

Special Issue Article

The bacterial sulfur cycle in expanding dysoxic and euxinic marine waters

Daan M. van Vliet ¹, F.A. Bastiaan von Meijenfeldt,²
Bas E. Dutilh,² Laura Villanueva,³
Jaap S. Sinninghe Damsté,^{3,4} Alfons J.M. Stams^{1,5}
and Irene Sánchez-Andrea ^{1*}

¹Laboratory of Microbiology, Wageningen University and Research, Stippeneng 4, 6708WE, Wageningen, Netherlands.

²Theoretical Biology and Bioinformatics, Science for Life, Utrecht University, Padualaan 8, 3584 CH, Utrecht, Netherlands.

³Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research (NIOZ), Utrecht University, Landsdiep 4, 1797 SZ, 't Horntje (Texel), Netherlands.

⁴Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Princetonlaan 8A, 3584 CB, Utrecht, Netherlands.

⁵Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

Dysoxic marine waters (DMW, < 1 μ M oxygen) are currently expanding in volume in the oceans, which has biogeochemical, ecological and societal consequences on a global scale. In these environments, distinct bacteria drive an active sulfur cycle, which has only recently been recognized for open-ocean DMW. This review summarizes the current knowledge on these sulfur-cycling bacteria. Critical bottlenecks and questions for future research are specifically addressed. Sulfate-reducing bacteria (SRB) are core members of DMW. However, their roles are not entirely clear, and they remain largely uncultured. We found support for their remarkable diversity and taxonomic novelty by mining metagenome-assembled genomes from the Black Sea as model ecosystem. We highlight recent insights into the metabolism of key sulfur-oxidizing SUP05 and *Sulfurimonas*

bacteria, and discuss the probable involvement of uncultivated SAR324 and BS-GSO2 bacteria in sulfur oxidation. Uncultivated *Marinimicrobia* bacteria with a presumed organoheterotrophic metabolism are abundant in DMW. Like SRB, they may use specific molybdoenzymes to conserve energy from the oxidation, reduction or disproportionation of sulfur cycle intermediates such as S^0 and thiosulfate, produced from the oxidation of sulfide. We expect that tailored sampling methods and a renewed focus on cultivation will yield deeper insight into sulfur-cycling bacteria in DMW.

Introduction

Oxygen deficiency is a rather common phenomenon in marine waters caused by microbial aerobic respiration coupled to the degradation of organic matter, combined with insufficient supply of oxygen through water circulation or diffusion (Canfield *et al.*, 2005). Oxygen-minimum zones (OMZs) are waters in the open ocean containing < 20 μ M oxygen occurring between 100 and 1,500 m depth. The largest OMZs are found in the Eastern Tropical North Pacific (ETNP), the Eastern Tropical South Pacific (ETSP) and the Arabian Sea (Fig. 1, Table S1). Together, OMZs amount to 10 million km³ or approximately 1% of the ocean's volume (Paulmier and Ruiz-Pino, 2009). When there is sufficient input of sinking phytoplankton biomass, oxygen concentrations in OMZs can drop to below the common detection level of 1 μ M and were considered anoxic and described as 'anoxic marine zones' (Ulloa *et al.*, 2012). However, using a highly sensitive STOX oxygen sensor, Revsbech and colleagues (2009) and Thamdrup and colleagues (2012) showed that the supposedly anoxic OMZ waters, which will here be termed 'OMZ core', still may contain traces of oxygen (< 50 nM). Yet, these sensors have not yet been widely applied in marine sampling campaigns. Coastal waters can similarly experience oxygen deficiency and anoxia, for example in the Namibian Upwelling, Chesapeake Bay and the Pacific South-American coastal waters (Fig. 1, Table S1).

Received 30 October, 2019; revised 3 September, 2020; accepted 28 September, 2020. *For correspondence. E-mail irene.sanchezandrea@wur.nl; Tel. +317 483 486.

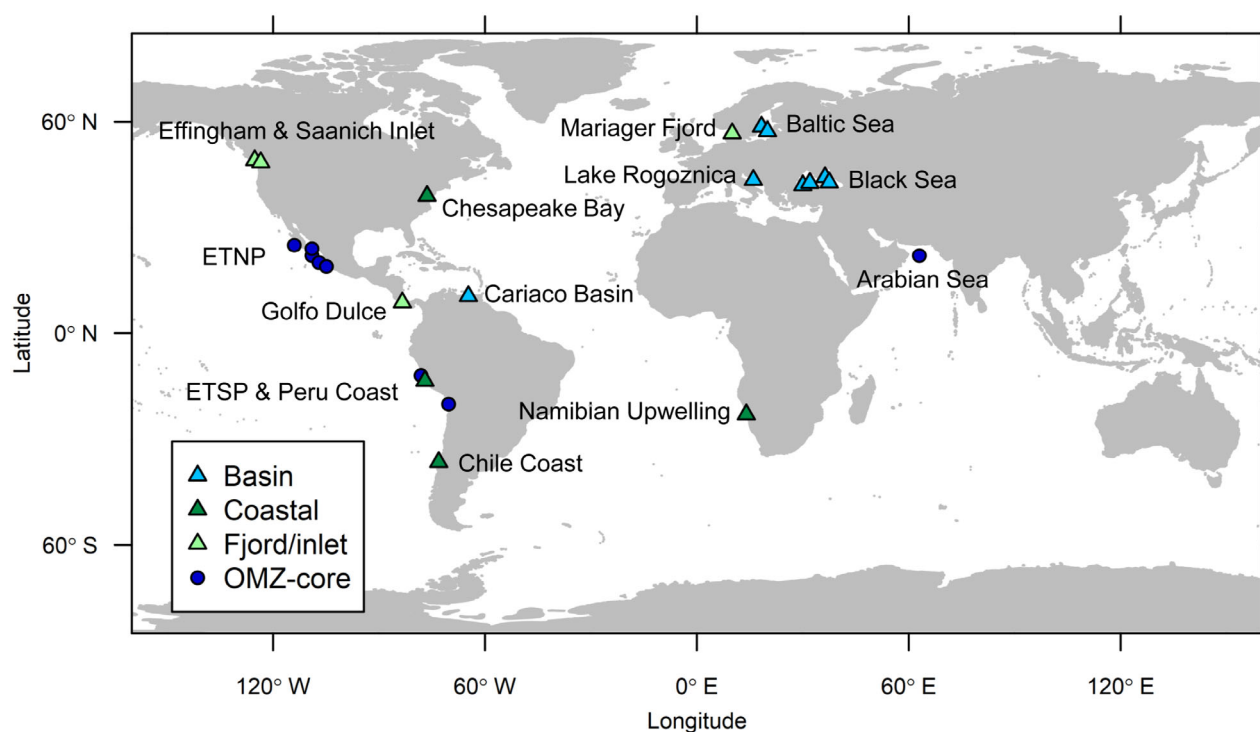


Fig. 1. Dysoxic marine waters studied with respect to microorganisms driving the sulfur cycle. Triangles indicate locations that are permanently, seasonally or incidentally euxinic. For a list of studies per location, see Table S1. ETNP, Eastern Tropical North Pacific; ETSP, Eastern Tropical South Pacific; OMZ, oxygen-minimum zone. [Color figure can be viewed at wileyonlinelibrary.com]

In enclosed marine basins and fjords, as well as in coastal waters, stratification is a common factor in the development and persistence of oxygen deficiency (Canfield *et al.*, 2005). Stratification can even lead to euxinia (Meyer and Kump, 2008), here defined as anoxic conditions with $> 0.1 \mu\text{M}$ sulfide. Several euxinic marine basins, fjords and inlets have been studied with respect to their sulfur cycle and the associated microorganisms (Fig. 1, Table S1). The development of euxinia in OMZs is prevented by a relatively high advection of oxygenated water compared to enclosed environments, and by a negative feedback loop centered around nitrogen loss. Denitrifying and anaerobic ammonia-oxidizing bacteria in the OMZ convert fixed forms of nitrogen such as ammonium and nitrate into N_2 at such high rates that OMZs are responsible for 30% to 50% of the total loss of fixed nitrogen from the ocean (Lam and Kuypers, 2011). This causes surface phytoplankton to be limited in nitrogen, which in turn limits the input of organic matter to the OMZs, preventing the depletion of nitrate and nitrite (Canfield, 2006; Boyle *et al.*, 2013). Hence, the ensuing development of euxinia is halted, as denitrifying bacteria outcompete sulfate-reducing ones (Froelich *et al.*, 1979; Chen *et al.*, 2017).

OMZ core waters, despite being dominated by nitrogen cycling, can also harbor an active sulfur cycle, which has

long been overlooked due to the absence of detectable sulfide (Canfield *et al.*, 2010; Johnston *et al.*, 2014; Carolan *et al.*, 2015). Similar conditions are found in some stratified environments where the oxic and euxinic zones are separated by a suboxic zone (Murray *et al.*, 1989; Lavik *et al.*, 2009; Hawley *et al.*, 2014; Findlay *et al.*, 2017), here defined to contain no detectable oxygen ($< 1 \mu\text{M}$) or sulfide ($< 0.1 \mu\text{M}$) using standard methods. The exact concentration of oxygen in suboxic zones is still unclear. However, the detection of sulfur-cycling microorganisms suggests an active sulfur cycle in suboxic zones, for instance in the Black Sea and Cariaco Basin (Neretin *et al.*, 2007; Rodriguez-Mora *et al.*, 2016). For the purpose of this review, we use the term 'dysoxic marine water' (DMW, $< 1 \mu\text{M}$ of oxygen) to describe all marine suboxic zones, OMZ core waters, anoxic waters and euxinic waters (Box 1).

Over the last 60 years, DMW has expanded in volume more than fourfold (Schmidtke *et al.*, 2017) because of oceanic warming – reducing oxygen solubility – and eutrophication (reviewed by Breitburg *et al.*, 2018). This process is expected to continue. In addition, Ulloa and colleagues (2012) have predicted that the deposition of anthropogenically fixed nitrogen will cause OMZ cores to develop euxinia, since it counteracts the nitrogen-loss-

Box 1. Glossary of definitions for marine oxygen-deficient environments.

Environmental terminology	Definition
Dysoxic water	< 1 μM oxygen
Anoxic water	Not containing oxygen
Euxinic water	Anoxic, > 0.1 μM sulfide
Oxygen-minimum zone (OMZ)	Open ocean water with < 20 μM oxygen
OMZ core, also known as 'anoxic marine zone'	Open ocean water with < 50 nM oxygen
Suboxic zone	< 1 μM oxygen, < 0.1 μM sulfide, in between oxic and euxinic zones of stratified waters

based negative feedback loop. Potential long-term, global consequences of expanding marine dysoxia and euxinia include changes in availability of key nutrients (iron, phosphorus, etc.) and trace metals (cadmium, copper, zinc, etc.), and loss of fishery stocks, affecting coastal economies and food security (Breitburg *et al.*, 2018). Furthermore, since DMW environments are biogeochemical hotspots for microbial production of the greenhouse gas nitrous oxide (Naqvi *et al.*, 2010), their expansion provides a feedback loop that in turn contributes to global warming. In the geological past, the rise of euxinic conditions has led to several mass extinction events such as during the end-Permian (Meyer and Kump, 2008) and the mid-Cretaceous (Kamyshny *et al.*, 2009).

The biogeochemical sulfur cycle in DMW consists of abiotic and biologically mediated reactions (Fig. 2; Ehrlich *et al.*, 2015), with the latter providing energy to many different microorganisms. Sulfate-reducing bacteria (SRB) reduce sulfate (SO_4^{2-}) to sulfide (HS^-), coupled to the oxidation of small organic compounds or H_2 (Muyzer and Stams, 2008). Most of this sulfide is re-oxidized by oxidized metals or sulfur-oxidizing bacteria (SOB), either completely to sulfate (Jørgensen *et al.*, 1991) or to different sulfur cycle intermediates (SCIs) including elemental sulfur (S^0), polysulfides (HS_n^-), thiosulfate ($\text{S}_2\text{O}_3^{2-}$), tetrathionate ($\text{S}_4\text{O}_6^{2-}$) and sulfite (SO_3^{2-} ; Zopfi *et al.*, 2001; Kamyshny *et al.*, 2011; Findlay, 2016). These SCIs can be used as electron donor or acceptor by various microorganisms including SRB and SOB (Rabus *et al.*, 2013; Han and Perner, 2015; Dahl, 2017).

The detection and analysis of sulfur-cycling genes, transcripts and proteins in DMW yield a powerful perspective on the diversity and activity of sulfur-cycling microorganisms (Fig. 2), and more so when applied to metagenome-assembled genomes (MAGs) or single-cell amplified

genomes (SAGs). Various 'omics' studies have yielded insight into the dominant SOB in DMW (Lavik *et al.*, 2009; Walsh *et al.*, 2009; Callbeck *et al.*, 2018; Plominsky *et al.*, 2018). However, only few studies have addressed the broader diversity of sulfur-cycling microorganisms (Canfield *et al.*, 2010; Stewart *et al.*, 2012; Schunck *et al.*, 2013; Hawley *et al.*, 2014), without tapping into the larger potential of genome-centric metagenomics and the available metagenome data. To fill this knowledge gap, we screened MAGs from DMW environments for sulfur-cycling marker genes (Supporting Information Methods). Part of the MAGs was assembled from metagenomes of the Arabian Sea and ETSP OMZ cores produced by Tara Oceans (Parks *et al.*, 2017; Tully *et al.*, 2018). Other MAGs were assembled from metagenomes of 15 different water depths of the Black Sea (Villanueva *et al.*, 2020; Suominen *et al.*, 2019; Supporting Information Methods), which served as model for enclosed DMW environments.

Despite the central role of the sulfur cycle in DMW, current biogeochemical and microbiological knowledge has not been comprehensively reviewed so far. Therefore, we herein provide an overview of sulfur cycle processes in DMW, and we discuss the diversity, metabolism and physiology of the bacteria involved in these processes. In the following section, we will discuss current knowledge on SRB, who form an essential part of the sulfur cycle through the production of sulfide. The subsequent section treats SOB, covering well-studied groups such as SUP05 and *Sulfurimonas*, less explored groups such as BS-GSO2, and putative sulfur oxidizers such as SAR324 members. The final section discusses which bacteria could be involved in sulfur reduction or disproportionation.

Sulfate-reducing bacteria

The presence and activity of SRB in the dysoxic water column have been demonstrated through sulfate reduction rate measurements with isotopically labelled sulfate ($^{35}\text{SO}_4^{2-}$) performed in euxinic settings such as the Black Sea with sulfate reduction rates up to $36 \text{ nmol l}^{-1} \text{ day}^{-1}$ (Sorokin, 1972; Jørgensen *et al.*, 1991; Albert *et al.*, 1995; Pimenov *et al.*, 2000) and Mariager Fjord with rates up to $140 \text{ nmol l}^{-1} \text{ day}^{-1}$ (Sørensen and Canfield, 2004), but also in the ETSP OMZ core with rates up to $16.9 \text{ nmol l}^{-1} \text{ day}^{-1}$ (Canfield *et al.*, 2010). More extensively conducted taxonomic marker studies point to a universal presence of SRB in DMW since the 16S rRNA genes of canonical SRB lineages of the class *Deltaproteobacteria* have been widely detected (Madrid *et al.*, 2001; Vetriani *et al.*, 2003; Lin *et al.*, 2006; Fuchsman *et al.*, 2011; Wright *et al.*, 2012; Schunck *et al.*, 2013; Ganesh *et al.*, 2014; Rodríguez-Mora *et al.*, 2015; Suter *et al.*, 2018; Callbeck *et al.*, 2019).

