The Energetic Balance of Microbial Exploitation of Pelagic Redox Gradients

G. Jost and F. Pollehne

Abstract In marine environments and especially in marginal seas, pelagic redox gradients with underlying sulfidic water layers are a known phenomenon. General explanations for the observed spatial distribution and amount of chemolithoautotrophic carbon dioxide fixation within these redox zones persisted for decades. Here, we try to combine the assessment of fluxes of electron acceptors and donors which fuel chemolithoautotrophy, including energetic aspects of different reactions with observations on the microbial taxonomic structure within marine redox gradients. Although modern molecular techniques help to identify the acting organisms and verify chemolithoautotrophy on the process level, there are still gaps that need to be solved. Within the energetic frame of contributing reactions, there is still the option of the presence of hitherto undescribed physiological pathways. In this environment, characterized by strong gradients, new approaches need verification by incubation-independent methods to eliminate artifacts.

Keywords Carbon dioxide fixation, Chemolithoautotrophy, Electron acceptors, Electron donors, Microbial communities, Pelagic redox zones

Contents

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Abbreviations

DAPI	4′,6-Diamidino-2-phenylindole, a fluorescence stain that binds strongly to nucleic acids
MICRO-CARD-FISH	Fluorescence in situ hybridization combined with micro-autoradiography
OMZ	Oxygen minimum zone

1 Introduction

Organisms living below the euphotic zone in marine environments are dependent on the organic matter supplied to this environment. Here, the quantity and quality of organic carbon is in most cases the limiting factor for heterotrophic growth. In some cases, also oxygen becomes limiting for metabolic processes in deeper layers, mostly as an effect of reduced water circulation. These oxygen minimum zones (OMZ) are well-known phenomena in oceanography. It is assumed that the extension of hypoxic zones will increase especially in coastal marine waters due to both eutrophication and global warming [1] with severe effects on these ecosystems. The supply of both oxygen and organic carbon to layers below the euphotic zone of most marine systems is essential to maintain lives of higher organisms, as nearly all living organisms beyond the prokaryotic level depend on oxygen as terminal electron acceptor generating energy by respiration of organic material.

OMZs in the open ocean like in the Arabian Sea or in the central Pacific are under recent conditions not exhausting the total oxygen and nitrate inventory, as the organic input from the oligotrophic surface layer is comparatively low. Hypoxia in coastal waters (oxygen concentration below 2 mL L^{-1} or <90 μM) has a higher capacity of exhausting these resources of oxidants, and the system might turn to sulfate as electron acceptor because of higher biomasses, metabolic rates, concentrations of organics, and turnover of nutrients.

This shift is common within sediments, particularly in coastal areas [2]. Due to the reduced mobility of sedimentary pore-waters and the constant oxidation in surface sediments, this process remains largely unnoticed, since in most cases only the depth of the oxic–anoxic interface moves upward under reduced supply of electron acceptors. Most of the produced H_2S in sediments will be buried as pyrite in anoxic sediments [3] as long as iron is in good supply, but certain amounts of it will diffuse upward. Depending on the organic load of the sediment and the reduced flux of electron acceptors from supernatant water, which is already free of oxygen, the export of reduced compounds from sediments can push the redoxcline into the open water. This leads to higher H_2S concentrations in the overlying water than sulfate reduction processes within this water body could provide.

Pelagic redox gradients differ from benthic gradients, as they allow particulate phases to be sinking into deeper layers and therefore are selectively separating

elements. In a lot of coastal and marginal seas, pelagic redox zones are unstable on a regular seasonal basis or triggered by hydrographic events. This sometimes leads to the transport of oxygen-free or even sulfidic water into the surface layer and provokes undesired effects on higher organisms including humans. The occurrence of hydrogen sulfide in the water column documents an advanced stage in the development of hypoxia due to a larger imbalance in the fluxes of electron acceptors and electron donors. The depths, where such pelagic chemoclines [defined as the first appearance of H_2S (e.g., [4, 5])] are observed, depend on the equilibrium between the transport of electron acceptors and organic matter.

Although the term "suboxic" is widely used in oceanographic literature, the exact boundaries in terms of oxygen concentrations are rather diffuse. Values for the Black Sea range from 4.5 μM O_2 (e.g., [6]) to 15 μM O_2 [7]. More often a value of 10 μM is used [8]. Canfield and Thamdrup [9] argue for a stricter definition of this term, which is the base for a differentiation of biogeochemical processes. We advocate the value of about 10 μM O_2 thereby integrating water layers with enhanced nitrification from upward flux of ammonia, so that the major coupled nitrogen conversion processes are included. Here, we define the upper border of a pelagic redox zone as the layer, where denitrification is not detectable (which otherwise is often marked by a secondary nitrite peak) and nitrification is the dominant chemolithotrophic process. This usually is the case at oxygen concentrations of around 10 μM. The lower boundary is within the sulfidic layer at a concentration of about 10–15 μM hydrogen sulfide. This sulfide concentration seems to be the upper limit for a peak of enhanced carbon dioxide fixation [10–12], which is supposed to have a functional relation to the redox gradient. The absolute vertical extension of the pelagic redox gradient varies between the different ecosystems and shows a seasonal variability. This is due to the different strength of vertical mixing that merges sulfide from the deeper and oxygen from the surface layers.

Seasonal hypoxia often occurs at ocean margins dominated by large rivers, in estuaries and in brackish marginal seas [13]. Since decades, pelagic chemoclines have been quite well investigated in marine areas such as the Black Sea [14–16], the Cariaco Basin [17–19], the deep basins of the Baltic Sea [20–22], and several fjords like Framvaren [23, 24]. One of the largest hypoxic areas, besides the central Black Sea, is found in the Baltic [25, 26]. Here, changing areas of hypoxic bottom water are accompanied by often perennial anoxic bottom waters in the central basins.

Pelagic redox gradients are much more affected by lateral transport processes than their sedimentary images. Dependent on the basin morphology of different marine areas with pelagic redox zones, there are distinct depths at which lateral intrusions of water can occur, which then cause fluctuations in the depth of the oxycline or instabilities above the redox zone. Examples of these lateral intrusions are reported as inflow events into the western Black Sea [27, 28], in the Gotland Basin of the Baltic Sea [29], and in the Cariaco Basin [30].

