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EDITED BY

Gordon T. Taylor,
Stony Brook University, United States

REVIEWED BY

Cui-Yun Zhang,
Chinese Academy of Geological Sciences,
China

Aina Astorch-Cardona,
Swiss Federal Institute of Technology
Lausanne, Switzerland

*CORRESPONDENCE

Dirk de Beer

✉ dbeer@mpi-bremen.de

†PRESENT ADDRESS

Marit R. van Erk,
Department of Microbiology, Radboud
Institute for Biological and Environmental
Sciences, Radboud University, Nijmegen,
Netherlands
Gilad Antler,
The Interuniversity Institute for Marine
Sciences, Eilat, Israel

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Microbial diversity, autotrophic- and heterotrophic processes in Mn-rich marine sediments

Sebastian Silva-Solar¹, Marit R. van Erk^{1†}, Gilad Antler^{2†},
Katrin Knittel¹, Subhayit Basu¹ and Dirk de Beer^{1*}

¹Max-Planck-Institute for Marine Microbiology, Department of Microbial Ecology, Bremen, Germany,

²Department of Earth and Environmental Sciences, Ben Gurion University, Beer Sheva, Israel

Marine microbial ecology aims to link microbial communities to the geochemical processes they drive. Molecular techniques have provided a view of the huge complexity of microbial communities and their metabolic potential, however, the information these approaches give about the geochemical processes is limited. Here, we combined geochemical and 16S rRNA amplicon sequencing analyses to spatially link dominant microbial taxa with geochemical processes in deep-sea sediments of the Gulf of Aqaba. The fine-grained aeolian sediments are diffusion controlled, allowing precise localization of the stratified geochemical processes in well-defined zones by transport-reaction modeling. Geochemical depth profiles and fluxes showed aerobic heterotrophy, nitrification, aerobic Mn-oxidation, denitrification, and anaerobic Fe oxidation to occur in sequence from upper to deeper sediment layers. Although chemoautotrophic processes were significant, 90% of the redox processing was driven by heterotrophy on organic carbon input. The microbial community composition also showed a clear stratification. The oxic zone was dominated by typical aerobes, while anaerobes were largely absent, as expected. Remarkably, the anoxic zone was inhabited by many aerobic bacteria, in addition to the anaerobic community. A narrow brown band enriched in solid-phase Mn-oxide was observed at 2–3 cm depth, where downward diffusing O₂ and upward diffusing Mn²⁺ overlapped. Here, members of the family *Ca. Scalinduaceae* peaked, suggesting involvement in Mn oxidation or -reduction. Dominant families above the band were *Woeseiaceae*, *Nitrospiraceae*, and *Nitrosococcaceae*, whereas below the band *Desulfosarcinaceae*, *Desulfatiglandaceae*, *Methylospiraceae*, and *Anaerolineaceae* dominated. While *Ca. Scalinduaceae* peaked in the Mn-oxide band, none of the other dominant families were exclusively linked to zones with specific geochemical processes. Our approach did not resolve relations between the geochemical processes and the dominant members of the community unambiguously, but characterized a unique habitat and leads to the formulation of new hypotheses, including the occurrence of cryptic cycling.

KEYWORDS

autotrophy/heterotrophy, Mn oxidation, geochemistry, microbial community diversity, ROS, cryptic cycles, spatial resolution, deep sea sediment

1 Introduction

1.1 General question

Microbial ecology, the study of microbial communities and their interactions with the natural environment, relies on analyzing community composition, diversity, and abundance. Ideally, these analyses should be coupled with what is known about the metabolisms and physiological activities of the dominant taxonomic groups, to better understand the relationships of the microorganisms with each other and with the environment. Enormous progress has been made with molecular and -omics techniques to characterize microbial communities, especially of the non-cultivated fraction. However, the information these methods can give about the actual geochemical processes happening in the environment is rather indirect. Detected species, families or higher taxonomic groups might not be active, metabolisms inferred from DNA sequences require translation steps and even the presence of certain enzymes requires knowledge of their kinetics as well as data on the substrate availability to assess if a process really happens. These methods should therefore be complemented by more direct process analyses. Approaches to link microbiota to geochemical processes include to combine microscopy and measured physiology of enrichments (Schulz et al., 1996; Schulz and Schulz, 2005; Boetius et al., 2000; Otte et al., 1999), combine hyperspectral analysis and microsampling (Klatt et al., 2015a, 2015; Merz et al., 2021) or to compare, in well stabilized sediments or microbial mats, the communities and main biogeochemical conversions with high spatial resolution (Ramsing et al., 1993; Teske et al., 1998; Santegeeds et al., 1998; Schramm et al., 1996). These studies were all done in habitats with high activities and unique geochemistry.

1.2 Sample location

The sea floor of the Gulf of Aqaba in the Red Sea may offer a natural laboratory. This habitat is characterized geochemically by only a few studies. These Mn- and Fe-rich sediments showed deep oxygen penetration and low sulfate reduction rates (Blonder et al., 2017; Boyko et al., 2018). The Red Sea is highly oligotrophic and its sea bottom has little organic input, hence chemoautotrophy may be relatively important (Middelburg, 2011). From deeper sediments NH_4^+ , Mn^{2+} and Fe^{2+} diffuse upwards, fueling chemoautotrophic processes (Boyko et al., 2019, 2018; Blonder et al., 2017; Steiner et al., 2019). The sediments are aeolian and have a low sedimentation rate of 0.4–0.7 mm per year (Blonder et al., 2017). Bioturbation, inferred from isotope distributions (Steiner et al., 2016), is limited. The bioturbation coefficients at the surface were estimated to be in the order of $10^{-12} \text{ (m}^2\text{s}^{-1}\text{)}$, 3 orders of magnitude lower than the diffusion coefficients of the solutes in the porewater, and rapidly decreasing with depth (Steiner et al., 2016). Therefore, the sediments are considered vertically stratified. Advection in these very fine-grained sediments can be ignored, allowing rates to be calculated from porewater profiles using diffusion-reaction modeling. These physico-chemical characteristics make these

sediments a useful system to assess the reflection of the biogeochemical profiles on the community structure.

1.3 Aims and hypotheses

In this contribution, we provide a detailed description of the geochemistry and microbial community composition of sediments from a deep site in the Gulf of Aqaba. We hypothesize that the microbial community is as stratified as the biogeochemical profile, which would allow us to infer which taxa are responsible for the main geochemical conversions: oxidation of Mn^{2+} , Fe^{2+} and NH_4^+ . We used a combination of centimeter resolution 16S rRNA gene community analysis, geochemical depth profile analyses and flux calculations to comprehensively describe these sediments.