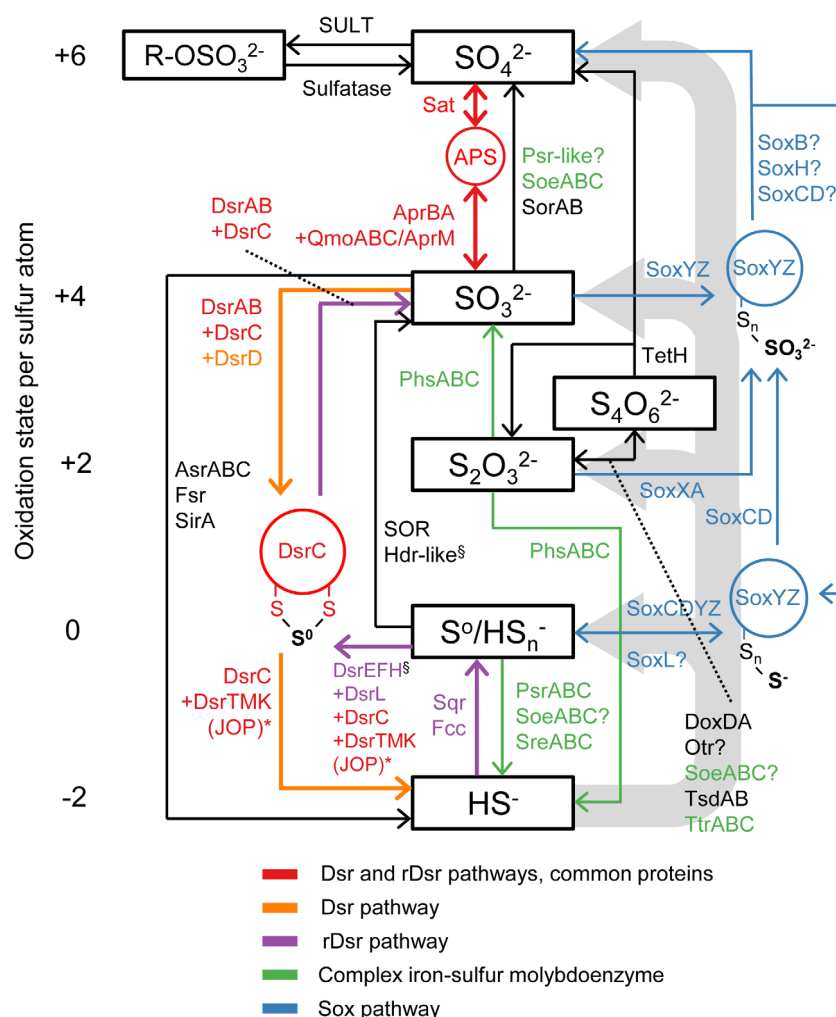


Fig. 2. The dissimilatory conversions within the marine sulfur cycle. The oxidation state of the inorganic species is indicated at the left. Abiotic and assimilatory reactions are not indicated, except for the abiotic oxidation of sulfide which is illustrated by wide grey arrows. The S^0 in DsrC-trisulfide is considered zero-valent (Santos *et al.*, 2015). The sulfur atom in APS has an oxidation state of +6, and those in tetrathionate have an oxidation state of +2.5. A question mark symbol (?) shows that involvement is uncertain. The asterisk symbol (*) indicates that DsrT is required for sulfide oxidation in green sulfur bacteria (Holkenbrink *et al.*, 2011), but is also found in SRB. Protein complexes other than DsrTMK(JOP) can also transfer electrons to DsrC to enable this reaction (Venceslau *et al.*, 2014). The section symbol (§) indicates that the rhodanese sulfurtransferases Rhd-TusA-DsrE2 are also essential in the reaction mediated by this complex (Dahl, 2017). Apr, APS reductase; Asr, anaerobic sulfite reductase; Dox, thiosulfate:quinone oxidoreductase; Dsr, dissimilatory sulfite reductase; Fcc, flavocytochrome *c* sulfide dehydrogenase; Fsr, F_{420} -dependent sulfite reductase; Hdr, heterodisulfide reductase; Otr, octaheme tetrathionate reductase; Pds, thiosulfate reductase; Pds, polysulfide reductase; Qmo, quinone-interacting membrane-bound oxidoreductase; Sat, sulfate adenylyltransferase; Sir, sulfite reductase; Soe, sulfite-oxidizing enzyme; SOR, sulfur oxygenase/reductase; Sor, sulfite-acceptor oxidoreductase; Sox, sulfur-oxidizing multienzyme complex; Sqr, sulfide:quinone oxidoreductase; Sre, sulfur reductase; SULT, sulfotransferase; Tet, tetrathionate hydrolase; Tsd, thiosulfate dehydrogenase; Ttr, tetrathionate reductase. [Color figure can be viewed at wileyonlinelibrary.com]

Presence and diversity

All known SRB reduce sulfate through the dissimilatory (bi)sulfite reductase (Dsr) pathway (Rabus *et al.*, 2015). This consistency has facilitated functional marker investigations of the ecology of SRB. Such a functional marker approach is more appropriate than 16S rRNA gene surveys, as it does not require metabolic assumptions based on taxonomy. Although the core proteins of the Dsr pathway, Sat, AprBA and DsrAB, are also present in the reversed Dsr (rDsr) sulfur oxidation pathway (Dahl, 2017; Fig. 2), their reductive and oxidative versions are phylogenetically distinguishable (Meyer and Kuever, 2007; Loy *et al.*, 2009; Müller *et al.*, 2015; Pelikan *et al.*, 2016). Reductive Dsr genes have been identified throughout suboxic and euxinic waters of the Black Sea (Neretin *et al.*, 2007) and the Cariaco Basin (Rodriguez-Mora *et al.*, 2016), in sulfidic coastal waters off Peru (Schunck *et al.*, 2013), in the core of the ETNP and ETSP OMZs (Canfield *et al.*, 2010; Carolan *et al.*, 2015), and in the Gdansk Deep within the Baltic Sea (Korneeva

et al., 2015). Moreover, reductive Dsr genes were shown to be transcribed into mRNA (Stewart *et al.*, 2012; Ulloa *et al.*, 2012; Schunck *et al.*, 2013; Rodriguez-Mora *et al.*, 2016; Saunders *et al.*, 2019), providing evidence for activity of SRB. Although powerful, the results of Dsr markers should be cautiously interpreted (Anantharaman *et al.*, 2018), as the Dsr pathway does not only facilitate dissimilatory sulfate reduction but can also mediate dissimilatory reduction and disproportionation of SCIs (Rabus *et al.*, 2015; Florentino *et al.*, 2017), and in some rare cases sulfur oxidation (Sigalevich and Cohen, 2000; Slobodkina *et al.*, 2017; Thorup *et al.*, 2017). Thus, although bacteria with reductive Dsr pathways are core members of the microbial community of DMW, their sulfur metabolism is not necessarily restricted to dissimilatory sulfate reduction. We therefore refer to them as putative SRB.

Surveys based on 16S rRNA or functional marker genes and metagenomic studies of DMW have revealed a high diversity of putative SRB, most of which are only

distantly related to described species. The deltaproteobacterial putative SRB detected in 16S rRNA gene data sets are rarely affiliated with established genera (Fuchsman *et al.*, 2011; Wright *et al.*, 2012; Ganesh *et al.*, 2014; Rodriguez-Mora *et al.*, 2015; Suter *et al.*, 2018). Of all canonical SRB lineages, *Desulfobacteraceae* species are thought to be dominant due to the prevalence of their sequences in 16S rRNA data sets (Fuchsman *et al.*, 2011; Wright *et al.*, 2012; Rodriguez-Mora *et al.*, 2015; Suter *et al.*, 2018) and metagenomic data sets (Canfield *et al.*, 2010; Schunck *et al.*, 2013). However, *Desulfobulbaceae* species have also been detected, specifically including the 16S rRNA genes of the genera *Desulfocapsa* and *Desulforhopalus* (Neretin *et al.*, 2007; Canfield *et al.*, 2010; Fuchsman *et al.*, 2011; Fuchsman *et al.*, 2012; Rodriguez-Mora *et al.*, 2015; Suter *et al.*, 2018). Furthermore, bacteria related to the genus *Desulfatiglans* seem widespread in DMW since *Desulfatiglans*-related sequences were retrieved from the Black Sea (16S rRNA genes; Vetriani *et al.*, 2003; Neretin *et al.*, 2007), coastal DMW off Peru (metagenomics; Schunck *et al.*, 2013), the Gdansk Deep in the Baltic Sea (*dsrB* fragments; Korneeva *et al.*, 2015) and the Cariaco Basin (*dsrA* fragments; Rodriguez-Mora *et al.*, 2016). Functional marker gene surveys indicated an even larger diversity of putative SRB beyond the *Deltaproteobacteria*, including *Thermodesulfobivrio*-related bacteria in the ETSP OMZ core (Canfield *et al.*, 2010) and diverse unknown putative SRB in euxinic basins (Korneeva *et al.*, 2015; Rodriguez-Mora *et al.*, 2016).

The *dsrD* gene, encoding a small protein with a possible regulatory function (Mizuno *et al.*, 2003; Venceslau *et al.*, 2014), is an alternative functional marker gene for detection of SRB (Mussmann *et al.*, 2005). It has been used to investigate bacterial genomes with *dsrAB* genes lacking a clear oxidative/reductive affiliation (Anantharaman *et al.*, 2018), since *dsrD* forms a reliable marker for the reductive Dsr pathway when present together with other Dsr genes (Rabus *et al.*, 2015). We detected the *dsrD* gene – in the context of other *dsr* genes – in metagenomes and MAGs from the Black Sea throughout the euxinic and suboxic zones (Fig. 3A,B), confirming previously reported distributions of putative SRB (Neretin *et al.*, 2007). We could not obtain any assembled reductive *dsrA* genes from publicly available OMZ metagenomes (Canfield *et al.*, 2010; Ganesh *et al.*, 2014; Fuchsman *et al.*, 2017; Tully *et al.*, 2018; Saunders *et al.*, 2019), likely due to sampling bias (see following subsection) and insufficient sequencing depth. However, many complete *dsrA* genes could be retrieved from the Black Sea metagenome (Supporting Information Methods). An analysis of these *dsrA* sequences supported the view emerging from previous studies: a

large diversity of putative SRB, with a somewhat distant relationship to canonical SRB belonging to *Desulfobacula*, *Desulfococcus*, *Desulfocapsa*, *Desulfatiglans* and *Thermodesulfobivrio*, and to non-canonical lineages other than the *Deltaproteobacteria* or *Nitrospirae* (Fig. 4). This view mirrors the overly large diversity of SRB that can generally be found in marine sediments (Muyzer and Stams, 2008; Müller *et al.*, 2015). Despite the wealth of knowledge on the metabolism of SRB, the ecophysiological causes behind this diversity are currently poorly understood.

Physiology and metagenomics

We have a considerable understanding of the physiology of SRB from marine sediments, owing to a rich diversity of isolated SRB that are available for laboratory research (Muyzer and Stams, 2008; Rabus *et al.*, 2015). In contrast, no SRB have been isolated from DMW, except for two subspecies of *Desulfobivrio oceani* from the ETSP OMZ (Finster and Kjeldsen, 2010). It can be reasonably assumed that most of the deltaproteobacterial putative SRB detected in DMW adhere to the general metabolism of dissimilatory reduction of sulfate and oxidation of small organic compounds or H₂. However, the lack of closely related described SRB does not allow further constraining of metabolic niches based on taxonomic affiliation, nor does it allow hypotheses on the many other variable physiological aspects.

These challenges can be addressed by genome-centric metagenomics, exemplified by recent explorations of the potential metabolism of *Desulfatiglans*-related SAGs from marine sediments (Jochum *et al.*, 2018) and of the identity and potential metabolism of non-canonical putative SRB from various environments (Wasmund *et al.*, 2016; Anantharaman *et al.*, 2018; Hausmann *et al.*, 2018; Thiel *et al.*, 2018; Meier *et al.*, 2019). With these aims, we mined metagenome data to obtain 13 MAGs of putative SRB from the Black Sea, encoding complete or incomplete reductive Dsr pathways (Figs 3A, B and 5, Table S2, Supporting Information Methods). In agreement with previous diversity studies, most of the putative SRB MAGs were affiliated with *Desulfobacterales*, *Desulfobulbales* and *Nitrospirae* but did not classify within established genera, except for *Desulfobacula maris*. Four of the putative SRB MAGs from the Black Sea (52%–93% complete, 1%–3% contaminated) affiliated with the non-canonical phyla *Nitrospirae*, *Chloroflexi* and candidate phylum AAMB5-125-24. Other novel putative SRB within the phylum *Nitrospirae*, ‘*Candidatus* *Sulfobium* mesophilum’ (Zecchin *et al.*, 2018) and ‘*Candidatus* *Nitrobium* versatile’ (Arshad *et al.*, 2017) were only distantly related to *Nitrospirae* MAG NIOZ-UU55 [< 51% amino acid identity (AAI), Fig. 5, Table S2]. Another phylogenetically related

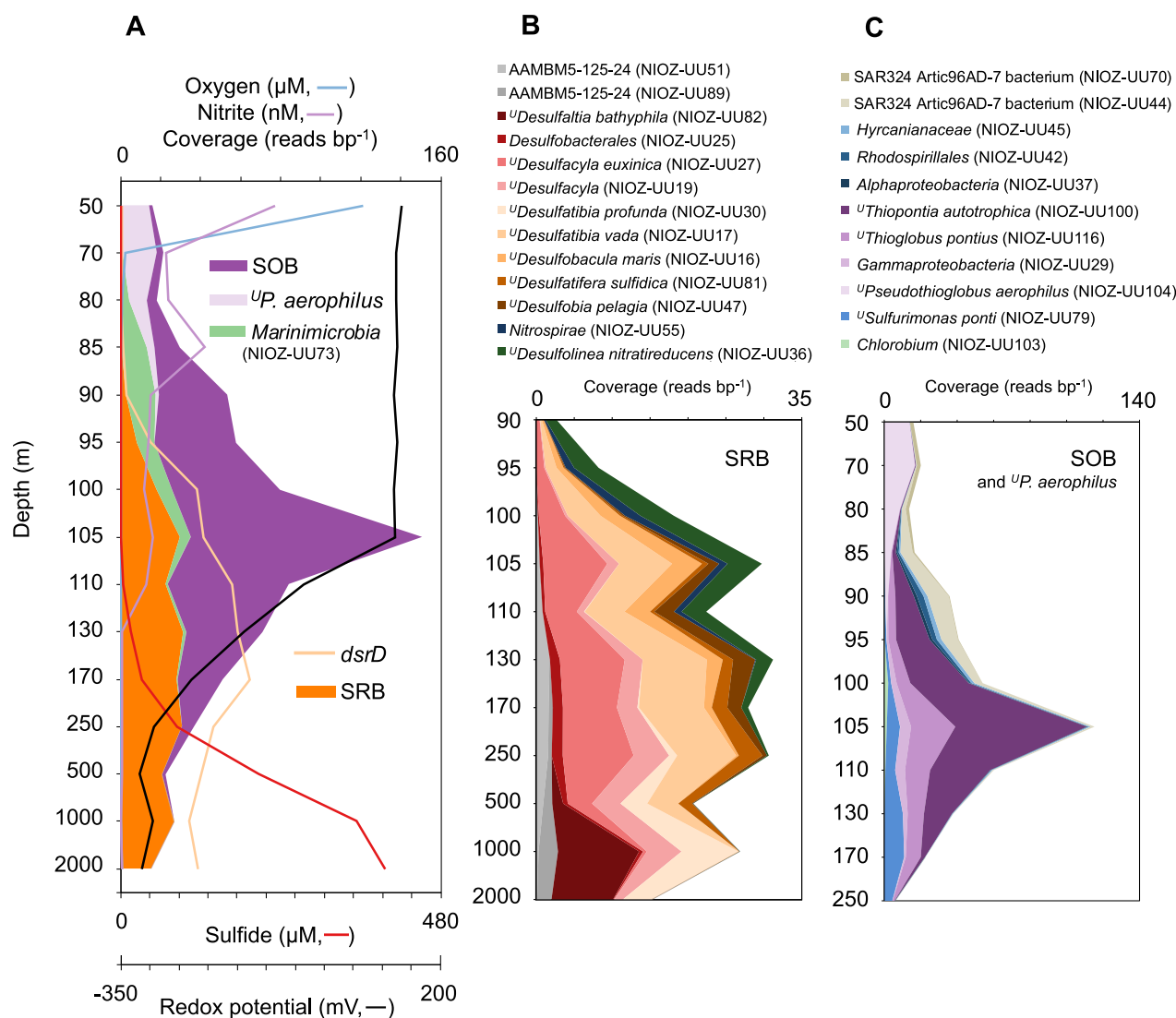


Fig. 3. Black Sea water column distribution of metagenome-assembled genomes (MAGs) of sulfur-cycling bacteria based on their genetic capacity.

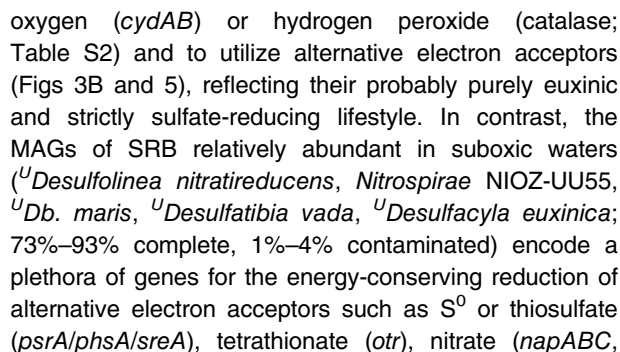
A. Physicochemical measurements and normalized cumulative metagenome coverage of all MAGs of putative sulfur-oxidizing bacteria (SOB) combined, *U.P. aerophilus* (NIOZ-UU104), *Marinimicrobia* (NIOZ-UU73), all *dsrD* genes combined and all MAGs of putative sulfate-reducing bacteria (SRB) combined in samples of 15 different depths of the Black Sea. The oxygen, nitrite and sulfide data correspond to the PHOXY cruise of June–July 2013 (Sollai *et al.*, 2019). The Black Sea metagenome was also constructed from samples taken during this cruise as detailed in Supporting Information Methods and Villanueva and colleagues (2020). Redox potential was measured during the 64PE408 NESSC/SIAM cruise of January–February 2016 from samples with a closely agreeing sulfide profile (Fig. S1).

B,C. Relative abundances of MAGs of (B) putative SRB and (C) putative SOB and *U.P. aerophilus* were based on normalized metagenome coverage. See Supporting Information Methods for details on the methodology and data processing. [Color figure can be viewed at wileyonlinelibrary.com]

SAG containing sulfate-reducing genes (AAMBM5-125-24) has been retrieved from the euxinic Zodletone Spring (Fig. 5; Youssef *et al.*, 2019). Despite the novelty revealed by this genome-centric approach, it should be noted that it did not encompass the complete diversity of putative SRB in the Black Sea detected according to the *dsrD* (Fig. 3A) and *dsrA* diversity (Fig. 4).

