

Supplementary Information for

Persistent seasonal deoxygenation alters benthic metabolic processes and accelerates hypoxia feedbacks in the Baltic Sea

Shuba Varshini Alampalli^{1§}, Stefanie Böhnke-Brandt^{2§}, Nicole Adam-Beyer², Katja Laufer-Meiser², Klaus Wallmann³, Hermann W. Bange⁴, Helmke Hepach⁵, Matthias Thiele², Florian Scholz⁶, Emanuel Wyler⁷, Markus Landthaler^{7,8}, Isabel Diercks⁹, Janina Fuss¹⁰ and Mirjam Perner^{2*}

¹ Core Unit Bioinformatics, Berlin Institute of Health at Charité-Universitätsmedizin, Berlin, Germany

² Geomicrobiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

³ Benthic Biogeochemistry, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

⁴ Chemical Oceanography, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

⁵ Biological Oceanography, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

⁶ Institute of Geology, University Hamburg, Germany

⁷ Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin Institute for Medical Systems Biology (BIMSB), Berlin, Germany

⁸ Institut für Biologie, Humboldt-Universität zu Berlin, Berlin, Germany

⁹ Marine Mineral Resources, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

¹⁰ Competence Centre for Genomic Analysis (CCGA), Institute of Clinical Molecular Biology (IKMB), Kiel University, Kiel, Germany

§ equally shared first authorship

This file contains Supplementary Information:

Material and Methods Text S1-S5

Results Text S6

Figures S1-S16

Data S8

Supplementary Material and Methods Text S1: Geochemistry of the water column, sediments, and pore waters

Water samples were collected with a CTD/Rosette equipped with 5L Niskin bottles on 02. February, 02. March, 12. April, 03. May, 12. July, 30. August, 28. September, 11. October, 9. November and 8. December 2022, and 26. January, 15. February and 20. March 2023 at 0 m, 5 m, 10 m, 15 m, 20 m and 25 m water depths. Nutrient (nitrate, nitrite, phosphate, silicate) and O₂ concentrations were measured according to the standard methods ¹. Methane concentrations were measured with a gas chromatograph equipped with a flame ionization detector ².

Sediment cores were collected with a minicorer (MIC, KUM Kiel, Germany), equipped with four acrylic core liners with 10 cm diameter, on 13. January, 24. February, 15. March, 13. April, 05. May, 14. July, 31. August, 05. October, 25. October 2022 and 25. January, 15. February and 15. March 2023. The bottom water overlying the sediment in the MIC liners was collected in a plastic tube and filtered for subsequent analyses. Core slicing was done under ambient temperatures at atmospheric O₂ concentrations using plastic spoons and a plastic plate. Sediment was sliced in 1 cm sections from 0-4 cm and in 2 cm sections below. For each slice, at first around 1 ml sediment was sampled into pre-weighed plastic cups for determining water content and porosity. Afterwards, the remaining sediment of the section was homogenized and aliquots of the sediment were sampled into centrifuge tubes for porewater analysis, Fe-extractions and RNA extractions. Samples for Fe-extractions were frozen at -20°C and samples for RNA extractions at -80°C. Porewater was separated from the solid phase by centrifuging sediment samples for 15 minutes at 4,000 rpm. The supernatant was filtered (0.2 µm cellulose-acetate filters) under argon atmosphere in a glove bag. Subsamples for metal analyses were acidified with concentrated nitric acid. Total alkalinity was analyzed by titration of 0.25 - 1 ml pore water with 0.02 M HCl according to Ivanenkov and Lyakhin ³. The sample was degassed during titration by continuous nitrogen bubbling to remove any generated CO₂ and sulfide. Results were calibrated against IAPSO seawater solution. Hydrogen sulfide concentrations were determined photometrically using the methylene blue method ⁴. Ammonia was measured via photometry ⁵, Fe²⁺ via the Ferrozine assay ⁶. Main elements were determined via inductively coupled plasma optical emission spectroscopy (ICP-OES) and sulfate, chloride and bromide via ion chromatography (IC).

Sequential endpoint extractions with HCl were performed to separate the poorly crystalline (0.5 M HCl, 1 h, room temperature, shaking at 150 rpm in the dark) from the crystalline (6 M HCl, 24 h, 70°C, in the dark) Fe²⁺ and Fe³⁺ in the sediment. In the HCl extractions, only Fe³⁺ extracted by 0.5M HCl is defined as reactive, in the sense of being easily available for Fe(III)-reducing bacteria ^{7,8,9}. All extraction solutions were made anoxic by bubbling with N₂ and all preparatory and sampling work was done in an anaerobic tent (Coy Laboratories) to prevent oxidation of Fe²⁺ during the extraction. Between 50-100 mg of wet sediment was weighed into a 10 ml glass Exetainer (Labco, UK), the exact mass of sediment determined, and 5 ml extraction solution was added. Each extraction step was stopped by centrifugation (15 min, 5,000 rpm) and a subsample of the extract was sampled into 1 M HCl (final concentration). The concentrations of Fe²⁺ and total Fe (after reduction of all Fe³⁺ to Fe²⁺ with hydroxylamine hydrochloride) were quantified with the Ferrozine assay ⁶.

Supplementary Material and Methods Text S2: Measured metabolic rates

Fluxes to and from the sediment were determined by benthic chamber incubations using a MiniChamber Lander system (Unisense, Aarhus, Denmark) on the 14. January, 15. March, 13. April, 05. May, 14. July, 11. and 31. August, 05. October, 25. October and 23. November 2022 as well as 25. January, 15. February, 15. March 2023 and 29. August 2023. This lander system is equipped with a square benthic chamber (30 x 30 cm), which partly sinks into the sediment and harbors sensors in the chamber lid to monitor geochemical parameters in the bottom water. Sensors included optodes for oxygen, temperature (model 4330DW) and conductivity (model 4319A DW) measurements (both Aandera, Bergen, Norway) as well as a miniaturized amperometric sensor for H₂S (H2S-500; Unisense). An additional H₂S sensor was mounted to the lander frame in order to obtain reference measurements of the ambient, non-incubated bottom water. Prior to deployment, all sensors were calibrated according to the manufacturer's instructions. Optode and hydrogen sulfide sensor data were logged throughout the incubations at a frequency of one data point per second. Water samples for further geochemical analyses were taken by means of a syringe sampling device carrying 11 syringes for sample retrieval as well as one for the injection of a KBr solution (2 M), used for determining the water volume trapped in the chamber during the respective incubation. Lander deployments were carried out according to the following scheme: The system was started while still on deck and lowered to the seafloor with an open chamber lid and constant stirring to displace surface water in the chamber. After sinking into the sediment (approx. 10 cm) and constant stirring for approx. 30 minutes, the chamber lid was closed and a first set of syringe samples (t_0) was taken. During the following 2 hours incubation (with continuous stirring of the trapped bottom water), O₂, hydrogen sulfide concentrations as well as conductivity were constantly monitored, while additional water samples were taken after 1 hour and at the end of the incubation. Once the last syringe set was released, 55 ml of a 2 M KBr solution was injected into the chamber. After further 10 minutes incubation to achieve an even dispersion of the KBr, another water sample was taken to later calculate the chamber volume based on the KBr dilution. The lander system was recovered within 30 minutes of the last sampling. Upon arrival on deck, the syringe sample sets were pooled according to the respective sampling time point, subsampled and fixed for geochemical analyses, encompassing IC, ICP-OES, Fe²⁺, and sulfide. The salinity of the chamber water was calculated from conductivity and temperature measurements using the practical salinity 1978 (PSS 78)¹⁰. In-situ O₂ consumption rates were calculated using linear regression: the slope was determined from O₂ concentration in the benthic chamber (optode data) versus incubation time, considering the water content inside the chamber as well as the covered sediment area. Rates are displayed as total O₂ uptake per day and m² of sediment.

