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Interplay between microbial community composition and chemodiversity of dissolved organic matter throughout the Black Sea water column redox gradient

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Abstract

Large quantities of carbon are stored in marine dissolved organic matter (DOM), and its recycling has a major effect on the carbon cycle. Microbes are responsible for turnover of DOM. Little is known about how the complex pool of DOM shapes microbial communities and vice versa, especially in anoxic systems. In this study, we characterized the DOM pool with high-resolution Fourier transform ion cyclotron resonance mass spectrometry and analyzed the microbial community composition with 16S rRNA gene amplicon sequencing across a redox gradient in the Black Sea. The chemical stratification of the water column was clearly reflected in the microbial community, with different putative autotrophic taxa abundant across redox zones. The nitrate maximum was characterized by a high abundance of Thaumarchaeota, the suboxic zone by Gammaproteobacteria Chromatiales, while Epsilonbacteraeota Campylobacterales were abundant at the onset of the sulfidic zone. Compared to the variance in the microbial community, the molecular composition of DOM was relatively uniform across the sampled depths. However, underlying differences in the oxidation state of the DOM molecular formulas showed distinct changes that were linked to the redox zones, possibly connecting autotrophic metabolisms to changes in the DOM composition. In addition, known heterotrophs like Planctomycetes Phycisphaerae and Chloroflexi Anaerolineales were linked to more oxidized molecular forms of DOM, and not to the identified redox zones, suggesting that these fermentative organisms are reliant on newly formed carbon molecules. Our study suggests that the metabolism of autotrophic microbes influences the composition of DOM across the Black Sea water column.

Marine dissolved organic matter (DOM) forms one of the largest reservoirs of carbon on the planet (Hedges 1992; Hansell et al. 2009). Microbial metabolism in the ocean is intricately linked to its production, degradation and recycling (Kujawinski 2011), especially in environments with low oxygen availability, as energy is driven toward lower trophic levels (Wright et al. 2012). Complex dynamics in relation to organic carbon degradation characterize unusual microbial ecosystems in oxygen-depleted marine water columns. On the one hand, oxygen is depleted because of respiration linked to the heterotrophic degradation of organic matter (OM) (Yakushev et al. 2007); on the other hand, the depletion of oxygen defines strict thermodynamic limits to microbial metabolism (Jørgensen 2006). This dynamic results in marine redoxclines, where a cascade of microbial metabolisms results in geochemically defined zones of different chemical composition (Jørgensen 2006). Despite the extent to which OM and microbial metabolisms shape the marine environment in these areas, little is known about the effect that the chemical composition of the organic carbon pool has on the microbial community and vice versa. This in turn hinders our understanding of the driving forces controlling the cycling of carbon in oxygen-depleted conditions.

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The Black Sea is an ideal model system to study OM and microbial community dynamics across a strong redox gradient, as it is the largest anoxic and sulfidic water mass on the planet, with a redoxcline spanning tens of meters. The Black Sea is permanently stratified with high salinity water originating from the Mediterranean Sea, overlaid by freshwater inflowing from rivers. Due to the density differences, and the enclosed nature of the Black Sea, these two water masses present very limited mixing, causing a unique stratified profile of the water column (Murray et al. 1991). DOM accumulates in the Black Sea; its dissolved organic carbon (DOC) concentrations are ca. 2.5 times higher than open ocean values (Ducklow et al. 2007; Margolin et al. 2016). This DOC originates from autochthonous production, marine intrusion via the Bosporus Strait and downward export of riverine terrigenous material. Recent estimates suggested that < 25% of the accumulated DOC have originated from terrestrial sources (Gomez-Saez et al. 2021). Likewise, dissolved organic nitrogen (DON) concentrations are high, with a relatively constant DOC : DON ratio across depth (Ducklow et al. 2007). DOM is an extremely complex organic mixture made of thousands of individual compounds, and is conceptually divided into different fractions based on residence times in the oceans (Zark et al. 2017). The major standing stock of DOM in the oceans has a resident time of thousands of years (Williams and Druffel 1987) and is therefore considered unavailable to microbial degradation (Dittmar 2015). Nevertheless, the more labile fractions of DOM are recycled in a matter of minutes to days and are constantly influenced by microbial production and consumption as well as other interactions like signaling (Carlson and Hansell 2014, and references therein). Despite surface production and chemoautotrophic sources in the Black Sea (Karl and Knauer 1991; Kaiser et al. 2017), net removal of DOM across the water column points toward active heterotrophic communities throughout depth (Margolin et al. 2016). However, the compositional changes in DOM caused by microbial metabolisms are difficult to constrain especially in anoxic conditions, where the reasons behind the apparent preservation of OM in relation to oxic conditions are still not well characterized (Keil et al. 2016; Jessen et al. 2017). One hypothesis is that the preservation is in fact linked to the thermodynamic properties of DOM molecules themselves (LaRowe and Van Cappellen 2011; Boye et al. 2017), indicating that the molecular composition of available pool of DOM would be an important factor in determining carbon cycling dynamics.

In oxic oceanic waters, microbial community members are specialized to degrade specific molecular forms of OM (Gómez-Consarnau et al. 2012; Nelson and Carlson 2012). Recent studies in the Black Sea water column (Suominen et al. 2019), as well as in anoxic sediments (Jessen et al. 2017; Orsi et al. 2018), point toward similar substrate effects under anoxic conditions. In addition to microbial degradation of DOM, compounds produced by microorganisms and found in their exometabolomes have been identified as major constituents of the DOM pool (Lechtenfeld et al. 2015). Comparisons between molecular characterizations of DOM and microbial community composition have been previously made across gradients of salinity (Osterholz et al. 2018), rice paddy fields across China (Li et al. 2018), a sediment profile (Oni et al. 2015), and across time in the surface ocean (Lucas et al. 2016), as well as in a microbial degradation experiment (Wu et al. 2018). While the relationship between these datasets has been complex, depending on the specific environment and comparisons made, specific molecular formulas or changes in DOM composition have been linked to the simultaneous change in microbial community composition in each case. However, little is known about how the effect of the redox state of water columns influences DOM composition. The study by Oni et al. (2015) across an anoxic sediment profile showed microbial populations were defined by the total organic carbon content of sediments, and there was a change of DOM to more reduced formulas in deeper sediments. This study indicated that oxygen (O)-rich aromatic and highly unsaturated compounds were important for microbial metabolisms across the sediment profile. Across rice paddy fields in China, variability in DOM compound composition was the factor that explained most of the variation in microbial community, and was significantly correlated with specific microbial metabolic genes (Li et al. 2018). Therefore, while environmental conditions can be linked to changes in DOM composition, it is possible that this link is indirect and ultimately controlled by the microbial metabolic potential.

Due to technological advances, it is now possible to characterize DOM more thoroughly at the molecular level. With the development of ultra-high-resolution mass spectrometry methods, research is advancing the understanding of the dynamics of the DOM pools in the environment. Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is one of the methods that can be used to identify the elemental composition of thousands of DOM compounds in seawater (Kujawinski 2002). Insights from DOM compositional studies indicate that DOM as a whole is remarkably stable across the world's deep oceans (Hansman et al. 2015; Zark and Dittmar 2018), but also reveals internal variability of the DOM pool (Flerus et al. 2012; Zark et al. 2017). In addition, the detailed genetic description of environmental microbial communities has become increasingly available with the continuous development of next-generation sequencing methodologies. By using untargeted analytical methods, we can detect how compositional changes in the abundant DOM pool may affect the composition of microbial communities and vice versa.

In this study, we hypothesized that there is an intimate link between DOM composition and the microbial communities residing in marine waters. We investigate, for the first time, this relationship across a strong redox gradient and in high resolution to unravel the effect of oxygen availability on the microbial processing of DOM.

Materials and methods

Sample collection

Samples were collected from the western basin of the Black Sea in spring 2017 during cruise number 64PE418 from Sta. 2 (R/V Pelagia; 42°53.8′N, 30°40.7′E). Water samples were collected with a rosette system, which was equipped with among others a SBE3plus thermometer, SBE4 conductivity sensor, and SBE43 dissolved oxygen sensor (Sea-Bird Electronics). Samples for the analysis of NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-} were filtered through a 0.2 μm Acrodisc syringe filter with a Supor membrane (Pall Corporation) and frozen at $-20°C$ in a pre-rinsed pony vial. Samples for DIC measurements were likewise filtered and preserved at $+4°C$. About 20 mL of water was collected and filtered with a 0.2 μm Acrodisc syringe filter with a Supor membrane (Pall Corporation) for DOC and TDN measurements into pre-combusted glass vials.

For DOM analyses 4 liters of filtrate from 0.2 μm Sterivex™ (Merck Millipore®) filtration were collected into acid-washed 2-liter sampling bottles at 15 different depths (5, 55, 60, 70, 85, 90, 95, 100, 105, 110, 130, 150, 250, 1000, and 2000 m). The water was acidified with concentrated HCl to a pH of 2–3 and the samples stored at $+4°C$ in the dark until DOM extraction in Spring 2018. For microbial community analysis, the Sterivex filters from these filtrations were immediately stored at $-80°C$ for further processing.