More so than taxonomic affiliation, functional gene annotation offers insight into the possible energy

metabolism(s) of these microorganisms. Metagenome mining yielded three *Desulfobacterales* MAGs from the Arabian Sea OMZ core (95%–96% complete, 0.7% contaminated) with a complete Dsr pathway but lacking the reductive marker *dsrD* (Fig. 5, Table S2, Supporting Information Methods). Moreover, they harbored oxidative instead of reductive *dsrA* genes possibly horizontally transferred from *Chlorobia* or SAR324 bacteria (Data S1). Together with the absence of *dsrD* and the presence



© 2020 The Authors. *Environmental Microbiology* published by Society for Applied Microbiology and John Wiley & Sons Ltd.,
Environmental Microbiology, **23**, 2834–2857

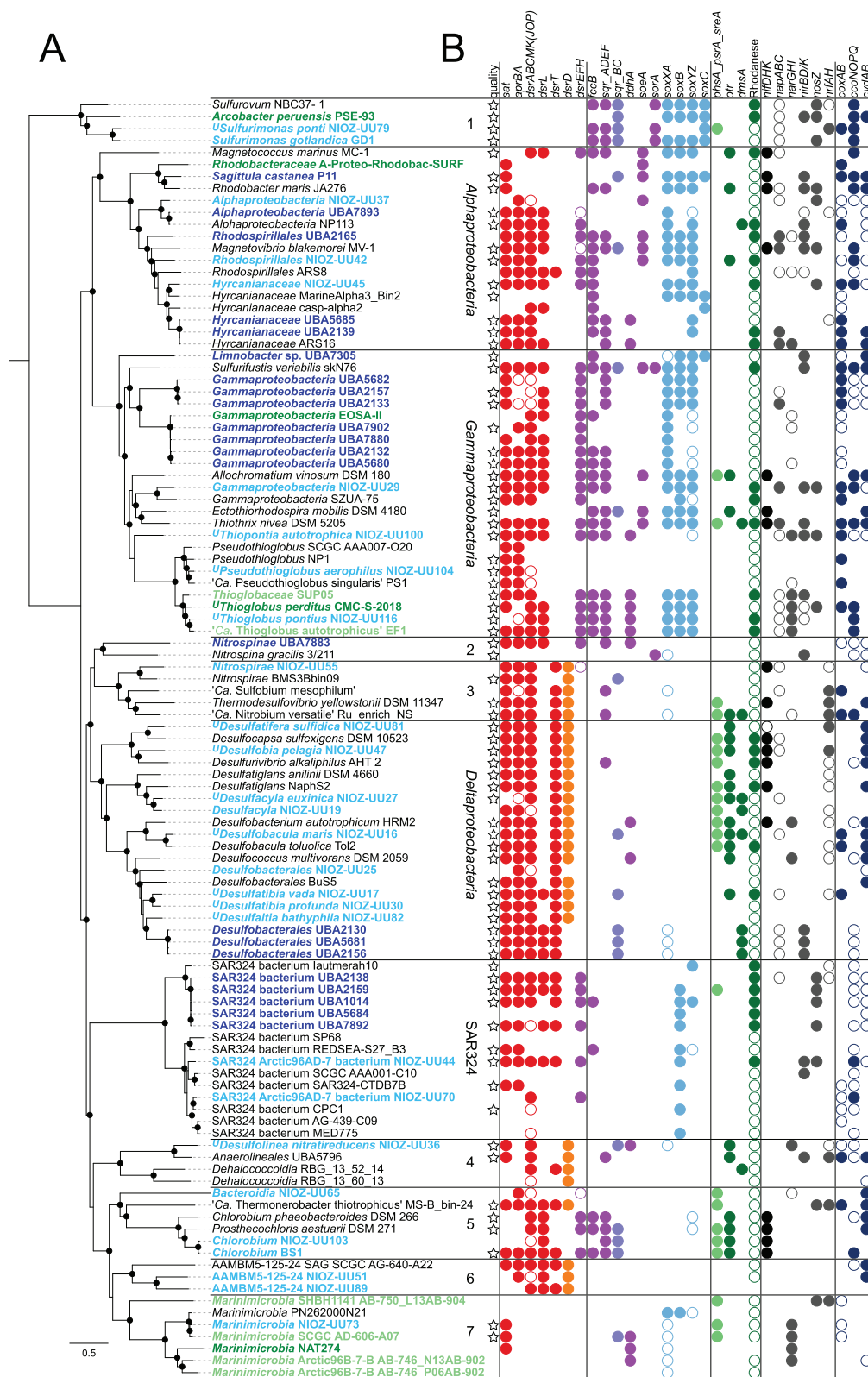


Fig. 5. A phylogenomic and genetic overview of important microbial players in the marine sulfur cycle of dysoxic marine water (DMW) environments.

narGHI) and nitrite (*otr*, *nirBD*, *nirK*, *nrfAH*). They also encode terminal oxidases (*coxAB*, *ccoNOPQ*, *cydAB*; Figs 3B and 5), which could be part of complete oxygen respiratory chains. These metabolic potentials are in line with a complex sulfur cycle interlinked with nitrogen cycling and oxygen intrusions (see the following sections). Whether the same SRB species as herein detected in the Black Sea are also present in other DMW requires additional research. However, the metagenomic data from the Black Sea offer a basis for such investigations ranging from 16S rRNA surveys to genome-centric genomics. Expression studies are required to investigate the metabolism of SRB in DMW, and whether they shift their metabolism in response to changing conditions. For instance, the expression of *Desulfocapsa*-related nitrogen fixation (*nif*) genes in the suboxic and upper euxinic zones of the Black Sea (Kirkpatrick et al., 2018) suggests that the *nif*-encoding SRB *^UDesulfatiferia sulfidica* (99% complete, 2% contaminated) and *^UDesulfobia pelagia* (96% complete, no contamination) could be actively fixing nitrogen. These genes are also encoded by *Nitrospirae* NIOZ-UU55 (73% complete, 1% contaminated). This supports a growing body of evidence for nitrogen fixation by putative SRB in DMW (Jayakumar et al., 2012; Bonnet et al., 2013; Loescher et al., 2014; Christiansen and Loescher, 2019).

Particles as microhabitat

Sulfate is thermodynamically an inferior electron acceptor to nitrate and nitrite (Table S3), implying that denitrifying microorganisms will outcompete SRB for electron donors in suboxic waters and OMZ cores. How then is dissimilatory sulfate reduction sustained, especially in nitrite- and nitrate-rich OMZ cores? It has been postulated that SRB

occupy microhabitats inside organic particles, in which nitrate and nitrite have already been depleted (Fuchsman et al., 2011; Wright et al., 2012). These organic particles or 'marine snow' (Alldredge and Silver, 1988) are particularly abundant in suboxic waters (Karl and Knauer, 1991; Taylor et al., 2001; Sorokin, 2002) and OMZs (Whitmire et al., 2009; Roullier et al., 2014), compared to other regions of the oceanic water column. When oxygen levels drop below ~25 µM, particles of the predominant size range (100–200 µm in diameter; Roullier et al., 2014) develop inner anoxic microhabitats due to limitation of oxygen diffusion (Shanks and Reeder, 1993; Klawonn et al., 2015; Ploug and Bergkvist, 2015). This implies that in nitrate-rich dysoxic waters, particles will develop a nitrate-depleted core, as nitrate rarely exceeds a concentration of 25 µM in DMW and diffuses slower than oxygen (Fuchsman et al., 2019). Thus, sulfate-reducing microhabitats may be abundant in non-sulfidic DMW.

SRB in seawater seem to be more abundant in particles than in free suspension. The 16S rRNA gene sequences of canonical deltaproteobacterial SRB were found to be predominantly particle-associated (> 30 µm) in marine suboxic zones (Fuchsman et al., 2011; Suter et al., 2017, 2018) and the ETNP OMZ core (Fuchsman et al., 2017). Moreover, reductive *dsrA* genes in the ETNP OMZ core were almost exclusively detected in the particle (> 30 µm) fraction, whereas oxidative *dsrA* genes showed no specific particle association (Saunders et al., 2019). This particle-bound lifestyle causes SRB to be significantly underrepresented in some molecular ecological studies. It is common practice to employ a pre-filter step for the collection of biomass to remove eukaryotes (1.6–10 µm pore size cut-off), thus also removing particles and particle-associated SRB. This methodology has been applied for investigations in DMW (Canfield et al., 2010; Stewart et al., 2012; Ulloa et al., 2012;

A. An unrooted phylogenomic maximum-likelihood tree constructed from a concatenated alignment of 120 single-copy household genes (Supporting Information Methods). Phylogenetic clades were identified, with numbers indicating the following lineages: 1, *Campylobacterota*; 2, *Nitrospinae*; 3, *Nitrospirae*; 4, *Chloroflexi*; 5, *Bacteroidetes*; 6, candidate phylum AAMBM5-125-24; 7, *Marinimicrobia*. Black dots indicate support by > 95% out of 1,000 ultra-fast bootstraps. The scale bar indicates substitutions per site. The tree includes data from all available genomes (March 2020) from DMW sites (bold, coloured by environment following the colour code of Fig. 1) that contain dissimilatory sulfur genes, and relevant reference genomes (black). The superscript prefix '*U*' indicates uncultured species for which a taxonomy has been proposed based on a high-quality genome, functional annotation and environmental distribution (Konstantinidis et al., 2017) with the genome sequences as type material (Chuvpochina et al., 2019; Murray et al., 2020; Supporting Information Protologue).

B. An overview of the presence of functional genes enabling conversions of sulfur, nitrogen and oxygen, following the colour scheme of Fig. 2. Shortly, red indicates core genes of the Dsr/rDsr pathways, orange indicates *dsrD*, purple indicates *dsrEFH* and various oxidative sulfur genes, light blue indicates *sox* genes, light-green indicates *phs/psr/sre* genes, dark-green indicates various (potentially) reductive sulfur genes, black/dark grey indicates nitrogen genes, dark blue indicates oxygen reduction genes. The presence of the indicated functional genes or gene clusters is shown with filled circles; open circles reveal incomplete gene clusters. For 'Rhodanese', filled circles indicate 10 or more rhodanese domains (Supporting Information Methods). Stars distinguish the high-quality genomes (> 80% complete, < 5% contaminated) from the medium-quality genomes (> 50% complete, < 10% contaminated) analysed. Only two low-quality metagenome-assembled genomes, that is, that of *Dehalococcoidia* RBG_13_52_14 (35% complete, 2% contaminated) and the population genome of *Gammaproteobacteria* EOSA-II composed of multiple combined single-cell amplified genomes (63% complete, 21% contaminated), were also included. A comprehensive overview of genome origin, quality, classification, annotation and average amino acid identity (AAI) between genomes can be found in Table S2. [Color figure can be viewed at wileyonlinelibrary.com]

Ganesh *et al.*, 2014; Hawley *et al.*, 2014), which may explain why SRB sequences were present in low abundance or absent. In contrast, studies omitting a pre-filter have identified SRB sequences in substantial proportion (Neretin *et al.*, 2007; Fuchsman *et al.*, 2011; Carolan *et al.*, 2015; Rodriguez-Mora *et al.*, 2016; Saunders *et al.*, 2019). Particle sinking in standard Niskin sampling bottles also caused bias against particles and SRB sequences (Suter *et al.*, 2017). Thus, complete circumvention of these potential biases requires *in situ* filtration devices, which have occasionally been used in DMW (Lavik *et al.*, 2009; Marschall *et al.*, 2010; Sollai *et al.*, 2019). *In situ* filtration was also applied for obtaining the Black Sea MAGs presented herein, indeed resulting in higher estimated relative abundances of SRB than found by Neretin and colleagues (2007) in both the suboxic zone (< 13% vs. < 2% of all bacteria) and the euxinic zone (< 20% vs. < 5%, Supporting Information Methods). Since these estimated fractional abundances may still be biased by DNA extraction methods, ideally an extraction-independent method should also be applied such as fluorescence *in situ* hybridization of functional genes (Barrero-Canosa *et al.*, 2017). To achieve comparability between different studies, similar sampling methodology with minimal bias is essential and should be carefully evaluated.

Sulfur-oxidizing bacteria

The oxidative part of the sulfur cycle starts with the competition between SOB and abiotic reactions for sulfide (Luther *et al.*, 2011). Depending on the sulfide oxidation route, a range of possible sulfide oxidation products can be formed (Fig. 2), having a significant effect on the rest of the biogeochemistry in DMW. Oxidized metals such as manganese oxide (MnO₂) or ferric (oxy)hydroxides are so efficient in catalyzing sulfide oxidation (Yao and Millero, 1993; Ma *et al.*, 2006) that even at micromolar concentrations MnO₂ is thought to be abiotically responsible for the bulk of the sulfide oxidation in systems with broad stable chemoclines and low oxygen flux such as the Black Sea (Jørgensen *et al.*, 1991; Kononov *et al.*, 2003; Trouwborst *et al.*, 2006; Stanev *et al.*, 2018) and Cariaco Basin (Ho *et al.*, 2004). Chemical oxidation of sulfide produces SCIs such as S⁰ and thiosulfate, which are commonly detected in euxinic marine waters (Jørgensen and Bak, 1991; Zopfi *et al.*, 2001; Li *et al.*, 2008; Kamysny *et al.*, 2013; Findlay *et al.*, 2014). Like sulfide, SCIs can be converted by SOB to sulfate as energy source.

Microorganisms that oxidize sulfur compounds possess widely differing metabolisms, including autotrophy or heterotrophy, and chemotrophy or phototrophy (Dahl, 2017). Some SOB oxidize a wide range of sulfur

compounds as primary energy source, whereas others facultatively oxidize specific sulfur compounds such as thiosulfate as supplementary energy source (Sorokin, 2003). Photolithotrophic SOB – green or purple sulfur bacteria – can become dominant if euxinic marine waters overlap with the photic zone in shallow waters (Findlay *et al.*, 2015; Pjevac *et al.*, 2015; Findlay *et al.*, 2017; Pjevac *et al.*, 2019). The deeper the chemocline, the smaller the population and role of phototrophic SOB, exemplified by low-light-adapted *Chlorobium* bacteria in the Black Sea (approximately 100 m depth; Overmann *et al.*, 1992; Manske *et al.*, 2005; Marschall *et al.*, 2010). They are outnumbered by chemolithotrophic SOB, with gammaproteobacterial SUP05 bacteria (Lavik *et al.*, 2009; Canfield *et al.*, 2010; Glaubitz *et al.*, 2013) and sulfur-oxidizing *Campylobacteriales* bacteria such as *Sulfurimonas* species (Grote *et al.*, 2008; Schunck *et al.*, 2013; Callbeck *et al.*, 2019) as foremost examples. However, the biogeochemical impact on element cycling is not always related to cellular abundance (Pester *et al.*, 2012; Hausmann *et al.*, 2019), exemplified by magnetotactic *Magnetococcus*-related bacteria that shuttle the scarcely available phosphate from the Black Sea chemocline into the euxinic zone (Schulz-Vogt *et al.*, 2019), which suggests a sulfur-oxidizing physiology akin to other *Magnetococcus* species (Bazylnski *et al.*, 2013). This underscores the importance of using multiple approaches when studying functional groups of microorganisms, including sulfur-cycling bacteria. Here, we will mainly discuss well-studied chemolithotrophic SOB specifically abundant in OMZ core waters and other deep DMW.

SUP05 bacteria

Based on 16S rRNA gene surveys, specific gammaproteobacterial bacteria belonging to the SUP05 clade and closely related to known sulfur-oxidizing symbionts have been identified as abundant putative SOB in DMW (reviewed by Wright *et al.*, 2012). The capacity of these bacteria for chemolithoautotrophic nitrate reduction – most probably coupled to the oxidation of sulfide and/or SCIs – in DMW was strongly indicated by stable isotope probing experiments with labelled inorganic carbon (Grote *et al.*, 2008; Glaubitz *et al.*, 2010) and correlations with rate measurements of nitrate reduction and dark carbon fixation (Lavik *et al.*, 2009; Schunck *et al.*, 2013). Direct cell counts with fluorescent probes showed that SUP05 bacteria may form a dominant group of the microbial community; they comprised up to 50% of the microbial population in euxinic shelf waters off Namibia and Peru (< 3 × 10⁶ cells ml⁻¹; Lavik *et al.*, 2009; Callbeck *et al.*, 2018), up to 17% in the ETSP OMZ core (5 × 10⁵ cells ml⁻¹; Callbeck *et al.*, 2018), up to 30% at an oxic–

euxinic interface in the Baltic Sea (4×10^5 cells ml⁻¹, Landsort Deep) and up to 10% and 13% in the suboxic and euxinic zone of the Black Sea respectively ($< 7 \times 10^4$ cells ml⁻¹; Glaubitz et al., 2013).

The presumed physiology of SUP05 bacteria was supported by the presence in their MAGs of genes for sulfide oxidation (*sqr*, *fccAB*), the 'Sox' thiosulfate oxidation pathway (*soxXABYZ*), the rDsr sulfur oxidation pathway (*sat*, *aprBA*, *dsrABCMK*, *dsrEFH*; Figs 2 and 5), nitrate reduction (*narGHIJ*) and inorganic carbon fixation through the Calvin–Benson–Bassham cycle (Walsh et al., 2009; Canfield et al., 2010; Murillo et al., 2014; Callbeck et al., 2018). This physiology has been confirmed by cultivation experiments of the only current SUP05 isolate, 'Candidatus Thioglobus autotrophicus' (Shah et al., 2017), which showed growth with sulfide, thiosulfate, thiotaurine and stored S⁰ as energy source (Shah et al., 2019). The four available SUP05 genomes from DMW show variation in the presence of other nitrogen-respiration genes (*nirK*, *nirS*, *nirBD*, *norCB*, *nosZ*; Fig. 5) and oxidative phosphorylation genes (*coxBAC*, *ccoNOPQ*, cytochrome *bc₁* complex genes; Fig. 5), indicating metabolic diversification of the strains within this clade. Corresponding with their high abundance, SUP05 bacteria generally dominate the detection of rDsr pathway genes, transcripts and proteins in DMW (Canfield et al., 2010; Stewart et al., 2012; Hawley et al., 2014). The sister clade ARCTIC96BD-19 is represented by 'Candidatus Thioglobus singularis' (Marshall and Morris, 2013), but ARCTIC96BD-19 genomes are too divergent from SUP05 genomes to consider them the same genus (63%–66% AAI, Table S2). 'Ca. T. singularis' has an organoheterotrophic aerobic lifestyle and does not oxidize sulfur (Spietz et al., 2019). These features are probably representative of all ARCTIC96BD-19 bacteria, based on the absence of most sulfur oxidation genes in their genomes (Swan et al., 2011; Fig. 5) and a preference for oxic waters (Wright et al., 2012; Fig. 3A; *U. aerophilus*). To reflect their distinct taxonomy and physiology, we suggest to rename the ARCTIC96BD-19 clade to *Pseudothioglobus* (Supporting Information Protologue).