NH_4^+ oxidation is relatively well confined to described microbial taxa (Beman et al., 2011; De Boer and Kowalchuk, 2001; Martinez-Garcia et al., 2008). Mn^{2+} and Fe^{2+} oxidation is much less defined. Chemical Fe^{2+} oxidation by O_2 occurs under pH neutral conditions very fast, thus most biological processing is anaerobic, driven by light (Widdel et al., 1993), Mn-oxides (Jørgensen and Nelson, 2004) or NO_3^- (Wang et al., 2020). Mn^{2+} oxidation is thought to be mainly biological by a wide group of microorganisms (Hansel, 2017). Mn-oxide reduction can be both biological and chemical driven by many reduced species such as Fe^{2+} , sulfide and NH_4^+ (Johnson et al., 2016). The active oxidation and reduction processes can drive rapid Mn cycling in sediments. An important complication of Fe and Mn oxidation is their potential to generate reactive oxygen species (ROS) (Hansel, 2017; Luther, 2010; Dixon and Stockwell, 2014; Koppenol, 1993). These develop upon single electron transfer leading to reduction of O_2 to superoxide ($\text{O}_2^{\cdot-}$) (Luther, 2010). This radical can be degraded by superoxide dismutase to form the less reactive peroxide H_2O_2 , that subsequently is converted by catalase to O_2 and water. ROS species can be highly destructive for life (e.g. the hydroxyl radical $\text{OH}^{\cdot}$) or less toxic (e.g. $\text{O}_2^{\cdot-}$ and H_2O_2). Although ROS are short-living, their presence in sediments was demonstrated and shown to significantly influence microbial degradation rates (Van Erk et al., 2023). Consequently, Mn and Fe oxidation could have lethal side-effects for microbes without effective ROS defense. The ROS presence was investigated by H_2O_2 micro-sensors and porewater analysis by a chemiluminescent method.

In summary, the aim of this study was to link the various spatially separated geochemical processes, inferred from vertical porewater profiles and solid phase chemistry, to the microbial community analyzed by 16S rRNA amplicon sequencing on horizontally sliced sediment cores.

2 Materials and methods

2.1 Study site and sampling

Sampling was conducted in March 2022 and June 2023 in the Gulf of Aqaba, northern Red Sea, aboard the R/V Sam Rothberg. The sampling site was located at 29°28'28.8"N, 34°55'20.2"E, at a

water depth of approximately 700 m (Figure 1). A detailed overview of the site and its sediments is described previously (Boyko et al., 2022). Sediments were collected using a multicorer from the ship (MC-400 Hedrick/Marrs Multi-Corer, Ocean Instruments), the coreliners were scrubbed and rinsed with freshwater before each use. Bottom water temperature was $\sim 21^\circ\text{C}$ —comparable to ambient conditions in the on-site laboratory where all sample processing was conducted. NH_4^+ , dissolved Mn, solid-phase Mn, TOC, O_2 , pH and H_2O_2 profiles were determined on sediments sampled in March 2022. Dissolved Fe^{2+} and microbial analyses were conducted on sediments sampled in June 2023. Because the fixed microbial samples from 2022 had leaked during transport to Bremen, the DNA quality was unsatisfactory and sampling for microbiology was repeated in 2023.

2.2 Sediment and porewater analyses

Sediment cores were collected using liners pre-drilled with holes for porewater extraction using Rhizons (Rhizosphere Research Products, The Netherlands). These were used for microsensor-based and chemiluminescent H_2O_2 analyses, as well as for Fe^{2+} , Mn^{2+} , NO_3^- , NH_4^+ , dissolved inorganic carbon (DIC), total organic carbon (TOC). For pore water extraction Rhizons were used at a depth resolution of 1–2 cm, and another core was sliced and centrifuged for solid phase and pore water analysis at depth resolution of 0.5 cm. Porewater was filtered ($0.22\ \mu\text{m}$) and immediately collected into prepared tubes for the respective analyses. O_2 and pH profiles were measured using microsensors. O_2 and H_2O_2 microprofiles were obtained using home-built microsensors as described previously (Van Erk et al., 2023; Revsbech, 1989). pH microprofiles were measured using a glass microelectrode (MI-413, Microelectrodes Inc., USA) on the same setup.

For dissolved and solid-phase Mn analyses, three sediment cores were sliced at 0.5 cm intervals from 0 to 5 cm depth. The sediment slices were transferred into 50 mL centrifuge tubes, flushed with N_2 (99.999%) and centrifuged for 10 minutes at

9000 rpm (Soto et al., 2023). The resulting supernatant was filtered through a $0.22\ \mu\text{m}$ syringe filter. For dissolved Mn analysis, 100 μL of the supernatant was mixed with 5 mL of 0.1 N HNO_3 . Following centrifugation, sediments were stored at -20°C .

For solid-phase Mn, sediment was dried at 80°C , ground, combusted at 450°C , and digested in 6 M HCl (100x dilution). Pore water and solid phase Mn concentrations were measured by microwave plasma atomic emission spectrometry (MP-AES, Agilent).

For two of the cores, supernatant for NH_4^+ analysis and NO_3^- analyses was stored at -20°C and later transported to the Max Planck Institute in Bremen, where analyses were carried out following previously described protocols (Billerbeck et al., 2006a).

For Fe^{2+} measurements, porewater was directly extracted through Rhizons (Rhizosphere Research Products) and mixed directly with an equal volume of Ferrozine reagent (0.29 g Ferrozine in 2 mL Milli-Q water) that was pre-filled in the syringes. Samples were stored at 4°C until spectrophotometric measurement at a wavelength of 562 nm.

A separate core was used for analyzing methane (CH_4) profiles with 1 cm resolution in porewater as described previously (Sivan et al., 2014). The limit of detection was 4 nM.

For chemoluminescent H_2O_2 analyses, porewater was extracted through pre-drilled holes in eight sediment cores using Rhizons (Rhizosphere Research Products, The Netherlands). Directly after collection, 100 μL porewater was transferred to a 2 mL tube pre-filled with 100 μL Ferrozine solution (0.29 g Ferrozine in 2 mL MQ water). Subsequently, chemoluminescent H_2O_2 detection was performed with a home-build system, as described earlier (Van Erk et al., 2023). Daily calibrations of the chemiluminescent H_2O_2 detection method were performed using a standard range, and catalase was added to selected subsamples to confirm signal specificity to H_2O_2 .