For quantification of sulfate reduction rates (SRR) by the whole-core injection method^{10, 11}, sediments were collected using a MIC and were subsampled with 2.5 cm diameter acrylic core-liners aboard the ship. Before the tracer was injected, the sub-cores were pre-incubated for 24h at in-situ temperature in the home laboratory. The 2.5 cm diameter core-liners had pre-drilled holes that were sealed with polyurethane-based elastic sealant (Sikaflex®-11FC+, Sika), through which 50 kBq of a carrier-free ³⁵S-SO₄²⁻ tracer were injected at 1 cm depth intervals. Afterwards, the cores were incubated for 16-24 hours at in-situ temperature. The incubation was stopped by slicing the sediment cores into 1 cm slices and adding the sediment to Falcon tubes containing 10 mL of 20% Zn-acetate. The Falcons containing the Zn-acetate were weight

before, so that the mass of the sampled sediment could be determined. Samples were stored frozen at -20 °C until further processing. Radiolabeled total reduced inorganic sulfur (TRIS) was separated from the sample using the cold chromium method^{11, 12} and the evolved H₂S was trapped as ZnS in 5 mL of 5% Zn-acetate solution. Radioactivity in the sulfate and TRIS pools was analyzed using scintillation counting, and SRR were calculated according to Jørgensen¹³.

Supplementary Material and Methods Text S3: Modeled rates

The seasonal data set was evaluated using a 1-D non-steady-state reactive transport model developed in Perner et al.¹⁴. The model simulates sediment temperature as well as time-dependent concentrations of dissolved chloride, sulfate, sulfide and ammonium over sediment depths ranging from 0 to 30 cm. It considers temperature-dependent molecular diffusion and bio-irrigation where time-dependent irrigation rates were derived by fitting the model to the seasonal chloride data set. The model generates time- and depth-dependent rates for the following biogeochemical processes: particulate organic matter (POM) degradation, sulfate reduction (SR), pyrite formation and sulfide oxidation. In-situ oxygen flux data were used to constrain rates of aerobic POM degradation whereas rates of anaerobic POM degradation via SR were calculated using sulfate and ammonium porewater data. Rates of pyrite formation were estimated assuming that 50 % of the sulfide formed by sulfate reduction was precipitated as pyrite. Sulfide porewater profiles were employed to derive rates of sulfide oxidation. More details on the model set-up are given in Perner et al. (2022)¹⁴.

Supplementary Material and Methods Text S4: Cell counts and 16S rRNA gene amplicons

DAPI Cell Counts. To quantify total microbial cell numbers 0.5 ml sediment from each depth horizon was fixed on board in formaldehyde/PBS with a 4% final formaldehyde concentration. After incubation at 4 °C overnight, sediments were washed twice by centrifugation (4,500 rpm, 10 min, 4 °C), and the resulting pellets were first resuspended in 1.5 ml sterile PBS, then in 1.5 ml PBS/ethanol (1:1) and stored at -20°C until further processing. To detach cells from sediment, 75 µl of the fixed sediment sample were diluted in 675 µl PBS/ethanol (1:40), sonicated on ice (2 times: 20%, 20 cycles, 20 seconds), and further diluted 1:4,000 with PBS. One milliliter of this final dilution was concentrated onto polycarbonate filters (0.2µm pore size) with 5 ml PBS as carrier using vacuum filtration and filters were stored at -20°C. For DAPI staining one quarter of each filter was passed through a drop of DAPI solution (1 µg/ml DAPI in Citiifluor/Vectashield [1:4]), placed on a microscope slide and then covered with another drop of the same DAPI solution. A coverslip was placed on top, edges were cleaned with ethanol and sealed with nail polish. Slides were stored at -20 °C and incubated at least overnight before counting cells using the Zeiss Axio Imager. M2 epifluorescence microscope equipped with a DAPI filter set. For each sample, a minimum of 20 fields of view and at least 500 DAPI-stained cells were counted to ensure representative quantification.

Sample processing for 16S rRNA gene analyses from RNA. Total RNA was extracted from approximately 500 mg of sediment using the NucleoBond® RNA Soil Mini Kit (Macherey-Nagel, Düren, Germany). To eliminate genomic DNA contamination, a DNase I treatment was performed using the RapidOut DNA Removal Kit (Thermo Fisher Scientific, Waltham, MA,

USA). The purified RNA was reverse-transcribed into complementary DNA (cDNA) using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Life Technologies™, Darmstadt, Germany), followed by an additional purification step using the Zymo Clean & Concentrator-5 Kit (Zymo Research, Irvine, CA, USA). For amplicon library preparation, the bacterial hypervariable V3–V4 regions of the 16S rRNA gene were amplified using the primers S-D-Bac-0341-b-S-17 and S-D-Bac-0785-a-A-21¹⁵, while the archaeal hypervariable V4–V5 regions were targeted using two primer pairs: Arch_519F/Arch_915R¹⁶ and Arch_524F/Arch_958R¹⁷. Sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, USA) in 2 × 300 bp paired-end mode in collaboration with the Competence Centre for Genomic Analysis (CCGA) in Kiel. Amplicon data processing was performed within the Qiime2 environment¹⁸. Raw reads were quality-filtered, trimmed, and merged using the DADA2 plugin with default parameters, including removal of primer sequences¹⁹. Taxonomic classification was conducted with a SILVA 138-based classifier²⁰, pre-trained for the applied primer pairs^{21,22}, using the classify-sklearn method from the feature-classifier plugin²². The phylogeny was calculated using the align-to-tree-mafft-fasttree pipeline²³. Downstream statistical analyses were carried out in R (R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org/>) using the “microeco” package²⁴.

The analyses clustered 6.41×10^6 reads into 35,931 ASVs (Amplicon Sequence Variants), assigned to 67 phyla. Of these, 1,447 were unassignable (7.66×10^4 reads), 762 ASVs were grouped on phylum level only (6.95×10^4 reads) and 35,922 ASVs (5.45×10^6 reads) grouped into low abundance reads ($\leq 1\%$).

Supplementary Material and Methods Text S5: Metagenomic and transcriptomic analyses - Caveats related to the metagenomic and transcriptomic data. Gene abundances were estimated using Salmon v1.10.3, which employs a variational Bayesian framework to assign reads to gene sequences and infer their relative abundances³⁷. Rather than computing exact posterior probabilities -which is computationally intractable - Salmon approximates the posterior distribution over gene abundances by maximizing the evidence lower bound (ELBO), a function that balances the likelihood of the observed data under a probabilistic model with a regularization term that penalizes divergence from prior expectations [<https://github.com/COMBINE-lab/salmon/issues/514>]. In cases where the reference is incomplete - such as when using genes predicted from MAGs - reads originating from missing transcripts are forcefully assigned to the most similar available gene sequence. This can lead to inflated or underrepresented abundance estimates, as Salmon adjusts count values to best explain the observed data within the constraints of the provided reference, potentially redistributing abundance across homologous or partially matching sequences. Consequently, completeness and accuracy of the transcriptome index are critical for reliable quantification in metatranscriptomic contexts. To address these limitations, we included eukaryotic (and unknown) MAGs along with the genes predicted from bacterial and archaeal contigs when quantifying the metatranscriptome data.