A unique property of oxic/sulfidic pelagic redox zones is a pronounced peak in dark carbon dioxide fixation which was found in any of the investigated gradients. First, Sorokin [14] measured carbon dioxide fixation in the redox zone of the Black Sea and attributed it to bacterial chemosynthesis fueled by oxidation of reduced

sulfur compounds. It took about 15 years until this feature was observed in Cariaco Basin [31]. Earlier investigations by the same authors showed the occurrence of sulfide- and thiosulfate-oxidizing bacteria at the redox zones of the Black Sea and the Cariaco Basin, and also their potential to use thiosulfate for dark fixation of carbon dioxide [32]. But up to now any attempt to explain the high rates of carbon dioxide fixation quantitatively failed. We therefore want to review and discuss the existing data and hypotheses concerning this process focusing on three topics:

(a) Microbial communities across pelagic redox gradients
(b) Flux of electron acceptors and donors as source of energy
(c) Observed carbon dioxide fixation and quantitative explanation

2 Microbial Communities Across Pelagic Redox Gradients

Although in most pelagic redox zones gradients of temperature and salinity provide the density gradient, which inhibits vertical mixing, these gradients itself are rarely so strong that they exert direct effects on biological diversity and activity. The resulting gradients of the different electron acceptors and donors are much more directly influencing the abundance of bacteria across these water layers. There is, however, not a general pattern in bacterial biomass and diversity. Ho et al. [33] reported a significant increase in bacterial numbers at the suboxic/anoxic interface which at least in some cases showed more than twice the number of cells compared to the layers above or below. Similar conditions were later reported by [34, 35]. Bacterial abundances in the pelagic redox zone of the Black Sea are much more variable compared to the layers above and below. Bird and Karl [36] could only find an "absence of a general enrichment in total microbial or bacterial biomass across the oxic-to-anoxic boundary". Sorokin et al. [37] reported a stock of bacterial numbers and biomass within the redox zone, which was nearly twice as high as that in the water layer between redox and thermocline (40–100 m). Sometimes, the differences in bacterial numbers between these layers do not seem to be so high [38–40]. In pelagic redox zones of the Cariaco Basin and the Black Sea, bacterial cell numbers between 0.3 and 0.5 $\times$ 10^6 mL^{-1} were most often found, although these numbers range from less than 0.2 to more than 1 $\times$ 10^6 mL^{-1} for the Black Sea and the Cariaco Basin, respectively. Bacterial numbers are about twice as high for Baltic Sea redox gradients (mean between 0.8 and 1.2 $\times$ 10^6 mL^{-1}, ranging from 0.5 to more than 2 $\times$ 10^6 mL^{-1}) (e.g., [41–44]).

During transition from oxic-to-anoxic conditions in the Gotland Basin, also higher cell volumes of bacteria with increasing depths were reported [37, 41]. Especially in the Black Sea, the occurrence of filaments at the redox zone was reported [39], but this was already observed in samples from sulfidic water layers by Kriss [45].

Table 1 Bacterial growth estimations around redox zones of the Black Sea, Cariaco Basin, and Baltic Sea

Location	Bacterial production (μgC L^{-1} day^{-1})	Growth rate (day^{-1})	References
Baltic Sea	0.3–3.4[a]	0.04–0.4[b]	[46]
Black Sea	3.2–18.6	0.2–0.6	[37]
Black Sea	1.6–6		[47]
Black Sea	0,15	0.02–0.15	[39]
Cariaco Basin	0.02–1.2	0.02–0.2	[34]

[a]Assuming 20 fg C per cell
[b]Calculated from given ranges of biomass and production

Estimates of bacterial production in the different redox zones were published infrequent and also performed with quite different methods. From the values provided in Table 1, we conclude that the earlier estimations [37, 47] were probably too high. Growth rates of about 0.1 day^{-1} seem to be in the mean range. In the Baltic Sea, the bacterial production and the growth rates were higher as the values reported more recently from the Black Sea and Cariaco Basin (Table 1; [46]).

The DOC concentration is slightly decreasing with depth in the Black Sea [48], the Cariaco Basin [33], and the Baltic Sea [49]. Since most of the prokaryotic organisms are heterotrophs, they gain decreasing amounts of energy over the redox gradient by respiration of the same amount of dissolved organic matter, because of the decreasing energy gain by using different electron acceptors. This combination should result in at least slightly decreasing bacterial productivity with depth.

Within all three pelagic oxic–anoxic transition zones, mentioned above, pronounced shifts in the composition of bacterial communities are observed [4, 19, 50, 51]. Within the prokaryotic organisms, *Eubacteria* are dominant. Also common for all of these areas are higher proportions of *Epsilonproteobacteria* at the redox zone, accounting for up to 27% and 20% of the DAPI counts for the Cariaco Basin and the Black Sea, respectively [50]. For the Baltic Sea redox zone, up to 16% of the DAPI counts were related to the *Epsilonproteobacteria* [5]. For the Cariaco Basin and the Black Sea, comparable, but low abundances of *Gammaproteobacteria* of around 2–4% of the DAPI counts were reported [50], whereas in both regions peaks of around 8% were detected just above the chemocline. Using fingerprinting techniques, Labrenz et al. [51] could detect bands from *Alpha-*, *Gamma-*, *Delta-*, and *Epsilonproteobacteria* from the suboxic to the sulfidic layer at the central Baltic redox zone.

In many marine systems, also *Crenarchaeota* are at least partly involved in ammonia oxidation. *Crenarchaeota* dominate the variable proportions of archaea in all basins [50, 51]. Especially in the suboxic part of the redox zone, these *Crenarchaeota* seem to be mainly composed of nitrifying organisms related to the *Candidatus* "Nitrosopumilus maritimus" [52, 53]. These nitrifying *Crenarchaeota* seem to be well adapted to low oxygen conditions in marine environments [54–56].

Their contribution to ammonia oxidation was proven for the Black Sea [6, 52] and indicated for the Cariaco Basin [50]. In the suboxic part of the Baltic Sea redox zone, a peak of transcripts of amoA from *Crenarchaeota* just above the chemocline supports the importance of these organisms versus ammonia-oxidizing bacteria

[53]. These *Crenarchaeota* were recently detected in nearly all, especially suboxic, marine waters [54, 57, 58].

The existence of chemolithoautotrophic bacteria in pelagic redox zones is acknowledged since decades. Already Kriss [45] described the filamentous bacteria found in sulfidic waters as autotrophs oxidizing hydrogen sulfide. He, however, assumed that they used the energy from radioactive disintegrations. First measurements of chemosynthetic production by autotrophs at the pelagic redox zone of the Black Sea were reported by Sorokin [14], who addressed it to thio-oxidizing bacteria. Tuttle and Jannasch [59] reported the isolation of bacteria from the Black Sea oxidizing different reduced sulfur compounds, capable for autotrophic growth as well as for surviving under anoxic conditions. They later repeated that with material from the pelagic redox zone of the Cariaco Basin [32].

Although at least since the late 1980s a peak of elevated dark carbon dioxide fixation at the redox zone of the Baltic Sea is known [60, 61]. Until the work of Labrenz et al. [62] resulted in enrichment cultures, there are no attempts known to isolate these chemolithoautotrophic bacteria.

