Detoxification of sulphidic African shelf waters by blooming chemolithotrophs

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This file includes:

1). Supplementary Table

Table S1 Monitoring data

Date	Sulphide (µM) at WW23002 ~3.5 km offshore at 45 m water depth	Sulphide (µM) at WW23005 ~8 km offshore at 75 m water depth
16-Jan-04	0	2
1-Mar-04	0	0
29-Mar-04		0
5-May-04	34	68
1-Jun-04	0	6
29-Jun-04	0	8
5-Aug-04	0	0
31-Aug-04	0	0
29-Sep-04	0	0
2-Nov-04	6	2
30-Nov-04	0	4
11-Feb-05	0	0
8-Mar-05	0	9
13-May-05	1	52
1-Jul-05	43	30
2-Sep-05	0	0
8-Nov-05	0	0
1-Dec-05	0	0

2). Supplementary Discussion

Water column sulphate reduction as a potential cause of sulphidic shelf waters

Direct ^{35}S-incubations with anoxic bottom waters from near this site during an RV/Alexander von Humboldt cruise (1st to 12th March, 2004; SI) indicated that sulphate reduction rates were only 1.6±0.2 pmol l^{-1} d^{-1}, which is insignificant compared to the average rate of sulphide increase (2.2 µmol l^{-1} d^{-1}).

Gas/sediment eruptions as a potential cause of sulphidic shelf waters

In the case that eruptive gas or sediment release was responsible for the observed event[1,2] in January 2004, sulphide would be expected to reach the surface waters within a short time (hours to days). At 24°S, sulphide concentrations ~2 m above the sediment increased from 5 µM on January 10, through 19 µM on January 15, to 25 µM on January 18 (Supplementary Fig. 1b). During this entire period, no elemental sulphur was visible in the surface waters. A small patch of weakly discoloured surface waters, which was attributable to colloidal elemental sulphur, was visible from space about one week after the AHAB1-cruise (Fig 1c)[3]. In the case that these events were related, there appears to be a delay of 2 to 3 weeks from the detection of sulphide in the subsurface shelf waters until the occurrence of colloidal sulphur in the surface waters. Furthermore, surface sediments (down to ~75 cm) at and around the center of the sulphide plume (WWW24010) were not gas saturated and the sediments showed no evidence of disturbance indicative of past gas eruptions (i.e. pockmarks)[4]. The sulphide-containing shelf waters observed during January 2004 occurred over ~7,000 km² and contained more than 16,000 tons of sulphide (assuming a 15 m-thick layer of sulphidic bottom waters with an average sulphide concentration of 5 µM), in addition to several thousand tons of elemental sulphur at the end the AHAB1 cruise. If gas eruptions caused the recorded event (~16,000 tons of sulphide) in January 2004, this would have required the release of all the sulphide contained in ~3.7x10^{7} m³ of sediments (based on a pore water sulphide concentration of 15 mM[4]. However, there was no indication of enhanced turbidity from the CTD casts in the sulphidic bottom waters, relating the presence of sulphide to

suspended sediments. Moreover, the biggest "pockmark" reported released $4.9x10^5$ m^3 of sediments corresponding to less than 2% of the observed sulphide in January 2004 [4]. All of these features are difficult to reconcile with the mechanism of a sudden, violent hydrogen sulphide/methane gas eruption as a cause for this sulphidic event[1,2] and, rather, indicate a diffusive flux of sulphide from the sulphidic sediments.

The role of the benthic microbial community in sulphide oxidation

The observed bloom of two discrete populations of Gamma- and Epsilonproteobacteria could be more effectively triggered if some of these chemolithotrophs were already present in the sediment. To check whether these organisms were present in surface sediments, 16S rRNA gene clone libraries were generated from surface sediments at nearby stations before and after the sulfidic event. However, these clone libraries did not contain sequences belonging to these organisms (E. Julies and V. Brüchert, unpublished data) nor did we detect these organisms with FISH analyses specifically targeting these bacteria.

The benthic sulphide flux can also be attenuated by the activity of the large nitrate-storing benthic sulphide-oxidizing bacteria *Beggiatoa* and *Thiomargarita*[4,5]. However, these bacteria are only present in considerable abundances north of 22°S and do not appear to thrive in the area of recurrent sulphidic shelf waters (23-25°S) and in the oxic shelf waters south of 25°S. This limits their impact as an oxidation buffer for the sedimentary sulphide flux to the suboxic shelf waters from 23-19°S.