Given the sparse nature of the metatranscriptomic data, interpreting the differential expression of functional genes was challenging⁴⁰. To investigate changes in functional profiles over time, pathway enrichment analysis was performed using FRY, implemented in the R package limma.

FRY is a fast approximation to ROAST, which accounts for gene-wise correlation and can be applied to any experimental design. Whereas ROAST estimates p-values through Monte Carlo rotations by simulating many randomized versions of the data to build a null distribution, FRY assumes equal gene-wise variances across samples and analytically computes the equivalent of an infinite number of rotations⁴¹. Nonetheless, the sparsity and variability in the data can reduce the stability of these p-value estimates and inflate FDR values, meaning that the nominal FDR may overstate the statistical evidence in this context. For this reason, and to avoid over-interpretation, the FDR of FRY was not considered in figures but is shown in the supplementary tables.

For selected energy metabolism, i.e. sulfur and nitrogen metabolism, gene names that were not included in KEGG modules due to lack of enzyme number were included for taxonomic and heatmap representation of the respective metabolism (data S2). In analysis like FRY using KEGG modules as IDs, the module names were not changed; for example, enzymes associated to “methane metabolism” were annotated to bacterial taxonomies – although bacteria are not known to produce methane -. However, since same enzymes are also associated with bacterial acetogenesis/acetate oxidation they were labeled accordingly^{42, 43, 44} in Figure 4. Module names are retained, while showing FRY analysis like in Figure S12. Gene names for iron cycling⁴⁵, Manganese-oxidizing mechanisms⁴⁶, Manganese transporter [searched by gene description and enzyme identifiers], Arsenic cycle⁴⁵ and [FeFe]- and [NiFe]-hydrogenase⁴⁷ were annotated into this study from the cited referenced studies. Peptidase were annotated using MEROPS database⁴⁸ [<https://www.ebi.ac.uk/merops/>] and CAZymes were annotating using the subfam_EC_mapping.tsv file from dbCAN2 database⁴⁹ [https://bcb.unl.edu/dbCAN2/download/Databases/subfam_EC_mapping.tsv].

Photorespiration, photosystems I & II and photosystem I assembly protein Ycf3, are understudied pathway modules, labeled as „O₂ respiration“ in Figure 4 since these modules are associated with genes for oxygen respiration/utilization and cytochrome functions.

We attempted to classify the assembled MAGs as either bacteria or archaea using GTDB-tk, but we had not initially considered the possibility that the metatranscriptome may also contain reads from smaller bacterioplankton genomes⁵⁰ with similar protein families. For example, the CAM (Crassulacean Acid Metabolism) pathway module includes four enzymes: pyruvate phosphate dikinase, NADP-dependent malic enzyme, malate dehydrogenase, and phosphoenolpyruvate carboxylase. This module appears upregulated in February compared to October (Figure 5). However, since bacteria do not carry out photosynthesis, we want to clarify that pathway annotations and module names should not be over-interpreted. Instead, such results should be understood as reflecting the overall transcriptomic profile of the marine sediments, irrespective of the specific microbes involved.

Supplementary Results Text S6: Microbial communities and metabolisms over a seasonal cycle

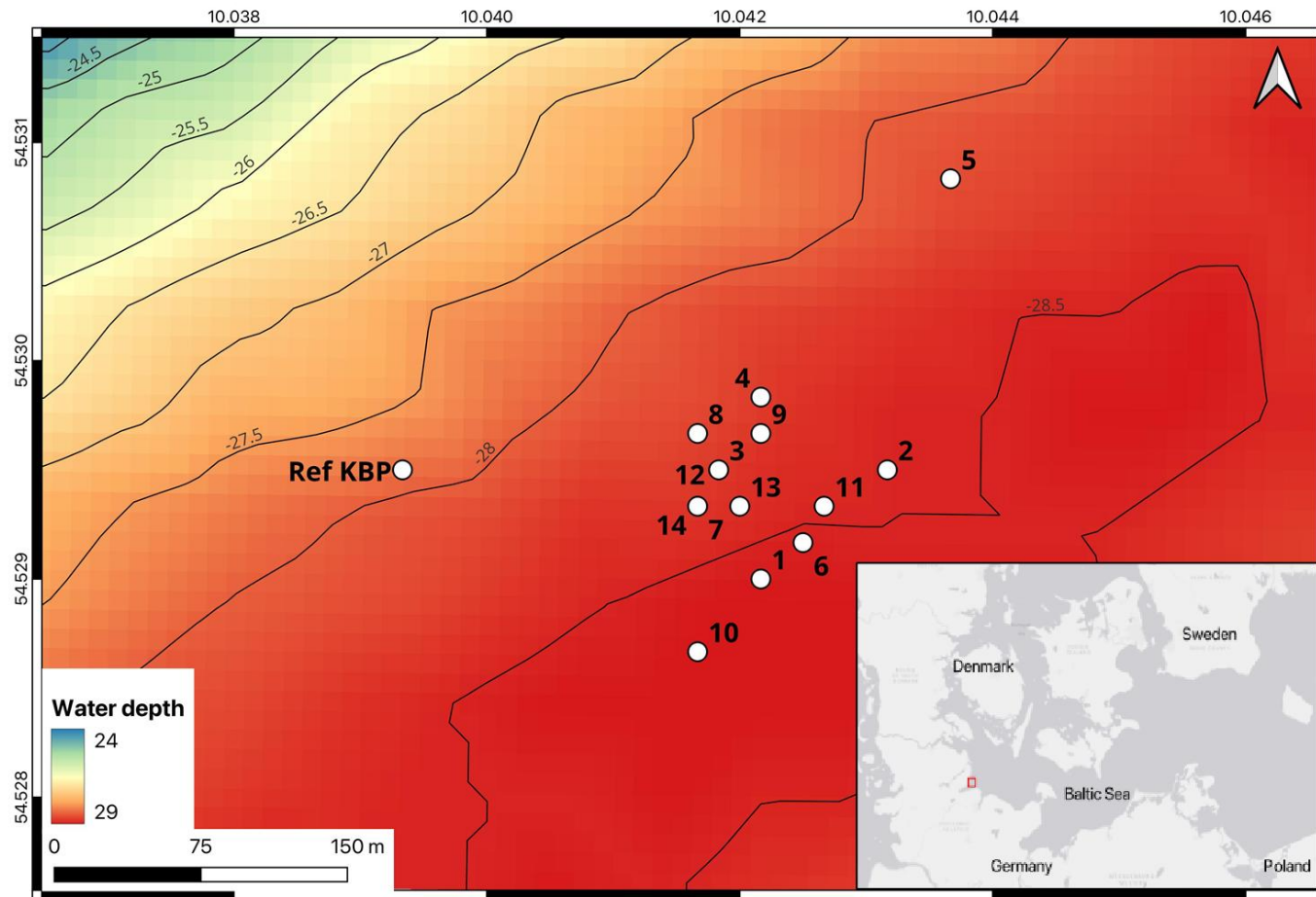
In late winter 2022 SOB in surface sediments were largely dominated by *Beggiatoaceae* (68%). However, in late winter 2023 *Beggiatoaceae* co-existed with *Sulfurimonas* spp. and *Candidatus*

Electrothrix (cable bacteria) but *Beggiatoaceae* were outcompeted by *Sulfurimonas* towards March (Figure S10B).