Since only a few metazoans are adapted to very low oxygen concentrations or even anoxic waters, protozoa seem to be the only organisms left to fulfill the function of grazers, preying on either other protozoa, flagellates, or prokaryotes [7]. Their abundance decreases with increasing concentrations of H_2S. In the central Baltic redox zone, ciliates instead of heterotrophic nanoflagellates seem to be the major grazers of bacteria within the suboxic part of the redox zone showing a peak at the oxic–anoxic interface [63]. Already Detmer et al. [61] reported a peak of ciliates at the chemocline followed by a drastic decrease further downward in the sulfidic water. This results in a decrease in grazing pressure on the prokaryotic populations over the pelagic redox zone, which might at least compensate the effect of reduced bacterial productivity on the standing stock of prokaryotes. A more pronounced increase in prokaryote abundance below the redox zone might be limited by increased mortality due to bacteriophages [46]. This might be also supported by a higher ratio of virus-like particles to bacteria as found in the anoxic part of the Cariaco Basin [34].

3 Flux of Electron Acceptors and Donors as Source of Energy

A shift in the composition of bacterial communities across oxic–anoxic transition zones is not surprising since the main electron acceptor oxygen and is gradually replaced by other oxidants. The next energetically favorable electron acceptor is nitrate, followed by oxidized iron, manganese, and, finally, sulfate. This microbial redox sequence based on the energy gained by the different electron acceptors describes principally the temporal succession of the exhausted electron acceptors and their spatial disappearance [2]. While such cascades in sediments happen often

within several millimeters or centimeters, we have to assume spacial scales of decimeters for strong gradients like in several fjords [24] to several meters like in the Cariaco Basin [11]. The availability of the electron acceptors will cause a transition from aerobic via facultative anaerobic to anaerobic microbial metabolisms. Following this succession, not only the energy gain for bacterial growth by alternative electron acceptors will be reduced drastically, but also alternative bacterial metabolism becomes dominant as reflected by the shift in the community structure.

Below we will focus our attention on compounds that are known to fuel chemolithoautotrophic growth of microorganisms. A number of different bacteria are specialized to gain their energy by oxidation of inorganic or one-carbon compounds and assimilating carbon dioxide [64]. The number of compounds that could react as electron acceptors and/or as electron donors for microbial carbon dioxide fixation is relatively limited. A list of the most probable compounds used by chemolithotrophic microorganisms is shown in Table 2. We will focus our attention on oxygen and nitrate as the most important electron acceptors. Less-oxidized nitrogen compounds can be detected in significantly lower concentrations during different redox transitions as intermediates and can therefore be included in the nitrate inventory. Particulate manganese oxides are important due to their different transport behavior. In contrast to dissolved substances that are dependent on enhanced eddy diffusion for their vertical distribution, these manganese particles are transported much faster by sinking [22, 65]. The highest energy gain can be achieved by chemolithoautotrophic oxidation of hydrogen or methane by oxygen or even nitrate [64], but both compounds can only be found close to the detection limit (hydrogen) or in the nMol range at pelagic chemoclines ([66], Heyer, personal communication). Therefore, the contribution of these oxidation pathways can be considered a minor contribution to the observed carbon dioxide fixation. According to the measured concentration gradients and calculated fluxes, this leaves only

Table 2 Inorganic electron acceptors and/or donors which could enable chemolithoautotrophic bacterial growth

Electron acceptors	Electron donors
Oxygen	Hydrogen
Nitrate	Methane
Nitrite[a]	Ammonia
Other partly oxidized nitrogen compounds[a]	Nitrite[a]
Oxidized iron	Other partly oxidized nitrogen compounds[a]
Oxidized manganese	Reduced iron
Carbon monoxide	Reduced manganese
Thiosulfate[a]	Hydrogen sulfide
Elemental sulfur[a]	Thiosulfate[a]
Other partly oxidized sulfur compounds[a]	Elemental sulfur[a]
Sulfate	Other partly oxidized sulfur compounds[a]

Underlined are the compounds (most oxidized/reduced) assumed to be most important

[a]Intermediate compounds, which could be further oxidized or reduced depending on environmental conditions

ammonia, sulfide, and manganese to contribute substantially to chemolithotrophy. Other more oxidized sulfur compounds such as elemental sulfur and thiosulfate can be included in the sulfide budget since they remain most probably intermediates within the redox zone. According to the vertical profiles, a vertical transport of these compounds from sediments to the pelagic redox zone in the Baltic Sea [67] or in the Cariaco Basin [68] is rather unlikely.

Biological turnover in the redox gradient is largely dependent on the downward flux of the most oxidized and upward flux of the most reduced chemical species into the pelagic redox zone. Intermediates that can be used as either electron donor or acceptor are mainly involved in cyclic processes within the system and not relevant for import or export.

The successive disappearance of the different electron acceptors with depth following the decreasing amount of energy gained by their use is well known from sediments. Like in sediments, the decline of oxygen with depth in pelagic redox zones is as well followed by a decline of nitrate. Denitrification already starts under suboxic conditions. For oceanic denitrification, oxygen concentrations above around 2 μM seem to be inhibiting [69, 70]. In the suboxic water of the Gotland Basin, denitrification was detectable at oxygen concentrations below 9 μM [71]. But already Brettar and Rheinheimer [21] reported that heterotrophic denitrification in the suboxic water column was hard to measure and assumed that the main nitrogen loss process is coupled to oxidation of reduced sulfur compounds. They concluded that heterotrophic denitrification is limited especially by the quality of available organic matter [21, 72]. The importance of chemolithotrophic denitrification as the main loss factor for nitrogen at pelagic redox zones of the Baltic Sea was also stressed by Hannig et al. [73].

This lack of electron acceptors leads to a decrease in denitrification capacity of the ecosystem. In sedimentary environments with high organic loads, nitrate penetrates from the bottom water into the sediment where it is reduced at the depth where oxygen becomes limited. Even ammonia produced during remineralization will be oxidized within the sediment before it will be released as nitrate or denitrified to dinitrogen. In most pelagic redox zones, the lack of available organic material for heterotrophic denitrification is also the limiting factor for microbial heterotrophic activities. This might, at least in the central Baltic, explain why there are no drastic changes in bacterial numbers and growth rates over the whole redox zone, which should be expected by the drastic changes of energy yield in the use of different electron acceptors. It as well implies the importance of chemolithoautotrophy for life at pelagic redox zones.

Many organisms are known to oxidize manganese and in theory it should be sufficient to support chemolithoautotrophic growth. Real proof is lacking, but as genes for CO_2 fixation were found in a manganese oxidizing bacterium [74], this pathway seems very likely. The energy gain will, however, be relatively low. Under oxic conditions, these microorganisms should be outcompeted by others using oxygen more efficiently for growth, although the indicated lack of degradable organic matter (lacking heterotrophic denitrification) would impede both denitrifiers and other heterotrophic bacteria. There are other possible competitors

Table 3 Examples of redox reactions which could fuel chemolithoautotrophy within different layers of the redox zone