For total organic carbon (TOC), two additional sediment cores were sliced at 1 cm intervals (0–10 cm). The 0–1 cm and 5–10 cm slices were stored frozen and analyzed at MPI Bremen following previously established protocols (Billerbeck et al., 2006b). DIC samples were fixed (5 μL saturated HgCl_2 per mL), brought to the

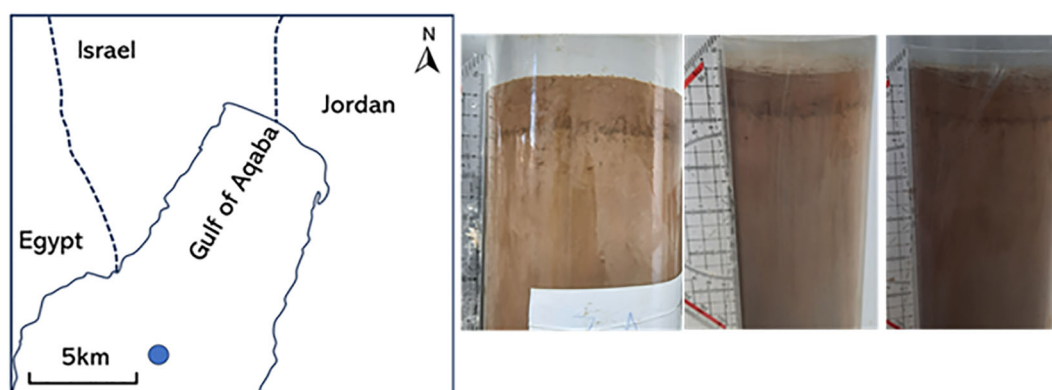


FIGURE 1

Sample location and sediment characteristics. The sampling station of the sediments, located at $29^\circ28'28.8''\text{N}$ $34^\circ55'20.2''\text{E}$ in the Gulf of Aqaba (left) and several cores showing a clear brown manganese oxide band at 2 cm bsf.

MPI Bremen in glass vials with septum (Exetainer, Labco, UK) and analyzed as described previously (Billerbeck et al., 2006b).

The chemicals for these analyses were analytical grade and obtained from Sigma-Aldrich, H₂O₂ (3% Beyvers GmbH, Berlin) was obtained from a pharmacy.

2.3 H₂O₂ degradation

H₂O₂ was added experimentally to high concentrations (100 µM - 1 mM) in the water column, from where it diffused into the sediment, and O₂ and H₂O₂ concentrations were measured using microsensors. This was done on (1) intact sediments to study the effects on sediments above the brown band, (2) with the oxic sediment removed to expose the brown band, and (3) with also the brown band removed to study the effects of H₂O₂ additions on the anoxic sediment below the brown band.

2.4 Flux and rate calculations

Local fluxes were calculated from the porewater profiles using Fick's law of diffusion: $J = D_s \times dC/dx$ with D_s the diffusion coefficient of the solutes corrected for salinity, temperature and porosity (Van Stroe-Biezen et al., 1993; Li and Gregory, 1974; De Beer et al., 2006), C the concentration and x the depth. The values for D_s used were (in m²s⁻¹): Fe²⁺ 2.63×10^{-9} , Mn²⁺ 2.6×10^{-9} , NO₃⁻ 7.3×10^{-9} , O₂ 9.5×10^{-10} , NH₄⁺ 7.6×10^{-9} , H₂O₂ 5.9×10^{-10} . Conversion rates at specific sediment depths were calculated from the profiles as previously described (Gieseke and De Beer, 2004).

2.5 Microscopy and cell counts

Three sediment cores were sliced at 2 cm intervals, and each sediment layer was preserved in 50% ethanol. To detach bacteria from sediment grains, samples were sonicated on ice with a Sonopuls GM Mini20 probe equipped with a microtip MS 2.5 (Bandelin, Berlin, Germany). Samples were sonicated for 30 s, at an amplitude of 86% and pulse of 0.2 s, sediment was removed using a table centrifuge with 2 ml eppendorf tubes and the supernatant was subsequently diluted 1:100. After dilution, cells were filtered on polycarbonate filters pre-stained with Irgalan Black. Filters were stained with 2 mL of acridine orange, rinsed with sterile-filtered citric acid buffer, and mounted onto glass slides with Cargille immersion oil. Additional staining attempts using DAPI, SYBR Green, and SYBR Gold were unsuccessful due to excessive background fluorescence, which prevented cell identification. Cells were counted using an epifluorescence microscope.

2.6 16S rRNA gene sequencing and community composition analysis

Three sediment cores were collected and sectioned at 1 cm intervals from the surface downward. Each sediment slice (3 mL of sediment) was preserved in 12 mL of LifeGuard[®] solution to stabilize

nucleic acids. One DNA extraction per sample was done using the PowerSoil[®] Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. PCR amplification was performed for 34 cycles using the primers Bac-341F (CCTACGGGNGGCWGCAG) and Bac-805R (GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011), and purified using AMPure XP beads (Beckman Coulter, California, USA). Sequencing was conducted on an Illumina NextSeq 2000 platform, generating 2 × 300 bp paired-end reads at the Max Planck Genome Center Cologne. All samples were sequenced in the same run.

Raw sequences were demultiplexed with Cutadapt v1.15 and processed using DADA2 v1.3 (Callahan et al., 2016) for quality filtering, denoising, and amplicon sequence variant (ASV) inference. Raw sequences were filtered based on length (reads shorter than <200 base pairs were removed) and maximum number of expected errors (2 for forward reads, and 3 for reverse reads). Taxonomic assignment was done using the SILVA database (silva_nr99_v138.1_train_set.fa.gz). Before any analysis, sequences classified as chloroplasts and mitochondria were removed, and only bacterial reads classified at the phylum level were kept. ASVs were filtered by length (>396 and <430 bp), and those that were observed in only one sample were removed, regardless of their abundance. We suspect those sequences to be largely PCR artifacts and preferred to have a rather conservative analysis when it comes to community composition and diversity. The proportion of ASV retained after each step is shown in Supplementary Table 1. Once the dataset was filtered, diversity analyses were done at the ASV level using the iNEXT package (Hsieh et al., 2016), standardizing for 99% sample coverage, which was the achieved coverage for most samples (for rarefaction curves, see Supplementary Figure 6S). Community composition was remarkably similar between cores with most sequences not being classified at the genus level. Therefore, the community composition analysis was done at the family level over the average community between the three cores (community composition at the genus level for each core is shown in Supplementary Figure 7S). Since our objectives regarding community analysis were mostly descriptive and exploratory, no statistical tests were applied. Instead, data is presented to show trends of the microbial community across depth.