The affinity of SUP05 bacteria for sulfide is higher than reported for any other bacterium or substrate (Crowe et al., 2018), demanding a re-evaluation of the existing definition of euxinia. Currently, the sulfide concentration threshold to distinguish non-sulfidic from euxinic conditions commonly falls in the range of 0.5–1 µM. This threshold is similar to the in vitro Michaelis–Menten half-saturation constant (K_m) of 2 µM of purified high-affinity Sqr proteins (Schutz et al., 1997; Brito et al., 2009) and the K_m found for phototrophic SOB (> 0.8 µM; Van Gemerden, 1984). However, the estimated K_m of SUP05 bacteria is much lower (25–340 nM; Crowe et al., 2018).

The most widely used method for determining sulfide concentrations has a sulfide detection limit of 0.1 µM (Cline, 1969; Jørgensen et al., 1991; Zopfi et al., 2001), hence falling within this estimated range. Thus, we suggest it is biologically sound and practically feasible to use a sulfide threshold of at most 0.1 µM to define euxinia, at least in marine environments. However, an even lower threshold would be more accurate, as SUP05 bacteria consume sulfide at < 5 nM (Crowe et al., 2018). Such low concentrations can be detected and quantified with sensitive voltammetric sensors (Luther et al., 1991; Luther et al., 2008). The findings of Crowe and colleagues (2018) illustrate the value of studies that quantify properties such as substrate affinity and should motivate further investigation, for instance with respect to sulfide toxicity or substrate affinity for sulfide of other key SOB. Such studies are required to advance biogeochemical models that explicitly take microbial community composition and function into account by integrating omics data (Reed et al., 2014; Louca et al., 2016).

Campylobacterota

The SOB of the phylum *Campylobacterota* (formerly *Epsilonproteobacteria*; Waite et al., 2017, 2018) are more diverse and environment-specific than the common SUP05 bacteria. The most widespread *Campylobacterota* genus in DMW is *Sulfurimonas*. Members of this genus dominate the upper euxinic zone of the Black Sea and Baltic Sea at a count of 15% to 30% of all microorganisms ($< 2 \times 10^5$ cells ml⁻¹) and outnumber SUP05 bacteria (Brettar et al., 2006; Grote et al., 2008; Glaubitz et al., 2010). Members of another *Campylobacterota* genus, *Arcobacter*, are generally less abundant in euxinic basins (Glaubitz et al., 2008; Fuchsman et al., 2012; Rodriguez-Mora et al., 2013), but proliferate in euxinic shelf waters during sulfidic events ($< 25\%$ of all cells; $< 1 \times 10^6$ cells ml⁻¹; Lavik et al., 2009; Schunck et al., 2013; Callbeck et al., 2019). Four *Campylobacterota* isolates have thus far been obtained from DMW, all facultative anaerobes capable of sulfur oxidation and nitrate reduction: *Sulfurimonas gotlandica* (Grote et al., 2012) and 'Candidatus Sulfurimonas baltica' (Henkel, 2019), both from a redoxcline in Gotland Basin; 'Candidatus Sulfurimonas marisnigri' from the Black Sea euxinic zone (Henkel et al., 2019); and *Arcobacter peruensis* from euxinic coastal waters off Peru (Callbeck et al., 2019). *A. peruensis* was only demonstrated to use sulfide as energy source (Callbeck et al., 2019), whereas the *Sulfurimonas* species were grown with sulfide, SCIs and H₂ (Labrenz et al., 2013; Henkel, 2019). As is common in *Campylobacterota* members, the genomes of *S. gotlandica* and *A. peruensis* lack rDsr genes and these SOB are presumed to oxidize

sulfur compounds through a variant of the Sox pathway encoded by two operons (*soxXY₁Z₁AB* and *soxCY₂Z₂*; Meier *et al.*, 2017; Pjevac *et al.*, 2018; Götz *et al.*, 2019; Figs 2 and 5). We recovered a *Sulfurimonas* MAG from the Black Sea (*^USulfurimonas ponti*), which also lacks most Sox genes despite being virtually complete (97% completeness, 4% contamination, Fig. 5). *^US. ponti* may oxidize sulfide incompletely to S⁰, or use an alternative route such as the Hdr-like sulfur oxidation pathway (Boughanemi *et al.*, 2016).

The heterotrophic *A. peruensis* was not capable of fixing inorganic carbon and requires an organic carbon source such as acetate (Callbeck *et al.*, 2019), whereas the autotrophic *Sulfurimonas* species fixed inorganic carbon for growth, presumably through the reverse tricarboxylic acid cycle (Grote *et al.*, 2008; Henkel, 2019). In euxinic coastal waters, sufficient organic carbon can be available to allow *A. peruensis* to successfully compete for sulfur substrate with autotrophic SOB by achieving a higher carbon assimilation rate and therefore probably also a higher growth rate (Callbeck *et al.*, 2019). *Sulfurovum* species of the *Campylobacterota* phylum were highly abundant during sulfidic events in coastal waters (Lavik *et al.*, 2009; Schunck *et al.*, 2013; Callbeck *et al.*, 2019) and possibly outnumber *Sulfurimonas* in Cariaco Basin (Rodriguez-Mora *et al.*, 2013; Rodriguez-Mora *et al.*, 2016; Taylor *et al.*, 2018). Previous cultivation- and metagenomics-based studies of *Sulfurovum* members have primarily addressed hydrothermal vent habitats. They revealed metabolic similarity to *Sulfurimonas* species with respect to sulfur oxidation, carbon fixation, and nitrate reduction (Yamamoto *et al.*, 2010; Giovannelli *et al.*, 2016; Jeon *et al.*, 2017; Meier *et al.*, 2017; Mori *et al.*, 2018). However, one notable exception is *Sulfurovum aggregans*, which cannot oxidize sulfur but instead reduces it (Mino *et al.*, 2014). As such, multiple biochemical roles are possible for *Sulfurovum* species in DMW.

Sulfur-oxidizing autotrophs such as *Sulfurimonas* and SUP05 bacteria compete for very similar niches through different strategies. The metabolically specialized, streamlined (< 1.5 Mbp genomes) and non-motile SUP05 bacteria prefer stable conditions, while the motile and more adaptable *Sulfurimonas* species benefit from a less stable chemocline with more mixing of sulfide, nitrate and oxygen (Rogge *et al.*, 2017; Taylor *et al.*, 2018). Furthermore, SUP05 bacteria are most abundant at low-sulfidic conditions (< 5 µM; Glaubit *et al.*, 2013; Rogge *et al.*, 2017), which may be due to their unparalleled high affinity for sulfide (Crowe *et al.*, 2018) and their capability to store S⁰ for later usage when external substrates are absent (Shah *et al.*, 2019). In euxinic basins, *Sulfurimonas* species can thrive simultaneously with SUP05 bacteria, but have a relative abundance peak in slightly

deeper, more sulfidic waters (median 17 µM; Fig. 3C; Rogge *et al.*, 2017). Here, the electron acceptors oxygen and nitrate are irregularly available (Konovalov *et al.*, 2003; Glaubit *et al.*, 2010; Glaubit *et al.*, 2013). *Sulfurimonas* species have adapted to these conditions through motility and chemotaxis towards nitrate-rich conditions (Grote *et al.*, 2012), which is sustained by energy storage in the form of polyphosphate (Möller *et al.*, 2019). Furthermore, *Sulfurimonas* species probably conserve more energy from nitrate than SUP05 bacteria, since instead of partial denitrification to nitrite (Shah *et al.*, 2017) or possibly nitrous oxide (Walsh *et al.*, 2009; Hawley *et al.*, 2014), *S. gotlandica* can perform complete denitrification to nitrogen gas (Labrenz *et al.*, 2013) and *^US. ponti* could perform ammonification (*nrfAH*, Fig. 5).

Intriguingly, 'Ca. *S. marisnigri*' is the first bacterium demonstrated to couple sulfur oxidation to the reduction of MnO₂ to Mn²⁺ for growth (Henkel *et al.*, 2019). This trait could be highly beneficial in euxinic basins such as the Black Sea since in contrast to oxygen and nitrate, MnO₂ is in constant supply – albeit at low concentrations – since it is particulate and sinks (Tebo, 1991; Konovalov *et al.*, 2004; Trouwborst *et al.*, 2006). This metabolic capacity could also answer the long-pending question of how high carbon fixation rates are sustained in euxinic waters without sufficient nitrate, nitrite or oxygen (Jørgensen *et al.*, 1991; Taylor *et al.*, 2001; Ho *et al.*, 2004; Jost *et al.*, 2010; Kirkpatrick *et al.*, 2018). Indeed, a reaction–diffusion model by Yakushev and colleagues (2007) required coupling of MnO₂ reduction to carbon fixation to reproduce the observed chemical profiles. There are indications that the reaction rates of abiotic and microbial sulfide oxidation by Mn in euxinic basins are in the same order of magnitude (Jørgensen *et al.*, 1991; Sorokin *et al.*, 1995; Henkel, 2019). However, there is currently no insight into the *in situ* abundance of 'Ca. *S. marisnigri*'. Like *S. gotlandica* (Grote *et al.*, 2012), it does not affiliate with the locally abundant *Sulfurimonas* GD17 subclade (95%–96% 16S rRNA gene sequence similarity). Further investigation through molecular studies is currently challenging, as the enzymatic pathway allowing MnO₂ reduction is unknown. Nevertheless, these findings have large consequences for our view on euxinic biogeochemistry, as sulfide-driven denitrification and nitrogen loss may effectively be bypassed. Mn-dependent sulfide oxidation could even result in a fixed nitrogen gain, since both *S. marisnigri* and *S. baltica* can apparently fix nitrogen (Henkel, 2019), confirming a recently published hypothesis (Kirkpatrick *et al.*, 2018).

Other sulfur-oxidizing lineages

As described above, the physiology of some of the key SOB has been explored in some detail, but other

microbial players are waiting to be described. Predominantly genomic studies point to a wide phylogenetic diversity of poorly studied SOB for which important ecological or biogeochemical roles in DMW have been demonstrated or are strongly indicated. The class *Gammaproteobacteria* probably contains relevant SOB that do not affiliate with the SUP05 clade, notwithstanding their key role. Firstly, genomes of the EOSA-II lineage were retrieved from coastal waters and the OMZ in Southern Pacific waters (Fig. 5), actively expressing sulfur oxidation genes in the ETSP OMZ core (Plominsky et al., 2018). Secondly, the gammaproteobacterial BS-GSO2 clade was detected in the Black Sea as autotrophic lineage with a peak in relative abundance at the euxinic interface together with SUP05, suggesting it uses sulfur as energy source (Glaubitx et al., 2010). This clade is especially noteworthy since sequencing studies indicate that BS-GSO2 bacteria may outnumber SUP05 bacteria in the Black Sea (Fuchsman et al., 2011; Kirkpatrick et al., 2018; Fig. 3C) and Cariaco Basin (Suter et al., 2018; Taylor et al., 2018). The only currently available BS-GSO2 MAG is that of *Thiopontia autotrophica* obtained from the Black Sea metagenomes analyzed herein (NIOZ-UU100, 93% complete, 0.1% contamination) with 99% 16S rRNA gene identity with the original BS-GSO2 sequence reported by Glaubitx and colleagues (2010). Indeed, *T. autotrophica* possesses the genes for sulfur oxidation (rDsr) and the Calvin–Benson–Bassham cycle, but it differs from SUP05 bacteria in lacking most Sox genes and encoding a complete denitrification pathway (Fig. 5). It thus seems the BS-GSO2 clade has been overshadowed by SUP05, yet may successfully compete for the same niche.

Bacteria of the uncultivated SAR324 candidate phylum have been abundantly and ubiquitously detected in DMW (Fuchsman et al., 2011; Wright et al., 2012; Beman and Carolan, 2013; Lüke et al., 2016; Suter et al., 2018; Fig. 3C). These SAR324 bacteria encode the rDsr sulfur oxidation pathway, which could enable them to oxidize sulfur for energy (Swan et al., 2011; Sheik et al., 2014; Fig. 5). Notably, they may couple this process to the reduction of the greenhouse gas nitrous oxide as they encode nitrous oxide reductase genes (*nosZ*, Fig. 5). The uncultured alphaproteobacterial family *Hyrcanianaceae* also harbors putative SOB with genomes retrieved from hydrothermal vent plumes (Zhou et al., 2020), the Arabian Sea OMZ core, and the Black Sea (Fig. 5). Low-abundance SOB could still significantly alter their environment, for instance through diazotrophy or N₂ fixation. Examples of such SOB are the heterotrophic alphaproteobacterium *Sagittula castanea* isolated from euxinic Peru shelf waters (Martínez-Pérez et al., 2018) or photolithoautotrophic *Chlorobium* strains (Overmann et al., 1992; Manske et al., 2005; Marschall et al., 2010;

nifDHK; Figs 3C, 5). Finally, marine *Nitrospinae* members have been shown to oxidize nitrite (Lücker et al., 2013; Sun et al., 2019; Kitzinger et al., 2020), but the presence of a complete rDsr pathway in a *Nitrospinae* MAG from the ETSP OMZ core (UBA7883, 97% complete, 1% contaminated) opens up the possibility that some members may use sulfur as additional or alternative energy source (Fig. 5).

Sulfur-reducing and sulfur-disproportionating bacteria

The SCIs formed or introduced in DMW could form the substrate for further oxidation by SOB, but could also be used as electron acceptor by sulfur-reducing microorganisms, or as substrate for disproportionation (Fig. 2), thus shortcutting the sulfur cycle as has been suggested for other aquatic ecosystems (Tonolla et al., 2004; Wilbanks et al., 2014; Bhatnagar et al., 2020). Similar to abiotic sulfide oxidation, SOB may introduce SCIs in DMW through oxidation of sulfide to S⁰ (Dahl, 2017), which is stored intracellularly by the abundant SUP05 bacteria (Shah et al., 2019). Part of this S⁰ may be released into the environment due to grazing of SUP05 bacteria by protists (Lin et al., 2007; Glaubitx et al., 2008; Anderson et al., 2013) or due to lysis by SUP05-infecting viruses (Cassman et al., 2012; Anantharaman et al., 2014; Roux et al., 2014; Roux et al., 2016). Additionally, S⁰ has been observed to be introduced into OMZs through the drifting off of S⁰ produced in coastal waters experiencing sulfidic events (Callbeck et al., 2018). These findings highlight the importance of considering full models of the sulfur cycle and avoiding simplified two-reaction representations consisting only of dissimilatory sulfate reduction and chemolithotrophic re-oxidation of sulfide to sulfate (Ulloa et al., 2012; Hawley et al., 2014). The extent of the other fluxes is currently a major unknown factor in DMW, with a large impact on the routes of sulfur-driven carbon fixation and on the occurrence of other linkages with the carbon and nitrogen cycles.

Sulfur-reducing bacteria and Marinimicrobia

The consumption of SCIs in DMW is thought to proceed through a combination of oxidation, reduction and disproportionation (Sorokin et al., 1995; Zopfi et al., 2001; Sørensen and Canfield, 2004). The relative importance of these consumption routes is currently unknown. Sulfur isotope fractionation studies offer little insight, since the measurements in various euxinic marine waters can be explained by sulfate reduction as well as sulfur reduction or disproportionation (Li et al., 2010; Kamysny et al., 2011). The reduction or disproportionation of S⁰ and thiosulfate is more exergonic than sulfate reduction under the conditions found in DMW (Supporting Information

Methods, Table S3), implying that SRB could gain more energy through these reactions. Many cultured SRB are able to reduce or disproportionate thiosulfate (Rabus *et al.*, 2015) through the Dsr pathway and thiosulfate reductase (PhsABC; Fig. 2; Burns and DiChristina, 2009) and may prefer this electron acceptor over sulfate (Jørgensen, 1990). Many anaerobic microorganisms use S^0 as electron acceptor (Rabus *et al.*, 2013) mediated by polysulfide reductase (PsrABC) or sulfur reductase (SreABC; Fig. 2; Laska *et al.*, 2003; Sorokin *et al.*, 2015). These three protein complexes (Phs, Psr, Sre) are complex iron–sulfur molybdoenzymes with such a close phylogenetic relationship and with so few characterized representatives, that distinction based on sequence is currently impossible (Hedderich *et al.*, 1998; Hinsley and Berks, 2002; Laska *et al.*, 2003; Duval *et al.*, 2008; Burns and DiChristina, 2009). Furthermore, various SOB also encode genes with similarity to *psrABC* (Wright *et al.*, 2014), of which the resulting enzymes may well act in reverse (Eddie and Hanson, 2013; Weissgerber *et al.*, 2013). Thus, the presence of *psr*-like genes in a genome suggests the capability of some form of dissimilatory sulfur conversion, but this requires further investigation.