Condition	Redox reactions	ΔG^0 (kJ mol^{-1})	References
Oxygen available	(1) Ammonia oxidation $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$	−272	[75]
	(2) Nitrite oxidation $2NO_2^- + O_2 \rightarrow 2NO_3^-$	−75	[75]
	(3) Incomplete sulfide oxidation $2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$	−145	[76]
	(4) Incomplete zero-valent sulfur oxidation $S^0 + O_2 + H_2O \rightarrow SO_3^{2-} + 2H^+$	−249	[76]
	(5) Manganese oxidation $2Mn_2^+ + O_2 + 2H_2O \rightarrow 2MnO_4 + 4H^+$	−81	[77]
	(6) Iron oxidation $4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 + 8H^+$	−88	[78]
Nitrite/nitrate available	(7) Anaerobic sulfide oxidation $5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$	−745	[79]
	(8) Anaerobic thiosulfate oxidation $5S_2O_3^{2-} + 8NO_3^- + H_2O \rightarrow 10SO_4^{2-} + 2H^+ + 4N_2$	−751	[76]
	(9) Anammox $NH_4^+ + NO_2^- \rightarrow N_2 + H_2O$	−360	[75]
Anoxic, without nitrite/nitrate	(10) Anaerobic sulfur oxidation $S^0 + 3MnO_2 + 4H^+ \rightarrow 3Mn^{2+} + 2H_2O + SO_4^{2-}$	−384	[80]
	(11) Anaerobic sulfide oxidation $HS^- + 4MnO_2 + 7H^+ \rightarrow SO_4^{2-} + 4Mn^{2+} + 4H_2O$	−525	[80]
	(12) Thiosulfate disproportionation $S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+$	−6	[76]

for oxygen-nitrifying bacteria, manganese oxidizers, and other chemolithotrophs which oxidize reduced sulfur compounds, as they are not in need of organic substrates (Table 3). In Black Sea studies, a coexistence of reduced sulfur and oxygen is often negated [81]. In this case, there should be differences in manganese oxidation rates, as the concurrent process of sulfur oxidation is omitted. Rates of Mn oxidation should be higher in the Black Sea, as compared to the Baltic Sea and the Cariaco Basin, where the overlap between oxygen and reduced sulfur compounds is the standard situation, and therefore more favorable options for the exploitation of oxygen are present.

According to our present knowledge of chemolithoautotrophic bacteria and the distribution of electron acceptors, we would expect their main activity around the oxic–anoxic interface. Since oxidation pathways with nitrogen species provide nearly the same energy yield as oxygen-based processes and due to the fact that these compounds are also dissolved, they should be depleted within a relative short distance after the disappearance of oxygen. The only possible electron

acceptors which could enter deeper layers by sedimentation can be oxidized iron and manganese particles.

4 Observed Carbon Dioxide Fixation and Quantitative Explanation

The gradient system of oxidized and reduced inorganic compounds within redox zones seems to be a perfect place for chemolithotrophic organisms. Most of them use either oxygen or oxidized nitrogen compounds as terminal electron acceptors oxidizing reduced compounds. Therefore, we can expect their peak in activity at the oxic–anoxic interface until oxidized nitrogen compounds disappear. There are few reports from different pelagic redox zones that support this hypothesis [31, 61, 82]. But more often the maximum of the carbon dioxide fixation is located further down in the anoxic part of the redox zone [11, 12, 14, 83]. This vertical structure as well as the amount of carbon dioxide fixation was explained quite differently. At first, Jørgensen et al. [10] recognized the difficulty to explain the values of chemolithotrophic carbon dioxide fixation quantitatively. Although they mentioned the possible contribution of anoxygenic phototrophic bacteria, they ruled out that they could contribute a substantial part. Since in most of these pelagic redox zones light is of minor importance (except shallow fjords), we might rule out the importance of photosynthesis as an important process in the major marine pelagic redox zones of the Cariaco Basin, the Black Sea and the Baltic Sea. Even if there are some reports about photosynthetic activity of anaerobic bacteria at the chemocline of the Black Sea [84–86], their contribution to the measured carbon fixation (e.g., [10, 14, 37]) can only be of minor importance.

There are still two more options to explain dark CO_2 fixation. Heterotrophic bacteria might take up carbon dioxide by anaplerotic reactions. It is assumed that this could account for up to 8% of bacterial carbon fixation [87, 88]. Under anaerobic conditions, this percentage seems even to be slightly higher [89]. As in all pelagic redox zones, rates of dark CO_2 fixation are not coupled to heterotrophic bacterial production and often show comparatively low values in the suboxic part, where the heterotrophic bacterial production is higher than in the anoxic part of the redox zone, and anaplerotic CO_2 fixation cannot explain the observed rates.

The only remaining choice of probable agents for this process is then narrowed down to chemolithoautotrophic organisms. Isolation of chemolithoautotrophic bacteria from the respective layers can proof the presence of this type of organisms but not their quantitative role. First evidence for the importance of these bacteria came from the Baltic Sea. Jost et al. [44] demonstrated the existence of about 20–40% of chemolithoautotrophic bacteria within the redox zone fixing between 3.5 and more than 20 fg C per cell per day. According to their calculations, 0.8 fg C should be the upper limit, which could be fixed by heterotrophic bacteria within these samples. At least one group of chemolithoautotrophic bacteria could be easily discerned

from others by relatively high 90° light side scatter signals in their flow cytometric signature. This unique cluster of bacteria just appeared at the lower transition of the chemocline to sulfidic waters [44]. Casamayor et al. [90] observed such a shift in the flow cytometric signature of phototrophic bacteria when they accumulated internal elemental sulfur. Such a morphological signature may also explain the appearance of the optically active bacterial cluster below the chemoclines of the Baltic Sea, which was also observed in the Black Sea (unpublished results). The existence of chemolithoautotrophic bacteria was also detected by MICRO-CARD-FISH analyses at redox zones of the Baltic Sea and Black Sea [43]. This investigation verified not only the importance of chemolithoautotrophic bacteria, but also showed that their activity was nearly 100% restricted to *Eubacteria* below the chemoclines. As already suggested by Lin et al. [19], it could be proven that *Epsilonproteobacteria* play a major role in CO_2 fixation at pelagic redox zones. Between 70 and nearly 100% of the detected chemolithoautotrophic bacteria could be identified as *Epsilonproteobacteria* by MICRO-CARD-FISH probes [43]. Studies by Glaubitz et al. [82, 91] render it likely that the remaining chemolithoautotrophs belong to *Gammaproteobacteria* both in the Baltic and in the Black Sea. Although their contribution to the estimated CO_2 fixation was less than half of the amount fixed by *Epsilonproteobacteria*, the chemolithoautotrophic *Gammaproteobacteria* were more diverse than the *Epsilonproteobacteria* [82]. At least one group of these *Gammaproteobacteria* is closely related to a symbiont, and it is also known that sulfide-oxidizing bacteria appear as ectosymbionts of protozoa in sulfidic environments [92–95]. The observed transfer of the CO_2 fixation by bacteria to protozoa could be related not only to unspecified grazing but also to grazing of own ectosymbionts [91]. The closely related *Epsilonproteobacteria* belong to *Sulfurimonas* sp. and proved them to be versatile key players within pelagic redox zones by microdiversity analyses [5, 43, 82, 91]. These approaches for direct detection and identification of chemolithoautotrophic organisms were used in layers showing enhanced CO_2 fixation. For these layers, a clear dominance of the oxidation of reduced sulfur compounds to fuel chemolithoautotrophy could be demonstrated. Here, it can only be speculated, whether this is also the case for the upper suboxic part of the redox zone. In this environment, at least the presence of *Epsilonproteobacteria* was demonstrated [5], and according to Kamyshny et al. [67] the presence of reduced sulfur compounds is as well proven.

