increase in both cell numbers and the abundance of genes encoding rDsrA at 23° S, the regular size and shape of the cells supports a bloom-like growth of these chemolithotrophs (Fig. 3b, c). Pure cultures of nitrate-reducing, sulphide-oxidizing bacteria have doubling times as short as ~1.5 h (ref. 22), indicating that these chemolithotrophs are capable of creating blooms under the correct conditions. The combined results indicate that the observed bloom of chemolithotrophs was sufficient to create a buffer zone between the oxic environment—where fish and other nekton live—and the toxic sulphidic Namibian shelf waters, by oxidizing sulphide with nitrate.

The findings from a series of monitoring cruises to the same area during 2004–2005 revealed that the sulphidic event observed in January 2004 was not a singular event and that sulphidic bottom waters frequently occur at the Namibian shelf (Fig. 1c, Supplementary Table 1). During 18 one-day monitoring cruises to two sites off Walvis Bay (23° S; Fig. 1c), sulphide was detected three times at the inshore coastal station (3.5 km off shore) and nine times

at the more distant station (~8 km off shore). Owing to the limited spatial and temporal resolution of the monitoring cruises, several other sulphidic events may have remained undetected. Remote satellite sensing is generally used to monitor large ocean areas on a day-to-day basis and, for 2004–2005, 16 larger (>200-km^2 surface area) events of colloidal sulphur were recorded by satellite imaging (Fig. 1c). Owing to the superior temporal (day versus month) and spatial resolution (22° S–27° S versus 23° S) of the satellite observations, a nearly complete record of all events would be expected. However, five of the nine occasions on which sulphide was detected in the shelf waters could not be related to colloidal sulphur clouds in the surface waters (Fig. 1c). On the basis of these observations, sulphide must be consumed in the subsurface shelf waters; the temporal bloom of pelagic sulphur-oxidizing bacteria reported in this study could account for this.

Current estimates postulate that the occurrence of shelf hypoxia, due to both human-induced eutrophication[3,24] and global warming[25,26], will strongly increase in the coming decades. In fact, hypoxic events, and the episodic occurrence of hydrogen sulphide, have been reported in other coastal waters, for example those of western India, Europe and California and the Gulf of Mexico[2–5,24,27]. On the basis of our results from Namibia, we postulate that many sulphidic events in coastal waters may go unnoticed because bacteria consume sulphide before it reaches the surface.

METHODS SUMMARY

Water samples for biogeochemical and microbiological analyses were obtained using a pump conductivity–temperature–depth (pump-CTD) system and Niskin bottles. Nutrient and sulphide concentrations were determined on board with an autoanalyser immediately after sampling. Sulphide, ammonium and nitrate fluxes and rates of sulphide oxidation and nitrate reduction were calculated from the concentration profiles and a vertical diffusion coefficient (K_z) with the program Profile[12]. To determine denitrification and anammox rates, Namibian shelf water collected from specific water depths with the pump-CTD system was incubated at *in situ* temperatures after the addition of ^{15}N-labelled substrates. ^{35}S-sulphate-tracer experiments were performed to determine water-column sulphate-reduction rates.

Particulate organic matter for phylogenetic and catalysed-reporter-deposition FISH (CARD-FISH) analyses was collected from specific water depths by onboard filtration, and *in situ* filtration of large volumes of water through glass-fibre filters was used to obtain DNA for quantitative PCR. In the laboratory, 16S rRNA, *aprA* and *rdsrA* were amplified and cloned and the obtained sequences were subsequently used to reconstruct phylogenetic trees. The rDsrA-encoding gene was quantified using quantitative PCR with a specifically designed primer set. CARD-FISH with specific horseradish peroxidase-labelled oligonucleotide probes was used to determine the abundance of members of the GSO and *Arcobacter* clusters in the Namibian waters.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Sequences for the 16S rRNA, *aprA* and *rdsrA* genes obtained in this study have been submitted to GenBank under the accession numbers FM246507–FM246787, FM246819–FM246832 and FM246788–FM246818, respectively. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.M.M.K. (mkuypers@mpi-bremen.de).

METHODS

Chemical analysis. A pump-CTD system and Niskin bottles (Hydrobios) were used to obtain nutrient samples. Nitrate, nitrite and ammonium concentrations (respective detection limits, 0.1, 0.02 and 0.3 μM) were determined onboard with a TRAACS 800 autoanalyser. For online sulphide measurements, the water flow from the pump-CTD was directly connected to the autoanalyser. For discrete samples, sulphide concentrations (detection limit, 0.1 μM) were measured by spectrophotometry[30].

For elemental sulphur analysis (detection limit, ~1 μM), 1 ml seawater was added to 0.5 ml 1% zinc chloride solution and 9 ml 100% methanol. Samples were stored cold until analysis. Aliquots were filtered through 0.2-μm syringe tip filters (nylon Acrodisc filters) and analysed by reverse-phase high-performance liquid chromatography (Sykam UV-Vis detector S3200 at 265 nm; eluent, 100% methanol; pump rate, 1 ml min^{-1}) after separation on a Zorbax C18 reverse-phase column (Agilent) at room temperature.

^{15}N incubations and analysis. ^{15}N-labelling experiments were performed in gas- and light-tight bags[31]. 250-ml bags were filled directly with water from the pump-CTD system; care was taken to avoid contact with atmospheric oxygen and the bags were maintained at *in situ* temperatures. ^{15}N-nitrate (final concentration, 20 μM) was injected through the rubber stoppers, which were used to close the glass outlets of the bags, and 15-ml aliquots of water were extracted from each bag with a gas-tight syringe at each time point (after 0, 6, 12, 24 and 48 h) and transferred to gas-tight exetainers containing 100 μl saturated mercuric chloride. The samples were stored upside down with a 2-ml helium headspace pending analyses. $^{14}N^{15}N$:$^{14}N^{14}N$ and $^{15}N^{15}N$:$^{14}N^{14}N$ ratios were determined by GC-IRMS (VG Optima).