Distinct genes associated with iron oxidation - *ccoN1* and *cyc1*⁴⁵ - as well as genes involved in iron reduction, including *mtrB* and *omcS*⁵¹ were recognized in the metagenomes. In addition, several genes with unclear roles in iron redox processes, such as *appC*, *cbaA/B*, *ccpA*, *cydA*, *fbhH*, *fdx*⁵¹, *feoB*⁵², *petC*, *qrcA*, and *yceJ*⁴⁵ were detected (Figures 6C). However, from all these genes only *omcS* was upregulated in different seasons.

Intriguingly, the bacterial community in July appeared relatively homogenous throughout the sediment, contrasting the vertical gradients in the community composition of sediments from other months. Porewaters showed extremely high dissolved ammonium and low sulfate concentrations (Figure S8) indicating elevated OM degradation rates via SR throughout the core. Such patterns could reflect intense and deep-reaching turbation that mixed reactive OM into deeper layers, consistent with the lack of major community gradients (Figure S10A). Although no storm events were documented for this time period (Figure S16), other physical disturbances like bottom trawling may have contributed to sediment reworking and redistribution of reactive substrates that would otherwise be stratified with depth⁵³.

Figure S1. Map showing the sampling sites at Boknis Eck during the 15-month time series study. The sampling area is marked by a red square in the overview map and station numbers correspond to the list in Data S8.



Ref KBP denotes the long-term Boknis Eck study site. The station numbers for the Boknis Eck 15-month benthic time series compare to coordinates in Data S8.

Figure S2. A) Quality assessment of metagenome-assembled genomes (MAGs) using CheckM. CheckM estimates the completeness, contamination, and strain heterogeneity of MAGs based on the presence and copy number of lineage-specific single-copy marker genes. Completeness reflects the proportion of expected marker genes detected in each bin, while contamination is inferred from the presence of multiple copies of these markers. Strain heterogeneity is assessed by analyzing sequence variation among the duplicated marker genes. These metrics provide an overall assessment of MAG quality and reliability. B) Quality control of gene abundances between metagenome and metatranscriptome samples. Raw gene counts were normalized to library size using Counts per Million (CPM), and low-count genes were filtered out. The plot shows the relationship between gene abundance in the metagenome and mean gene expression in the metatranscriptome for the four time points.

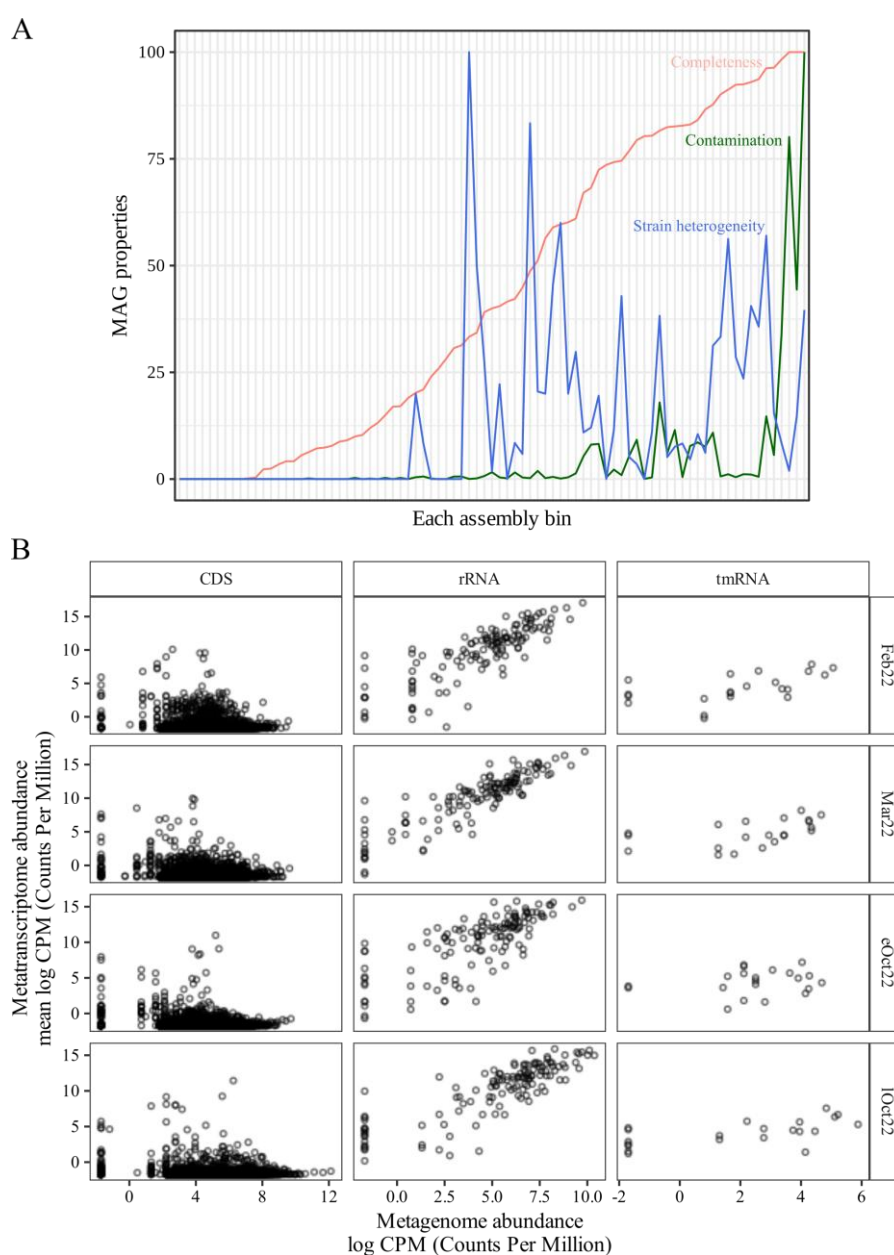


Figure S3. Taxonomic abundance analysis (Z-scores) revealed distinct seasonal responders across the metagenome and metatranscriptome. There have been very prominent representations of “unclassified archaea” or “unclassified bacteria” in each individual season with high abundance in the respective season. Feb-March samples were defined by high transcriptional activity in Woeseiales and unclassified Bacteria, consistent with the degradation of fresh organic matter. In contrast, the hypoxic early October phase showed a specific activity peak in unclassified Archaea and Cyanobacteriia. Following re-oxygenation in late October, we observed a coordinated increase in transcripts from Desulfatiglandales, Desulfobacterales, and Polyangiales. Comparisons of the coefficient of variation (CV) indicate these shifts were often driven by transcriptional upregulation (high metatranscriptome CV) rather than changes in genomic abundance.