In the suboxic part of the redox zone, alternative reactions allow chemolithoautotrophy. The importance of nitrifying bacteria within suboxic environments is well known [96]. Ammonia oxidation is restricted to at least suboxic environments. In the Baltic Sea, this process is indicated by the appearance of a nitrate peak below the halocline [51, 97], which should be related to highest ammonia oxidation activities. In this layer, CO_2 fixation rates are, however, near the detection limit (Jost, unpublished results) and can be assigned only to the activity of chemolithoautotrophic ammonia oxidizers [12]. This view will probably be modified by new findings that *Crenarchaeota* are as well involved in the marine nitrogen cycle. It is assumed that the marine *Crenarchaeota* are also autotrophs [54, 98], and for the Black Sea it was suggested that both *Gammaproteobacteria* and *Crenarchaeota*

contribute to nitrification [6]. Because of different pathways of carbon fixation in bacterial and archaeal metabolism [99], their fixation rate per oxidation equivalent might slightly differ. Even if these organisms were just partly autotrophic or mixotrophic, as was assumed from crenarchaeotal uptake of amino acids [100, 101], their respective metabolism cannot change the overall budget within the suboxic layers of pelagic redox zones substantially, as CO_2 fixation rates are generally low. The calculated rates of carbon dioxide fixation by chemolithoautotrophic ammonia-oxidizing bacteria are not only in good agreement with the low rates measured in the suboxic part of the pelagic redox zone of the Baltic Sea, but can also explain the disappearance of ammonia around the chemocline [12].

The contribution of anammox bacteria (oxidation of NH_4 with NO_2 to N_2 and H_2O) to CO_2 fixation seems as well to be of minor importance, as their energy gain is in the same range as that of aerobic ammonia oxidizers [102]. Since these bacteria use nitrate or nitrite under anoxic conditions with an energy yield as low as nitrifying bacteria, they cannot contribute more to CO_2 fixation than the nitrifiers. Earlier, it was assumed that anammox would be restricted to anoxic and non-sulfidic environments, since it was completely inhibited already at oxygen concentrations as low as 2 μM [103]. But then it was found that anammox still proceeds under low oxygen concentrations [104], though under suboxic conditions anammox rates are lower than under anoxic conditions [105]. Lam et al. [6] postulated a short circuit between nitrification and anammox for the suboxic to upper anoxic layers of the redox zone of the Black Sea since they could not detect any denitrification. This implies that anammox bacteria could outcompete denitrifying bacteria. Since the energy yield of the anammox bacteria per mol nitrite/nitrate is about ten times less than that of heterotrophic denitrifiers or even chemolithoautotrophic denitrifiers using reduced sulfur compounds, this is not very probable in a concurrent situation. In case it occurs, it indicates a lack of suitable organic matter or reduced sulfur compounds. Under these conditions, the highest rates of CO_2 fixation should then be found in the deeper part of the suboxic layer and within the upper part of the anoxic layer. But here enhanced fixation rates of chemolithoautotrophic denitrifiers are not probable because of lacking nitrite/nitrate. Such a scenario seems, however, quite possible in an environment like the Black Sea, where an extended anoxic layer without detectable hydrogen sulfide seems to be existing at least temporarily [106].

In the Baltic Sea anammox could only be detected occasionally, rates were in the range of 0.05–0.005 μmol N L^{-1} day^{-1} [73]. This was attributed to higher Mn(IV) concentrations, which developed after intense salt water inflow led to a short period of fully oxygenated bottom water in the Gotland Basin. In a newly establishing pelagic redox zone extending from the sediment, relatively large amounts of sinking manganese oxide and its potential to oxidize sulfide seemed to be able to establish a temporarily zone, free of oxygen and H_2S. It should, however, be noted that these anammox measurements are based on the addition of nitrate to anoxic water samples without detectable in situ nitrate concentrations. Therefore, induction or at least an enhancement of the measured rates must be assumed.

The basic process that fuels all chemolithoautotrophic activities is basically driven by the stepwise oxidation of sulfide with oxygen over the total redox

gradient. But all calculations to explain the measured carbon dioxide fixation by sulfide oxidation failed so far [10–12, 24]. First, there is a lack of sulfide reaching the zone of enhanced carbon dioxide fixation by upward transport in all of the investigated systems, and second, the peak of the fixation zone is often within the sulfidic part of the redox zone without detectable amounts of oxygen and oxidized nitrogen, which are the most important electron acceptors for chemolithoautotrophic sulfur-oxidizing bacteria.

Although even in suboxic parts of pelagic redox zones sulfate-reducing bacteria were detected in the Baltic Sea [51], the Black Sea [50, 107], and the Cariaco Basin [35, 50], it seems very unlikely that these bacteria supplied enough, if any, sulfide to produce the required amount of sulfide to fuel the process. Albert et al. [108] measured production rates of up to 3.5 nmol H_2S L^{-1} day^{-1} below the pelagic redox zone of the Black Sea, but also deeper than the zone of elevated carbon dioxide fixation. Within this zone, Jørgensen et al. [10] measured values between 3 and 36 nmol H_2S L^{-1} day^{-1}. Since these rates were two orders of magnitude lower than measured sulfide oxidation rates within the same layer, sulfate reduction cannot provide an important source for sulfide [10]. Jost et al. [12] suggested a recycling of sulfide from only partly oxidized sulfur compounds like elemental sulfur or thiosulfate. Although the calculated turnover time of the sulfide pool seems with about 1 day comparatively low, this still needs to be verified. The most critical point in this argumentation is to explain the origin of the organic matter for the heterotrophic bacteria using thiosulfate or elemental sulfur as electron acceptors, as the quality and amount of organic matter in layers only several meters above are not even sufficient to fuel heterotrophic denitrification.

Since oxidized nitrogen compounds can only explain a small part of the observed carbon dioxide fixation by sulfur oxidizers, the question for alternative electron acceptors within the sulfidic environment remains open. Most likely seems to be the involvement of particulate iron and manganese [10–12]. But the amount of oxidized metals which could reach these anoxic layers would also by far be not sufficient to explain the observed carbon dioxide fixation rates [12]. It is well known that humic substances could mediate the electron transfer between particulate metal oxides and bacteria [109]. It has also been shown that humic substances could be used as electron acceptors for the oxidation of organic substances [110]. It is, however, unknown whether these substances could also be used to oxidize reduced sulfur compounds by chemolithoautotrophic bacteria. But even if this would be possible, these electron acceptors would needed to be recycled, e.g., by metal oxides, as otherwise their capacity to accept electrons would degrade quickly.