3 Results

3.1 Geochemistry data

The sediments consisted of very fine light colored clay with a porosity of 0.7 and a grain size of 45 µm (Rasheed et al., 2003). No faunal activity or worm burrows were observed in any of the cores. Due to the fine grain size the transport of porewater solutes was purely diffusional (Wilson et al., 2008; Shepherd, 1989). In all sampled cores a characteristic brown band was observed at 2 cm below the surface (Figure 1). This coincides with the Mn solid phase maximum (Figure 2).

Porewater depth profiles (Figure 2) and fluxes derived from these porewater profiles (Table 1) show occurrence of various aerobic and

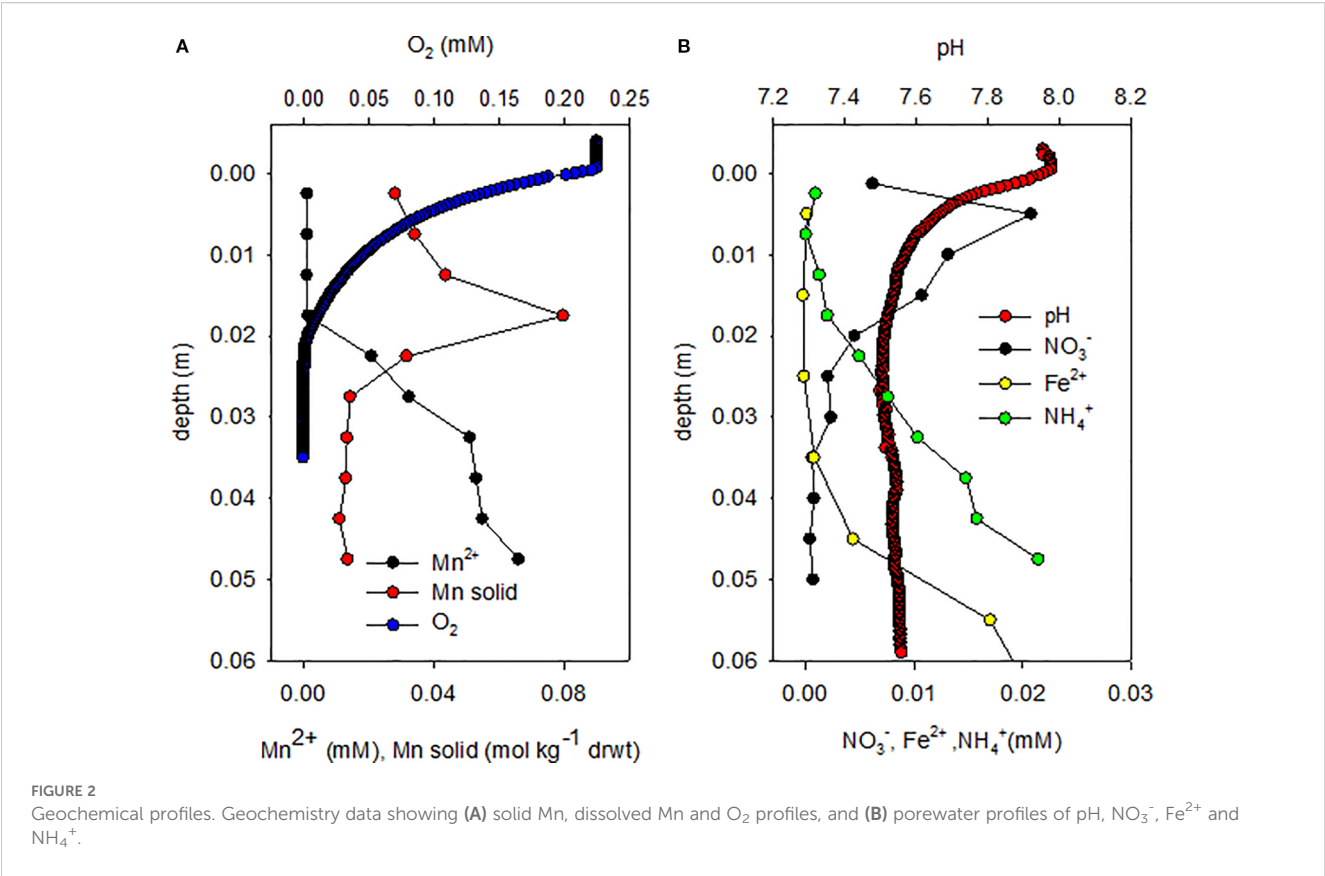


FIGURE 2
Geochemical profiles. Geochemistry data showing (A) solid Mn, dissolved Mn and O_2 profiles, and (B) porewater profiles of pH, NO_3^- , Fe^{2+} and NH_4^+ .

anaerobic microbial processes. O_2 diffuses from the water column downwards. From larger depths NH_4^+ , Mn^{2+} and Fe^{2+} diffuse upwards and are oxidized at slightly different depths. NH_4^+ diffusing upwards from the anoxic zone penetrates 1 cm into the oxic zone. Mn^{2+} disappears in the brown band where also the

TABLE 1 Fluxes of Mn^{2+} and O_2 , and Fe^{2+} and NO_3^- , resp. into their overlapping zones.