Microbial community analysis

Microbial cells in sea water were collected with Sterivex filters during the sampling campaign in 2017 and extracted with the Powersoil DNA isolation kit (MoBio, QIAGEN) according to manufacturer's instructions, but with an elution step with 50 μL instead of 100 μL TE. The V4 region of the 16S SSU rRNA gene was amplified using the forward primer 515F-Y and the reverse primer 806RB as described in Suominen et al. (2019). 16S rRNA gene copy numbers were estimated with quantitative polymerase chain reaction (PCR) like in Suominen et al. (2019). It should be noted that application of these primers may miss some divergent 16S rRNA genes from bacterial groups belonging to the candidate phyla radiation, which are known to be important in anoxic conditions (Brown et al. 2015). PCRs were made in Phusion HF buffer with 0.2 mM dNTPs, 800 μg mL^{-1} BSA, 0.6 μM primers, 1 unit Phusion High-Fidelity Taq polymerase (ThermoFisher Scientific) and 5 μL of DNA template in a total of 50 μL. The conditions were $98°C$ for 30 s, 25 rounds of $98°C$ for 10 s, $50°C$ for 20 s, $72°C$ for 30 s, and finally 7 min of extension at $72°C$. All reactions were performed in triplicate and negative controls were performed in each round. PCR products were run on 1% agarose gels for 50 min at 80 V with SmartLadder small fragment (100–1000 bp, Kaneka Eurogentec S.A.) as a size marker. Target bands were cut out of the gel, and DNA was purified with the QIAquick Gel Extraction Kit (QIAGEN). Samples were combined at equal concentrations, concentrated with the

MinElute PCR Purification Kit (QIAGEN), and sequenced at the University of Utrecht (the Netherlands) on an Illumina Miseq platform as paired-end reads of 250 bp.

Illumina amplicon data was analyzed with an in-house pipeline. Sequence quality was checked at several process points with FastQC (v. 0.11.3, Andrews 2010). PEAR was used to extend paired-end reads (v. 0.9.8, Zhang et al. 2013) with a minimum length of reads of 20, minimum overlap set at 7 and a *p*-value threshold of 0.05. Barcodes were removed (rules: extract_barcodes --bc1_len 12 and correct_barcodes –bc_missmatch 2) and libraries were split (rule: split_libraries, max unacceptable phred –q 10, and maximum number of consecutive low base calls –r 5) and reads outside of 250–350 bp length were discarded (rule: remove_short_long_reads) with QIIME (v. 1.9.1; Caporaso et al. 2010). Uclust was used to cluster operational taxonomic units (OTUs) at 97% similarity and the most common sequence of each OTU cluster was picked as the representative one. We assigned taxonomies to the representative sequences using uclust at a 75% cutoff against the SILVA database release 128 (Quast et al. 2013). As many sequences do not have more detailed taxonomic classification in the database, a cutoff of 75% for phylum level taxonomy was used (Yarza et al. 2014). Finally, singletons and OTUs below 0.005% in relative abundance (Bokulich et al. 2013) were removed prior to analysis. All further analyses were carried out in the R package Phyloseq (v. 1.22.3; McMurdie and Holmes 2013) and MixOmics (v. 6.3.2; Le Cao et al. 2016; Rohart et al. 2017).

DOC and DOM analyses

Triplicate samples for DOC measurements were taken from the filtered and acidified 4 liter DOM samples before solid-phase extraction. DOC was analyzed with high-temperature catalytic combustion using a Shimadzu TOC-VCPH/CPN Total Organic Carbon Analyzer. The samples as well as two acidified (pH 2) ultrapure water extraction blanks were solid-phase extracted (SPE) using modified styrene divinyl benzene copolymer cartridges (PPL, Agilent) as specified in Dittmar et al. (2008). Briefly, before the extraction, the cartridges were activated with methanol and rinsed twice with ultrapure water, once with methanol and once with ultrapure water at pH 2. The samples were extracted at a rate of about 10 mL min^{-1} and desalinated by rinsing the cartridge twice with ultrapure water at pH 2. The cartridges were dried with argon gas and finally the SPE-DOM was extracted with an addition of methanol and stored at $-20°C$. The DOC was measured again from the SPE fraction to determine extraction efficiencies in terms of carbon. To ensure maximum comparability of samples, the DOC samples were diluted to the same concentration before analysis with FT-ICR-MS (5 ppm, 1 : 1 MilliQ : methanol). Because of this adjustment step, no observable relationship between total ion current (TIC) and DOC concentrations is retained. In addition, TIC was stable (within the range of random analytical fluctuations) and did

not show any systematic trend among the samples. This stability of TIC indicates minor differences in ionization among samples. A 15 Tesla Solarix electrospray ionization Fourier-transform ion cyclotron resonance mass-spectrometer (ESI-FT-ICR-MS) (Bruker Daltonics) was used for ultra-high resolution mass-spectrometry analysis as specified in Osterholz et al. (2018). The negative ionization mode was applied to the ESI unit with the needle voltage set to -4 kV at a flow rate of 120 μL h^{-1}. DOM samples in duplicates, blanks, and two controls of North Equatorial Pacific Intermediate Water (NEqPIW) were directly infused in methanol : water. Acquired masses were matched across samples and assigned molecular formulas and "molecular categories" with ICBM in-house Matlab (MathWorks) routines as described in Riedel and Dittmar (2014). Peaks in blanks and all formulas that were found in only one of the duplicates were removed. The normalized relative abundances of the identified formulas were multiplied by the concentration of the initial SPE-DOC (Osterholz et al. 2018). Molecular indices were calculated for all samples from the acquired molecular formulas identified by FT-ICR-MS. The double bond equivalence index (DBE) was used to estimate the degree of unsaturation, and the modified aromaticity index (AI$_{mod}$) (Koch and Dittmar 2016) to estimate the presence of aromatics in the DOM. Moreover, the nominal oxidation state of carbon (NOSC; calculated following LaRowe and Van Cappellen 2011) was calculated to estimate the thermodynamic potential of each formula. To investigate the changes in DOM molecular formulas across the samples, we also calculated the molar ratios, including O/C, H/C, N/C, S/C, and P/C. A categorization of formulas into rough compound categories was used to identify changes in major DOM constituents. These included highly unsaturated (AI$_{mod}$ < 0.5, H/C < 1.5), aromatic (AI$_{mod}$ >0.5) which are then divided into polyphenols (0.66 > AI$_{mod}$ > 0.5) and condensed aromatics (AI$_{mod}$ > 0.67). Besides, these DOM constituents also included unsaturated aliphatic (2.0 ≥ H/C > 1.5, N = 0), unsaturated formulas with N (2.0 > H/C ≥ 1.5, N > 0), and saturated fatty acids (H/C > 2.0, O/C < 0.9) (Rossel et al. 2016). In addition, the highly unsaturated, polyphenol, and unsaturated aliphatics formulas were subdivided into oxygen-rich (O/C > 0.5) and oxygen-poor (O/C ≤ 0.5) compound classes. We acknowledge that these compound classes are estimations as each formula can account for multiple isomers, and therefore several different functional molecular categories (Zark et al. 2017). However, they are useful in defining the compound groups with different saturations and heteroatoms, as well aromatic moieties.

Indicators are weighted average numbers of DOM formulas or elemental ratios as well as NOSC. These weighted-normalized averages values were calculated for each sample by taking the average value across all formulas weighed by the estimated abundance. Van Krevelen diagrams (VKDs), which are based on the O/C and H/C values, were used to depict the variation in all molecular formulas encountered for DOM

(Fig. S1). DOM composition was compared to the NEqPIW sample representative of deep oceanic recalcitrant DOM (Green et al. 2014).

Statistical analysis

The relationship of sample compositions with each other were analyzed by multivariate methods for both microbial and DOM datasets. Distance matrices were calculated using Phyloseq (v. 1.22.3; McMurdie and Holmes 2013) for microbial community data with weighted Unifrac, while for DOM data Bray–Curtis dissimilarity calculations were used. Principal coordinate analyses (PCoA) were made using the distance matrices to understand the relationship of diversity of samples across the water column. Environmental measurements were projected on to the PCoA analyses using the envfit function from vegan R package (v. 2.5.3; Oksanen et al. 2018). To find covarying OTUs with DOM formula, we performed partial least squares (PLS) analysis similar to Rossel et al. (2016) with the MixOmics R package (v. 6.3.2; Le Cao et al. 2016). PLS analysis maximizes covariance between two datasets, finding the axes that explain the most variance in both datasets. The strength of a PLS analysis is that it can handle multiple collinear or missing variables and works well when the number of variables is much larger than the number of samples. It is therefore suitable for finding covarying variables in two highly complex datasets.