At the moment, we cannot provide a satisfying explanation for the extent of the carbon dioxide fixation rates especially in the anoxic part of the redox zones. Lateral intrusions were often assumed to supply the redox gradients with additional oxygen [5, 11, 28]. But even if this amount would be large enough, the capacity of the dissolved metal inventory would not be sufficient to transport it as equivalents into the anoxic part, which is characterized by enhanced carbon dioxide fixation.

Although a suite of precautionary measures were taken to eliminate oxygen contamination of the incubation samples by all groups engaged in these

experiments, an ultimate guarantee cannot be given. Even the observed progression of the carbon dioxide fixation maximum in the anoxic layer could be explained by assuming that every sample might have been contaminated with about the same amount of oxygen. Then, depending on the amount of reduced sulfur compounds, an increasing amount of carbon dioxide may be fixed, as long as enough microbes capable of oxidizing reduced sulfur compounds are present. In deeper layers with higher sulfide concentrations, their cell numbers will either decline or the bacteria might need longer lag phases to reestablish their physiology. Due to the fact that intense care has been taken, to keep oxygen out of the experiments, this scenario is not very probable but cannot be ruled out completely.

All published results showing enhanced carbon dioxide fixation within anoxic environments without nitrite/nitrate are based on incubations of water samples which have been transferred through oxic environments before incubation with ^{14}C- or ^{13}C-bicarbonate solution. Incubation-independent measurements like the estimation of stable isotopes within fatty acids show significant changes only at the layer around the chemocline and not in the sulfidic part [91]. At present, the trade-off of these arguments leaves us to conclude that there is at least the capacity for anoxic carbon dioxide fixation. The rates might, however, be overestimated by contaminations during incubation procedures. We therefore advocate the improvement of techniques toward in situ rate measurements and the integration of in situ molecular approaches like metatranscriptomics or -proteomics regarding carbon dioxide fixing key enzymes.

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References

1. Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926–929
2. Fenchel T, King GM, Blackburn TH (1998) Bacterial biogeochemistry: the ecophysiology of mineral cycling. Academic, London
3. Morse J, Thomson H, Finneran D (2007) Factors controlling sulfide geochemistry in subtropical estuarine and bay sediments. Aquat Geochem 13:143–156
4. Vetriani C, Tran HV, Kerkhof LJ (2003) Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the black sea. Appl Environ Microbiol 69:6481–6488
5. Grote J, Labrenz M, Pfeiffer B et al (2007) Quantitative distributions of *Epsilonproteobacteria* and a *Sulfurimonas* subgroup in pelagic redoxclines of the central Baltic Sea. Appl Environ Microbiol 73:7155–7161
6. Lam P, Jensen MM, Lavik G et al (2007) Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proc Natl Acad Sci USA 104:7104–7109
7. Zubkov MV, Sazhin AF, Flint MV (1992) The microplankton organisms at the oxic-anoxic interface in the pelagial of the Black Sea. FEMS Microbiol Ecol 101:245–250
8. Murray JW, Stewart K, Kassakian S et al (2005) Oxic, suboxic and anoxic conditions in the Black Sea. In: Gilbert A, Yanko-Hombach V, Panin N (eds) Climate change and coastline migrationas factors in human adaptation to the Circum-Pontic region: from past to forecast. Kluwer, New York, pp 437–452

9. Canfield DE, Thamdrup B (2009) Towards a consistent classification scheme for geochemical environments, or, why we wish the term 'suboxic' would go away. Geobiology 7:385–392
10. Jørgensen BB, Fossing H, Wirsen CO et al (1991) Sulfide oxidation in the anoxic Black Sea chemocline. Deep Sea Res 38:S1083–S1103
11. Taylor GT, Iabichella M, Ho T-Y et al (2001) Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic carbon production. Limnol Oceanogr 46:148–163
12. Jost G, Martens-Habbena W, Pollehne F et al (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
13. Rabouille C, Conley DJ, Dai MH et al (2008) Comparison of hypoxia among four river-dominated ocean margins: the Changjiang (Yangtze), Mississippi, Pearl, and Rhône rivers. Cont Shelf Res 28:1527–1537
14. Sorokin JI (1964) On the primary production and bacterial activities in the Black Sea. J Cons Int Explor Mer 29:41–60
15. Repeta D (1993) Chemocline of the Black Sea. Nature 366:415–416
16. Wakeham SG, Amann R, Freeman KH et al (2007) Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study. Org Geochem 38:2070–2097
17. Deuser WG (1973) Cariaco Trench: oxidation of organic matter and residence time of anoxic water. Nature 242:601–603
18. Zhang J-Z, Millero FJ (1993) The chemistry of the anoxic waters in the Cariaco Trench. Deep Sea Res I 40:1023–1041
19. Lin X, Scranton MI, Varela R et al (2007) Compositional responses of bacterial communities to redox gradients and grazing in the anoxic Cariaco Basin. Aquat Microb Ecol 47:57–72
20. Fonselius S (1981) Oxygen and hydrogen sulphide conditions in the Baltic Sea. Mar Pollut Bull 12:187–194
21. Brettar I, Rheinheimer G (1991) Denitrification in the central Baltic: evidence for H_2S-oxidation as motor of denitrification at the oxic-anoxic interface. Mar Ecol Prog Ser 77: 157–169
22. Neretin LN, Pohl C, Jost G et al (2003) Manganese cycling in the Gotland Deep, Baltic Sea. Mar Chem 82:125–143
23. Millero FJ (1991) The oxidation of H_2S in Framvaren Fjord. Limnol Oceanogr 36:1007–1014
24. Zopfi J, Ferdelman TG, Jørgensen BB et al (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
25. Conley DJ, Humborg C, Rahm L et al (2002) Hypoxia in the Baltic Sea and basin-scale changes in phosphorus biogeochemistry. Environ Sci Technol 36:5315–5320
26. Conley DJ, Björck S, Bonsdorff E et al (2009) Hypoxia-related processes in the Baltic Sea. Environ Sci Technol 43:3412–3420
27. Özsoy E, Rank D, Salihoglu I (2002) Pycnocline and deep mixing in the Black Sea: stable isotope and transient tracer measurements. Estuar Coast Shelf Sci 54:621–629
28. Konovalov SK, Luther GW, Friederich GE et al (2003) Lateral injection of oxygen with the Bosporus plume – fingers of oxidizing potential in the Black Sea. Limnol Oceanogr 48: 2369–2376
29. Jost G, Clement B, Pakhomova S et al (2007) Field studies of anoxic conditions in the Baltic Sea during the cruise of R/V *Professor Albrecht Penck* in July 2006. Oceanology 47: 590–593
30. Astor Y, Muller-Karger F, Scranton MI (2003) Seasonal and interannual variation in the hydrography of the Cariaco Basin: implications for basin ventilation. Cont Shelf Res 23: 125–144
31. Tuttle JH, Jannasch HW (1979) Microbial dark assimilation of CO_2 in the Cariaco Trench. Limnol Oceanogr 24:746–753