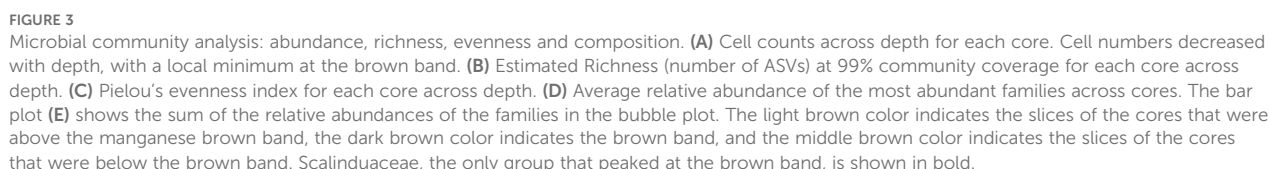
Depth zone	Solute	J (mol m ² s ⁻¹)	Stoichiometry
brown band 1.75–2 cm bsf	O_2	-2.83×10^{-9} (n=3)	$J_{O_2}/J_{Mn^{2+}}$ 0.55
	Mn^{2+}	5.16×10^{-9} (n=3)	
3–5 cm bsf	Fe^{2+}	2.18×10^{-9} (n=3)	$J_{Fe^{2+}}/J_{NO_3^-}$ 4.7
	NO_3^-	-4.63×10^{-10} (n=1)	
Oxic zone	O_2 in at 0	-4.95×10^{-8} (n=3)	$J_{O_2}/J_{NH_4^+}$ 34
	NH_4^+ in at 2.5 cm	1.47×10^{-9} (n=1)	

The considered reactions are:
 $Mn^{2+} + 0.5O_2 \rightarrow MnO_2 + 2H^+$
 $5Fe^{2+} + NO_3^- + 12 H_2O \rightarrow 5Fe(OH)_3 + 0.5N_2 + 4H^+$
The acidification is visible in the brown band, but not in the Fe oxidation zone (Figure 2). The stoichiometry of the Mn/ O_2 fluxes in the brown band and the Fe^{2+} and nitrate fluxes are compatible with Mn and Fe oxidation, resp. The NH_4^+ and O_2 fluxes are far from stoichiometric.

maximum in solid phase Mn is found. Fe^{2+} diffusing upwards disappears 1–2 cm below the brown band, in the anoxic zone.

The brown band was colored by Mn-oxides, as exactly at that depth the solid Mn content showed a maximum (Figures 2A; Supplementary Figure 2S). O_2 diffusing downwards meets Mn^{2+} diffusing upwards (Figures 2A; Supplementary Figure 2S) in the brown band, driving aerobic Mn^{2+} oxidation. Porewater solute fluxes were calculated using Fick's law of diffusion (Table 1). The O_2 flux into the band was half of the Mn^{2+} influx into this zone, close to the stoichiometry of aerobic Mn^{2+} oxidation ($Mn^{2+} + 0.5O_2 \rightarrow MnO_2 + 2H^+$). Thus, the brown band is the location where all upwards diffusing Mn^{2+} is oxidized aerobically.

The curvature of the oxygen profile indicates a homogeneously distributed oxygen consumption in the oxic zone above the Mn^{2+} containing zone, by both NH_4^+ diffusing upwards from the anoxic zone (Figure 2B) and organic matter deposited from the water column. The O_2 flux from the sediment surface downward is approximately 30x higher than the upward NH_4^+ flux into the oxic zone (Table 1). This is far above the stoichiometry of nitrification ($NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$). Hence all NH_4^+ diffusing into the oxic zone can be oxidized by nitrification. Nitrification in the oxic zone results in elevated NO_3^- , with a maximum just below the sediment surface. From its maximum NO_3^- diffuses partially downwards into the anoxic zone below the brown band, and partially upwards into the water column. Besides NH_4^+ , additional e-donor is needed to explain the O_2 consumption in the oxic zone, which is probably organic matter. Indeed TOC



The oxidation of metals, like Mn^{2+} and Fe^{2+} , can result in the formation of reactive oxygen species (ROS) (Luther, 2010; Jomova et al., 2012). ROS was shown to significantly decrease organic

From the profiles the kinetic parameters of H_2O_2 degradation were estimated using diffusion-reaction modeling (Gieseke and De

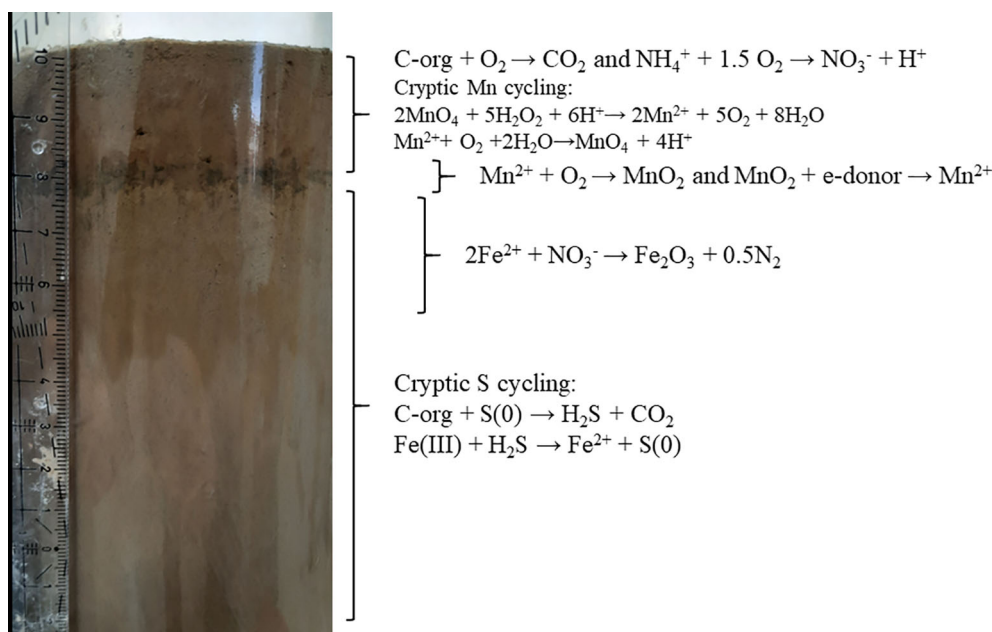


FIGURE 4

A summary of the distribution of the measured and inferred processes. The cryptic S cycling was documented previously (Blonder et al., 2017; Boyko et al., 2022).

Beer, 2004). Each profile represents a range of concentrations and local degradation rates. The rates were linearly proportional to the concentration, as occurs under first order kinetics (Supplementary Figure 5S). The first order reaction rate constant C was 0.00206 (s⁻¹) above the brown band and 0.00288 (s⁻¹) within the band. The rates in the zone above the bands showed larger variations than in the band due to variable MnO₂ content.

3.2 Microbial community analysis

The cell numbers decreased from 2×10^9 cells mL⁻¹ at the sediment surface to approximately half of this value at 8–9 cm depth (Figure 3A). Notably, a local minimum was observed at the brown band in all 3 cores, below which cell numbers increased again.