Results

Physicochemical conditions across the water column

Oxygen was depleted at about 70 m depth at the time of sampling (Fig. 1). CTD measurements showed the position of the chemocline separating surface waters to more saline bottom waters at about 50 m at the sampling cast (Fig. 1; Supplementary Fig. S2). According to previous campaigns in the Black Sea water column at the same sampling site, a suboxic zone with no discernible oxygen or sulfide follows across about 100 m, after which sulfide accumulates in the deep waters (Sollai et al. 2019). There was no difference in the potential density profile (Fig. 1D) with that obtained in the sampling campaign in 2013 (Sollai et al. 2019), further confirming that the physical conditions at this site are relatively stable. Across the redox zone there was a peak in the nitrate concentration at 55 m (5.07 μM) and the nitrite concentration at 85 m (0.051 μM). There was a minimum of phosphate concentration at 70–80 m (avg. 0.63 $\pm$ 0.002 μM) and a peak at the bottom of the suboxic zone (95–105 m, avg. 6.60 $\pm$ 0.3 μM) after which phosphate concentrations gradually increased again in the sulfidic zone (500–2000 m, avg. 7.6 $\pm$ 0.7 μM). Sulfate concentrations were lower in the oxic waters (avg. 16.0 $\pm$ 0.7 μM), but stable across the remaining water column (avg. 17.5 $\pm$ 0.4 μM). The total dissolved nitrogen increased from a mean of 9.8 $\pm$ 1.9 μM in the oxic samples (5–70 m) to a mean of 68 $\pm$ 28 μM in the sulfidic water samples (500–2000 m).
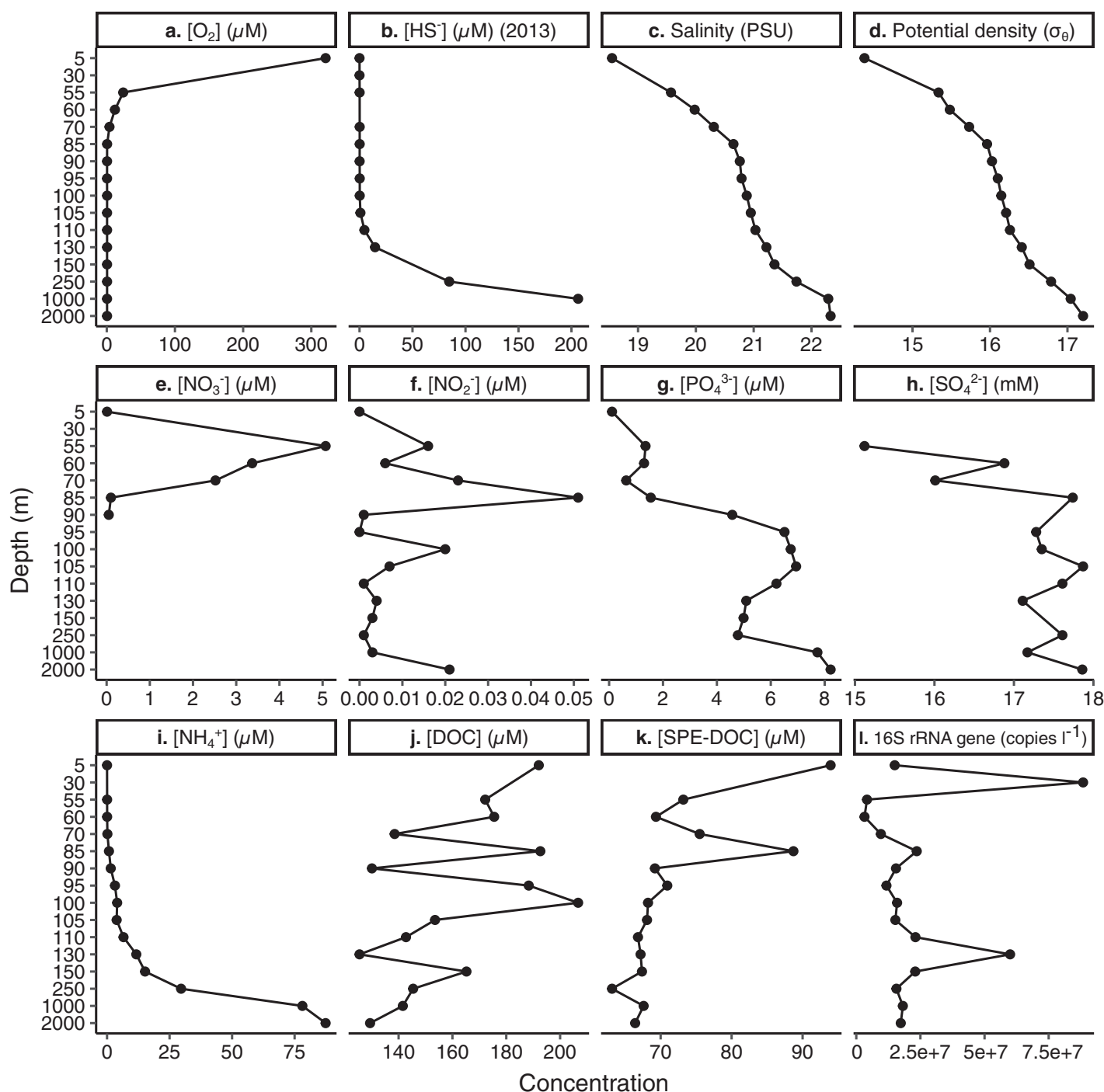
Fig. 1. Measured chemical physical and biological parameters across the Black Sea water column. All values are in μmol L^{-1} except potential density σ_θ and 16S rRNA gene copies L^{-1}. All parameters were measured in 2017 during the sampling campaign, except sulfide, which was measured at the same site in 2013. DOC and SPE-DOC is the concentration of DOC extracted with SPE. Note that the depth axis is not to scale.

This was mainly caused by an increase of ammonium concentration, which accumulated with depth in the water column (0.05 $\pm$ 0.06 μM in oxic samples to 62 $\pm$ 26 μM in sulfidic samples). DOC concentration peaked at the surface (190 μM) and at 100 m (207 μM) and varied across the remaining water column with no clear trend with depth (avg. 160 $\pm$ 25 μM).

Microbial community composition across the water column

We collected 15 samples across the water column, with an increased sampling resolution around the redoxcline, and we determined the microbial community composition (Fig. 2).

For that aim, a fragment of the 16S rRNA gene was amplified and sequenced. A total of ca. 2 million sequences with an average read length of 304 base pairs were characterized into ca. 240 thousand OTUs at 97% sequence similarity. After filtering out low abundance OTUs, ca. 42,500 OTUs remained
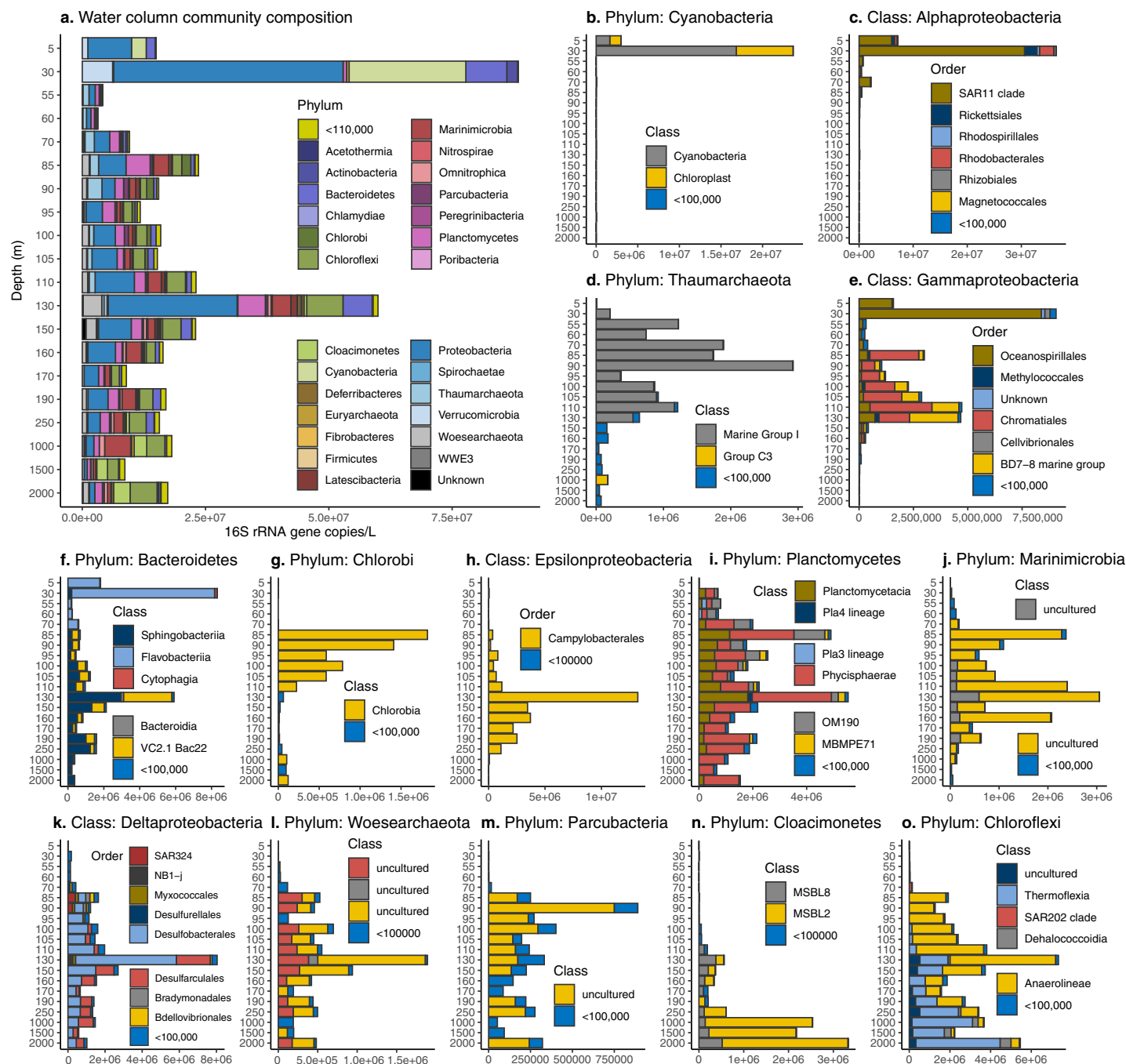
Fig. 2. The absolute abundance of 16S rRNA genes (in copies L^{-1}) grouped by taxonomy across the Black Sea water column revealing the diversity of the microbial community. (**a**). All microbial phyla, (**b**) to (**o**) taxonomic groups of microbes shown separately, either at phylum or class level and colored by the next lower taxonomic rank, that is, class or order. Taxonomic groups with low abundance were clustered if they amounted to less than 100,000/110,000 16S rRNA gene copies L^{-1}.