The reduction of SCIs in DMW was used as energy metabolism by an organoheterotrophic *Shewanella* strain isolated from the Black Sea (Perry *et al.*, 1993). However, related microorganisms are unlikely to play a big role in DMW, as they have not been detected in microbial ecology studies. SRB remain probable candidates for mediating sulfur reduction, as several genomes of putative SRB retrieved from the Black Sea encoded *psr*-like genes and a tetrathionate reductase gene (*otr*) in addition to their sulfate-reducing genes (Fig. 5). Other MAGs from DMW metagenomes also showed possibly reductive *psr*-like genes, such as *Bacteroidia* NIOZ-UU65 from the Black Sea, a MAG of uncultivated clade SAR324 from the Arabian Sea OMZ core and three *Marinimicrobia* genomes (Fig. 5). Bacteria of the uncultivated candidate phylum *Marinimicrobia* (formerly known as Marine Group A and clade SAR406) are prevalent in DMW and contain genomic signatures of organoheterotrophy (Wright *et al.*, 2014; Bertagnoli *et al.*, 2017; Hawley *et al.*, 2017), a metabolism supported by DNA-based stable isotope probing incubations from the Black Sea (Suominen *et al.*, 2019). It has been suggested that *Marinimicrobia* may reduce S^0 based on the presence of *psrABC* genes (Wright *et al.*, 2014; Hawley *et al.*, 2017). As explained, we think it would be more accurate and unambiguous to broaden the hypothesis to *Marinimicrobia* having an unspecified dissimilatory sulfur metabolism. Furthermore, the preference for shallow waters with relatively oxidizing conditions by *Marinimicrobia* NIOZ-UU73 (Fig. 3A; 90% complete, no contamination) suggests that for this specific member, a facultative sulfur-oxidizing lifestyle is

more likely. Another *Marinimicrobia* MAG (PN262000N21, 98% complete, no contamination) encodes an almost-complete 'Sox' pathway conferring the potential for dissimilatory thiosulfate oxidation (Figs 2 and 5). The most straightforward path to revealing the energy metabolism of these uncultivated bacteria would be cultivation, isolation and characterization. Like genomes of SAR11 and SUP05 bacteria, *Marinimicrobia* genomes are extensively streamlined (Hawley *et al.*, 2017) implying that these bacteria are highly adapted to *in situ* conditions. Hence, cultivation may require natural seawater as medium, or recently designed synthetic alternatives (Henson *et al.*, 2016).

Sulfur-disproportionating bacteria

Sulfur disproportionation is the simultaneous oxidation and reduction of an SCI, typically leading to the production of both sulfate and sulfide, which is an uncommon microbial trait (Finster, 2008; Slobodkin and Slobodkina, 2019). The biochemistry of sulfur disproportionation is unresolved and may involve the Dsr pathway in *Deltaproteobacteria* such as *Desulfurivibrio alkaliphilus* (Thorup *et al.*, 2017) and *Desulfocapsa sulfexigens* (Finster, 2008; Finster *et al.*, 2013), and *psr*-like molybdoenzymes and rhodanese sulfurtransferases in other microorganisms such as *Desulfurella amilsii* (Florentino *et al.*, 2019). Although uncommon, disproportionation is probably influential in euxinic marine waters, as microorganisms growing through disproportionation of S^0 or thiosulfate could be cultivated from the euxinic Mariager Fjord (Sørensen and Canfield, 2004), and a diffusion–reaction model of Chesapeake Bay required the inclusion of S^0 disproportionation or reduction to explain the observed S^0 concentration profiles (Findlay *et al.*, 2017). In euxinic basins *Desulfocapsa* species could be involved, as their 16S rRNA genes were detected in the Cariaco Basin (Rodriguez-Mora *et al.*, 2015) as well as the Black Sea (Neretin *et al.*, 2007; Fuchsman *et al.*, 2011; Fuchsman *et al.*, 2012). This hypothesis is difficult to test with genomic data due to the unclear biochemistry behind S^0 disproportionation. Out of the MAGs obtained from the Black Sea, *^UDf. sulfidica* is the most closely related to *Desulfocapsa*. Yet, it has a markedly different gene repertoire than *Dc. sulfexigens*, without molybdoenzymes or high numbers of rhodanese genes. However, plenty other sulfur-disproportionating bacterial candidates with Dsr pathways, molybdoenzymes and high numbers of rhodanese genes remain (Fig. 5). Like *Dv. alkaliphilus* (Thorup *et al.*, 2017), some might oxidize sulfide in a disproportionation-dependent pathway including sulfide oxidation by Sqr (*^UDb. maris*, *^UDb. vada*, *^UDi. nitratireducens*, Arabian Sea OMZ core *Desulfobacterales*).

Since most characterized sulfur-disproportionating microorganisms can grow autotrophically (Finster *et al.*, 1998; Florentino *et al.*, 2016; Mardanov *et al.*, 2016; Slobodkin and Slobodkina, 2019), their presence could spell a role in the high rates of carbon fixation that are generally observed within euxinic marine waters just below the euxinic interface (Jørgensen *et al.*, 1991; Taylor *et al.*, 2001). This phenomenon is commonly attributed solely to sulfur oxidation by chemolithoautotrophic SOB (Grote *et al.*, 2008; Glaubitz *et al.*, 2010). Our hypothesis is in agreement with the stimulation of carbon fixation measured upon the addition of thiosulfate or polysulfide to euxinic samples from the Baltic Sea (Labrenz *et al.*, 2005; Jost *et al.*, 2010). However, these findings could be influenced by artificial introduction of oxygen (De Brabandere *et al.*, 2012) and are in need of further testing. In general, more dedicated experimental work is needed to quantitatively constrain SCI-consuming reactions, such as was done for a freshwater lake (Findlay and Kamysny, 2017). Another unexplored factor in the marine sulfur cycle is the cycling of organic sulfur compounds, which has been highlighted for marine sediments (Wasmund *et al.*, 2017). Such processes may be important in DMW, as dimethylsulfide oxidation genes (*ddhA*) were detected in genomes of SUP05 bacteria, *U. autotrophica* and

Marinimicrobia, and dimethylsulfoxide reductase genes (*dmsA*) in MAGs of *Desulfacyla* species, *U. Db. maris* and OMZ *Desulfobacterales*. Future studies should address to what extent reactions of SCIs and organic sulfur compounds contribute to the overall sulfur cycle, and whether this is affected by environmental conditions.

Conclusions and future perspectives

This review has presented a compendium of the current insights into sulfur-cycling bacteria in DMW (Fig. 6). Based on current experimental evidence, it is difficult to investigate *in situ* sulfur reactions beyond sulfide oxidation and sulfate reduction. Euxinic marine waters are thought to host a complex network of reactions, whereas this remains more uncertain for suboxic waters and OMZ cores. Molecular studies have revealed a high diversity of putative SRB and SOB, which we expect to be explored further and consolidated over the coming years, specifically with the use of improved genome-centric metagenomics. Sampling methods without bias against particle-associated microorganisms can give an accurate and intercomparable view on diversity and abundance across DMW, specifically of SRB. With this in mind, it could be evaluated whether the community of novel

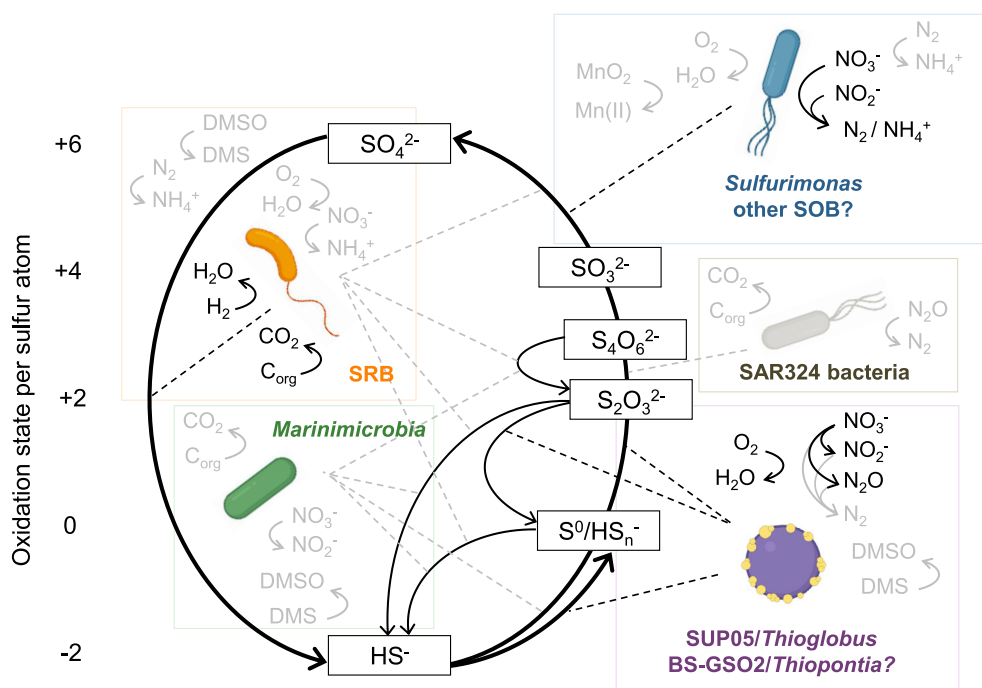


Fig. 6. Conceptual ecophysiological model of the sulfur cycle and the involved microorganisms in DMW.

Question marks and the light gray colour are used when there are indications for involvement of specific microorganisms and/or processes but definitive proof is lacking. Bacterial images were created with BioRender. [Color figure can be viewed at wileyonlinelibrary.com]

putative SRB genomically revealed by us in the Black Sea is representative of euxinic marine basins and perhaps DMW in general. Together, the diverse sulfur-cycling bacteria form a myriad of connections with other elemental cycles. SUP05 bacteria fix inorganic carbon with energy from very low sulfide concentrations, warranting a biologically meaningful reshaping of our concept of euxinia. These insights into sulfide affinity are crucial building blocks for biogeochemical modelling efforts, which could be further improved by estimations of critical biological parameters including biomass yield and sulfide tolerance. Metatranscriptomics and meta-proteomics experiments might play a role in testing under which conditions SRB use their genomic potential for respiration of oxygen and diverse nitrogen compounds. Notably, genomic classifications of some bacteria as 'SRB' are for now putative, as the Dsr pathway on which this is based could also confer other forms of sulfur metabolism, such as sulfur reduction, disproportionation or oxidation. Similarly, the lack of fundamental insight into the relation of sequence and function of prevalent Psr-related molybdoenzymes hinders metabolic predictions. Thus, genomic-based research can find strong support in cultivation experiments and the *in vitro* study of heterologously expressed sulfur enzymes. The power of cultivation has been showcased by the isolation of SUP05, *Sulfurimonas* and *Arcobacter* bacteria, and specifically that of the Mn-reducing and probably nitrogen-fixing 'Ca. *S. marisnigri*'. The cultivation and isolation of SRB from DMW is also feasible (Teske *et al.*, 1996; Zopfi *et al.*, 2001; Sørensen and Canfield, 2004; Finster and Kjeldsen, 2010), but more challenging than cultivation from sediment due to rapid oxidation of sampled water. These efforts could be facilitated by genome-guided cultivation (Gutleben *et al.*, 2018) or a reverse genomic approach (Cross *et al.*, 2019). Finally, the factors controlling nitrogen fixation by SOB and SRB require further investigation, as this may be an important factor in the expected development of open-ocean euxinia (Ulloa *et al.*, 2012). The advances as summarized and predicted herein will enable the construction of biogeochemical models of the sulfur cycle from meta-omics data, as has been done for the nitrogen cycle in the Arabian Sea (Reed *et al.*, 2014) and in the Saanich Inlet (Louca *et al.*, 2016). In the future, such endeavors could aid in predicting the biogeochemical response to expanding dysoxia and euxinia.

Acknowledgements

We would like to thank all crew and scientific party of the 64PE371 and 64PE408 Black Sea cruises aboard R/V Pelagia for sampling, the sulfur thesis ring of the

Wageningen University Laboratory of Microbiology and Department of Environmental Technology for constructive proofreading, Dr. Karthik Anantharaman for freely sharing his HMMs before peer-reviewed publication, and prof. Friedrich Widdel for helpful discussion on the sulfur cycle and autotrophy. This research was supported through SIAM Gravitation grant 024.002.002 to AJMS and JSSD of the Netherlands Ministry of Education, Culture and Science and the Netherlands Organisation for Scientific Research (NWO). BED and FABvM were supported by the NWO Vidi grant 864.14.004. BED was supported by the European Research Council (ERC) Consolidator grant 865694: DiversiPHI.

Conflict of interest

The authors declare no conflict of interest.

References

- Albert, D.B., Taylor, C., and Martens, C.S. (1995) Sulfate reduction rates and low-molecular-weight fatty-acid concentrations in the water column and surficial sediments of the Black Sea. *Deep Sea Res Part I Oceanogr Res Pap* **42**: 1239–1260.
- Aldredge, A.L., and Silver, M.W. (1988) Characteristics, dynamics and significance of marine snow. *Prog Oceanogr* **20**: 41–82.
- Anantharaman, K., Duhaime, M.B., Breier, J.A., Wendt, K.A., Toner, B.M., and Dick, G.J. (2014) Sulfur oxidation genes in diverse deep-sea viruses. *Science* **344**: 757–760.
- Anantharaman, K., Hausmann, B., Jungbluth, S.P., Kantor, R.S., Lavy, A., Warren, L.A., *et al.* (2018) Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. *ISME J* **12**: 1715–1728.
- Anderson, R., Wylezich, C., Glaubitz, S., Labrenz, M., and Jurgens, K. (2013) Impact of protist grazing on a key bacterial group for biogeochemical cycling in Baltic Sea pelagic oxic/anoxic interfaces. *Environ Microbiol* **15**: 1580–1594.
- Arshad, A., Dalcin Martins, P., Frank, J., Jetten, M.S.M., Op den Camp, H.J.M., and Welte, C.U. (2017) Mimicking microbial interactions under nitrate-reducing conditions in an anoxic bioreactor: enrichment of novel *Nitrospirae* bacteria distantly related to *Thermodesulfovibrio*. *Environ Microbiol* **19**: 4965–4977.
- Barrero-Canosa, J., Moraru, C., Zeugner, L., Fuchs, B.M., and Amann, R. (2017) Direct-geneFISH: a simplified protocol for the simultaneous detection and quantification of genes and rRNA in microorganisms. *Environ Microbiol* **19**: 70–82.
- Bazylnski, D.A., Williams, T.J., Lefevre, C.T., Berg, R.J., Zhang, C.L., Bowser, S.S., *et al.* (2013) *Magnetococcus marinus* gen. nov., sp. nov., a marine, magnetotactic bacterium that represents a novel lineage (Magnetococcaceae fam. nov., Magnetococcales ord. nov.) at the base of the Alphaproteobacteria. *Int J Syst Evol Microbiol* **63**: 801–808.
- Beman, J.M., and Carolan, M.T. (2013) Deoxygenation alters bacterial diversity and community composition in the