Production of N_2 through denitrification and anammox was calculated from the excess concentrations of $^{14}N^{15}N$ and $^{15}N^{15}N$ and the ^{15}N-labelling of the NO_3^- pool (F), according to[32]

$$D = {}^{15}N^{15}N \times F^{-2}$$

$$A = {}^{14}N^{15}N \times F^{-1} - [D \times 2 \times (1 - F)]$$

where D and A denote production of N_2 through denitrification and anammox, respectively.

Sulphate reduction rates. Water-column sulphate reduction rates were determined with duplicate 100-ml Winkler bottles at station MPI-3 (22° 38.3′ S, 14° 18.27′ E, 70-m water depth, occupied 5 March 2004), MPI-5 (23° 45.08′ S, 14° 15.8′ E, 112 m, occupied 6 March 2004), and MPI-9 (24° 55.115′ S, 14° 25.248′ E, 122 m, occupied 9 March 2004). To each bottle, 100 μl of carrier-free ^{35}S-sulphate (total activity, 10 MBq per bottle) were added. Bottles were incubated in the dark at 12 °C for 48 h. Subsequently, the contents were transferred to 40 ml 20% zinc acetate. At each station, zinc acetate was added to one Winkler bottle before the tracer addition to determine a procedural blank. The fixed samples were stored frozen until analysis. In the laboratory, the samples were acid-distilled with 6N HCl after the addition of 5 ml 1 mM ZnS to enhance recovery of the evolved ^{35}S-H_2S gas. The radioactivity of ^{35}S-H_2S and ^{35}S-sulphate was determined on a Packard TR100 scintillation counter. Sulphate reduction rates were calculated according to a previously described protocol[33] accounting for the blanks of ^{35}S-sulphate (15 cpm; $n = 3$) and ^{35}S-H_2S (35–65 cpm; $n = 3$).

Phylogenetic analysis. Amplification and cloning of 16S rRNA and *aprA* genes was performed as described previously[34,35]. Partial *rdsrA* genes were amplified using primers 240F: GGNTAYTGGAARGGNGG and 403R: ARCCANCCY-TGNGTRTG. The following PCR conditions were applied: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 55.5 °C for 1 min, elongation at 72 °C for 3 min, 35 cycles. The 16S rRNA clone library was generated from pooled DNA samples (83–103 m at 24° S) whereas the *aprA* and *rdsrA* sequences were obtained from 95-m water depth at 24° S. The recovered *rdsrA* fragments comprised ~1,680 bp, of which 984 bp were used for phylogenetic analysis using ARB[29]. For the 16S rRNA phylogeny, only sequences longer than 1,200 bp were considered for tree reconstruction. Partial sequences (Chilean OMZ and Baltic Sea) were added with the Quick add function in ARB.

Quantitative polymerase chain reaction (qPCR). Water was filtered on to combusted glass-fibre filters (GF/F, 142- or 292-mm diameter; Whatman) with *in situ* pumps (McClane). DNA was extracted from a 2-cm-diameter subsample of the GF/F filters following the protocol of ref 36 and was further purified with the Wizard DNA Clean-up System (Promega). Total DNA concentrations were determined using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). The rDsrA-encoding gene was quantified by qPCR with a specially designed primer set: rdsr393f (5′-ACAGGCGGCATTACGTACC-3′) and rdsr810r (5′-AGTACGCTTTCCACCCATG-3′). The thermal cycling conditions were as follows: initially 2 min at 50 °C, 10 min at 95 °C, followed by 50 cycles of 30 s at 95 °C, 1 min at 53 °C, 2 min at 72 °C and fluorescence detection at 76.9 °C. The correct sizes of qPCR products were verified by melting analysis and gel electrophoresis after amplification. For qPCR standards, partial sequences of *rdsrA* genes were amplified from sequenced plasmid DNA isolated from the corresponding clone library. The PCR products were then purified with QIAquick Spin Columns (Qiagen), and their sizes and corresponding concentrations were accurately determined with a DNA1000 chip on a Bioanalyser 2100 (Agilent). A dilution series with the DNA standard was analysed in parallel during each qPCR run. All qPCRs were performed using PowerSYBR Green Master Mixes (Applied Biosystems) and an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories).

CARD-FISH. Water samples (30 ml) were fixed with paraformaldehyde (final concentration, 2%) for CARD-FISH analyses and were filtered through 0.22-μm polycarbonate filters. Filter sections were hybridized with horseradish peroxidase-labelled oligonucleotide probes[37]. Briefly, endogenous peroxidases were inactivated in a H_2O_2 solution (3%) for 7 min. The probe GSO477, designed specifically for detection of the entire GSO cluster (5′-CTAAAGTTAACGT-CAAGG-3′) was hybridized at 35 °C with 35% formamide. The probe ARC94 (5′-TTAGCATCCCCGCTTCGA-3′) was hybridized at 46 °C with 20% formamide as described elsewhere[38]. Washing, signal amplification and epifluorescence microscopy were conducted as described previously[39].

Flux modelling. Sulphide, ammonium and nitrate fluxes and rates of sulphide oxidation and nitrate reduction were calculated from the concentration profiles and a vertical diffusion coefficient (K_z) with the program Profile[12]. A K_z value of 0.4 cm^2 s^{-1} was reconstructed for the zone of overlapping nitrate and sulphide profiles from *in situ* density[40]. The model predicted zones of net ammonium and sulphide consumption at 110–80 m and net nitrate consumption at 78–105 m.

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