with an average of 6600 ± 2000 OTUs per sample. At the order level, the surface oxic water at 5 m was characterized by a majority of Alphaproteobacteria SAR11 (40.1%), Bacteroidetes Flavobacteria (11.9%), Cyanobacteria (11%), and Gammaproteobacteria Oceanospirillales (10.1%). The waters at 55–85 m still had a high relative abundance of SAR11 sequences (18.4%), and additionally Thaumarchaeota Marine Group I (16.1%), and Planctomycetes Phycisphaerales (8.5%). Suboxic waters from 85- to 110-m water depth contained abundant sequences attributed to the Chloroflexi Anaerolineales (11.6%), Gammaproteobacteria Chromatiales (9.2%), Thaumarchaeota Marine Group I (7.2%), Marinimicrobia (7.1%), and Chlorobi Chlorobiales (5.2%). The total community across the upper sulfidic waters at 130 and 150 m depth had a high relative abundance of sequences related to the Epsilonproteobacteria Campylobacterales (18.5%), Chloroflexi Anaerolineales (8.5%), and Deltaproteobacteria Desulfobacterales (7.8%), while the deeper sulfidic water column (250–2000 m depth) was characterized by sequences of the order Chloroflexi Thermoflexales (17.7%), as well as of the phyla Cloacimonetes (10.5%), and Marinimicrobia (6.3%).

The abundance of 16S rRNA gene copies had two clear maxima, at 30 m (88.4×10^6 copies L^{-1}) and at 130 m (60.0×10^6 copies L^{-1}), and otherwise on average $13.9 \pm 6.3 \times 10^6$ copies L^{-1} (Fig. 1). DOM was not sampled at 30 m, and therefore we excluded this sample from further analyses. The estimated abundance of each OTU across the water profile was calculated based on their relative abundance in the 16S rRNA gene amplicon sequencing analysis multiplied by the total 16S rRNA gene copy abundance by quantitative PCR, estimating the occurrence of one 16S rRNA gene copy per genome. This allowed the comparison of OTU abundances between depths for the various orders (Fig. 2a). There were many taxonomic groups at the class level that were abundant across the whole water column or across several physicochemical zones. Most distinctly, these belonged to phyla Woesearchaeota, Parcubacteria, and Planctomycetes as well as the class Deltaproteobacteria. The distribution of the main taxa occurring across the water column is shown in Figs. 2b–o.

Based on the estimated abundance data, a principal coordinate analysis with weighted Unifrac distances was performed to analyze how the variance in the composition of the microbial community was related to depth (Fig. 3a). The first two principal components of this analysis explained 68.4% of the variance in the dataset and clearly revealed zones where the microbial diversity was most similar. The environmental parameters that were significantly related to these zones were analyzed with an envfit analysis. Oxygen concentrations were related to the surface sample at 5 m, while the nitrate concentration was significantly related to waters from 55, 60, and 70 m, which were also separated with the ordination analysis. Suboxic waters from the 85–110-m depth were grouped together and were not correlated with any environmental variable. The microbial community composition of waters at

130 and 150 m (the upper sulfidic zone) were well separated from those of the deeper sulfidic waters (at and below 250) (Fig. 3a), and the ammonium concentration was significantly related to this.

DOM molecular composition across the water column

The molecular composition of DOM from the same water depths as the microbial community was analyzed by FT-ICR-MS. An expanded section of the FT-ICR-MS is shown in Supplementary Fig. S3. Based on DOC concentrations, the extracted DOM represented $45\% \pm 6\%$ of total DOM (Fig. 1). In the extracted DOM, we identified ca. 3600 ± 700 molecular formulas at each water depth, ranging in molecular weight from 97 to 1000 daltons (Da). Of the total number of identified molecular formula, 1706 were shared across all samples in the water column, amounting to $74.1\% \pm 4.1\%$ of the relative abundance by sample. The bulk molecular composition of DOM did not change substantially across the water column with relatively uniform relative abundances of the molecular formulas containing the same atoms (i.e., CHO, CHON, CHOS, CHOP, CHONS, CHONP, and CHOSP) across the entire water column. Molecular formulas containing only carbon, hydrogen, and oxygen (CHO) were the most abundant making up $48.3\% \pm 2.0\%$ of the total DOM. Formulas containing also N (CHON) comprised $32.9\% \pm 1.2\%$, while the S-containing formulas (CHOS) formed $16.2\% \pm 2.7\%$ of the total relative abundance. Other formulas represented a minor fraction of the total DOM, namely $1.2\% \pm 0.62\%$ of P-containing formulas (CHOP), $1.3\% \pm 0.55\%$ of N- and S-containing formulas (CHONS), $0.08\% \pm 0.06\%$ N-and P-containing formulas (CHONP), and $0.055\% \pm 0.072\%$ S- and P-containing formulas (CHOSP). The corresponding values of a control representing open ocean DOM (see materials and methods) were 40.2% of CHO, 47.5% of CHON, 7.1% of CHOS, 3.4% CHOP, 1.3% CHONS, 0.26% CHONP, and 0.17% of CHOSP formulas.

The formulas can be further categorized into rough compound categories based on the number of DBE and the AI_{mod}. The majority of formulas were classified as highly unsaturated representing $78.5\% \pm 2.0\%$ of the total DOM. In addition, the unsaturated aliphatics and polyphenols were abundant with $13.0\% \pm 1.7\%$ and $6.2\% \pm 0.82\%$ of total relative abundance, respectively. The corresponding abundances of these categories in the open ocean DOM were 89.0% of highly unsaturated formulas, 4.7% of unsaturated aliphatics, and 4.4% of polyphenols. The aromatic molecular formulas ($AI_{mod} > 0.5$) made up $4.9\% \pm 0.69\%$ of the Black Sea DOM and 3.6% of the open ocean DOM. The O-poor highly unsaturated formulas in the Black Sea DOM were similar in abundance ($55.4\% \pm 2.8\%$) compared to open ocean DOM (54.7%), while the O-rich formulas were considerably less ($23.1\% \pm 4.1\%$ in the Black Sea and 34.3% in the control sample). Both categories of the unsaturated aliphatic were on average more abundant in the Black Sea DOM ($10.1\% \pm 1.6\%$ O-poor and $2.9\% \pm 0.45\%$
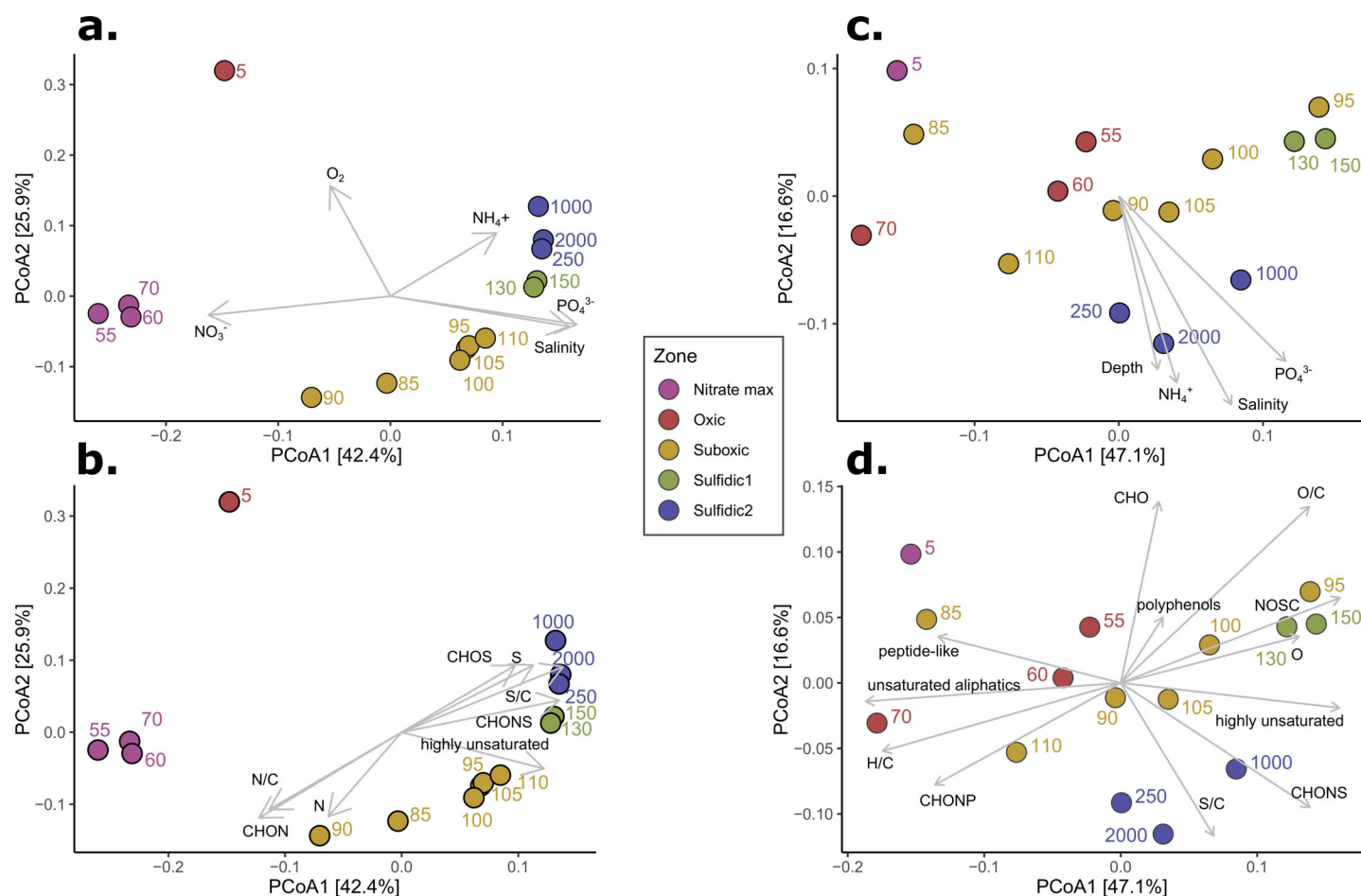
Fig. 3. Principal coordinate analysis (PCoA) of (**a** and **c**) microbial diversity and (**b** and **d**) DOM formula composition of the water column of the Black Sea. (**a/c**) The analysis is derived from weighted Unifrac distances of estimated microbial community abundance data at each depth based on 16S rRNA gene amplicon data (as shown in Fig. 2). (**b/d**) The analysis is derived from Bray–Curtis distances of estimated DOM formula data based on molecular formulas characterized by FT-ICR-MS at each depth. Zones are colored by groupings and numbers correspond to sampling depths. (**a/b**) The arrows correspond to fitted environmental variables that were significantly related to the PCoA axes. (**c/d**) The arrows correspond to fitted DOM composition indicators that were significantly related to the PCoA axes. Indicators are weighted average numbers of DOM formulas or elemental ratios as well as NOSC.