Phylogeny, diversity and abundance of AprA and rDsrAB gene sequences

Genes encoding two enzymes in the sulphur oxidation pathway, the adenosine 5'-phosphosulphate reductase (AprA) and the reverse dissimilatory sulphite reductase (rDsrAB) were amplified to identify organisms capable of oxidizing sulphur compounds in the Namibian shelf waters (sampling site at 24°S, pooled samples, 70 - 90 m water depth). The capacity to oxidize sulphur compounds is widely spread amongst prokaryotes and several pathways of sulphur oxidation are known[6]. To date the rDsrAB has been found in sulphur oxidizing organisms that form elemental sulphur/polysulphides as intermediate storage products[7,8] and has been shown a suitable phylogenetic marker for sulphur oxidizers[9]. The elemental sulphur/polysulphide is oxidized by the rDsrAB and associated proteins yielding sulphite[7,10], which is then further oxidized to adenosine 5'-

phosphosulphate catalyzed by the AprAB or directly to sulphate by other enzymes[8,11]. Sulphur oxidizing epsilonproteobacteria apparently do not utilize the rDsrAB and the AprAB for sulphur oxidation[12,13]. In agreement with 16S rRNA phylogeny of the GSO cluster, we identified both rDsrA and AprA encoding gene sequences that are most closely related to *Candidatus* Ruthia magnifica, the sulphide-oxidizing gill endosymbiont of the mussel *Calyptogena magnifica* inhabiting sulphide-rich vent and seep environments[14]. These endosymbionts are known to oxidize sulphide to elemental sulphur[15,16], and subsequently to sulphate using the rDsrAB and AprAB[17]. The rDsrA-encoding gene of the members of gammaproteobacterial GSO cluster identified in this study was quantified by quantitative polymerase chain reaction (qPCR) with a specifically-designed primer set in both sulphidic and non-sulphidic waters. While rDsrA gene sequences were present in substantial abundance (3,226±1,259 copies/µg DNA) in the sulphidic water at 24°S (90m; Fig. 2b), they were at or below detection limit (567±579 copies/µg DNA) in the suboxic waters south of the sulphidic waterbody (26°S at 93m). Further north at 23°S these rDsrA-encoding genes were at or below detection limit (270±290 copies/µg DNA) on January 8 when no sulphide was detected (95 m; Fig. 2f), but were abundant (2,448±1,409 copies/µg DNA) in the sulphidic waters (120 m; Supplementary Fig. 2b) 10 days later. This increase in rDsrA-encoding gene abundance agrees well with the rapid increase in gammaproteobacterial cell-numbers at 23°S (See also Main Text).We also recovered AprA sequences related to *Pelagibacter ubique*, an organism that is abundantly present in many marine coastal habitats, but which is not known to oxidize sulphur[18]. The rDsrA clone library contained additional gene sequences clustering within the Proteobacteria and Chlorobi group (Supplementary Fig. 4) indicating that members of these groups might also be involved in the detoxification of the sulphidic Namibian waters.

The combined cell abundance profiles, rDsrA-encoding gene abundance and the congruent phylogenies of three genes provide strong evidence for a substantial contribution of the members of gammaproteobacterial GSO cluster and epsilonproteobacterial genus *Arcobacter* to the detoxification of the sulphidic Namibian shelf waters. In fact, the cell abundance of these gamma- and epsilonproteobacterial

populations were sufficient to account for the observed sulphide oxidation rates (See also Main Text).

Effect on fisheries and benthic community

Even if sulphide does no get into the surface waters, the recurrent accumulation of hydrogen sulphide in the bottom waters of the Namibian shelf (Fig. 1 and 2; Supplementary Fig. 2), will create a hostile environment for higher life. This may explain why important marine resources inhabiting the shelf, such as shallow water hake, pilchard and rocklobsters, seem to largely avoid the region of the Namibian shelf with recurrent sulphidic bottom waters (Fig. 1)[19-21].