- ocean's largest oxygen minimum zone. *Nat Commun* **4**: 2705.
- Bertagnolli, A.D., Padilla, C.C., Glass, J.B., Thamdrup, B., and Stewart, F.J. (2017) Metabolic potential and in situ activity of marine *Marinimicrobia* bacteria in an anoxic water column. *Environ Microbiol* **19**: 4392–4416.
- Bhatnagar, S., Cowley, E.S., Kopf, S.H., Pérez Castro, S., Kearney, S., Dawson, S.C., et al. (2020) Microbial community dynamics and coexistence in a sulfide-driven phototrophic bloom. *Environ Microbiome* **15**: 3.
- Bonnet, S., Dekaezemacker, J., Turk-Kubo, K.A., Moutin, T., Hamersley, R.M., Grosso, O., et al. (2013) Aphotic N₂ fixation in the Eastern Tropical South Pacific Ocean. *PLoS One* **8**: e81265.
- Boughanemi, S., Lyonnet, J., Infossi, P., Bauzan, M., Kosta, A., Lignon, S., et al. (2016) Microbial oxidative sulfur metabolism: biochemical evidence of the membrane-bound heterodisulfide reductase-like complex of the bacterium *Aquifex aeolicus*. *FEMS Microbiol Lett* **363**: fnw156.
- Boyle, R.A., Clark, J.R., Poulton, S.W., Shields-Zhou, G., Canfield, D.E., and Lenton, T.M. (2013) Nitrogen cycle feedbacks as a control on euxinia in the mid-Proterozoic Ocean. *Nat Commun* **4**: 1533.
- Breitbart, D., Levin, L.A., Oschlies, A., Gregoire, M., Chavez, F.P., Conley, D.J., et al. (2018) Declining oxygen in the global ocean and coastal waters. *Science* **359**: eaam7240.
- Brettar, I., Labrenz, M., Flavier, S., Botel, J., Kuosa, H., Christen, R., and Hofle, M.G. (2006) Identification of a *Thiomicrospira denitrificans*-like epsilonproteobacterium as a catalyst for autotrophic denitrification in the Central Baltic Sea. *Appl Environ Microbiol* **72**: 1364–1372.
- Brito, J.A., Sousa, F.L., Stelter, M., Bandejas, T.M., Vonrhein, C., Teixeira, M., et al. (2009) Structural and functional insights into sulfide:quinone oxidoreductase. *Biochemistry* **48**: 5613–5622.
- Burns, J.L., and DiChristina, T.J. (2009) Anaerobic respiration of elemental sulfur and thiosulfate by *Shewanella oneidensis* MR-1 requires psrA, a homolog of the *phsA* gene of *Salmonella enterica* serovar typhimurium LT2. *Appl Environ Microbiol* **75**: 5209–5217.
- Callbeck, C.M., Lavik, G., Ferdelman, T.G., Fuchs, B., Gruber-Vodicka, H.R., Hach, P.F., et al. (2018) Oxygen minimum zone cryptic sulfur cycling sustained by offshore transport of key sulfur oxidizing bacteria. *Nat Commun* **9**: 1729.
- Callbeck, C.M., Pelzer, C., Lavik, G., Ferdelman, T.G., Graf, J.S., Vekeman, B., et al. (2019) *Arcobacter peruensis* sp. nov., a chemolithoheterotroph isolated from sulfide- and organic-rich coastal waters off Peru. *Appl Environ Microbiol* **85**: e01344–19.
- Canfield, D.E. (2006) Models of oxic respiration, denitrification and sulfate reduction in zones of coastal upwelling. *Geochim Cosmochim Acta* **70**: 5753–5765.
- Canfield, D.E., Kristensen, E., and Thamdrup, B. (2005) *Aquatic Geomicrobiology*. Oxford, UK: Gulf Professional Publishing.
- Canfield, D.E., Stewart, F.J., Thamdrup, B., De Brabandere, L., Dalsgaard, T., Delong, E.F., et al. (2010) A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast. *Science* **330**: 1375–1378.
- Carolan, M.T., Smith, J.M., and Beman, J.M. (2015) Transcriptomic evidence for microbial sulfur cycling in the eastern tropical North Pacific oxygen minimum zone. *Front Microbiol* **6**: 334.
- Cassman, N., Prieto-Davo, A., Walsh, K., Silva, G.G., Angly, F., Akhter, S., et al. (2012) Oxygen minimum zones harbour novel viral communities with low diversity. *Environ Microbiol* **14**: 3043–3065.
- Chen, J., Hanke, A., Tegetmeyer, H.E., Kattelmann, I., Sharma, R., Hamann, E., et al. (2017) Impacts of chemical gradients on microbial community structure. *ISME J* **11**: 920–931.
- Christiansen, C.F., and Loescher, C.R. (2019) Facets of diazotrophy in the OMZ off Peru revisited: what we could not see from a single marker gene approach. *bioRxiv*: 558072.
- Chuvochina, M., Rinke, C., Parks, D.H., Rappe, M.S., Tyson, G.W., Yilmaz, P., et al. (2019) The importance of designating type material for uncultured taxa. *Syst Appl Microbiol* **42**: 15–21.
- Cline, J.D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* **14**: 454–458.
- Cross, K.L., Campbell, J.H., Balachandran, M., Campbell, A. G., Cooper, S.J., Griffen, A., et al. (2019) Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. *Nat Biotechnol* **37**: 1314–1321.
- Crowe, S.A., Cox, R.P., Jones, C., Fowle, D.A., Santibanez-Bustos, J.F., Ulloa, O., and Canfield, D.E. (2018) Decrypting the sulfur cycle in oceanic oxygen minimum zones. *ISME J* **12**: 2322–2329.
- Dahl, C. (2017) Sulfur metabolism in phototrophic bacteria. In *Modern Topics in the Phototrophic Prokaryotes: Metabolism, Bioenergetics, and Omics*, Hallenbeck, P.C. (ed). Cham, Switzerland: Springer International Publishing, pp. 27–66.
- De Brabandere, L., Thamdrup, B., Revsbech, N.P., and Foadi, R. (2012) A critical assessment of the occurrence and extend of oxygen contamination during anaerobic incubations utilizing commercially available vials. *J Microbiol Methods* **88**: 147–154.
- Duval, S., Ducluzeau, A.L., Nitschke, W., and Schoepp-Cothenet, B. (2008) Enzyme phylogenies as markers for the oxidation state of the environment: the case of respiratory arsenate reductase and related enzymes. *BMC Evol Biol* **8**: 206.
- Eddie, B.J., and Hanson, T.E. (2013) *Chlorobaculum tepidum* TLS displays a complex transcriptional response to sulfide addition. *J Bacteriol* **195**: 399–408.
- Ehrlich, H.L., Newman, D.K., and Kappler, A. (2015) *Ehrlich's Geomicrobiology*. Boca Raton, FL: CRC Press.
- Findlay, A.J. (2016) Microbial impact on polysulfide dynamics in the environment. *FEMS Microbiol Lett* **363**: fnw103.
- Findlay, A.J., Bennett, A.J., Hanson, T.E., and Luther, G.W., 3rd. (2015) Light-dependent sulfide oxidation in the anoxic zone of the Chesapeake Bay can be explained by small populations of phototrophic bacteria. *Appl Environ Microbiol* **81**: 7560–7569.

- Findlay, A.J., Di Toro, D.M., and Luther, G.W. (2017) A model of phototrophic sulfide oxidation in a stratified estuary. *Limnol Oceanogr* **62**: 1853–1867.
- Findlay, A.J., Gartman, A., MacDonald, D.J., Hanson, T.E., Shaw, T.J., and Luther, G.W. (2014) Distribution and size fractionation of elemental sulfur in aqueous environments: the Chesapeake Bay and Mid-Atlantic Ridge. *Geochim Cosmochim Acta* **142**: 334–348.
- Findlay, A.J., and Kamysny, A. (2017) Turnover rates of intermediate sulfur species in anoxic freshwater and sediments. *Front Microbiol* **8**: 2551.
- Finster, K. (2008) Microbiological disproportionation of inorganic sulfur compounds. *J Sulfur Chem* **29**: 281–292.
- Finster, K., Liesack, W., and Thamdrup, B. (1998) Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens* sp. nov., a new anaerobic bacterium isolated from marine surface sediment. *Appl Environ Microbiol* **64**: 119–125.
- Finster, K.W., and Kjeldsen, K.U. (2010) *Desulfovibrio oceani* subsp. *oceani* sp. nov., subsp. nov. and *Desulfovibrio oceani* subsp. *galatae* subsp. nov., novel sulfate-reducing bacteria isolated from the oxygen minimum zone off the coast of Peru. *Antonie Van Leeuwenhoek* **97**: 221–229.
- Finster, K.W., Kjeldsen, K.U., Kube, M., Reinhardt, R., Mussmann, M., Amann, R., and Schreiber, L. (2013) Complete genome sequence of *Desulfocapsa sulfoexigens*, a marine deltaproteobacterium specialized in disproportionating inorganic sulfur compounds. *Stand Genomic Sci* **8**: 58–68.
- Florentino, A.P., Brienza, C., Stams, A.J.M., and Sanchez-Andrea, I. (2016) *Desulfurella amilsii* sp. nov., a novel acidotolerant sulfur-respiring bacterium isolated from acidic river sediments. *Int J Syst Evol Microbiol* **66**: 1249–1253.
- Florentino, A.P., Pereira, I.A.C., Boeren, S., van den Born, M., Stams, A.J.M., and Sanchez-Andrea, I. (2019) Insight into the sulfur metabolism of *Desulfurella amilsii* by differential proteomics. *Environ Microbiol* **21**: 209–225.
- Florentino, A.P., Stams, A.J., and Sanchez-Andrea, I. (2017) Genome sequence of *Desulfurella amilsii* strain TR1 and comparative genomics of *Desulfurellaceae* family. *Front Microbiol* **8**: 222.
- Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N. A., Heath, G.R., Cullen, D., et al. (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim Cosmochim Acta* **43**: 1075–1090.
- Fuchsman, C.A., Devol, A.H., Saunders, J.K., McKay, C., and Roca, G. (2017) Niche partitioning of the N cycling microbial community of an offshore oxygen deficient zone. *Front Microbiol* **8**: 2384.
- Fuchsman, C.A., Kirkpatrick, J.B., Brazelton, W.J., Murray, J. W., and Staley, J.T. (2011) Metabolic strategies of free-living and aggregate-associated bacterial communities inferred from biologic and chemical profiles in the Black Sea suboxic zone. *FEMS Microbiol Ecol* **78**: 586–603.
- Fuchsman, C.A., Murray, J.W., and Staley, J.T. (2012) Stimulation of autotrophic denitrification by intrusions of the bosporus plume into the anoxic black sea. *Front Microbiol* **3**: 257.
- Fuchsman, C.A., Paul, B., Staley, J.T., Yakushev, E.V., and Murray, J.W. (2019) Detection of transient denitrification during a high organic matter event in the Black Sea. *Global Biogeochem Cycles* **80**: 402–416.
- Ganesh, S., Parris, D.J., DeLong, E.F., and Stewart, F.J. (2014) Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone. *ISME J* **8**: 187–211.
- Giovannelli, D., Chung, M., Staley, J., Starovoytov, V., Le Bris, N., and Vetriani, C. (2016) *Sulfurovum riftiae* sp. nov., a mesophilic, thiosulfate-oxidizing, nitrate-reducing chemolithoautotrophic epsilonproteobacterium isolated from the tube of the deep-sea hydrothermal vent polychaete *Riftia pachyptila*. *Int J Syst Evol Microbiol* **66**: 2697–2701.
- Glaubit, S., Kiesslich, K., Meeske, C., Labrenz, M., and Jurgens, K. (2013) SUP05 dominates the Gammaproteobacterial sulfur oxidizer assemblages in pelagic redoxclines of the Central Baltic and Black Seas. *Appl Environ Microbiol* **79**: 2767–2776.
- Glaubit, S., Labrenz, M., Jost, G., and Jurgens, K. (2010) Diversity of active chemolithoautotrophic prokaryotes in the sulfidic zone of a Black Sea pelagic redoxcline as determined by rRNA-based stable isotope probing. *FEMS Microbiol Ecol* **74**: 32–41.
- Glaubit, S., Lueders, T., Abraham, W.R., Jost, G., Jürgens, K., and Labrenz, M. (2008) 13C-isotope analyses reveal that chemolithoautotrophic *Gamma*- and *Epsilon*proteobacteria feed a microbial food web in a pelagic redoxcline of the Central Baltic Sea. *Environ Microbiol* **11**: 326–337.
- Götz, F., Pjevac, P., Markert, S., McNichol, J., Becher, D., Schweder, T., et al. (2019) Transcriptomic and proteomic insight into the mechanism of cyclooctasulfur- versus thiosulfate-oxidation by the chemolithoautotroph *Sulfurimonas denitrificans*. *Environ Microbiol* **21**: 244–258.
- Grote, J., Jost, G., Labrenz, M., Herndl, G.J., and Jurgens, K. (2008) *Epsilonproteobacteria* represent the major portion of chemoautotrophic bacteria in sulfidic waters of pelagic redoxclines of the Baltic and Black Seas. *Appl Environ Microbiol* **74**: 7546–7551.
- Grote, J., Schott, T., Bruckner, C.G., Glockner, F.O., Jost, G., Teeling, H., et al. (2012) Genome and physiology of a model *Epsilonproteobacterium* responsible for sulfide detoxification in marine oxygen depletion zones. *Proc Natl Acad Sci USA* **109**: 506–510.
- Gutleben, J., Chaib De Mares, M., van Elsas, J.D., Smidt, H., Overmann, J., and Sipkema, D. (2018) The multi-omics promise in context: from sequence to microbial isolate. *Crit Rev Microbiol* **44**: 212–229.
- Han, Y., and Perner, M. (2015) The globally widespread genus *Sulfurimonas*: versatile energy metabolisms and adaptations to redox clines. *Front Microbiol* **6**: 989.
- Hausmann, B., Pelikan, C., Herbold, C.W., Kostlbacher, S., Albertsen, M., Eichorst, S.A., et al. (2018) Peatland *Acidobacteria* with a dissimilatory sulfur metabolism. *ISME J* **12**: 1729–1742.
- Hausmann, B., Pelikan, C., Rattei, T., Loy, A., and Pester, M. (2019) Long-term transcriptional activity at zero growth of a cosmopolitan rare biosphere member. *MBio* **10**: e02189–e02118.

- Hawley, A.K., Brewer, H.M., Norbeck, A.D., Pasa-Tolic, L., and Hallam, S.J. (2014) Metaproteomics reveals differential modes of metabolic coupling among ubiquitous oxygen minimum zone microbes. *Proc Natl Acad Sci USA* **111**: 11395–11400.
- Hawley, A.K., Nobu, M.K., Wright, J.J., Durno, W.E., Morgan-Lang, C., Sage, B., et al. (2017) Diverse *Marinimicrobia* bacteria may mediate coupled biogeochemical cycles along eco-thermodynamic gradients. *Nat Commun* **8**: 1507.
- Hedderich, R., Klimmek, O., Kroger, A., Dirmeier, R., Keller, M., and Stetter, K.O. (1998) Anaerobic respiration with elemental sulfur and with disulfides. *FEMS Microbiol Rev* **22**: 353–381.
- Henkel, J.V. (2019) Bacterial H₂S oxidation coupled to the reduction of MnO₂ studied on new isolated species of the genus *Sulfurimonas*. In *Leibniz-Institut für Ostseeforschung Warnemünde (IOW)*. Rostock, Germany: Universität Rostock, p. 109.
- Henkel, J.V., Dellwig, O., Pollehne, F., Herlemann, D.P.R., Leipe, T., and Schulz-Vogt, H.N. (2019) A bacterial isolate from the Black Sea oxidizes sulfide with manganese(IV) oxide. *Proc Natl Acad Sci USA* **116**: 12153–12155.
- Henson, M.W., Pitre, D.M., Weckhorst, J.L., Lanclos, V.C., Webber, A.T., and Thrash, J.C. (2016) Artificial seawater media facilitate cultivating members of the microbial majority from the Gulf of Mexico. *mSphere* **1**: e00028–16.
- Hinsley, A.P., and Berks, B.C. (2002) Specificity of respiratory pathways involved in the reduction of sulfur compounds by *Salmonella enterica*. *Microbiology* **148**: 3631–3638.
- Ho, T.Y., Taylor, G.T., Astor, Y., Varela, R., Muller-Karger, F., and Scranton, M.I. (2004) Vertical and temporal variability of redox zonation in the water column of the Cariaco Basin: implications for organic carbon oxidation pathways. *Mar Chem* **86**: 89–104.
- Holkenbrink, C., Barbas, S.O., Møllerup, A., Otaki, H., and Frigaard, N.-U. (2011) Sulfur globule oxidation in green sulfur bacteria is dependent on the dissimilatory sulfite reductase system. *Microbiology* **157**: 1229–1239.
- Jayakumar, A., Al-Rshaidat, M.M., Ward, B.B., and Mulholland, M.R. (2012) Diversity, distribution, and expression of diazotroph *nifH* genes in oxygen-deficient waters of the Arabian Sea. *FEMS Microbiol Ecol* **82**: 597–606.
- Jeon, W., Priscilla, L., Park, G., Lee, H., Lee, N., Lee, D., et al. (2017) Complete genome sequence of the sulfur-oxidizing chemolithoautotrophic *Sulfurovum lithotrophicum* 42BKT(T). *Stand Genomic Sci* **12**: 54.
- Jochum, L.M., Schreiber, L., Marshall, I.P.G., Jørgensen, B. B., Schramm, A., and Kjeldsen, K.U. (2018) Single-cell genomics reveals a diverse metabolic potential of uncultivated *Desulfatiglans*-related *Deltaproteobacteria* widely distributed in marine sediment. *Front Microbiol* **9**: 2038.
- Johnston, D.T., Gill, B.C., Masterson, A., Beirne, E., Casciotti, K.L., Knapp, A.N., and Berelson, W. (2014) Placing an upper limit on cryptic marine sulphur cycling. *Nature* **513**: 530–533.
- Jørgensen, B.B. (1990) A thiosulfate shunt in the sulfur cycle of marine sediments. *Science* **249**: 152–154.
- Jørgensen, B.B., and Bak, F. (1991) Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). *Appl Environ Microbiol* **57**: 847–856.
- Jørgensen, B.B., Fossing, H., Wirsén, C.O., and Jannasch, H.W. (1991) Sulfide oxidation in the anoxic Black Sea chemocline. *Deep Sea Res Part A Oceanogr Res Pap* **38**: S1083–S1103.
- Jost, G., Martens-Habben, W., Pollehne, F., Schmetzer, B., and Labrenz, M. (2010) Anaerobic sulfur oxidation in the absence of nitrate dominates microbial chemoautotrophy beneath the pelagic chemocline of the eastern Gotland Basin, Baltic Sea. *FEMS Microbiol Ecol* **71**: 226–236.
- Kamyshny, A., Borkenstein, C.G., and Ferdelman, T.G. (2009) Protocol for quantitative detection of elemental sulfur and polysulfide zero-valent sulfur distribution in natural aquatic samples. *Geostand Geoanal Res* **33**: 415–435.
- Kamyshny, A., Yakushev, E.V., Jost, G., and Podymov, O.I. (2013) Role of sulfide oxidation intermediates in the redox balance of the oxic–anoxic interface of the Gotland Deep, Baltic Sea. In *Chemical Structure of Pelagic Redox Interfaces: Observation and Modeling*. Berlin/Heidelberg, Germany: Springer, pp. 95–119.
- Kamyshny, A., Zerkle, A.L., Mansaray, Z.F., Ciglenecki, I., Bura-Nakic, E., Farquhar, J., and Ferdelman, T.G. (2011) Biogeochemical sulfur cycling in the water column of a shallow stratified sea-water lake: speciation and quadruple sulfur isotope composition. *Mar Chem* **127**: 144–154.
- Karl, D.M., and Knauer, G.A. (1991) Microbial production and particle flux in the upper 350 m of the Black Sea. *Deep Sea Res Part A Oceanogr Res Pap* **38**: S921–S942.
- Kirkpatrick, J.B., Fuchsman, C.A., Yakushev, E.V., Egorov, A.V., Staley, J.T., and Murray, J.W. (2018) Dark N₂ fixation: *nifH* expression in the redoxcline of the Black Sea. *Aquat Microb Ecol* **82**: 43–58.
- Kitzinger, K., Marchant, H.K., Bristow, L.A., Herbold, C.W., Padilla, C.C., Kidane, A.T., et al. (2020) Single cell analyses reveal contrasting life strategies of the two main nitrifiers in the ocean. *Nat Commun* **11**: 767.
- Klawonn, I., Bonaglia, S., Bruchert, V., and Ploug, H. (2015) Aerobic and anaerobic nitrogen transformation processes in N₂-fixing cyanobacterial aggregates. *ISME J* **9**: 1456–1466.
- Konovalov, S., Samodurov, A., Oguz, T., and Ivanov, L. (2004) Parameterization of iron and manganese cycling in the Black Sea suboxic and anoxic environment. *Deep Sea Res Part I Oceanogr Res Pap* **51**: 2027–2045.
- Konovalov, S.K., Luther, G.W., Friederich, G.E., Nuzzio, D. B., Tebo, B.M., Murray, J.W., et al. (2003) Lateral injection of oxygen with the Bosphorus plume – fingers of oxidizing potential in the Black Sea. *Limnol Oceanogr* **48**: 2369–2376.
- Konstantinidis, K.T., Rosselló-Móra, R., and Amann, R. (2017) Uncultivated microbes in need of their own taxonomy. *ISME J* **11**: 2399–2406.
- Korneeva, V.A., Pimenov, N.V., Krek, A.V., Tourova, T.P., and Bryukhanov, A.L. (2015) Sulfate-reducing bacterial communities in the water column of the Gdansk Deep (Baltic Sea). *Mikrobiologiya* **84**: 250–260.