O-rich) compared to the open ocean DOM (3.1% O-poor and 1.6% O-rich). However, the O-rich unsaturated aliphatic showed a decreasing trend with depth across the Black Sea water column, which was not evident in the O-poor category (Fig. S4). The distribution of all formulas based on H/C and O/C ratios and colored by elemental composition, formulas categories, and NOSC is shown in VKDs in Supplementary Fig. S1.

The differences in DOM composition between samples from different depths was estimated by multiplying the relative abundance of different formulas with SPE-DOC concentrations, which were estimated by the total DOC concentration multiplied by extraction efficiency in each sample (SPE-DOC; Fig. 1). This does not represent absolute abundances of DOM formulas, but it can be used to compare differences between samples. Based on the estimated abundance of different DOM formulas, a principal coordinate analysis based on Bray–Curtis distances of DOM compound abundance was performed in order to

determine how the chemical composition of DOM varied with depth. Despite the fact that the first two principal components of the analysis explained a total of 63.7% of the variance, there were few significant relationships with environmental variables, and the analysis did not reveal any clear groupings of samples with depth (Fig. 3b). Instead, molecular indices calculated from DOM composition were significantly related to the molecular variability of DOM (Fig. 3d), such as H/C, O/C, NOSC, and S/C.

In addition, some indicators of molecular composition of DOM revealed gradual changes with depth (Fig. 4). The weighted average number of CHON molecular formulas was significantly negatively correlated (Spearman's correlation, $p < 0.05$, $r > 0.6$) with depth (CHON $r = -0.65$). On the other hand, the molar S/C ratio and the abundance of CHONS formulas were significantly positively correlated with depth (S/C $r = 0.76$; CHONS $r = 0.76$). The distribution of other indicator values showed patterns in the DOM data that were not linearly
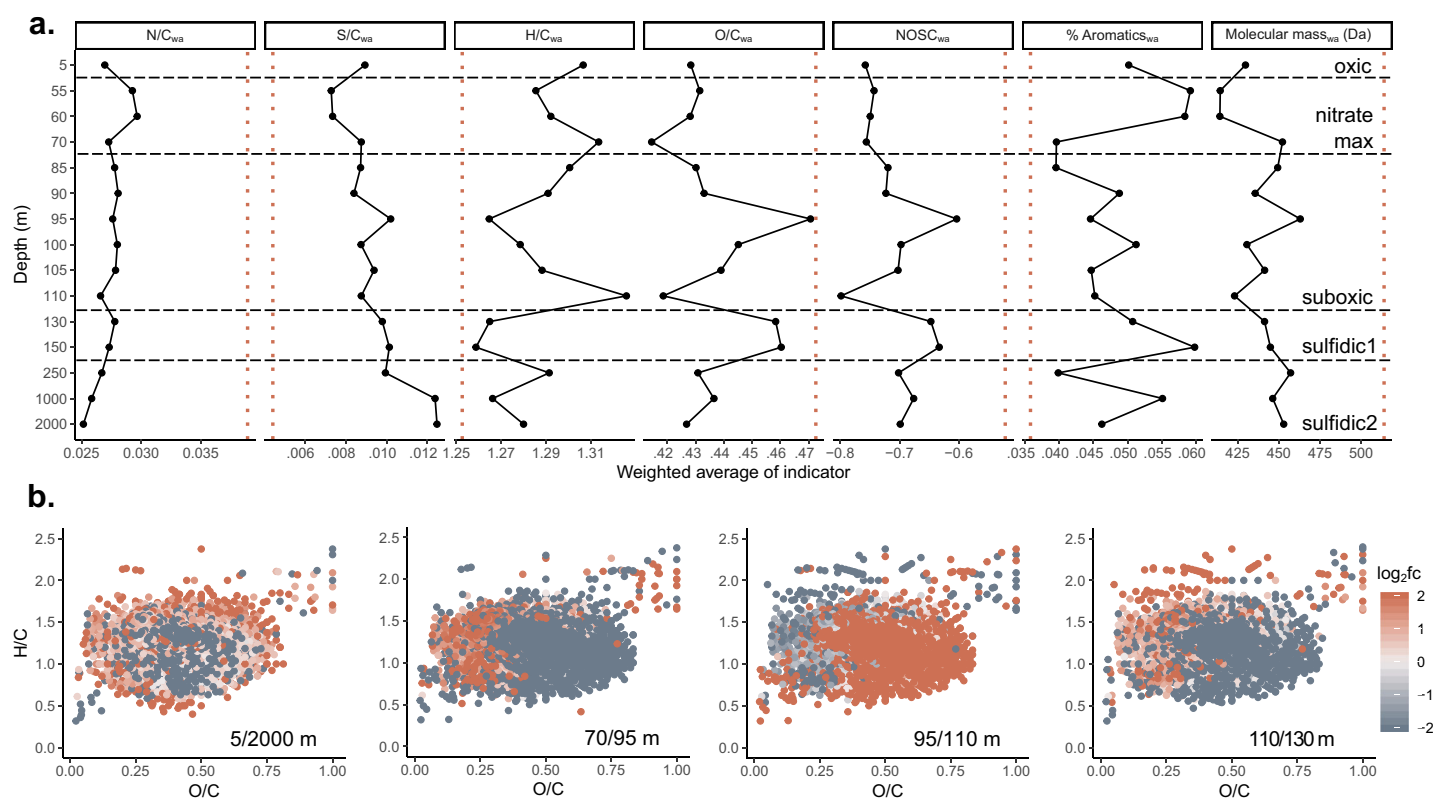
Fig. 4. Variation of the Black Sea water column DOM pool. (**a**) Changes in DOM abundance-weighted average molar ratios and NOSC, proportion of aromatic molecular formulas ($AI_{mod} > 0.5$) and mass to charge ratio (m/z) across depth in the water column. Note that *Y* axis is not to scale. Values calculated from the reference sample of NEqPIW are shown for comparison to typical open ocean values with the red dotted lines. (**b**) VKD of main changes in the DOM dataset across shallow to deep and the suboxic zone samples. Colors correspond to calculations of $\log_2$ fold change, from surface to deep; red means the compound was more abundant in the more shallow samples; while the blue color means it was more abundant in the more deep sample. Values at |2| are maximum (corresponding to a quadrupling in abundance), all values above |2| are estimated as 2.

related to any environmental variables (Fig. 4a), but explained sample distribution in the ordination analysis (Fig. 3d). The weighted averages of H/C and O/C ratios, the NOSC, the percentage of aromatics ($AI_{mod} > 0.5$), and the mass to charge (m/z) ratio of all ions in the FT-ICR spectrum are shown across the water column to describe these changes (Fig. 4a). The open ocean DOM control contained a higher average N/C (0.039), O/C (0.47), NOSC (-0.52), and m/z (513.8) value than the Black Sea water column DOM. Contrastingly, the S/C (0.0044) and H/C (1.25) ratios as well as the weighted average percentages of aromatics (0.036) were lower in the open ocean DOM control than in the Black Sea water column DOM (Fig. 4a). The differences between the open ocean control sample and the Black Sea water column samples are shown in a VKD in Supplementary Fig. S5. The changes in DOM molecular formula composition in the suboxic zone (90–130 m) revealed shifts in abundances of formulas with different O/C ratios as shown in VKD plots (Fig. 4b). The difference in abundances is shown as the $\log_2$ of the fold change between samples (i.e., 1 means a doubling in the abundance between samples), to show the relationship of the abundances on a linear color scale.