Estimated richness, defined as the number of ASVs at 99% community coverage, was between 1264–3566 (Figure 3B), and exhibited a slight increase below the brown band. Evenness, estimated by Pielou's evenness index, was exceptionally high and constant across depth with a mean value of 0.91 and a standard deviation of 0.014, with a slight decrease towards the deepest layers. It indicates a balanced community distribution and no dominant single taxonomic group at any depth.

All three cores were remarkably similar, and the community composition showed clear changes with depth (Figure 3D, see Supplementary Figure 8S for MDS plot on Jensen-Shannon distances between communities). Together, 13 families and 10 groups not classified at the family level showed more than 2% in at least one sample, and in total, they accounted for about 50% of the community at any given depth (Figure 3E). All of these groups were present at all depths, with some of them showing clear changes in their

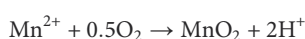
relative abundances with depth, while others remained more constant (Supplementary Figure 9S). *Woeseiaceae*, *Nitrospiraceae*, *Nitrosococcaceae*, and *Cyclobacteriaceae*, together with unclassified groups from *Ca. Actinomarinales*, 'Subgroup 21', 'AT-s2-59', and 'SAR202 clade' were more abundant above the brown band than below, while *Spirochaetaceae*, *Desulfatiglandaceae*, *Desulfosarcinaceae*, *Methylomirabilaceae*, and *Anaerolineaceae*, together with unclassified groups from *Aminicenantales*, *Thermodesulfobionia*, and *Syntrophobacterales*, were more abundant below the band than above. Sequences belonging to archaeal *Nitrosopumilus* were also found above the brown band, but were not included in the analysis (see Methods). *Ca. Scalduaceae* was the only group that peaked at the brown band. *Kiloniellaceae*, *Omnitrophaceae*, *Thermoanaerobaculaceae*, together with unclassified groups from "NB1-j", "BD2-11 terrestrial group", and "bacteriap25" showed fairly stable relative abundances, with little variation across depth.

4 Discussion

The biogeochemistry of the Gulf of Aqaba sediments pore water shows a well-defined stratification, consistent with the fine-grained clay deposits where solute transport is mainly driven by diffusion (Boudreau, 1996). This allows a one-dimensional interpretation of the geochemical data, as the stratification is homogeneous with depth (summarized in Figure 4). Oxygen penetration reaches 2 cm, NH₄⁺ oxidation occurs in the oxic zone, Mn²⁺ oxidation occurs in the brown band, at 2–3 cm bsf, and Fe²⁺ oxidation by NO₃⁻ takes place between 3–5 cm bsf. Organic matter degradation is slow and occurs gradually across both oxic and anoxic zones. This biogeochemistry is reflected in the community structure, where

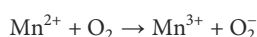
major families change in relative abundance and distribution with depth.

From the calculated fluxes (Table 1) we assessed the importance of autotrophy versus heterotrophy by assuming that O_2 is used directly or indirectly to oxidize the input of all reductants. We conclude that while Mn oxidation is indeed the most important autotrophic process, the O_2 influx is primarily used for the oxidation of organic input. Identified autotrophic substrates are Mn^{2+} , Fe^{2+} and NH_4^+ . Although Fe oxidation occurs via NO_3^- reduction, the NO_3^- is produced aerobically, hence also in the end is driven by O_2 . It was documented that the sulfur cycle is cryptic and occurs below the oxic zone (Blonder et al., 2017; Boyko et al., 2022), hence requires no net input of O_2 . The stoichiometries of the Mn^{2+} , Fe^{2+} and NH_4^+ oxidation processes are respectively 0.5, 0.25 and 2 for O_2 according to the reactions:



Multiplying the upward fluxes of the reductants with the O_2 stoichiometry results in their ultimate O_2 consumption. The most important autotrophic process for the sedimentary O_2 consumption is Mn oxidation, amounting to 2.6×10^{-9} ($\text{mol m}^{-2}\text{s}^{-1}$), followed by NH_4^+ oxidation, 7.4×10^{-10} ($\text{mol m}^{-2}\text{s}^{-1}$) and Fe oxidation, 5.4×10^{-10} ($\text{mol m}^{-2}\text{s}^{-1}$). The sum of these autotrophic processes is approximately 10% of the total O_2 flux into the sediment (Table 1). Thus 90% of the O_2 consumption must be heterotrophic, coupled to the oxidation of organic matter. Autotrophy was lower than expected, considering the highly oligotrophic nature of the Red Sea (Berninger and Wickham, 2005). However, the contribution of autotrophy to total O_2 respiration is higher than in the open ocean where the autotrophic contribution is on average 2% (Middelburg, 2011). In absence of external input of reduced substances, for example by seepage, chemoautotrophy depends on the supply of NH_4^+ , Fe^{2+} and Mn^{2+} from anaerobic heterotrophic degradation processes. These clearly continue in the anoxic zone, as it is a net supplier of these reductants. Chemoautotrophy may also occur as cryptic processes in the oxic zone, where most of the heterotrophic processing occurs.

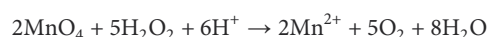
A notable feature of the sediment is the brown band, where most Mn^{2+} oxidation occurs. Mn oxidation is thought to lead to enhanced ROS formation (Hansel, 2017; Jofré et al., 2021), via the biological reaction.



while O_2^- can subsequently rapidly decompose to H_2O_2 (Hansel, 2017). However, accumulation of H_2O_2 in the band was not observed. We did detect ROS in minute but significant quantities, even in the anoxic zone (Supplementary Figure 3S). A peak was observed near the sediment surface, probably as by product of aerobic respiration. The pool is highly ephemeral, subjected to local production and consumption processes. We determined the H_2O_2 consumption kinetics which appeared first

order, thus allows to calculate the H_2O_2 turnover from the measured concentrations. Under first order kinetics, rates can be calculated by multiplying the concentration by the rate constant C [$R = C \times [H_2O_2]$ (Lehninger, 1970)]. The average concentration above the band was 0.74×10^{-3} (mol m^{-3}), the corresponding degradation rate was 1.5×10^{-6} ($\text{mol m}^{-3} \text{s}^{-1}$), resulting in a residence time of 485 (s) or approximately 8 minutes. This is much shorter than the reported turnover time in seawater, where concentrations are mostly 0.3×10^{-3} (mol m^{-3}) or less, and half-lives are in the order of hours to days (Morris et al., 2022). The short residence time indicates catalysis with a significant volumetric activity. The H_2O_2 consumption rate was half of the volumetric O_2 respiration rate of 2.86×10^{-6} ($\text{mol m}^{-3} \text{s}^{-1}$). The volumetric O_2 respiration rate was calculated from the influx (Table 1) divided by the penetration depth (2 cm). It should be noted that the electron flow per molecule of H_2O_2 is half that of O_2 , thus the H_2O_2 turnover is approximately 25% of the O_2 turnover, expressed per electron transfer. The H_2O_2 degradation by catalase and MnO_2 produces again O_2 , hence forms a closed cycle. Remarkably, below the brown band H_2O_2 was not degraded, and thus, it is a stable porewater component, at low concentrations.