- Labrenz, M., Grote, J., Mammitzsch, K., Boschker, H.T., Laue, M., Jost, G., *et al.* (2013) *Sulfurimonas gotlandica* sp. nov., a chemoautotrophic and psychrotolerant epsilonproteobacterium isolated from a pelagic redoxcline, and an emended description of the genus *Sulfurimonas*. *Int J Syst Evol Microbiol* **63**: 4141–4148.
- Labrenz, M., Jost, G., Pohl, C., Beckmann, S., Martens-Habben, W., and Jurgens, K. (2005) Impact of different in vitro electron donor/acceptor conditions on potential chemolithoautotrophic communities from marine pelagic redoxclines. *Appl Environ Microbiol* **71**: 6664–6672.
- Lam, P., and Kuypers, M.M. (2011) Microbial nitrogen cycling processes in oxygen minimum zones. *Ann Rev Mar Sci* **3**: 317–345.
- Laska, S., Lottspeich, F., and Kletzin, A. (2003) Membrane-bound hydrogenase and sulfur reductase of the hyperthermophilic and acidophilic archaeon *Acidianus ambivalens*. *Microbiology* **149**: 2357–2371.
- Lavik, G., Stuhmann, T., Bruchert, V., Van der Plas, A., Mohrholz, V., Lam, P., *et al.* (2009) Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. *Nature* **457**: 581–584.
- Li, X., Gilhooly, W.P., Zerkle, A.L., Lyons, T.W., Farquhar, J., Werne, J.P., *et al.* (2010) Stable sulfur isotopes in the water column of the Cariaco Basin. *Geochim Cosmochim Acta* **74**: 6764–6778.
- Li, X.N., Taylor, G.T., Astor, Y., and Scranton, M.I. (2008) Relationship of sulfur speciation to hydrographic conditions and chemoautotrophic production in the Cariaco Basin. *Mar Chem* **112**: 53–64.
- Lin, X., Scranton, M.I., Varela, R., Chistoserdov, A., and Taylor, G.T. (2007) Compositional responses of bacterial communities to redox gradients and grazing in the anoxic Cariaco Basin. *Aquat Microb Ecol* **47**: 57–72.
- Lin, X., Wakeham, S.G., Putnam, I.F., Astor, Y.M., Scranton, M.I., Chistoserdov, A.Y., and Taylor, G.T. (2006) Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea by use of fluorescence *in situ* hybridization. *Appl Environ Microbiol* **72**: 2679–2690.
- Loescher, C.R., Groskopf, T., Desai, F.D., Gill, D., Schunck, H., Croot, P.L., *et al.* (2014) Facets of diazotrophy in the oxygen minimum zone waters off Peru. *ISME J* **8**: 2180–2192.
- Louca, S., Hawley, A.K., Katsev, S., Torres-Beltran, M., Bhatia, M.P., Kheirandish, S., *et al.* (2016) Integrating biogeochemistry with multiomic sequence information in a model oxygen minimum zone. *Proc Natl Acad Sci USA* **113**: E5925–E5933.
- Loy, A., Duller, S., Baranyi, C., Mußmann, M., Ott, J., Sharon, I., *et al.* (2009) Reverse dissimilatory sulfite reductase as phylogenetic marker for a subgroup of sulfur-oxidizing prokaryotes. *Environ Microbiol* **11**: 289–299.
- Lücker, S., Nowka, B., Rattei, T., Spieck, E., and Daims, H. (2013) The genome of *Nitrospina gracilis* illuminates the metabolism and evolution of the major marine nitrite oxidizer. *Front Microbiol* **4**: 27.
- Lüke, C., Speth, D.R., Kox, M.A.R., Villanueva, L., and Jetten, M.S.M. (2016) Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone. *PeerJ* **4**: e1924.
- Luther, G.W., 3rd, Findlay, A.J., Macdonald, D.J., Owings, S. M., Hanson, T.E., Beinart, R.A., and Girguis, P.R. (2011) Thermodynamics and kinetics of sulfide oxidation by oxygen: a look at inorganically controlled reactions and biologically mediated processes in the environment. *Front Microbiol* **2**: 62.
- Luther, G.W., Church, T.M., and Powell, D. (1991) Sulfur speciation and sulfide oxidation in the water column of the Black Sea. *Deep-Sea Res Part A Oceanogr Res Pap* **38**: S1121–S1137.
- Luther, G.W., Glazer, B.T., Ma, S.F., Trouwborst, R.E., Moore, T.S., Metzger, E., *et al.* (2008) Use of voltammetric solid-state (micro)electrodes for studying biogeochemical processes: laboratory measurements to real time measurements with an *in situ* electrochemical analyzer (ISEA). *Mar Chem* **108**: 221–235.
- Ma, S., Noble, A., Butcher, D., Trouwborst, R.E., and Luther, G.W. (2006) Removal of H₂S via an iron catalytic cycle and iron sulfide precipitation in the water column of dead end tributaries. *Estuar Coast Shelf Sci* **70**: 461–472.
- Madrid, V.M., Taylor, G.T., Scranton, M.I., and Chistoserdov, A.Y. (2001) Phylogenetic diversity of bacterial and archaeal communities in the anoxic zone of the Cariaco Basin. *Appl Environ Microbiol* **67**: 1663–1674.
- Manske, A.K., Glaeser, J., Kuypers, M.M., and Overmann, J. (2005) Physiology and phylogeny of green sulfur bacteria forming a monospecific phototrophic assemblage at a depth of 100 meters in the Black Sea. *Appl Environ Microbiol* **71**: 8049–8060.
- Mardanov, A.V., Beletsky, A.V., Kadnikov, V.V., Slobodkin, A.I., and Ravin, N.V. (2016) Genome analysis of *Thermosulfurimonas dismutans*, the first thermophilic sulfur-disproportionating bacterium of the phylum *Thermodesulfobacteria*. *Front Microbiol* **7**: 950.
- Marschall, E., Jogler, M., Hessge, U., and Overmann, J. (2010) Large-scale distribution and activity patterns of an extremely low-light-adapted population of green sulfur bacteria in the Black Sea. *Environ Microbiol* **12**: 1348–1362.
- Marshall, K.T., and Morris, R.M. (2013) Isolation of an aerobic sulfur oxidizer from the SUP05/Arctic96BD-19 clade. *ISME J* **7**: 452–455.
- Martínez-Pérez, C., Mohr, W., Schwedt, A., Dürschlag, J., Callbeck, C.M., Schunck, H., *et al.* (2018) Metabolic versatility of a novel N₂-fixing *Alphaproteobacterium* isolated from a marine oxygen minimum zone. *Environ Microbiol* **20**: 755–768.
- Meier, D.V., Pjevac, P., Bach, W., Hourdez, S., Girguis, P. R., Vidoudez, C., *et al.* (2017) Niche partitioning of diverse sulfur-oxidizing bacteria at hydrothermal vents. *ISME J* **11**: 1545–1558.
- Meier, D.V., Pjevac, P., Bach, W., Markert, S., Schweder, T., Jamieson, J., *et al.* (2019) Microbial metal-sulfide oxidation in inactive hydrothermal vent chimneys suggested by metagenomic and metaproteomic analyses. *Environ Microbiol* **21**: 682–701.
- Meyer, B., and Kuever, J. (2007) Phylogeny of the alpha and beta subunits of the dissimilatory adenosine-5'-phosphosulfate (APS) reductase from sulfate-reducing prokaryotes—origin and evolution of the dissimilatory sulfate-reduction pathway. *Microbiology* **153**: 2026–2044.

- Meyer, K.M., and Kump, L.R. (2008) Oceanic Euxinia in earth history: causes and consequences. *Annu Rev Earth Planet Sci* **36**: 251–288.
- Mino, S., Kudo, H., Arai, T., Sawabe, T., Takai, K., and Nakagawa, S. (2014) *Sulfurovum aggregans* sp. nov., a hydrogen-oxidizing, thiosulfate-reducing chemolithoautotroph within the *Epsilonproteobacteria* isolated from a deep-sea hydrothermal vent chimney, and an emended description of the genus *Sulfurovum*. *Int J Syst Evol Microbiol* **64**: 3195–3201.
- Mizuno, N., Voordouw, G., Miki, K., Sarai, A., and Higuchi, Y. (2003) Crystal structure of dissimilatory sulfite reductase D (DsrD) protein – possible interaction with B- and Z-DNA by its winged-helix motif. *Structure* **11**: 1133–1140.
- Möller, L., Laas, P., Rogge, A., Goetz, F., Bahlo, R., Leipe, T., and Labrenz, M. (2019) *Sulfurimonas* subgroup GD17 cells accumulate polyphosphate under fluctuating redox conditions in the Baltic Sea: possible implications for their ecology. *ISME J* **13**: 482–493.
- Mori, K., Yamaguchi, K., and Hanada, S. (2018) *Sulfurovum denitrificans* sp. nov., an obligately chemolithoautotrophic sulfur-oxidizing epsilonproteobacterium isolated from a hydrothermal field. *Int J Syst Evol Microbiol* **68**: 2183–2187.
- Müller, A.L., Kjeldsen, K.U., Rattei, T., Pester, M., and Loy, A. (2015) Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. *ISME J* **9**: 1152–1165.
- Murillo, A.A., Ramírez-Flandes, S., DeLong, E.F., and Ulloa, O. (2014) Enhanced metabolic versatility of planktonic sulfur-oxidizing γ -proteobacteria in an oxygen-deficient coastal ecosystem. *Front Mar Sci* **1**: 18.
- Murray, A.E., Freudenstein, J., Gribaldo, S., Hatzenpichler, R., Hugenholtz, P., Kampfer, P., et al. (2020) Roadmap for naming uncultivated *Archaea* and *Bacteria*. *Nat Microbiol*, **5**: 987–994.
- Murray, J.W., Jannasch, H.W., Honjo, S., Anderson, R.F., Reeburgh, W.S., Top, Z., et al. (1989) Unexpected changes in the oxic anoxic interface in the Black Sea. *Nature* **338**: 411–413.
- Musmann, M., Richter, M., Lombardot, T., Meyerdierks, A., Kuever, J., Kube, M., et al. (2005) Clustered genes related to sulfate respiration in uncultured prokaryotes support the theory of their concomitant horizontal transfer. *J Bacteriol* **187**: 7126–7137.
- Muyzer, G., and Stams, A.J. (2008) The ecology and biotechnology of sulphate-reducing bacteria. *Nat Rev Microbiol* **6**: 441–454.
- Naqvi, S.W.A., Bange, H.W., Fariás, L., Monteiro, P.M.S., Scranton, M.I., and Zhang, J. (2010) Marine hypoxia/anoxia as a source of CH₄ and N₂O. *Biogeosciences* **7**: 2159–2190.
- Neretin, L.N., Abed, R.M., Schippers, A., Schubert, C.J., Kohls, K., and Kuypers, M.M. (2007) Inorganic carbon fixation by sulfate-reducing bacteria in the Black Sea water column. *Environ Microbiol* **9**: 3019–3024.
- Overmann, J., Cypionka, H., and Pfennig, N. (1992) An extremely low-light-adapted phototrophic sulfur bacterium from the Black Sea. *Limnol Oceanogr* **37**: 150–155.
- Parks, D.H., Rinke, C., Chuvochina, M., Chaumeil, P.-A., Woodcroft, B.J., Evans, P.N., et al. (2017) Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nat Microbiol* **2**: 1533–1542.
- Paulmier, A., and Ruiz-Pino, D. (2009) Oxygen minimum zones (OMZs) in the modern ocean. *Prog Oceanogr* **80**: 113–128.
- Pelikan, C., Herbold, C.W., Hausmann, B., Müller, A.L., Pester, M., and Loy, A. (2016) Diversity analysis of sulfite- and sulfate-reducing microorganisms by multiplex *dsrA* and *dsrB* amplicon sequencing using new primers and mock community-optimized bioinformatics. *Environ Microbiol* **18**: 2994–3009.
- Perry, K.A., Kostka, J.E., Luther, G.W., 3rd, and Nealson, K. H. (1993) Mediation of sulfur speciation by a black sea facultative anaerobe. *Science* **259**: 801–803.
- Pester, M., Knorr, K.H., Friedrich, M.W., Wagner, M., and Loy, A. (2012) Sulfate-reducing microorganisms in wetlands – fameless actors in carbon cycling and climate change. *Front Microbiol* **3**: 72.
- Pimenov, N.V., Rusanov, I.I., Yusupov, S.K., Fridrich, J., Lein, A.Y., Wehrli, B., and Ivanov, M.V. (2000) Microbial processes at the aerobic-anaerobic interface in the deep-water zone of the Black Sea. *Microbiology* **69**: 436–448.
- Pjevac, P., Dykstra, S., Goldhammer, T., Mujakić, I., Koblížek, M., Mußmann, M., et al. (2019) *In situ* abundance and carbon fixation activity of distinct anoxygenic phototrophs in the stratified seawater lake Rogoznica. *Environ Microbiol* **21**: 3896–3908.
- Pjevac, P., Korlevic, M., Berg, J.S., Bura-Nakic, E., Ciglenecki, I., Amann, R., and Orlic, S. (2015) Community shift from phototrophic to chemotrophic sulfide oxidation following anoxic holomixis in a stratified seawater lake. *Appl Environ Microbiol* **81**: 298–308.
- Pjevac, P., Meier, D.V., Markert, S., Hentschker, C., Schweder, T., Becher, D., et al. (2018) Metaproteogenomic profiling of microbial communities colonizing actively venting hydrothermal chimneys. *Front Microbiol* **9**: 680.
- Plominsky, A.M., Trefault, N., Podell, S., Blanton, J.M., De la Iglesia, R., Allen, E.E., et al. (2018) Metabolic potential and *in situ* transcriptomic profiles of previously uncharacterized key microbial groups involved in coupled carbon, nitrogen and sulfur cycling in anoxic marine zones. *Environ Microbiol* **20**: 2727–2742.
- Ploug, H., and Bergkvist, J. (2015) Oxygen diffusion limitation and ammonium production within sinking diatom aggregates under hypoxic and anoxic conditions. *Mar Chem* **176**: 142–149.
- Rabus, R., Hansen, T.A., and Widdel, F. (2013) Dissimilatory sulfate- and sulfur-reducing prokaryotes. In *The Prokaryotes*, Berlin, Heidelberg: Springer, pp. 309–404.
- Rabus, R., Venceslau, S.S., Wohlbrand, L., Voordouw, G., Wall, J.D., and Pereira, I.A. (2015) A post-genomic view of the ecophysiology, catabolism and biotechnological relevance of sulphate-reducing prokaryotes. *Adv Microb Physiol* **66**: 55–321.
- Reed, D.C., Algar, C.K., Huber, J.A., and Dick, G.J. (2014) Gene-centric approach to integrating environmental genomics and biogeochemical models. *Proc Natl Acad Sci USA* **111**: 1879–1884.
- Revsbech, N.P., Larsen, L.H., Gundersen, J., Dalsgaard, T., Ulloa, O., and Thamdrup, B. (2009) Determination of ultra-