Correlation between microbial diversity and DOM

To explore the relationship between the microbial diversity detected in the Black Sea water column with the DOM composition, a PLS regression analysis was performed (Fig. 5). PLS analysis is especially suitable for comparing the variation in two complex datasets as it can handle collinear variables and datasets where the number of samples is smaller than the number of variables (Tobias 1995; Wold et al. 2001). The first two components of our analysis that combined both datasets explained 28 and 12% of the variance in the DOM dataset (Fig. 5a). The first PLS component separated samples from the surface waters to the samples from the start of the sulfidic zone (Fig. 5a). The main variation in the DOM composition that caused these trends was detected through their relationship to specific indicator values. Indicators of DOM composition that were significantly positively correlated with PLS component 1 were weighted averages of elemental H/C ($r = 0.93$, $p = 0$), P/C ($r = 0.74$, $p = 0.009$), CHON ($r = 0.63$, $p = 0.03$), and CHONP ($r = 0.76$, $p = 0.007$). In contrast, negatively correlated with PLS component 1 were O/C ($r = -0.76$, $p = 0.006$), DBE ($r = -0.72$, $p = 0.01$), and NOSC ($r = -0.9$,
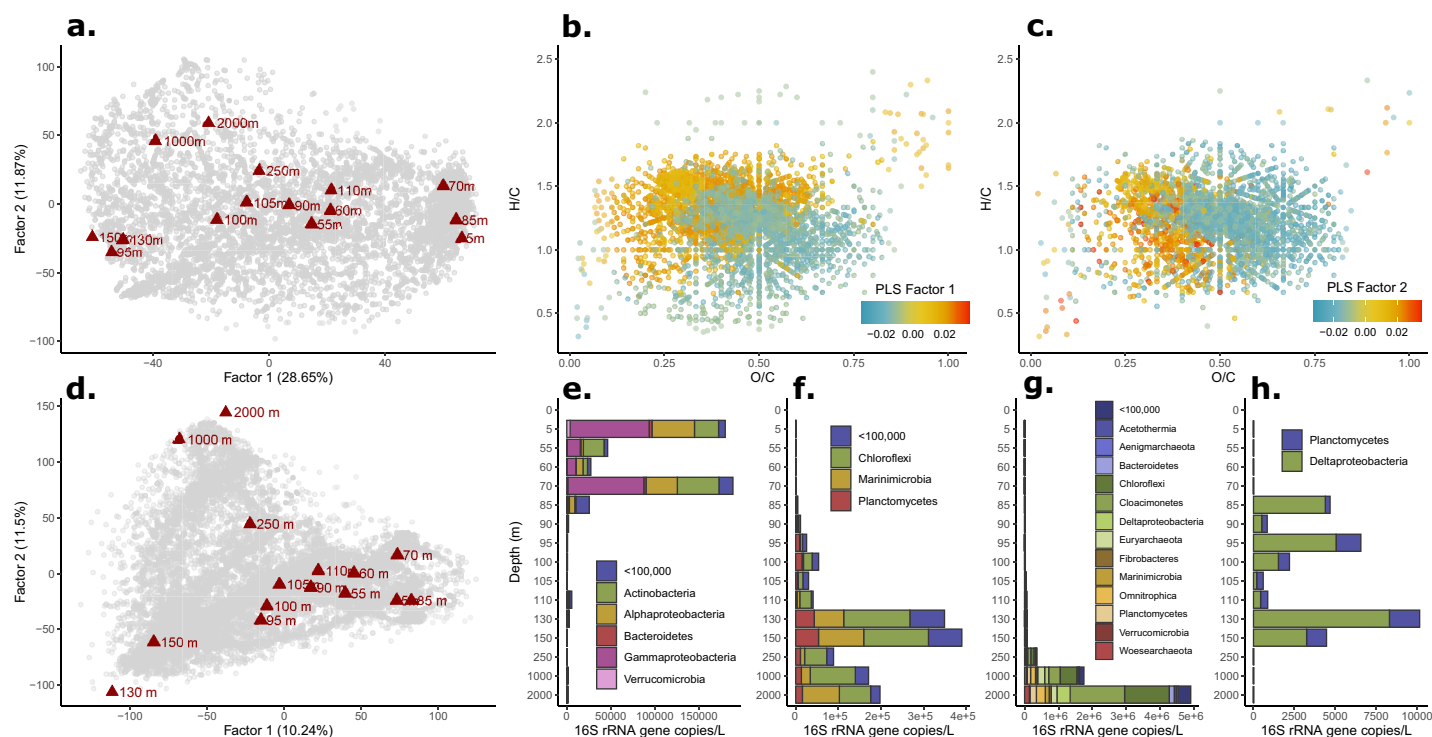
Fig. 5. PLS analysis combining DOM and microbial data. (**a**) spread of DOM samples based on PLS analysis. Explained variance in brackets, light grey dots are individual molecular formulas whose PLS loadings have been projected to show the spread of data. (**b**) A van Krevelen diagram (VKD) of DOM formulas colored based on loadings on PLS factor 1. (**c**) VKD of DOM formulas colored based on loadings on PLS factor 2. (**d**) Spread of microbial samples based on PLS analysis. Explained variance shown in brackets, light grey dots are individual OTUs whose PLS loadings have been inflated to show the spread of data. (**e**) OTUs most positively correlated with PLS factor 1 (loadings over 0.01). (**f**) OTUs most negatively correlated with PLS factor 1 (loadings under −0.01), respectively. (**g**) OTUs most positively correlated with PLS factor 2. (**h**) OTUs most negatively correlated with factor 2. Colors correspond to phylum level taxonomy, or class level for Proteobacteria, as shown in the legends.

$p = 0$). The second PLS component separated the deep sulfidic waters from those from the redoxcline (Fig. 5a). No measured environmental variables were significantly connected to the second PLS component. VKDs showed the spread of all of the molecular formulas by H/C and O/C values, which are colored by their relationship with the first (Fig. 5b) and second (Fig. 5c) PLS axes. This analysis shows that the molecular formulas most positively correlated with the first component were more aliphatic (high atomic H/C ratio) and reduced (low atomic O/C ratio), while the second component was mostly related to the degree of oxidation (i.e., O/C ratio) of the DOM.

The OTUs that were most related to the main changes in DOM data across the water column were also analyzed from their relation to the PLS components. The two first components explained 9.4% and 10.7% of variability in the OTU data (Fig. 5d). The main order level taxa that were most positively correlated with first PLS axis were affiliated to the Alphaproteobacteria SAR11 clade (33 OTUs), Bacteroidetes Flavobacteria (13 OTUs), Planctomycetes Phycisphaerales (10 OTUs), and Actinobacteria Acidimicrobiales (8 OTUs) (Fig. 5e). On the other hand, the most negatively connected to the first component of variation were Planctomycetes Phycisphaerae (28 OTUs), Deltaproteobacteria Desulfobacterales (14 OTUs),

Chloroflexi Anaerolineales (11 OTUs), and Thermoflexales (10 OTUs) (Fig. 5f). Component 2 was related to changes from the suboxic to the deep sulfidic water column and therefore selected positively correlated OTUs that were abundant in the deepest water column samples (Fig. 5g). These belonged to Chloroflexi Thermoflexales (186 OTUs), Omnitrophica (130 OTUs), Cloacimonetes (81 OTUs), and Planctomycetes Phycisphaerales (56 OTUs). On the contrary, the main taxonomic groups of negatively correlated OTUs belonged mostly to Deltaproteobacteria Desulfobacterales (5 OTUs), Woesearcheaota (2 OTUs), Chloroflexi Anaerolineales (2 OTUs), Planctomycetes Planctomycetales (2 OTUs), and Phycisphaerales (2 OTUs; Fig. 5h).

Discussion

Redox zonation reflected in the microbial community structure

The principal coordinate analysis performed on the microbial diversity data based on 16S rRNA gene amplicon sequencing revealed clustering of the microbial community with distinct redox zones. This clustering is well in accordance with previous studies characterizing the microbial community in the Black Sea water column (Vetriani et al. 2003; Lin et al. 2006;

Fuchsman et al. 2011, 2012*a*). The surface oxic zone was separated from all remaining samples (Fig. 3). This zone was characterized by a high abundance of Alphaproteobacteria SAR11, Bacteroidetes Flavobacteria, and Gammaproteobacteria Oceanospirillales. These surface layer organisms are ubiquitous marine bacteria found globally in oxic conditions, and are responsible for the heterotrophic degradation of OM fixed in the surface waters by photoautotrophic organisms (Lucas et al. 2016). In fact, the globally abundant SAR11 group has been shown to be remarkably significant for the recycling of labile DOM compounds (Malmstrom et al. 2004).

Samples from 55- to 70-m depth were significantly associated with the observed nitrate maxima (Fig. 3). At this depth range, a high abundance of SAR11 remained in the microbial community, likely explained by the presence of oxygen in trace concentration or by the recent identification of the nitrate-respiring capabilities of this group (Tsementzi et al. 2016). We also observed a high abundance of ammonia-oxidizing Marine Group I Thaumarchaeota that are known to fix inorganic carbon while oxidizing ammonium (Könneke et al. 2005). Several previous studies have identified the importance of these organisms to nitrogen cycling at the nitrate maximum in the Black Sea (Lam et al. 2007; Sollai et al. 2019 among others), indicating that the nitrate peak is clearly reflected in the microbial community and possible activities in this zone. While also nitrite-oxidizing bacteria are required for the nitrification process, these are known to be numerically less abundant than the ammonia-oxidizing archaea, either due to larger cell sizes or lower metabolic costs (Pachiadaki et al. 2017), hence this might be the reason why they were not as important in defining the variability of the samples.