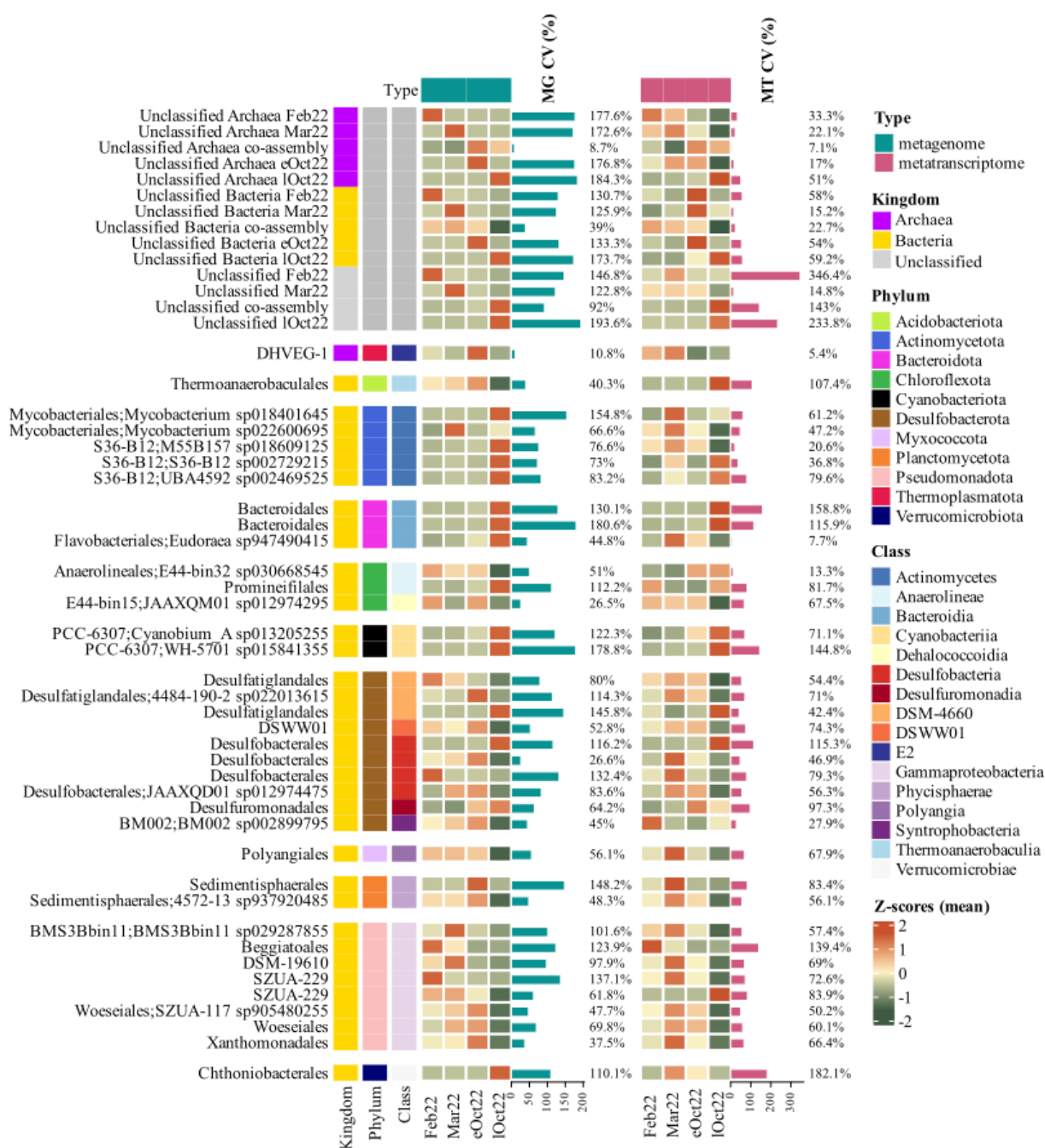


Figure S4. Visualization of read mapping across samples and genomic features. This figure visualizes the number of reads and their mapping percentages from each sample to MAGs and annotated genes. Time points are abbreviated as in the table (Feb22, Mar22, eOct22, lOct22). Total number of reads after QC (A), mapping percentages to all the MAGs (B) and genes annotated for bacteria and archaea (C), are depicted respectively. On average, 60% of metagenomic reads and 72% of metatranscriptomic reads mapped to the assemblies (B and Table S2). A total of 622,521 genes were annotated, spanning bacterial, archaeal, mitochondrial, and unknown prokaryotic sequences. On average, 7% of metagenomic reads and 27% of metatranscriptomic reads mapped to annotated genes (C; Table S2). Distribution of reads in MAGs and PCA analysis of gene abundances. Reads from metagenomes and metatranscriptomes collected in February, March, early October, and late October 2022 from the top 2 cm of sediment were mapped to assembled contigs/scaffolds and classified using the tool Tiara (D).