The H_2O_2 degradation occurred only in and above the brown band. This could indicate that the microorganisms do not produce catalase below the brown band. However, also below the brown band approximately 1 μM H_2O_2 was measured. Alternatively, the H_2O_2 degradation occurs by MnO_2 , a well-documented chemical process (Do et al., 2009):



Both catalase and MnO_2 degrade H_2O_2 in an identical first-order process, producing O_2 . The H_2O_2 degradation rates link very well with the solid phase Mn distribution. Below the brown band there was no catalase-like activity, the highest rates were in the brown band, while above the brown band the rates were lower and more variable. Thus, some of the H_2O_2 degradation may well be catalyzed by the MnO_2 . This process produces Mn^{2+} , that is instantly re-oxidized to below detection in the oxic zone. This implies a cryptic Mn cycle above the brown band.

The fixed microbial samples from 2022 had leaked during transport to Bremen, thus the DNA quality was unsatisfactory and sampling for microbiology was repeated in 2023. The cores taken in the different years were visually the same, were taken from the same location and in the same season, the sediment accumulation is ca 0.7 mm/year (much less than the spatial resolution of our slicing). The porewater Mn^{2+} profiles, measured in 2023 in cores parallel to those for microbial analyses (Supplementary Figure 2S), showed similar concentrations and also depletion in the upper 2 cm. Therefore, we assume good comparability between the geochemical data from 2022 and microbial data from 2023.

The microbial community is also well-stratified, be it less clear than the net geochemistry. The brown band exhibits relatively low cell numbers, despite their potential for enhanced primary production driven by aerobic Mn oxidation. The reduced number of cells does not appear to be caused by elevated ROS, at least not by

H_2O_2 , as no elevated H_2O_2 was detected. Instead, another inhibitor might be the reason, e.g., the intermediate of Mn oxidation Mn^{3+} , which is a strongly oxidizing compound (Morgan Chan et al., 2018; Hansel, 2017). The notable maximum of *Ca. Scalanduaceae* suggests that ANAMMOX (Van De Vossenberg et al., 2013; Woebken et al., 2008; Lodha et al., 2021) occurs at the brown band, as it is a well-characterized metabolism of this taxonomic group. Furthermore, it can be predicted that nitrifiers (e.g., *Nitrospiraceae*) and *Ca. Scalanduaceae* would compete for NO_2^- in the sub-oxic layer. If this is the case, ANAMMOX would not be confined to the MnO_2 brown band and in principle could happen below the band as well, which is also reflected in the relative abundance of *Scalinduaceae*. The reduction of MnO_2 might be coupled to the oxidation of NH_4^+ by ‘mangananammox’ (Avendaño et al., 2024; Cheng et al., 2022; Javanaud et al., 2011). Under this scenario, we suggest that *Ca. Scalanduaceae* would reduce MnO_2 , possibly with NH_4^+ and produce N_2 or NO_2^- , which would be oxidized to NO_3^- by *Nitrospiraceae* (Kuypers et al., 2018). Previously, close association between Mn-oxides and *Ca. Scalanduaceae* was observed in marine sediments (Shulga et al., 2022). Indeed, an enrichment of *Ca. Scalanduaceae* was shown to couple the reduction of MnO_2 to the oxidation of NH_4^+ (Yu et al., 2021). It thus seems that in this sediment *Ca. Scalanduaceae* is more likely to consume MnO_2 instead of producing it. The Mn reduction on NH_4^+ must be slow, as the profiles do not show a conspicuous NH_4^+ consumption at the brown band.

It should be emphasized that the MnO_2 pool in the brown band is not permanent, but in a steady state subjected to continuous oxidation and reduction processes (Johnson et al., 2016). The reduction processes cause the solid phase of Mn (e.g., MnO_2) to decrease below the brown band, while the oxidation processes cause the depletion of the soluble state of Mn (e.g., Mn^{2+}) above the brown band. With gradual sedimentation (approximately 0.7 mm per year), the brown band moves upwards in par with the rising sediment surface, resulting in an equal amount of MnO_2 and Mn^{2+} produced at the brown band and below the brown band, respectively. Obviously, the oxidation process in the brown band is so efficient that it prevents breakthrough of dissolved Mn into the oxic zone. The oxidation of Mn^{2+} must be biotically driven, as chemical oxidation is very slow (Morgan et al., 2021). Mn^{2+} oxidation can be performed by a very wide diversity of bacteria and eukaryotes (Wang et al., 2022; Zhou and Fu, 2020; Tebo et al., 2005). As mentioned above, the *Ca. Scalanduaceae* are thought to perform NH_4^+ oxidation by MnO_2 , thus driving the Mn-reduction. No other family of bacteria is associated with the brown band besides *Ca. Scalanduaceae*, thus we have no clear evidence which organisms drive the Mn oxidation in this sediment. As no other group was found in abundance in the brown band, it is tempting to propose that *Ca. Scalanduaceae* might do both, reduce MnO_2 and oxidize Mn^{2+} . Reversal of metabolic pathways have been shown before, e.g. sulfate reducers were shown to also be able to oxidize sulfide (while also producing ATP) (Cypionka, 2000) and anaerobic CH_4 oxidizers can also produce CH_4 (Lloyd et al., 2011). Of course, it is well possible that other families are involved in the Mn-oxidation. Further studies focusing on rigorous physiological

analysis, either on isolates and enrichments or using -omics data should be done in order to assign these metabolic pathways to specific members of the community.