Suboxic zone samples from 85- to 110-m depth were not significantly related to any single environmental parameter. Rather, the lack of oxygen and sulfide delineates a specific zone, often called the suboxic zone (Murray et al. 1989; Jørgensen et al. 1991). Its microbial community was characterized by a considerable abundance of sequences belonging to sulfide-oxidizing and phototrophic organisms. The Gammaproteobacterial OTUs in this water mass were almost exclusively from the Family Ectothiorhodospiraceae, genus Thiorhodospira, of which the only isolate is a purple sulfur bacterium (PSB) (Bryantseva et al. 1999). In addition, the green sulfur bacterium (GSB) Chlorobi Chlorobium was also abundant. GSB have previously been identified in the Black Sea suboxic zone (Repeta et al. 1989; Marschall et al. 2010 among others). Contrastingly, evidence for the existence of a population of PSB is less strong. They were reported to be present deeper (160 m) in the water column much further back in time by Dickman and Artuz (1978). However, specific PSB pigments were not detected in other studies (Repeta et al. 1989; Marschall et al. 2010). It is possible that fluctuations in the depth of the redoxcline cause differences in the photoautotrophic populations, or that the population of

Thiorhodospira could use an alternative metabolism in the dark. The most important factor for determining the composition of the microbial communities in this zone is the sulfide concentration and the minor nitrite maximum (Fig. 1). Additionally, light must play a role in enabling the growth of diverse phototrophic sulfur bacteria, although the level of their activity remains an open question (Marschall et al. 2010).

The upper sulfidic zone at 130 and 150 m, where sulfide started to accumulate, was slightly separated from other zones in the principal component analysis (Fig. 3). We detected a considerable population of Epsilonbacteraeota Sulfurimonas, which have previously been identified as key autotrophic sulfide oxidizers in the sulfide interface of the Black Sea (Glaubitz et al. 2010; Kirkpatrick et al. 2018 among others). The possible electron acceptors for their metabolism at these depths are unknown, but may be traces of nitrite (Fuchsman et al. 2012*a*) or sulfide oxidation is coupled to the reduction of metals as recently reported for an isolated member of this order (Henkel et al. 2019). The relatively high concentrations of iron and manganese species at this depth interval (Dijkstra et al. 2018) support this metabolism.

Generally, the separation of samples to zones that were associated with environmental parameters was defined by different autotrophic groups that form up to 20% of the total prokaryotic community. Simultaneously, taxa that according to existing knowledge are most probably heterotrophic were spread out across several of these zones. For example, the Planctomycetes Phycisphaerae has also been previously identified in the Black Sea (Fuchsman et al. 2012*b*), and described as a fermentative group related to the degradation of OM. Concurrently, sediment Chloroflexi are known as ubiquitous heterotrophic anaerobic bacteria also in the Black Sea (Jessen et al. 2017). Isolated strains of this group are slow-growing chemoorganotrophs and often need a syntrophic partner to sustain metabolism (Yamada and Sekiguchi 2009). Interestingly, in an anammox bioreactor, Chloroflexi-associated bacteria were responsible for the degradation of decaying biomass (Kindaichi et al. 2012), which could indicate a role in scavenging detrital material from other bacterial populations. Taken together, this would indicate that while autotrophic taxa are reliant on light availability as well as the chemical stratification and redox potential at each depth, heterotrophic organisms would be more independent of these gradients, possibly relying on a fermentative lifestyle supported by the labile compounds fixed by autotrophic microorganisms.

Interestingly, the microbial community showed only minor changes from 1000- to 2000-m depth, which is either caused by the slower turnover rates hypothesized under anoxic conditions in the Black Sea (Mopper and Kieber 1991), or could support our hypothesis that possibly fermenters of OM do not compete with other metabolic group that are reliant on additional redox pairs at these reduced depths.

Compositional changes in DOM across the water column

DOM in the oceans is comprised of a bulk stock of carbon that is retained for thousands of years. Recent molecular characterizations of DOM originating from different water samples indicate that a majority of DOM is in fact highly similar also at the structural level (Zark et al. 2017; Zark and Dittmar 2018). The shared structural component of DOM is thought to arise from the combination of gradual abiotic and microbial degradation processes, forming the DOM pool, which is stored for millennia in the marine water column. It is therefore no surprise that our bulk DOM analysis shows a highly similar molecular composition across the water column. When compared to the open ocean DOM control, however, there are some considerable differences. Black Sea DOM contained less nitrogen, more sulfur, and was more saturated and reduced (higher H/C, lower O/C) than deep ocean DOM. Sediment pore waters of the Black Sea compared to those of other freshwater and marine systems also contained more saturated DOM (Schmidt et al. 2017). Other differences compared to open ocean DOM are the degree of oxygen content in DOM formulas. Especially the decreased abundance of specifically oxygen-rich highly unsaturated formulas compared to the open ocean DOM control as well as a decrease in oxygen-rich aliphatic formulas with depth clearly shows this (Fig. S4).

Despite the uniform elemental composition of DOM across the water column, underlying changes could be identified across the different redox zones (Fig. 4). The main changes with depth were a decrease in N/C and an increase in S/C ratios (Fig. 4a). In contrast, other studies of anoxic waters and sediment pore waters have found an increase in N-containing molecular formulate in DOM with increasing degradation, possibly due to release of nitrogenous compounds from degrading biomass or particulate organic matter (POM) (Valle et al. 2018). A high proportion of DON in total DOM has been also detected in the hypoxic basins of the Baltic Sea water column (Seidel et al. 2017). However, there is evidence that N-containing organic compounds, like proteins, are actively degraded in oxygen-depleted water columns (Pantoja et al. 2009) and have been found to be preferred substrates over other biochemical classes of compounds in POM (Van Mooy et al. 2002; Engel et al. 2017). It is possible that the general decrease of the atomic N/C ratio with depth is caused by gradual microbial degradation of DOM, also reflected in the increasing ammonium concentrations detected (Fig. 1). The depth of the sulfidic water column in the Black Sea is 10-fold that of the Baltic, decreasing the amount of mixing of the water masses and allowing a longer residence time for POM in the deep waters of the Black Sea, possibly resulting in fewer processes remaining that would release high N/C OM. Consequently, the decrease in N/C content and DON molecular formulas would indicate active processing of the dissolved fraction of OM in the deep sulfidic water column.

On the other hand, the gradual increase in elemental S/C ratios and dissolved organic sulfur (DOS) formulas is probably tightly linked to the sulfidic nature of the Black Sea water column (Gomez-Saez et al. 2021). In sediments, it is known for over three decades that formation of organic sulfur compounds can happen through an abiotic sulfurization reaction where (poly)sulfide reacts with functional groups like alcohols, aldehydes and conjugated double bonds (Sinninghe Damsté and de Leeuw 1990). This reaction contributes to long-term carbon storage in anoxic sediments by deterring the microbial degradation processes, through for example the sulfurization of algal carbohydrates (Kok et al. 2000). The gradual increase of DOS compounds across the deep sulfidic waters could indicate that sulfurization processes with DOM take place in the Black Sea water column (Gomez-Saez et al. 2021), as shown for sulfidic sediments in specific lipids (Wakeham et al. 1995; Sinninghe Damsté et al. 2007) or with pore-water DOM (Jessen et al. 2017; Schmidt et al. 2017). In addition, in the S-containing formulas of the DOM in the water below the redoxcline there was a continued decrease in O-rich unsaturated aliphatic compounds, suggesting that these were available for microbial utilization. However, the major fraction of S-containing formulas was highly unsaturated and their abundance increased strongly in these deep waters, pointing toward an accumulation of S-containing highly unsaturated formula below the redoxcline. This is in accordance with sulfurization as a process that hinders microbial degradation and results in the enhanced preservation of OM (Sinninghe Damsté and de Leeuw 1990; Burdige 2007; Raven et al. 2021). Future research work in the deep-water column will be needed to understand the DON and DOS dynamics and its connection to microbial metabolism and activities.

The remaining DOM composition indicators had a more complex relationship across the water column. Underlying weighted averages of H/C, O/C, and NOSC showed fluctuations across the water column. Across sediments in the Black Sea, Schmidt et al. (2017) hypothesized that in the presence of terminal electron acceptors, OM would be oxidized, while in the methanogenic zone, fermentation would lead to a general decrease in O/C ratios and increase in saturation (H/C) of DOM molecules. A decrease in O/C ratios has also previously been detected in anoxic incubations of lake sediment pore waters (Valle et al. 2018), as well as when comparing DOM from surface to subsurface sediments (Oni et al. 2015). On the molecular level, this could be caused by the loss of carboxyl and hydroxyl groups from OM and the opening of unsaturated double bonds (Schmidt et al. 2017). The saturation of organic molecules can be assessed with H/C ratios as well the DBE and AI_{mod}. On the other hand, the NOSC of the OM represents the bioenergetic potential of a molecule (LaRowe and Van Cappellen 2011). Thermodynamic calculations regarding the limits of substrate composition in redox half-reactions (Boye et al. 2017) concluded that in oxygen-depleted conditions, compounds with a higher NOSC will be preferentially degraded, resulting in a general decrease in average NOSC values. Theoretical calculations state that the higher the NOSC

value, the easier it is for an electron to be removed from the organic compounds, and therefore it is more thermodynamically favorable for microbial degradation in anoxic conditions (LaRowe and Van Cappellen 2011; Boye et al. 2017). In this regard, an anoxic incubation study with OM from aquifer sediments, compounds with higher NOSC values were indeed preferentially degraded (Pracht et al. 2018). Across the Black Sea water column in our study, there were several local minima of the weighted average values of H/C and respectively local maxima of O/C and NOSC, possibly indicating less degraded OM. Interestingly, the zones defined by the microbial community composition were related to these patterns of change in the overall oxygenation and saturation of the organic compounds suggesting that the microbial community was indeed involved in altering the molecular composition of the DOM.