3). Supplementary Figure captions

Supplementary Figure 1 Changes in sulphide concentrations of the bottom waters at 23°S and 24°S between January 7 and January 23, 2004. The location of the sampling sites is shown in Fig. 1

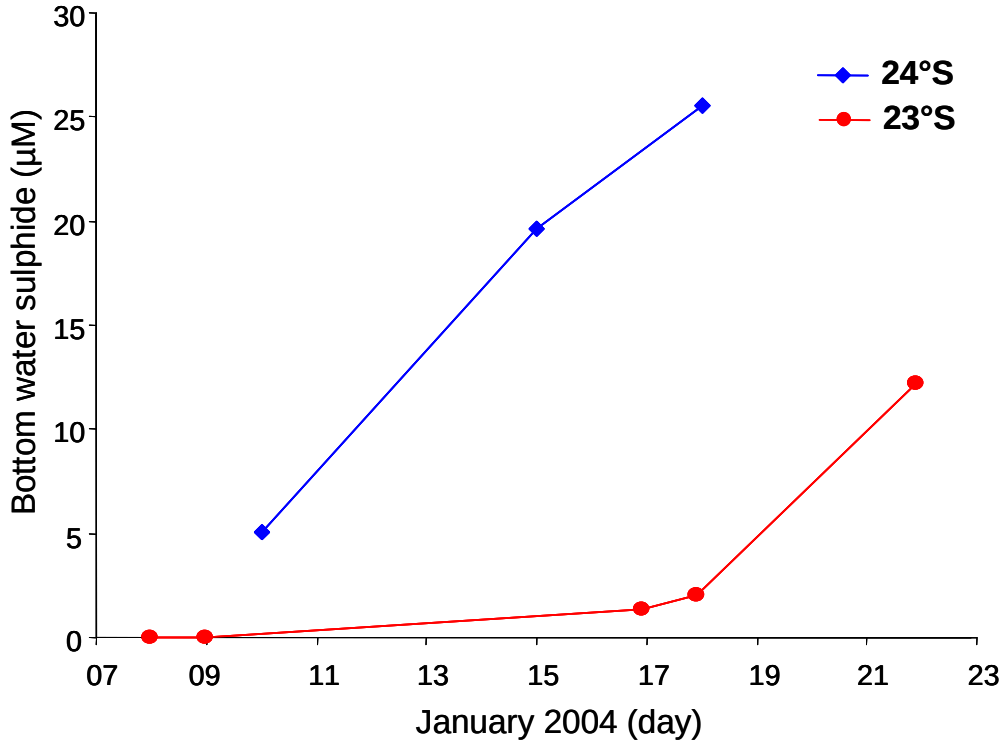
Supplementary Figure 2 Chemical zonation and distribution of indicators for bacterial sulphide-oxidation at 23°S on January 18, 2004 **(a-d)** and at 26°S on January 13, 2004 **(e-h). (a, e)** Oxygen and ammonium concentrations. **(b, f)** Nitrate and sulphide concentrations. **(c, g)** Denitrification and anammox rates (n.d.; depths where N_2-production was below detection) determined from *in situ* $^{15}NO_3^-$ incubations (right panels). **(d, h)** Epsilonproteobacterial cells hybridizing with the Arc94 oligonucleotide probe, gammaproteobacterial cells hybridizing with the GSO477 probe. The location of the sampling sites is shown in Fig. 1

Supplementary Figure 3 Sulphide concentrations at 24°S on January 15 (red circles) and 18 (black diamonds), and water density -1,000 at 24°S on January15, 2004 (blue diamonds). The location of the sampling site is shown in Fig. 1