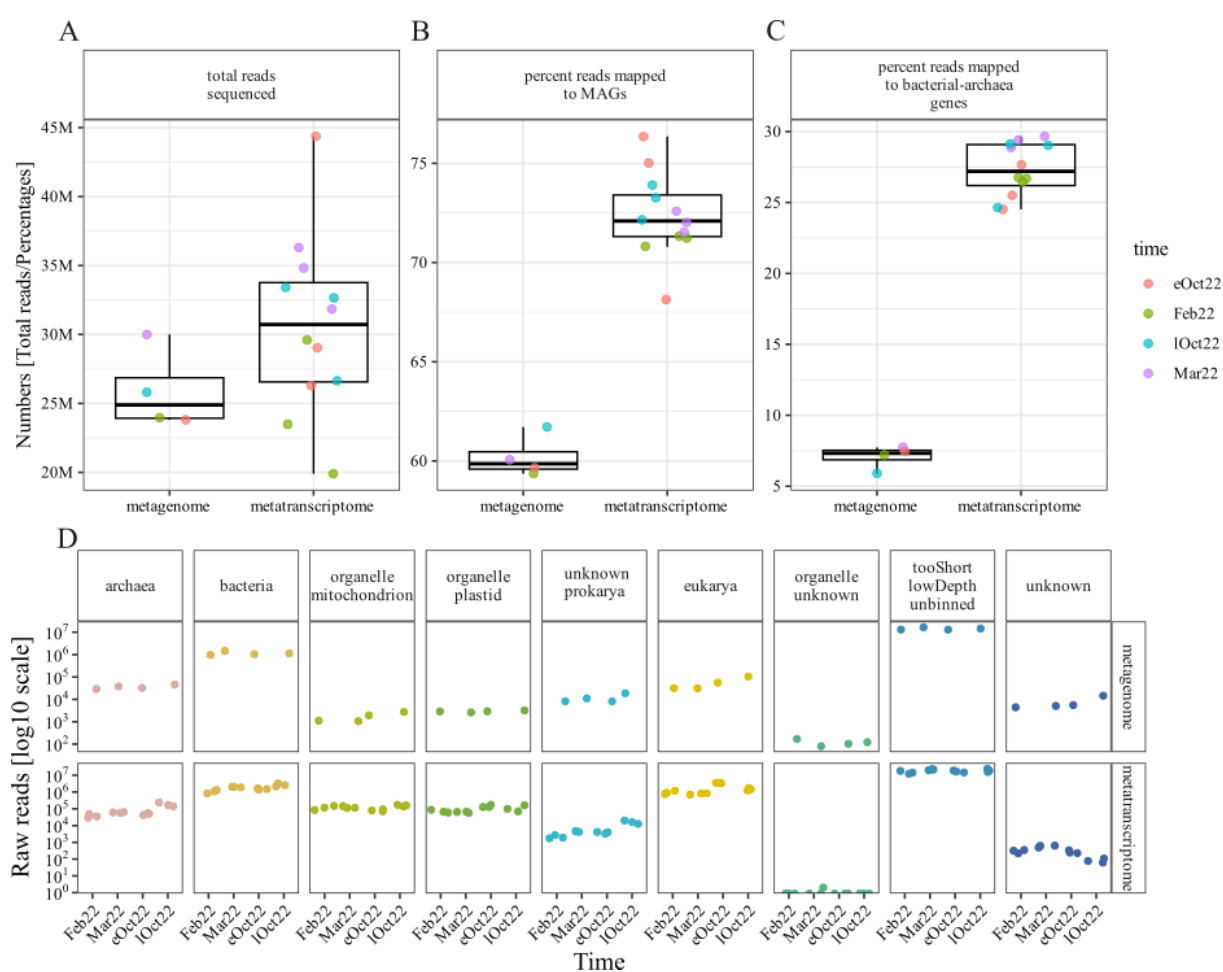
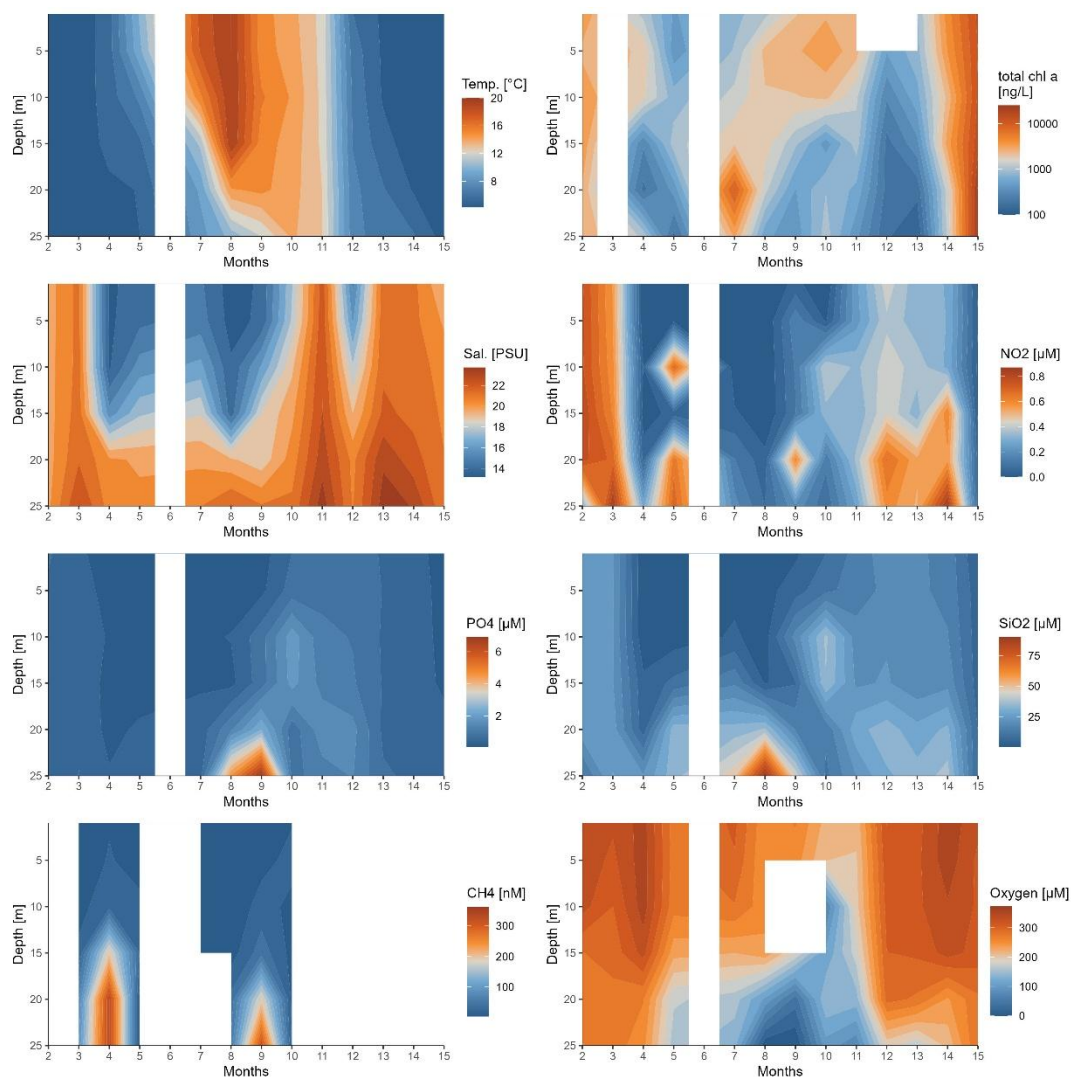
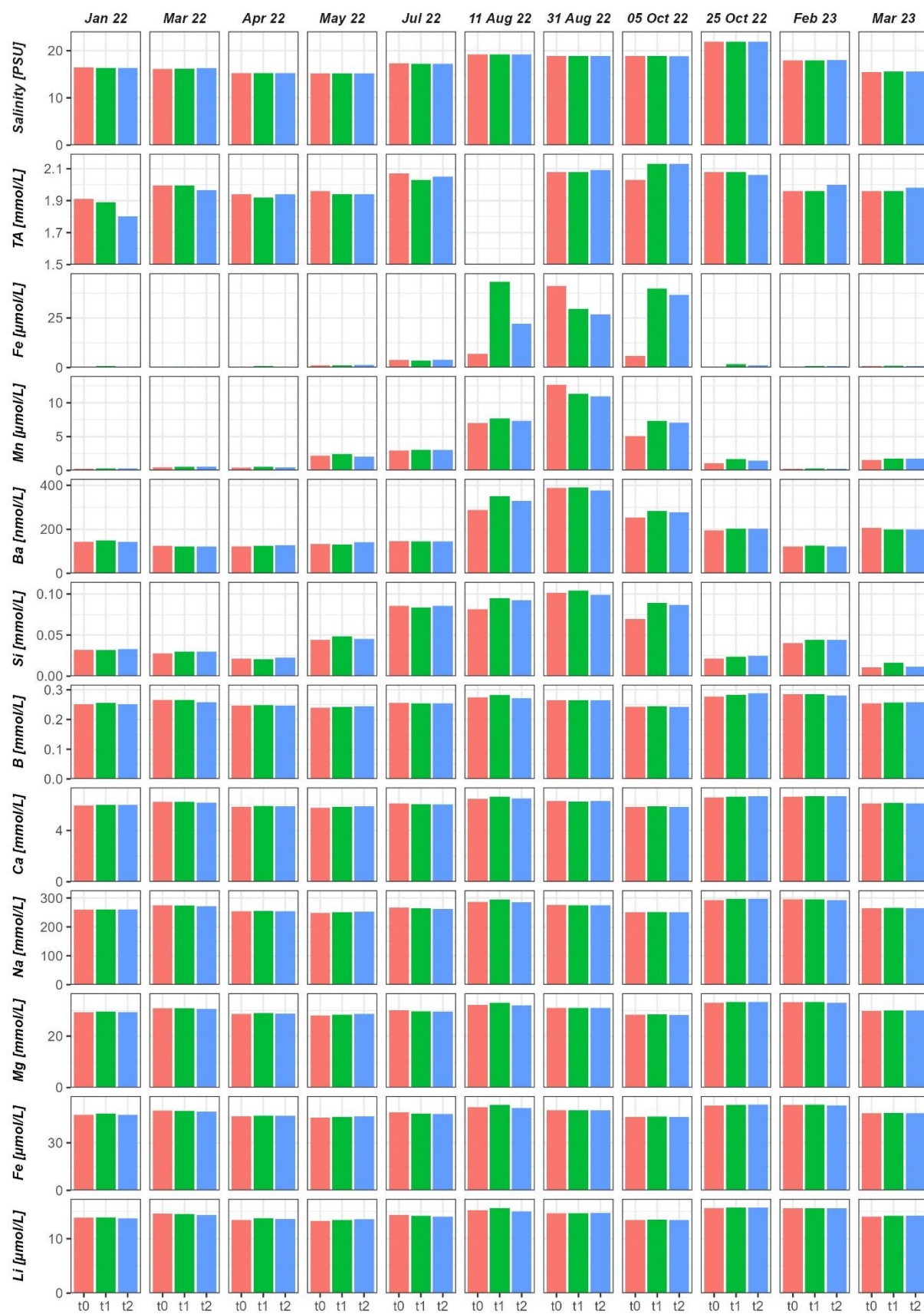