Especially the suboxic zone (i.e., 85–110 m) stood out, as this was part of the suboxic water column, where sampling was performed in higher resolution, and peak and minimum NOSC values were detected (Fig. 4). This zone was at the transition from suboxic to anoxic conditions recognized by isotopic measurements of POM as zones for chemoautotrophic production in the Black Sea in previous studies (Karl and Knauer 1991; Yilmaz et al. 2006; Kirkpatrick et al. 2018; Ediger et al. 2019). This is similar to the upper sulfidic zone (130 and 150 m) where NOSC increased again, and a considerable population of putative chemoautotrophic sulfide-oxidizing microbial population was present. We will discuss the possible reasons behind these dynamics in the following section. In addition, in the suboxic zone, there was also a peak in measured concentrations of DOC. However, the DOM extraction yield dropped substantially (Fig. 1), which might point to a presence of low molecular weight compounds that are not extracted with SPE (Hawkes et al. 2016). Previous measurements in the Black Sea have noted surprisingly high concentrations of low-molecular weight compounds at several points in the water column (Mopper and Kieber 1991; Albert et al. 1995) that could explain this discrepancy. Interestingly, the average abundance of unsaturated formulas with N has an opposite trend to the NOSC and increases slightly from 95 to 110 m (Fig. S4). These unsaturated formulas with N are thought to arise from microbial activity (Schmidt et al. 2009), and are possibly also linked to microbial activity at these depths of the water column.

Connection between the molecular composition of DOM and the structure of the microbial community

The changes across the water column in the microbial community and in the DOM composition were inherently different. The changes in the microbial zones were most strongly revealed by the marked changes in (chemo)autotrophic populations (Fig. 2) as discussed in the previous sections. It is possible that these (chemo)autotrophic populations influenced the composition of the DOM pool. The compound

types excreted by photoautotrophic producers in the surface ocean have been studied extensively (Obernosterer and Herndl 1995; Flynn et al. 2008), but few studies have been made for marine chemoautotrophs. A few observations suggest that chemoautotrophs in the Black Sea water column do affect DOM composition. First, in the nitrate maximum, at water depths of 55–70 m, characterized by an abundant population of Thaumarchaeota (Fig. 2d), we detect the highest values of average AI_{mod}, indicating a relatively higher abundance of aromatic moieties in the DOM pool. Bayer et al. (2019) identified a suite of compounds with aromatic properties like phenols and polyphenols accounting for about half of the total exometabolome released by these ammonia-oxidizing archaea. As the metabolism of Thaumarchaeota govern the dynamics of nitrogen metabolism in the surficial waters of the Black Sea (Lam et al. 2007), conceivably the effect of these metabolites could be seen also in the composition of the total DOM pool. Second, in the upper sulfidic zone of the Black Sea indications of chemoautotrophic metabolisms have been reported based on the stable isotope ratios of POM (Karl and Knauer 1991; Kaiser et al. 2017) as well as on dark carbon-fixation rates (Yilmaz et al. 2006; Ediger et al. 2019). In this zone (130–150 m), we detect abundant sulfur-oxidizing Epsilonbacteraeota (Fig. 2h), previously shown to be active sulfur-oxidizing chemolithotrophs in the Black Sea (Glaubitz et al. 2010; Fuchsman et al. 2012a; Kirkpatrick et al. 2018). In the upper sulfidic zone, the DOM was characterized by peak NOSC values and a minimum in the average H/C ratio. This could be an indication of compositional changes caused directly or indirectly by the sulfur-oxidizing chemoautotrophic bacteria. Rate measurements of dark carbon fixation did not show clear chemoautotrophic activity at this zone (Yilmaz et al. 2006), but anammox mRNA has been previously identified (Kirkpatrick et al. 2012), their activity (Jensen et al. 2008) as well as active manganese oxidation (Clement et al. 2009). More studies on the exact nature of metabolites excreted by especially anaerobic chemoautotrophic bacteria would help answer these questions. The varying trends of NOSC across the Black Sea water column indicates that the total DOM diversity across the Black Sea redoxcline was influenced by more than the intrinsic degradability of DOM compound types, and that the exometabolomes of chemoautotrophic microbial communities were likely influencing the molecular DOM composition. Chemoautotrophic metabolic pathways have previously been linked to DOM composition across paddy soils (Li et al. 2018), showing that autotrophic metabolisms can have a discernible impact on DOM compositional changes. Activity measurements and transcriptomic datasets in high resolution across the redoxcline would help answer this question.

To look at more detail if there were specific populations of microbes that were related to changes in DOM composition irrespective of the redox gradient, we performed a PLS analysis. We present these results from this analysis only to

generate hypotheses on possible relationships between the two datasets, since larger datasets will be needed to confirm these results. The PLS analysis finds components that maximize the covariance of the two datasets across the samples. By applying this analysis, we aimed to see if there were cooccurring changes in the two datasets that would reveal relationships between specific compound types and organisms. The taxa related to the more oxidized DOM molecular formulas (higher O/C and NOSC) recognized by both PLS components, belonged to subdominant groups like Chloroflexi classes Thermoflexia and Anaerolineales, as well as Planctomycetes Phycisphaerales, Planctomycetales, and Deltaproteobacteria Desulfobacterales. Particularly, these microbial groups identified by the PLS analysis were not related to the abundant chemoautotrophic populations. This has to do with the clear zonation of taxonomically distinct chemoautotrophic populations that were only observed across a few samples in the water column. An analysis like this, comparing changes across the entire depth profile, finds microorganisms that are connected to repeated changes in the DOM compound composition. Therefore, our analysis highlights those microorganisms that are abundant in the several NOSC peaks across the redox gradient. These microbial groups are thus not directly reliant on the redox potential, but rather, their abundances coincide with more oxidized compounds in DOM. It is possible that they are fermenters that utilize OM as both electron donors and acceptors in their metabolism. Fuchsman et al. (2012*b*) also identified anoxic classes of Planctomycetes that were present across both suboxic and sulfidic conditions, and therefore were most probably connected to fermentative metabolism, supporting our hypothesis. The metabolic role of the ubiquitous Chloroflexi is less clear, but the high number of genes related to organic carbon degradation in their genomes (Hug et al. 2013; Fullerton and Moyer 2016) suggests a capability to degrade released organic compounds. Desulfobacterales are known as sulfate-reducing oxidizers of hydrogen or small organic acids produced in anaerobic degradation (Na et al. 2015), and would also benefit from enhanced OM input and degradation. Taken together, our results indicate that ubiquitous fermenters in the Black Sea are reliant on oxidized DOM compounds made available by chemoautotrophic metabolism.

Conclusion

The Black Sea water column is characterized by strong chemical gradients, which are affected by microbial activities. This study revealed that the physical and chemical stratification of the Black Sea water column was clearly reflected in the taxonomic composition of the microbial communities. Some of the most abundant taxa defining the stratification were related to known chemoautotrophic taxa like Thaumarchaeota Marine Group I, Gammaproteobacteria Thiorhodospira, Chlorobi Chlorobium, and Epsilonbacteraeota Campylobacterales, indicating that available redox pairs in the Black Sea water column

could be predominantly utilized to fix carbon into new biomass.

The molecular composition of DOM on the other hand had a highly different variability across the water column than the microbial data (cf. Figs. 2, 3), with DOM showing few changes in the bulk elemental composition across the full water column. This is to be expected as majority of the DOM stock in oceans are molecules with a residence time of thousands of years, which are resistant against microbial degradation. Despite this apparent molecular inertness of the DOM composition, we identified subtle changes in the indicators of oxidation state of DOM molecular formulas that were apparently linked to the activity of abundant groups of chemoautotrophic organisms. In addition, we identified microbial taxa, whose distribution was not dependent on the chemical stratification of the water column. Organisms related to known fermentative lineages like Chloroflexi Anaerolineae and Planctomycetes Phycisphaerae were abundant across several of the identified redox zones, and linked to water masses with more oxidized DOM molecular formulas. A link between heterotrophic organisms and the oxidation state of DOM supports the notion that thermodynamic availability of OM molecular formulas can be a major factor that limits heterotrophic microbial degradation of OM in anoxic environments. More information on metabolic rates and excreted compounds of chemoautotrophic bacteria in the Black Sea would enable modeling-based studies to estimate the relevance of different metabolic pathways to the DOM pool described here. Therefore, our study highlights the importance of the availability of redox pairs in shaping the molecular DOM composition in a marine setting with a strong redoxcline. Ultimately, this will help define the processes controlling carbon cycling in anoxic marine systems.

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Conflict of Interest

None declared.