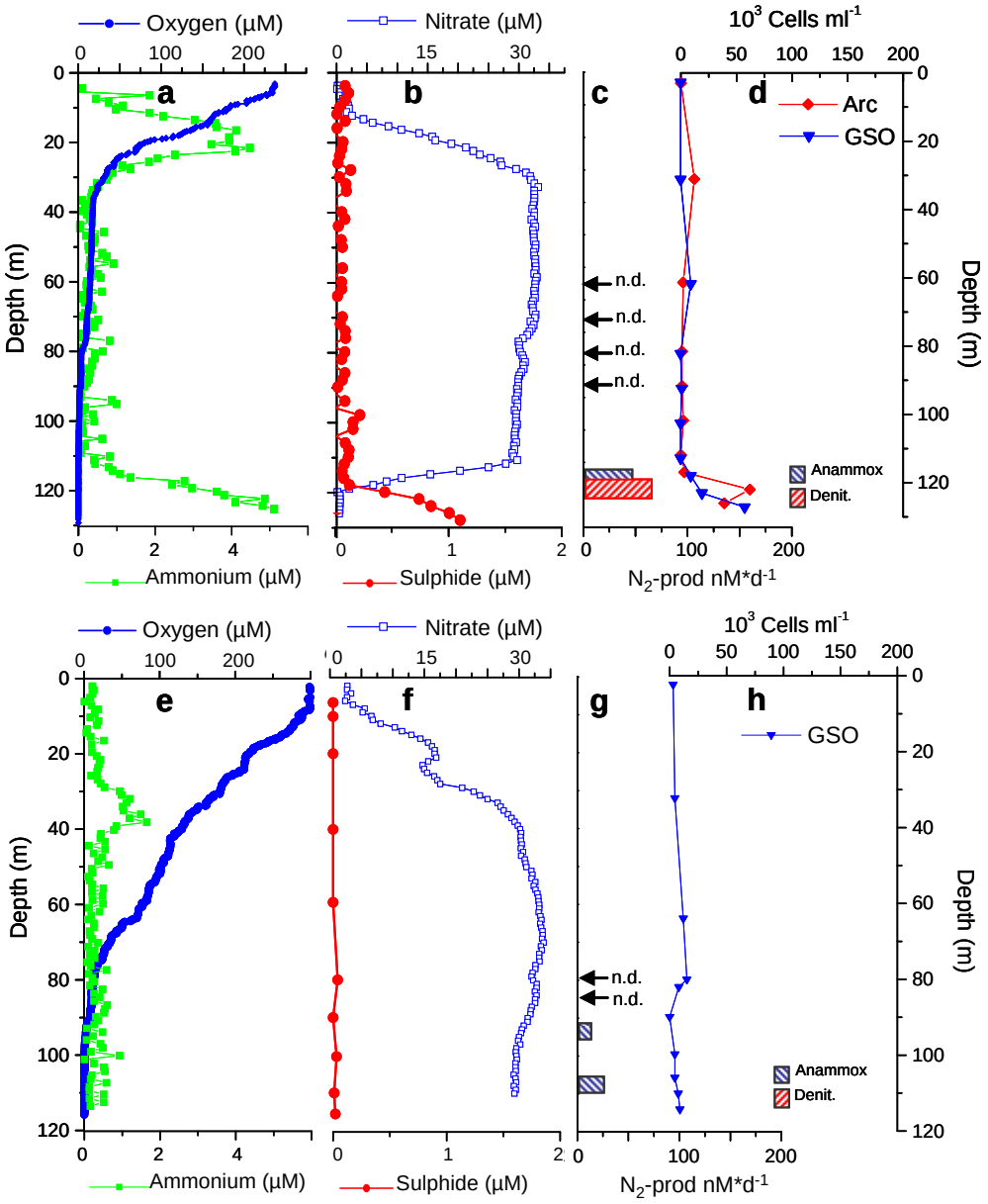
Supplementary Figure 4 (a) For the phylogenetic reconstruction of AprA a 30% conservation filter was applied considering 96 amino acid positions. From the sulphidic layer 14 *aprA* Sequences were derived, 10 of these clustered with *Pelagibacter ubique*, 4 with *Candidatus* Ruthia magnifica. **(b)** For the phylogenetic reconstruction of the rDsrA a 25% conservation filter was applied considering 328 amino acid positions. Maximum parsimony, neighbour joining and maximum likelihood methods were applied for phylogenetic reconstructions and the results were converted into a consensus tree by ARB[22]. The NAM III and IV clusters could not be assigned to known proteobacterial groups.