32. Tuttle JH, Jannasch HW (1973) Sulfide and thiosulfate-oxidizing bacteria in anoxic marine basins. Mar Biol 20:64–70
33. Ho TY, Scranton MI, Taylor GT et al (2002) Acetate cycling in the water column of the Cariaco Basin: seasonal and vertical variability and implication for carbon cycling. Limnol Oceanogr 47:1119–1128
34. Taylor GT, Hein C, Iabichella M (2003) Temporal variations in viral distributions in the anoxic Cariaco basin. Aquat Microb Ecol 30:103–116
35. Lin X, Scranton MI, Chistoserdov AY et al (2008) Spatiotemporal dynamics of bacterial populations in the anoxic Cariaco Basin. Limnol Oceanogr 53:37–51
36. Bird DF, Karl DM (1991) Microbial biomass and population diversity in the upper water column of the Black Sea. Deep Sea Res 38:S1069–S1082
37. Sorokin YI, Sorokin PY, Avdeev VA et al (1995) Biomass, production and activity of bacteria in the Black Sea, with special reference to chemosynthesis and the sulfur cycle. Hydrobiologia 308:61–76
38. Gorlenko VM, Mikheev PV, Rusanov II et al (2005) Ecophysiological properties of photosynthetic bacteria from the Black Sea chemocline zone. Microbiology 74:201–209
39. Morgan JA, Quinby HL, Ducklow HW (2006) Bacterial abundance and production in the western Black Sea. Deep Sea Res II 53:1945–1960
40. Mosharova I, Sazhin A (2007) Bacterioplankton in the northeastern part of the Black Sea during the summer and autumn of 2005. Oceanology 47:671–678
41. Gast V, Gocke K (1988) Vertical distribution of number, biomass and size-class spectrum of bacteria in relation to oxic/anoxic conditions in the Central Baltic Sea. Mar Ecol Prog Ser 45:179–186
42. Brettar I, Höfle MG (1993) Nitrous oxide producing heterotrophic bacteria from the water column of the central Baltic: abundance and molecular identification. Mar Ecol Prog Ser 94:253–265
43. Grote J, Jost G, Labrenz M et al (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
44. Jost G, Zubkov MV, Yakushev E et al (2008) High abundance and dark CO_2 fixation of chemolithoautotrophic prokaryotes in anoxic waters of the Baltic Sea. Limnol Oceanogr 53: 14–22
45. Kriss AE (1959) The role of microorganisms in the primary production of the Black Sea. J Cons Int Explor Mer 24:221–230
46. Weinbauer MG, Brettar I, Höfle MG (2003) Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. Limnol Oceanogr 48:1457–1465
47. Karl DM, Knauer GA (1991) Microbial production and particle flux in the upper 350 m of the Black Sea. Deep Sea Res 38:S921–S942
48. Ducklow HW, Hansell DA, Morgan JA (2007) Dissolved organic carbon and nitrogen in the Western Black Sea. Mar Chem 105:140–150
49. Schneider B, Nagel K, Struck U (2000) Carbon fluxes across the halocline in the eastern Gotland Sea. J Mar Syst 25:261–268
50. Lin X, Wakeham SG, Putnam IF et al (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
51. Labrenz M, Jost G, Jürgens K (2007) Distribution of abundant prokaryotic organisms in the water column of the central Baltic Sea with an oxic-anoxic interface. Aquat Microb Ecol 46: 177–190
52. Coolen MJL, Abbas B, van Bleijswijk J et al (2007) Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. Environ Microbiol 9:1001–1016
53. Labrenz M, Sintes E, Toetzke F et al (2010) Relevance of a crenarchaeotal subcluster related to *Candidatus* Nitrosopumilus maritimus to ammonia oxidation in the suboxic zone of the central Baltic Sea. ISME J 4:1496–1508

54. Kuypers MM, Blokker P, Erbacher J et al (2001) Massive expansion of marine archaea during a mid-Cretaceous oceanic anoxic event. Science 293:92–95
55. Woebken D, Fuchs BM, Kuypers MMM et al (2007) Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. Appl Environ Microbiol 73:4648–4657
56. Walker CB, de la Torre JR, Klotz MG et al (2010) *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proc Natl Acad Sci USA 107:8818–8823
57. Francis CA, Roberts KJ, Beman JM et al (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci USA 102:14683–14688
58. Beman JM, Popp BN, Francis CA (2008) Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. ISME J 2:429–441
59. Tuttle JH, Jannasch HW (1972) Occurrence and types of thiobacillus-like bacteria in the sea. Limnol Oceanogr 17:532–543
60. Gocke K (1989) Bakterielle Stoffaufnahme im aeroben und anaeroben Milieu der Ostsee. Ber Inst Meeresforsch 188:40–47
61. Detmer AE, Giesenhagen HC, Trenkel VM et al (1993) Phototrophic and heterotrophic pico- and nanoplankton in anoxic depths of the central Baltic Sea. Mar Ecol Prog Ser 99:197–203
62. Labrenz M, Jost G, Pohl C et al (2005) Impact of different in vitro electron donor/acceptor conditions on potential chemolithoautotrophic communities from marine pelagic redoxclines. Appl Environ Microbiol 71:6664–6672
63. Anderson R, Weber F, Wylezich C et al. (2011) Protist diversity, distribution and bacterivory in Baltic Sea pelagic redoxclines. ASLO 2011 Aquatic Sciences Meeting, San Juan, Puerto Rico
64. Shively JM, van Keulen G, Meijer WG (1998) Something from almost nothing: carbon dioxide fixation in chemoautotrophs. Annu Rev Microbiol 52:191–230
65. Yakushev EV, Debolskaya EI (2000) Particulate manganese as a main factor of oxidation of hydrogen sulfide in redox zone of the Black Sea. In: Oceanic fronts and related phenomena. Konstantin Fedorov memorial symposium, Pushkin, Saint-Petersburg, Russia, 18–22 May 1998, Proceedings, IOC Workshop Report No 159. Kluwer Academic Publishers, pp 592–597
66. Schmale O, Schneider von Deimling J, Gülzow W et al (2010) Distribution of methane in the water column of the Baltic Sea. Geophys Res Let 37:L12604
67. Kamyshny A Jr, Yakushev EV, Jost G et al (2011) Role of sulfide oxidation intermediates in the redox balance of the oxic-anoxic interface of the Gotland Deep, Baltic Sea. In: Yakushev EV (ed) Chemical structure of pelagic redox interfaces: Observation and modeling. Springer, Berlin/Heidelberg, doi 698_2010_83
68. Hayes MK, Taylor GT, Astor Y et al (2006) Vertical distributions of thiosulfate and sulfite in the Cariaco Basin. Limnol Oceanogr 51:280–287
69. Cline JD, Richards FA (1972) Oxygen deficient conditions and nitrate reduction in the eastern tropical North Pacific Ocean. Limnol Oceanogr 17:885–900
70. Devol AH (1978) Bacterial oxygen uptake kinetics as related to biological processes in oxygen deficient zones of the oceans. Deep Sea Res 25:137–146
71. Rönner U, Sörensson F (1985) Denitrification rates in the low-oxygen waters of the stratified Baltic proper. Appl Environ Microbiol 50:801–806
72. Brettar I, Rheinheimer G (1992) Influence of carbon availability on denitrification in the central Baltic Sea. Limnol Oceanogr 37:1146–1163
73. Hannig M, Lavik G, Kuypers MMM et al (2007) Shift from denitrification to anammox after inflow events in the central Baltic Sea. Limnol Oceanogr 52:1336–1345
74. Caspi R, Haygood MG, Tebo BM (1996) Unusual ribulose-1,5-bisphosphate carboxylase/oxygenase genes from a marine manganese-oxidizing bacterium. Microbiology 142: 2549–2559
75. Broda E (1977) Two kinds of lithotrophs missing in nature. Z Allg Mikrobiol 17:491–493
76. Kelly DP (1999) Thermodynamic aspects of energy conservation by chemolithotrophic sulfur bacteria in relation to the sulfur oxidation pathways. Arch Microbiol 171:219–229