- low oxygen concentrations in oxygen minimum zones by the STOX sensor. *Limnol Oceanogr Methods* **7**: 371–381.
- Rodriguez-Mora, M.J., Edgcomb, V.P., Taylor, C., Scranton, M.I., Taylor, G.T., and Chistoserdov, A.Y. (2016) The diversity of sulfide oxidation and sulfate reduction genes expressed by the bacterial communities of the Cariaco Basin, Venezuela. *Open Microbiol J* **10**: 140–149.
- Rodriguez-Mora, M.J., Scranton, M.I., Taylor, G.T., and Chistoserdov, A.Y. (2013) Bacterial community composition in a large marine anoxic basin: a Cariaco Basin time-series survey. *FEMS Microbiol Ecol* **84**: 625–639.
- Rodriguez-Mora, M.J., Scranton, M.I., Taylor, G.T., and Chistoserdov, A.Y. (2015) The dynamics of the bacterial diversity in the redox transition and anoxic zones of the Cariaco Basin assessed by parallel tag sequencing. *FEMS Microbiol Ecol* **91**: fiv088.
- Rogge, A., Vogts, A., Voss, M., Jurgens, K., Jost, G., and Labrenz, M. (2017) Success of chemolithoautotrophic SUP05 and *Sulfurimonas* GD17 cells in pelagic Baltic Sea redox zones is facilitated by their lifestyles as K- and r-strategists. *Environ Microbiol* **19**: 2495–2506.
- Roullier, F., Berline, L., Guidi, L., Durrieu De Madron, X., Picheral, M., Sciandra, A., et al. (2014) Particle size distribution and estimated carbon flux across the Arabian Sea oxygen minimum zone. *Biogeosciences* **11**: 4541–4557.
- Roux, S., Brum, J.R., Dutilh, B.E., Sunagawa, S., Duhaime, M.B., Loy, A., et al. (2016) Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. *Nature* **537**: 689–693.
- Roux, S., Hawley, A.K., Torres Beltran, M., Scofield, M., Schwientek, P., Stepanauskas, R., et al. (2014) Ecology and evolution of viruses infecting uncultivated SUP05 bacteria as revealed by single-cell- and meta-genomics. *Elife* **3**: e03125.
- Santos, A.A., Venceslau, S.S., Grein, F., Leavitt, W.D., Dahl, C., Johnston, D.T., and Pereira, I.A. (2015) A protein trisulfide couples dissimilatory sulfate reduction to energy conservation. *Science* **350**: 1541–1545.
- Saunders, J.K., Fuchsman, C.A., McKay, C., and Rocap, G. (2019) Complete arsenic-based respiratory cycle in the marine microbial communities of pelagic oxygen-deficient zones. *Proc Natl Acad Sci USA* **116**: 9925–9930.
- Schmidtke, S., Stramma, L., and Visbeck, M. (2017) Decline in global oceanic oxygen content during the past five decades. *Nature* **542**: 335–339.
- Schulz-Vogt, H.N., Pollehne, F., Jürgens, K., Arz, H.W., Beier, S., Bahlo, R., et al. (2019) Effect of large magnetotactic bacteria with polyphosphate inclusions on the phosphate profile of the suboxic zone in the Black Sea. *ISME J* **13**: 1198–1208.
- Schunck, H., Lavik, G., Desai, D.K., Grosskopf, T., Kalvelage, T., Loscher, C.R., et al. (2013) Giant hydrogen sulfide plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. *PLoS One* **8**: e68661.
- Schutz, M., Shahak, Y., Padan, E., and Hauska, G. (1997) Sulfide-quinone reductase from *Rhodobacter capsulatus*. Purification, cloning, and expression. *J Biol Chem* **272**: 9890–9894.
- Shah, V., Chang, B.X., and Morris, R.M. (2017) Cultivation of a chemoautotroph from the SUP05 clade of marine bacteria that produces nitrite and consumes ammonium. *ISME J* **11**: 263–271.
- Shah, V., Zhao, X., Lundeen, R.A., Ingalls, A.E., Nicastro, D., and Morris, R.M. (2019) Morphological plasticity in a sulfur-oxidizing marine bacterium from the SUP05 clade enhances dark carbon fixation. *mBio* **10**: e00216–e00219.
- Shanks, A.L., and Reeder, M.L. (1993) Reducing microzones and sulfide production in marine snow. *Mar Ecol Prog Ser* **96**: 43–47.
- Sheik, C.S., Jain, S., and Dick, G.J. (2014) Metabolic flexibility of enigmatic SAR324 revealed through metagenomics and metatranscriptomics. *Environ Microbiol* **16**: 304–317.
- Sigalevich, P., and Cohen, Y. (2000) Oxygen-dependent growth of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in coculture with *Marinobacter* sp. strain MB in an aerated sulfate-depleted chemostat. *Appl Environ Microbiol* **66**: 5019–5023.
- Slobodkin, A.I., and Slobodkina, G.B. (2019) Diversity of sulfur-disproportionating microorganisms. *Microbiology* **88**: 509–522.
- Slobodkina, G.B., Mardanov, A.V., Ravin, N.V., Frolova, A. A., Chernyh, N.A., Bonch-Osmolovskaya, E.A., and Slobodkin, A.I. (2017) Respiratory ammonification of nitrate coupled to anaerobic oxidation of elemental sulfur in deep-sea autotrophic thermophilic bacteria. *Front Microbiol* **8**: 87.
- Sollai, M., Villanueva, L., Hopmans, E.C., Reichart, G.J., and Sinninghe Damste, J.S. (2019) A combined lipidomic and 16S rRNA gene amplicon sequencing approach reveals archaeal sources of intact polar lipids in the stratified Black Sea water column. *Geobiology* **17**: 91–109.
- Sørensen, K.B., and Canfield, D.E. (2004) Annual fluctuations in sulfur isotope fractionation in the water column of a euxinic marine basin. *Geochim Cosmochim Acta* **68**: 503–515.
- Sorokin, D.Y. (2003) Oxidation of inorganic sulfur compounds by obligately organotrophic bacteria. *Microbiology* **72**: 641–653.
- Sorokin, D.Y., Kublanov, I.V., Gavrilov, S.N., Rojo, D., Roman, P., Golyshin, P.N., et al. (2015) Elemental sulfur and acetate can support life of a novel strictly anaerobic haloarchaeon. *ISME J* **10**: 240.
- Sorokin, Y.I. (1972) The bacterial population and the processes of hydrogen sulphide oxidation in the Black Sea. *Journal du Conseil* **34**: 423–454.
- Sorokin, Y.I. (2002) *The Black Sea: Ecology and Oceanography*. Amsterdam, The Netherlands: Backhuys Publishers.
- Sorokin, Y.I., Sorokin, P.Y., Avdeev, V.A., Sorokin, D.Y., and Ilchenko, S.V. (1995) Biomass, production and activity of bacteria in the Black Sea, with special reference to chemosynthesis and the sulfur cycle. *Hydrobiologia* **308**: 61–76.
- Spietz, R.L., Lundeen, R.A., Zhao, X., Nicastro, D., Ingalls, A.E., and Morris, R.M. (2019) Heterotrophic carbon metabolism and energy acquisition in *Candidatus* Thioglobus singularis strain PS1, a member of the SUP05 clade of marine *Gammaproteobacteria*. *Environ Microbiol* **0**: 2391–2401.
- Stanev, E.V., Poulain, P.M., Grayek, S., Johnson, K.S., Claustre, H., and Murray, J.W. (2018) Understanding the

- dynamics of the oxic-anoxic interface in the Black Sea. *Geophys Res Lett* **45**: 864–871.
- Stewart, F.J., Ulloa, O., and DeLong, E.F. (2012) Microbial metatranscriptomics in a permanent marine oxygen minimum zone. *Environ Microbiol* **14**: 23–40.
- Sun, X., Kop, L.F.M., Lau, M.C.Y., Frank, J., Jayakumar, A., L  cker, S., and Ward, B.B. (2019) Uncultured *Nitrospina*-like species are major nitrite oxidizing bacteria in oxygen minimum zones. *ISME J* **13**: 2391–2402.
- Suominen, S., Dombrowski, N., Sinninghe Damste, J.S., and Villanueva, L. (2019) A diverse uncultivated microbial community is responsible for organic matter degradation in the Black Sea sulphidic zone. *Environ Microbiol*. <https://doi.org/10.1111/1462-2920.14902>.
- Suter, E.A., Pachiadaki, M., Taylor, G.T., Astor, Y., and Edgcomb, V.P. (2018) Free-living chemoautotrophic and particle-attached heterotrophic prokaryotes dominate microbial assemblages along a pelagic redox gradient. *Environ Microbiol* **20**: 693–712.
- Suter, E.A., Scranton, M.I., Chow, S., Stinton, D., Medina Faull, L., and Taylor, G.T. (2017) Niskin bottle sample collection aliases microbial community composition and biogeochemical interpretation. *Limnol Oceanogr* **62**: 606–617.
- Swan, B.K., Martinez-Garcia, M., Preston, C.M., Sczyrba, A., Woyke, T., Lamy, D., et al. (2011) Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. *Science* **333**: 1296–1300.
- Taylor, G.T., Iabichella, M., Ho, T.Y., Scranton, M.I., Thunell, R.C., Muller-Karger, F., and Varela, R. (2001) Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic carbon production. *Limnol Oceanogr* **46**: 148–163.
- Taylor, G.T., Suter, E.A., Pachiadaki, M.G., Astor, Y., Edgcomb, V.P., and Scranton, M.I. (2018) Temporal shifts in dominant sulfur-oxidizing chemoautotrophic populations across the Cariaco Basin's redoxcline. *Deep Sea Res Part II Top Stud Oceanogr* **156**: 80–96.
- Tebo, B.M. (1991) Manganese (II) oxidation in the suboxic zone of the Black Sea. *Deep Sea Res Part A Oceanogr Res Pap* **38**: S883–S905.
- Teske, A., Wawer, C., Muyzer, G., and Ramsing, N.B. (1996) Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Appl Environ Microbiol* **62**: 1405–1415.
- Thamdrup, B., Dalsgaard, T., and Revsbech, N.P. (2012) Widespread functional anoxia in the oxygen minimum zone of the Eastern South Pacific. *Deep Sea Res Part I Oceanogr Res Pap* **65**: 36–45.
- Thiel, V., Garcia Costas, A.M., Fortney, N.W., Martinez, J.N., Tank, M., Roden, E.E., et al. (2018) '*Candidatus* Thermotrophicus', a non-phototrophic member of the *Bacteroidetes/Chlorobi* with dissimilatory sulfur metabolism in hot spring mat communities. *Front Microbiol* **9**: 3159.
- Thorup, C., Schramm, A., Findlay, A.J., Finster, K.W., and Schreiber, L. (2017) Disguised as a sulfate reducer: growth of the Deltaproteobacterium *Desulfurivibrio alkaliphilus* by sulfide oxidation with nitrate. *mBio* **8**: e00671–17.
- Tonolla, M., Peduzzi, S., Demarta, A., Peduzzi, R., and Hahn, D. (2004) Phototrophic sulfur and sulfate-reducing bacteria in the chemocline of meromictic Lake Cadagno, Switzerland. *J Limnol* **63**: 161–170.
- Trouwborst, R.E., Clement, B.G., Tebo, B.M., Glazer, B.T., and Luther, G.W., 3rd. (2006) Soluble Mn(III) in suboxic zones. *Science* **313**: 1955–1957.
- Tully, B.J., Graham, E.D., and Heidelberg, J.F. (2018) The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. *Sci Data* **5**: 170203.
- Ulloa, O., Canfield, D.E., DeLong, E.F., Letelier, R.M., and Stewart, F.J. (2012) Microbial oceanography of anoxic oxygen minimum zones. *Proc Natl Acad Sci USA* **109**: 15996–16003.
- Van Gernerden, H. (1984) The sulfide affinity of phototrophic bacteria in relation to the location of elemental sulfur. *Arch Microbiol* **139**: 289–294.
- Venceslau, S.S., Stockdreher, Y., Dahl, C., and Pereira, I.A. (2014) The 'bacterial heterodisulfide' DsrC is a key protein in dissimilatory sulfur metabolism. *Biochim Biophys Acta* **1837**: 1148–1164.
- Vetriani, C., Tran, H.V., and Kerkhof, L.J. (2003) Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the Black Sea. *Appl Environ Microbiol* **69**: 6481–6488.
- Villanueva, L., von Meijenf  ldt, F.A.B., Westbye, A.B., Yadav, S., Hopmans, E.C., Dutilh, B.E., and Sinninghe Damste, J.S. (2020) Bridging the membrane lipid divide: bacteria of the FCB group superphylum have the potential to synthesize archaeal ether lipids. *ISME J*, 1–15. <https://doi.org/10.1038/s41396-020-00772-2>.
- Waite, D.W., Vanwonterghem, I., Rinke, C., Parks, D.H., Zhang, Y., Takai, K., et al. (2017) Comparative genomic analysis of the class *Epsilonproteobacteria* and proposed reclassification to *Epsilonbacteraeota* (phyl. nov.). *Front Microbiol* **8**: 682.
- Waite, D.W., Vanwonterghem, I., Rinke, C., Parks, D.H., Zhang, Y., Takai, K., et al. (2018) Erratum: addendum: comparative genomic analysis of the class *Epsilonproteobacteria* and proposed reclassification to *Epsilonbacteraeota* (phyl. nov.). *Front Microbiol* **9**: 772.
- Walsh, D.A., Zaikova, E., Howes, C.G., Song, Y.C., Wright, J.J., Tringe, S.G., et al. (2009) Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. *Science* **326**: 578–582.
- Wasmund, K., Cooper, M., Schreiber, L., Lloyd, K.G., Baker, B.J., Petersen, D.G., et al. (2016) Single-cell genome and group-specific *dsrAB* sequencing implicate marine members of the class *Dehalococcoidia* (phylum *Chloroflexi*) in sulfur cycling. *mBio* **7**: e00266–16.
- Wasmund, K., Mussmann, M., and Loy, A. (2017) The life sulfuric: microbial ecology of sulfur cycling in marine sediments. *Environ Microbiol Rep* **9**: 323–344.
- Weissgerber, T., Dobler, N., Polen, T., Latus, J., Stockdreher, Y., and Dahl, C. (2013) Genome-wide transcriptional profiling of the purple sulfur bacterium *Allochrochromatium vinosum* DSM 180T during growth on different reduced sulfur compounds. *J Bacteriol* **195**: 4231–4245.
- Whitmire, A.L., Letelier, R.M., Villagran, V., and Ulloa, O. (2009) Autonomous observations of in vivo fluorescence and particle backscattering in an oceanic oxygen minimum zone. *Opt Express* **17**: 21992–22004.

- Wilbanks, E.G., Jaekel, U., Salman, V., Humphrey, P.T., Eisen, J.A., Facciotti, M.T., *et al.* (2014) Microscale sulfur cycling in the phototrophic pink berry consortia of the Sippewissett Salt Marsh. *Environ Microbiol* **16**: 3398–3415.
- Wright, J.J., Konwar, K.M., and Hallam, S.J. (2012) Microbial ecology of expanding oxygen minimum zones. *Nat Rev Microbiol* **10**: 381–394.
- Wright, J.J., Mewis, K., Hanson, N.W., Konwar, K.M., Maas, K.R., and Hallam, S.J. (2014) Genomic properties of Marine Group A bacteria indicate a role in the marine sulfur cycle. *ISME J* **8**: 455–468.
- Yakushev, E., Pollehne, F., Jost, G., Kuznetsov, I., Schneider, B., and Umlauf, L. (2007) Analysis of the water column oxic/anoxic interface in the Black and Baltic seas with a numerical model. *Mar Chem* **107**: 388–410.
- Yamamoto, M., Nakagawa, S., Shimamura, S., Takai, K., and Horikoshi, K. (2010) Molecular characterization of inorganic sulfur-compound metabolism in the deep-sea epsilonproteobacterium *Sulfurovum* sp. NBC37-1. *Environ Microbiol* **12**: 1144–1153.
- Yao, W., and Millero, F.J. (1993) The rate of sulfide oxidation by δMnO_2 in seawater. *Geochim Cosmochim Acta* **57**: 3359–3365.
- Youssef, N.H., Farag, I.F., Hahn, C.R., Jarett, J., Becraft, E., Elie-Fadrosh, E., *et al.* (2019) Genomic characterization of candidate division LCP-89 reveals an atypical cell wall structure, microcompartment production, and dual respiratory and fermentative capacities. *Appl Environ Microbiol* **85**: e00110–e00119.
- Zecchin, S., Mueller, R.C., Seifert, J., Stingl, U., Anantharaman, K., von Bergen, M., *et al.* (2018) Rice Paddy *Nitrospirae* carry and express genes related to Sulfate respiration: proposal of the new genus 'Candidatus Sulfobium'. *Appl Environ Microbiol* **84**: e02224-17.
- Zhou, Z., Tran, P.Q., Kieft, K., and Anantharaman, K. (2020) Genome diversification in globally distributed novel marine *Proteobacteria* is linked to environmental adaptation. *ISME J* **14**: 2060–2077.
- Zopfi, J., Ferdelman, T.G., Jorgensen, B.B., Teske, A., and Thamdrup, B. (2001) Influence of water column dynamics on sulfide oxidation and other major biogeochemical processes in the chemocline of Mariager Fjord (Denmark). *Mar Chem* **74**: 29–51.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Data S1. Maximum-likelihood phylogenetic reconstruction of *dsrA* genes in newick format. See Supporting Information Methods for methodology.

Table S1. Microbiological and biogeochemical studies of the sulfur cycle in dysoxic marine waters, grouped by environment. Volumes based on the work of Paulmier and Ruiz-Pino (2009) correspond to the estimated volume of waters containing > 0.5 μM nitrite. The maximum volume of anoxic water off the Namibian coast was calculated from the largest observed extent of sulfidic bottom waters (7000 km^2 ; Lavik *et al.*, 2009) and an assumed sulfidic layer thickness of 10 m.

Table S2. Origin, quality, classification, annotation, and average amino acid identity (AAI) of the analysed genomes in Fig. 5. Methods are described in Supporting Information Methods. The AAI values were calculated with an *enveomics* script using Diamond because of computational limitations, which could lead to significantly overestimated AAI values between 50% and 60% (<https://rodriguez-r.com/blog/aai-blast-vs-diamond/>). Following the thresholds proposed by Konstantinidis and colleagues (2017), values exceeding the 65% genus-level lower threshold are coloured green, and values exceeding the 45% family-level lower threshold are coloured yellow.

Table S3. Gibbs free energies [ΔG (kJ e^-)] of common dissimilatory conversions mediated by anaerobic microorganisms under conditions representative of the upper euxinic zone of the Black Sea and the core of the ETSP OMZ. Calculation methodology and variables used can be found in Supporting Information Methods.

Appendix S1. Supplementary Information Methods.

Appendix S2. Supplementary Information Protologue.