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The bacterial sulfur cycle in expanding dysoxic and euxinic marine waters

Daan M. van Vliet 1, F.A. Bastiaan von Meijenfeldt, Bas E. Dutilh, Laura Villanueva, Jaap S. Sinninghe Damsté, Alfons J.M. Stams, 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 μM 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 S0 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 μM 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 μM 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).
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 μM 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 N₂ at such high rates that OMZs are responsible for 30% to 50% of the total loss of fixed nitrogen from the ocean (Lam and Kuyers, 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 μM) or sulfide (< 0.1 μM) 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 μM 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 (Schmidtko 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-
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 Eocene-Oligocene (Han and Perner, 2015; Dahl, 2017). More extensively conducted taxonomic marker studies have been widely detected (Madrid et al., 2013; Ganesh et al., 2015; Suter et al., 2018), but also in the ETSP OMZ core with rates up to 16.9 nmol l$^{-1}$day$^{-1}$ (Canfield et al., 2010). The subsequent section discusses which bacteria could be involved in sulfur reduction or disproportionation.

**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 |

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 (S$_2$O$_3^{2-}$), tetrathionate (S$_4$O$_6^{2-}$), and sulfito (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 ($^{35}$SO$_4^{2-}$) performed in euxinic settings such as the Black Sea with sulfate reduction rates up to 36 nmol l$^{-1}$ day$^{-1}$ (Sorokin, 1972; Jørgensen et al., 1991; Albert et al., 1995; Pimenov et al., 2000) and Mariager Fjord with rates up to 140 nmol l$^{-1}$ day$^{-1}$ (Sørensen and Canfield, 2004), but also in the ETSP OMZ core with rates up to 16.9 nmol l$^{-1}$ 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; Rodriguez-Mora et al., 2015; Suter et al., 2018; Callbeck et al., 2019).
**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., 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; Rodríguez-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; Rodríguez-Mora et al., 2015; Suter et al., 2018). Furthermore, bacteria related to the genus Desulfatiflans seem widespread in DMW since Desulfatiflans-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; Rodríguez-Mora et al., 2016). Functional marker gene surveys indicated an even larger diversity of putative SRB beyond the Deltaproteobacteria, including Thermodesulfovibrio-related bacteria in the ETSP OMZ core (Canfield et al., 2010) and diverse unknown putative SRB in euxinic basins (Korneeva et al., 2015; Rodríguez-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 Thermodesulfovibrio, 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 Desulfovibrio oceanii 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 dissipatory 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 Desulfatiflans-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, Desulfobulales and Nitrospirae but did not classify within established genera, except for Desulfovibularia 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 AAMBM5-125-24. Other novel putative SRB within the phylum Nitrospirae, ‘Candidatus Sulfofibium mesophilum’ (Zecchin et al., 2018) and ‘Candidatus Nitrobitium 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

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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 Desulfobateriales 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...
of \textit{sqr} \cite{Fig. 5}, this suggests a sulfur-oxidizing rather than sulfate-reducing metabolism. These MAGs thus question the common assumption that all \textit{Desulfobacterales} reduce sulfate, and undermine taxonomy-based physiological assumptions in general. Habitat profiling can further support metabolic hypotheses based on functional annotation of the genes in different MAGs. For instance, the SRB that reside exclusively in the deeper euxinic waters of the Black Sea \cite{UDesulfatibia profunda, UDesulfaltia bathyphila, Desulfacyla NIOZ-UU19; 95\%–97\% complete} apparently lack the genes to detoxify oxygen \cite{cydAB} or hydrogen peroxide \cite{catalase; Table S2} and to utilize alternative electron acceptors \cite{Figs 3B and 5}, reflecting their probably purely euxinic and strictly sulfate-reducing lifestyle. In contrast, the MAGs of SRB relatively abundant in suboxic waters \cite{UDesulfolinea nitratireducens, Nitrospirae NIOZ-UU55, UDb. maris, UDesulfatibia vada, UDesulfacyla euxinica; 73\%–93\% complete, 1\%–4\% contaminated} encode a plethora of genes for the energy-conserving reduction of alternative electron acceptors such as S\textsubscript{0} or thiosulfate \cite{psrA/pshA/sreA}, tetrathionate \cite{otr}, nitrate \cite{napABC, Fig. 4. Maximum-likelihood phylogenetic reconstruction based on bacterial reductive DsrA proteins predicted from Black Sea MAGs and unbinned contigs (blue) and reference genomes (Anantharaman \textit{et al.}, 2018). Black dots indicate support of > 95\% out of 1,000 ultra-fast bootstraps. The scale bar indicates substitutions per site. See Supporting Information Methods for methodology and Data S1 for the full phylogenetic tree in Newick format. \[\text{Color figure can be viewed at wileyonlinelibrary.com}\] © 2020 The Authors. \textit{Environmental Microbiology} published by Society for Applied Microbiology and John Wiley & Sons Ltd., \textit{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.
shortly, red indicates core genes of the Dsr/rDsr pathways, orange indicates B. An overview of the presence of functional genes enabling conversions of sulfur, nitrogen and oxygen, following the colour scheme of Fig. 2.