77. De Schamphelaire L, Rabaey K, Boon N et al (2007) The potential of enhanced manganese redox cycling for sediment oxidation. Geomicrobiol J 24:547–558
78. Sobolev D, Roden EE (2004) Characterization of a neutrophilic, chemolithoautotrophic Fe(II)-oxidizing β-proteobacterium from freshwater wetland sediments. Geomicrobiol J 21:1–10
79. Megonigal JP, Hines ME, Visscher PT (2004) Anaerobic metabolism: linkages to trace gases and aerobic processes. In: Schlesinger WH (ed) Biogeochemistry. Elsevier-Pergamon, Oxford, pp 317–424
80. Aller RC, Rude PD (1988) Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. Geochim Cosmochim Acta 52:751–765
81. Murray JW, Jannasch HW, Honjo S et al (1989) Unexpected changes in the oxic/anoxic interface in the Black Sea. Nature 338:411–413
82. Glaubitz S, Labrenz M, Jost G et al (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
83. Pimenov NV, Neretin LN (2006) Composition and activities of microbial communities involved in carbon, sulfur, nitrogen and manganese cycling in the oxic/anoxic interface of the Black Sea. In: Neretin LN (ed) Past and present marine water column anoxia, vol 64. Springer, Dordrecht, pp 501–521
84. Overmann J, Cypionka H, Pfennig N (1992) An extremely low-light-adapted phototrophic sulfur bacterium from the Black Sea. Limnol Oceanogr 37:150–155
85. Overmann J, Manske AK (2006) Anoxygenic phototrophic bacteria in the Black Sea chemocline. In: Neretin LN (ed) Past and present marine water column anoxia, vol 64. Springer, Dordrecht, pp 523–541
86. Marschall E, Jogler M, Henßge U et al (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
87. Romanenko VI (1964) Heterotrophic assimilation of CO_2 by bacterial flora of water. Microbiologiya 33:610–614
88. Roslev P, Larsen MB, Jørgensen D et al (2004) Use of heterotrophic CO_2 assimilation as a measure of metabolic activity in planktonic and sessile bacteria. J Microbiol Meth 59: 381–393
89. Hesselsoe M, Nielsen JL, Roslev P et al (2005) Isotope labeling and microautoradiography of active heterotrophic bacteria on the basis of assimilation of $^{14}CO_2$. Appl Environ Microbiol 71:646–655
90. Casamayor EO, Ferrera I, Cristina X et al (2007) Flow cytometric identification and enumeration of photosynthetic sulfur bacteria and potential for ecophysiological studies at the single-cell level. Environ Microbiol 9:1969–1985
91. Glaubitz S, Lueders T, Abraham W-R et al (2009) ^{13}C-isotope analyses reveal that chemolithoautotrophic *Gamma-* and *Epsilonproteobacteria* feed a microbial food web in a pelagic redoxcline of the central Baltic Sea. Environ Microbiol 11:326–337
92. Bernhard JM, Buck KR, Farmer MA et al (2000) The Santa Barbara Basin is a symbiosis oasis. Nature 403:77–80
93. Vopel K, Thistle D, Ott J et al (2005) Wave-induced H_2S flux sustains a chemoautotrophic symbiosis. Limnol Oceanogr 50:128–133
94. Rinke C, Schmitz-Esser S, Stoecker K et al (2006) "*Candidatus* Thiobios zoothamnicoli," an ectosymbiotic bacterium covering the giant marine ciliate *Zoothamnium niveum*. Appl Environ Microbiol 72:2014–2021
95. Rinke C, Lee R, Katz S et al (2007) The effects of sulphide on growth and behaviour of the thiotrophic *Zoothamnium niveum* symbiosis. Proc R Soc B 274:2259–2269
96. Ward BB (2008) Nitrification in marine systems. In: Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds) Nitrogen in the marine environment. Academic, Amsterdam, pp 199–261

97. Brettar I, Moore ERB, Höfle MG (2001) Phylogeny and abundance of novel denitrifying bacteria isolated from the water column of the central Baltic Sea. Microb Ecol 42:295–305
98. Wuchter C, Schouten S, Boschker HTS et al (2003) Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiol Lett 219:203–207
99. Berg IA, Kockelkorn D, Ramos-Vera WH et al (2010) Autotrophic carbon fixation in archaea. Nat Rev Microbiol 8:447–460
100. Herndl GJ, Reinthaler T, Teira E et al (2005) Contribution of Archaea to total prokaryotic production in the deep Atlantic ocean. Appl Environ Microbiol 71:2303–2309
101. Teira E, Lebaron P, van Aken H et al (2006) Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. Limnol Oceanogr 51:2131–2144
102. Enrich-Prast A, Bastviken D, Crill P (2009) Chemosynthesis. In: Likens GE (ed) Encyclopedia of inland waters. Academic, Oxford, pp 211–225
103. Jetten MSM, Strous M, van de Pas-Schoonen KT et al (1999) The anaerobic oxidation of ammonium. FEMS Microbiol Rev 22:421–437
104. Kuypers MMM, Lavik G, Woebken D et al (2005) Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc Natl Acad Sci USA 102: 6478–6483
105. Jensen MM, Kuypers MMM, Lavik G et al (2008) Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea. Limnol Oceanogr 53:23–36
106. Murray JW, Yakushev E (2006) The suboxic transition zone in the Black Sea. In: Neretin LN (ed) Past and present marine water column anoxia, vol 64. Springer, Dordrecht, pp 105–138
107. Neretin LN, Abed RMM, Schippers A et al (2007) Inorganic carbon fixation by sulfate-reducing bacteria in the Black Sea water column. Environ Microbiol 9:3019–3024
108. Albert DB, Taylor C, Martens CS (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 I 42:1239–1260
109. Lovley DR, Coates JD, BluntHarris EL et al (1996) Humic substances as electron acceptors for microbial respiration. Nature 382:445–448
110. Bradley PM, Chapelle FH, Lovley DR (1998) Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene. Appl Environ Microbiol 64:3102–3105