Figure S5. Water column properties from surface to 25 m water depth were monitored from January 2022 to March 2023.



For some months no data is available as indicated by white overlays. Water column data was collected on the 02. February 2022, 02. March 2022, 12. April 2022, 03. May 2022, 12. July 2022, 30. August 2022, 28. September 2022, 11. October 2022, 09. November 2022, 08. December 2022, 26. January 2023, 15. February 2023 and 20. March 2023

Figure S6. Compounds emitting from sediments in the course of 2-hour incubations with the enclosed benthic chamber.



t0 = immediately after deployment of the chamber; t1 = after 1 hour incubation; t2 = after 2 hours incubation.

Figure S7. Temperature (blue) and oxygen (grey) concentration development during in-situ incubations with the MiniChamber Lander. Temperature and oxygen concentrations were measured inside the benthic chamber and are displayed for the whole deployment period. The vertical line marks the closing of the benthic chamber and thus the start of the incubation time. As a reference, temperature and oxygen concentration measured in bottom waters at the KBP reference site for the respective month are displayed as dark blue (temperature) and dark grey (oxygen) dots.

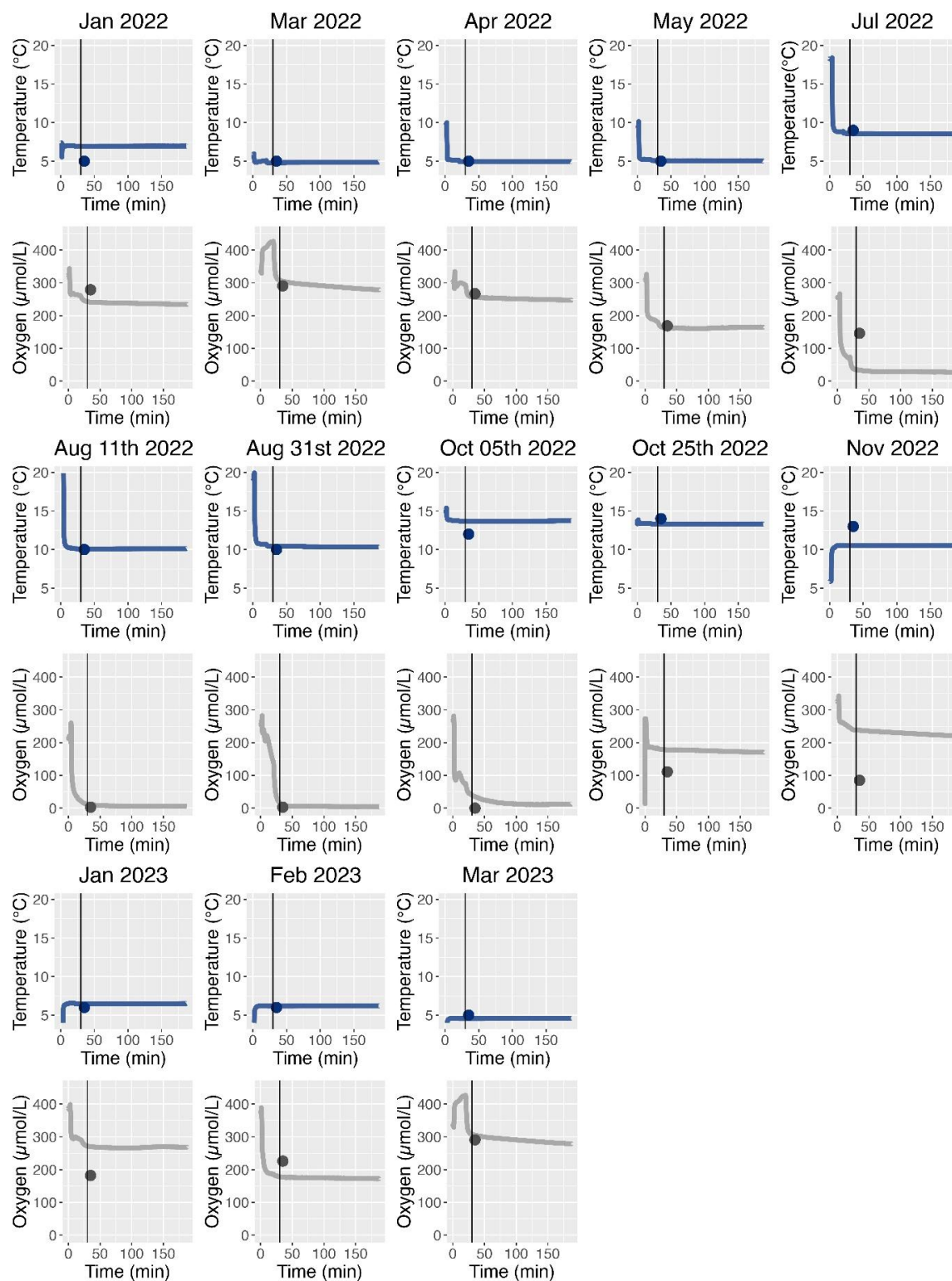
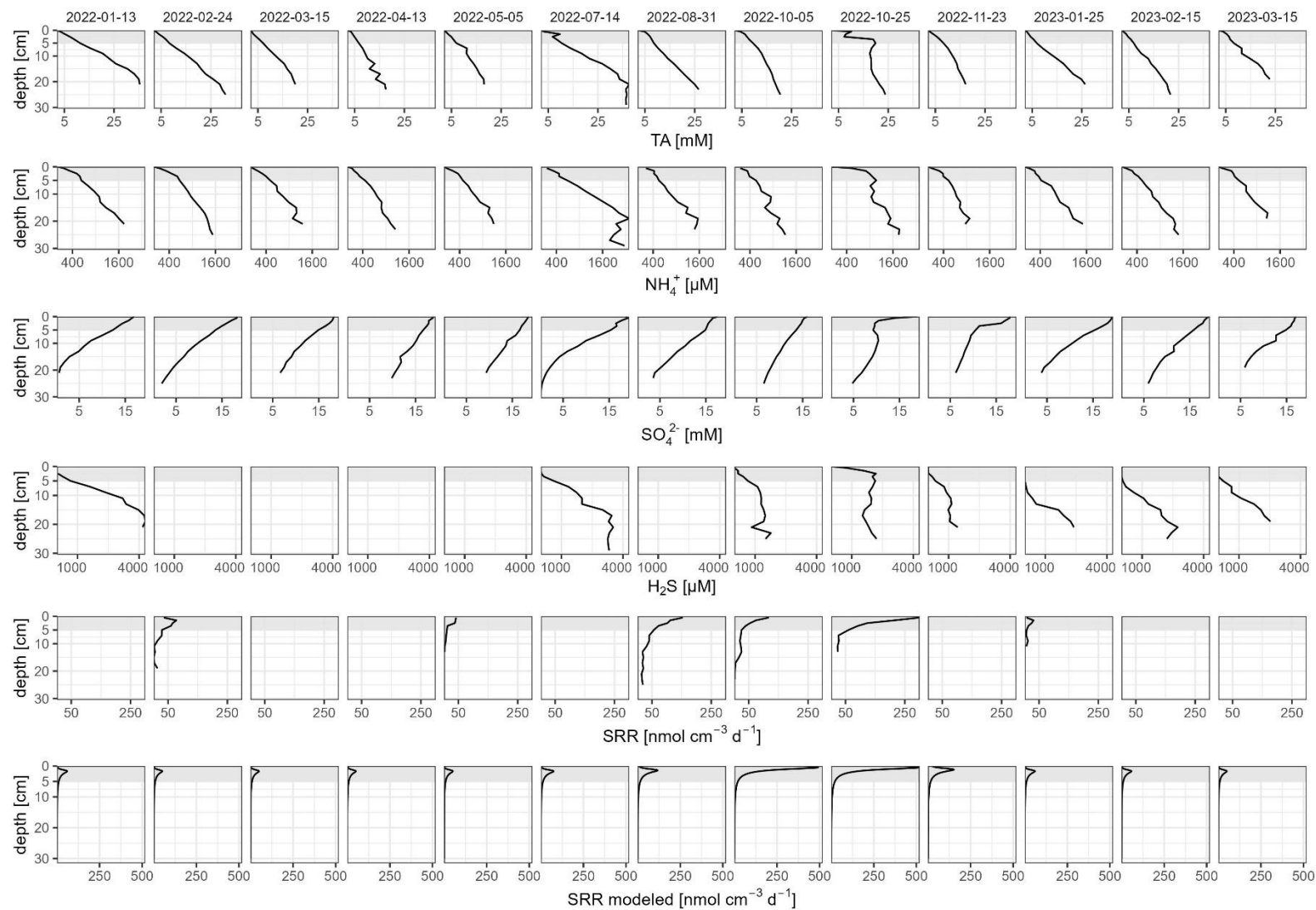