4). Supplementary Figures

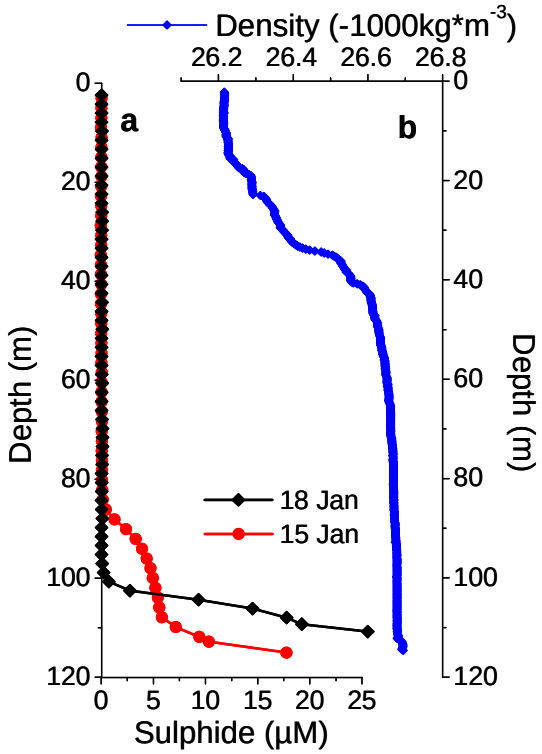
Supplementary Figure 1



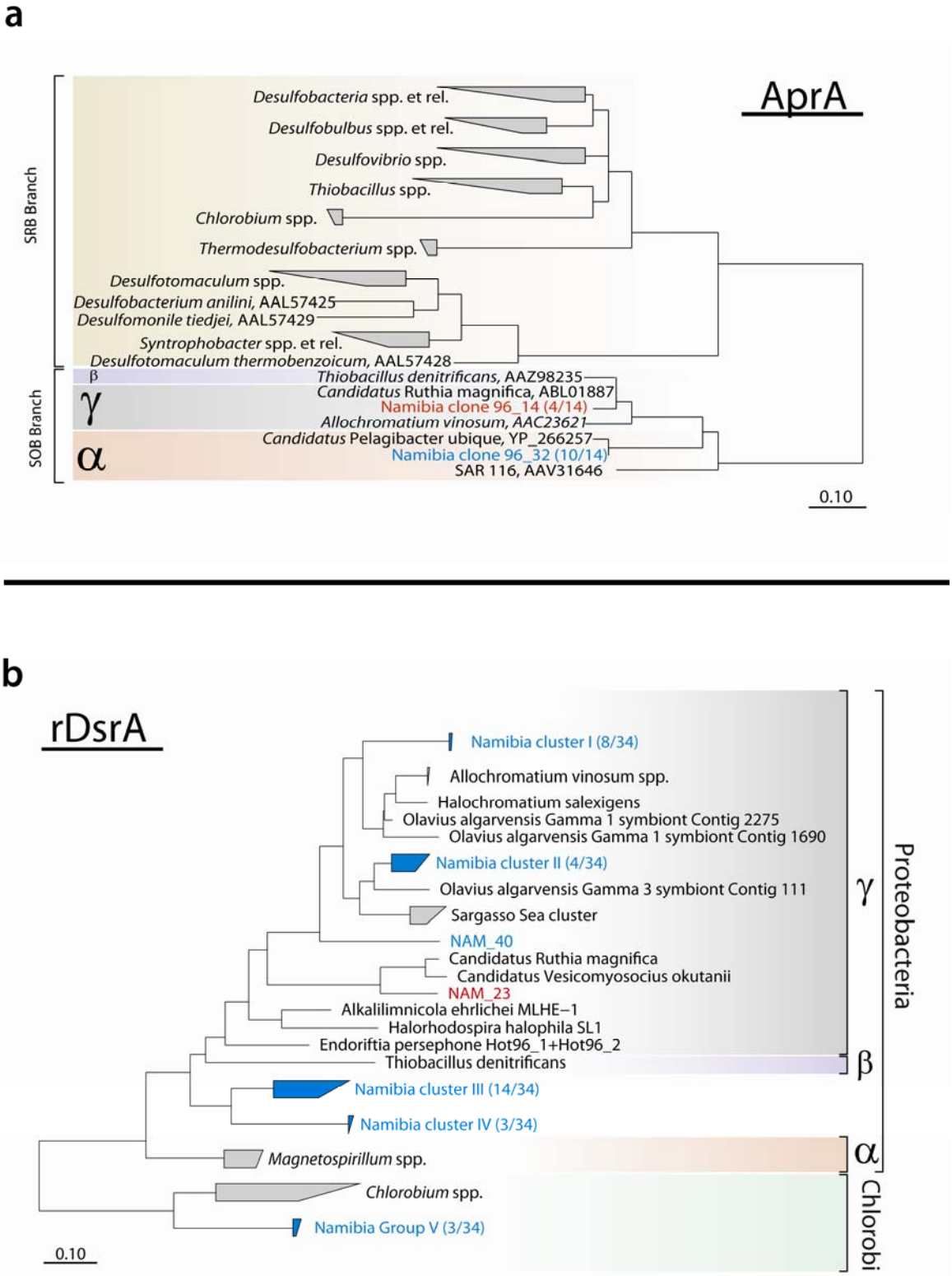
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



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