Above the brown band, where high concentrations of O_2 and NO_3^- are detected, *Woeseiaceae*, *Nitrospiraceae*, *Nitrosococcaceae*, *Cyclobacteriaceae*, and others groups of unclassified bacteria dominate. *Woeseiaceae* typically comprise aerobic chemoheterotrophic bacteria (Wiese et al., 2020) and are usually found in oxic sediment layers. *Nitrospiraceae*, a well-known group of nitrifiers (Kuypers et al., 2018), are thought to be producing the NO_3^- maximum right below the surface, which then diffuses to the water column and downwards in the sediment where denitrification should occur. The *Nitrosococcaceae* and *Nitrosopumilaceae* are thought to drive the aerobic oxidation of NH_4^+ (Kuypers et al., 2018) in the oxic layers. *Cyclobacteriaceae* was comprised mostly by unclassified sequences at the genus level, which challenges any inference on their metabolism. Same is the case for those groups not classified at the family (or order) level. This study, like many other surveys of remote habitats, highlights the need to improve microbial taxonomy and shows how much we still have to learn about the functions of microorganisms in the environment.

Below the brown band, where O_2 and NO_3^- are exhausted, and sediments are at least several decennia old, the microbial community changes completely. At this depth, cell numbers are half of those found at the surface, and groups characterized as strict anaerobes dominate. *Desulfosarcinaceae* and *Desulfatiglandaceae*, show a dramatic increase in their relative abundances, despite sulfate reduction rates being below detection limits (Boyko et al., 2022). *Anaerolineaceae*, whose relative abundance is the highest in the anoxic layers, along with *Spirochaetaceae* and *Thermoanaerobaculaceae*, are typically described as strictly anaerobic chemoheterotrophic bacteria and fermenters (Yamada and Sekiguchi), and it is therefore not surprising that they are found in higher relative abundances below the band (where oxygen is depleted). Groups whose relative abundance seem constant across depth (*Kiloniellaceae*, *Omnitrophaceae*, *Thermoanaerobaculaceae*, ‘bacteriap25’, ‘NB1-j’, and ‘BD2-11’) have been documented before in marine sediments, however, no specific metabolic characteristics have been described, and thus their ecological role remains elusive. *Omnitrophaceae*, has been proposed to reduce NO_3^- (Williams et al., 2021). *Omnitrophaceae* have magnetosomes (Kolinko et al., 2016) which is typical for motile cells, which explains their presence in the oxic zone. At deeper layers, the sediment does not show measurable biogeochemical activities, although cryptic sulfur and iron cycling are thought to occur (Boyko et al., 2022). A candidate for Fe oxidation is not evident from our data. Fe^{2+} can be oxidized by MnO_2 but it clearly occurred far below the MnO_2 band. Fe^{2+} and NO_3^- overlap at 3–5 cm bsf, where both are consumed. NO_3^- driven Fe oxidation requires microbial catalysis (Straub et al., 1996) but in the relevant zone between 3 and 5 cm bsf no especially abundant community was observed. The increased abundance at 8 cm bsf of *Methyloirabilaceae* is puzzling. The only characterized metabolism of this family is from *Methyloirabilis oxyfera* which can couple the oxidation of CH_4 with NO_3^- reduction (Wu et al., 2011). NO_3^- was certainly absent at that depth. CH_4 was below the

limit of detection (>4 nM) and can be considered absent. Even when accepting concentrations as low as 2 nM, CH_4 fluxes and thus metabolic CH_4 conversions will be effectively absent and cannot maintain a significant CH_4 cycling community. This indicates that the *Methylospirillaceae* in this sediment harbor alternative metabolic activities, or that these bacteria grew during a CH_4 event in the past and are highly resilient.

Remarkably, most of the groups present in the oxic layer still show relative abundances between 2–3% below the band, where oxygen is not detected. The opposite, however, is rarely the case (with the notable exception of the motile *Omnitrophaceae*): families that dominate the anoxic zone are rarely found in the oxic layers. This observation cannot be explained by the reported bioturbation (Steiner et al., 2016). Infauna may mix sediments near the surface, but bioturbation attenuates rapidly with depth. This results in an upward redistribution of sediment signatures or any geological marker, which is the opposite of what we observed: we see more aerobic microbes in the anoxic zone than anaerobes in the oxic zone. Using the equations proposed by Steiner (Steiner et al., 2016) sediment reworking decreases about 10 fold per 2 cm depth. The attenuation of the effects of fauna is even faster as bioturbation in anoxia, below the brown band, is unlikely. The observed absence of anaerobes in the oxic zone and presence of aerobes in the anoxic zone may be rather explained by the history of the upward moving anoxic zone during ongoing sedimentation: the anoxic sediments have an aerobic history during which an aerobic community established and they could apparently maintain for decennia after becoming anoxic. The oxic zone has never been anoxic, thus anaerobic microorganisms had no chance to establish there. At deep layers, diversity will not only reflect living and active communities, but also those that can simply survive at extremely low metabolic rates or are dormant (Bradley et al., 2019). Hence, translation of a community description to a functional analysis must be done with care.

5 Conclusion

The progress in taxonomic knowledge has surpassed the understanding of the metabolisms in natural habitats, but without knowing their associated functions taxonomic data have limited informative value. Our work aimed for a comprehensive *in situ* description of the microbial community from a biogeochemical and taxonomic perspective. However not straightforward, our approach allowed us to infer connections between the two. Clear patterns in the geochemical profiles and microbial community composition were identified above, in, and below the manganese-rich brown band. Even under such special physico-chemical conditions, complicating factors have to be taken into account: sediment mixing, motility of bacteria, unexpected metabolic flexibility, resilience and others. It must also be recognized that not all significant processes are easily detectable. Cryptic element cycling is virtually invisible by most geochemical analyses, but will drive local metabolic activities and development of communities. Examples of cryptic cycling are the reported S cycle in the anoxic zone (Boyko et al., 2022), and the H_2O_2 -driven Mn cycling in the

oxic zone. The history of the sediment layers should also be taken into consideration, as in deeper zones microbes might be buried, are dormant and no longer active, while still show up in a DNA analysis. We could define zones of specific activities, but not associate these with local microbial communities. Still, rather than giving unambiguous descriptions on the role of specific microbes in the environment, our approach serves a solid ground for new questions and hypotheses, and adds to the characterization of remote and unexplored habitats.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author. Sequences were deposited in the European Nucleotide Archive (ENA) under accession numbers PRJEB89874.

Author contributions

SS-S: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. MvE: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. GA: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. KK: Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. SB: Formal analysis, Methodology, Writing – review & editing. DdB: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2025.1639181/full#supplementary-material>.

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