light blue indicates putative SRB in DMW (Jayakumar et al., 2019). It has been postulated that SRB in suboxic waters and OMZ cores. How then is dissimilation 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]

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 dissimulatory 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 eukaryotic protists (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;
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; Konovalov 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; Kamyszny 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 Campylobacterales 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 (Bazylinski 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⁻¹⁻¹; Lavik et al., 2009; Callbeck et al., 2018), up to 17% in the ETSP OMZ core (5 × 10⁵ cells ml⁻¹⁻¹; Callbeck et al., 2018), up to 30% at an oxic–
euxinic interface in the Baltic Sea (4 × 10⁵ cells ml⁻¹, Landsort Deep) and up to 10% and 13% in the suboxic and euxinic zone of the Black Sea respectively (< 7 × 10⁴ 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 (spr, fccAB), the ‘Sox’ thiosulfate oxidation pathway (soxXABYZ), the rDsr sulfur oxidation pathway (sat, aprBA, dsrABCMK, dsrEFH; Figs 2 and 5), nitrate reduction (narGHJ) and inorganic carbon fixation through the Calvin–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., 2017). 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% AA1, Table S2). ‘Ca. T. singularis’ has an organoheterotrophic aerobic lifestyle and does not oxidize sulfur (Spiecz 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 (Kₘ) of 2 μM of purified high-affinity Sqr proteins (Schutz et al., 1997; Brito et al., 2009) and the Kₘ found for phototrophic SOB (> 0.8 μM; Van Gemerden, 1984). However, the estimated Kₘ 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 × 10⁵ 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 × 10⁵ 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: *Sulfurimonasgotlandica* (Grote et al., 2012) and *‘Candidatus Sulfurimonas balitica’* (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, SCls 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 (soxXY,Z;AB and soxCDY,Z;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 (*Sulfurimonas ponti*), which also lacks most Sox genes despite being virtually complete (97% completeness, 4% contamination, Fig. 5). *U. ponti* may oxidize sulfide incompletely to S²⁰, or use an alternative route such as theHdr-like sulfur oxidation pathway (Boughanemi et al., 2016).

The heterotrophic *A. peruenis* 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. peruenis* 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 chemoclone with more mixing of sulfide, nitrate and oxygen (Rogge et al., 2017; Taylor et al., 2018). Furthermore, SUP05 bacteria are most abundant at lower-sulfidic conditions (<5 μM; Glaubitz 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 S²⁰ 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; Glaubitz et al., 2010; Glaubitz 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 *U. ponti* could perform ammonification (nrtAH, 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 tRNA 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
Examples of such SOB are the heterotrophic alphaproteobacterium *Sagittula castanea* isolated from euxinic Peru shelf waters (Martínez-Pérez et al., 1992; Manske et al., 2005; Marshall 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 shortcircuiting 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 S^0^ (Dahl, 2017), which is stored intracellularly by the abundant SUP05 bacteria (Shah et al., 2019). Part of this S^0^ may be released into the environment due to grazing of SUP05 bacteria by protists (Lin et al., 2007; Glaubitz et al., 2008; Anderson et al., 2013) or due to lysis by SUP05-infecting viruses (Cassman et al., 2012; Antharaman et al., 2014; Roux et al., 2014; Roux et al., 2016). Additionally, S^0^ has been observed to be introduced into OMZs through the drifting off of S^0^ produced in coastal waters experiencing sulfide events (Cailbeck 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 reoxidation 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; Serensen 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; Kamysnhy et al., 2011). The reduction or disproportionation of S^0^ 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 Hansen, 2013; Weisgerber 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 SCls 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; Bertagnolli 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 sulfaxigens (Finster, 2008; Finster et al., 2013), and Psr-like molybdoenzymes and rhodanese sulfurtransferases in other microorganisms such as Desulfurella amilisii (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, *Df. sulfidica* is the most closely related to Desulfocapsa. Yet, it has a markedly different gene repertoire than *Dc. sulfaxigens*, 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 (*Df. maris*, *Df. vada*, *Df. 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, Ut. autotrophica and Marinimicrobia, and dimethylsulfide reductase genes (dmsA) in MAGs of Desulfaclya species, 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 metaproteomics 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.

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Conflict of interest

The authors declare no conflict of interest.

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**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 km2; 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.