Figure S8. Porewater geochemistry as well as modeled and measured SRR.



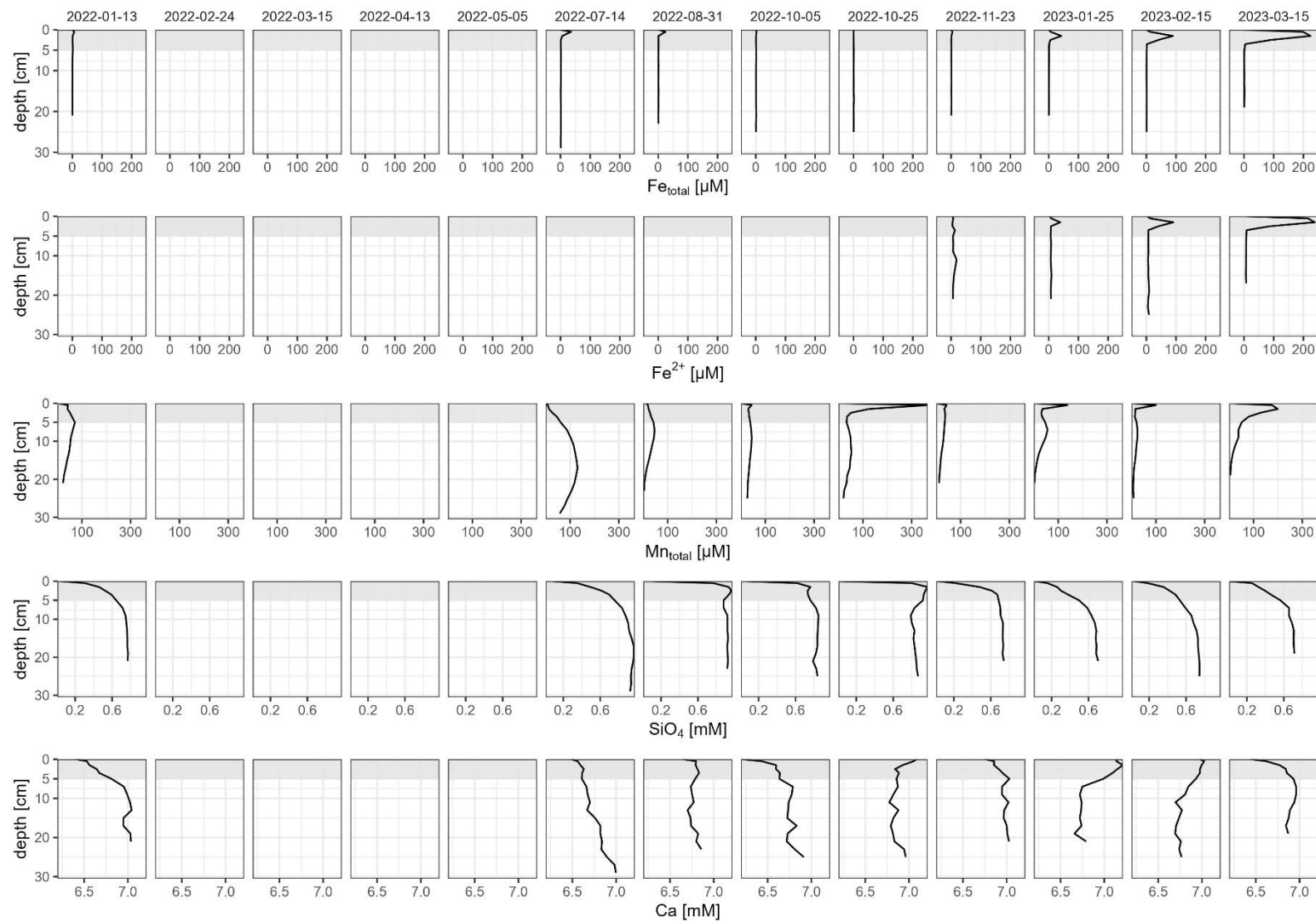


Figure S9. Cell numbers (cells ml^{-1} sediment) as determined for different sediments depths by DAPI staining.

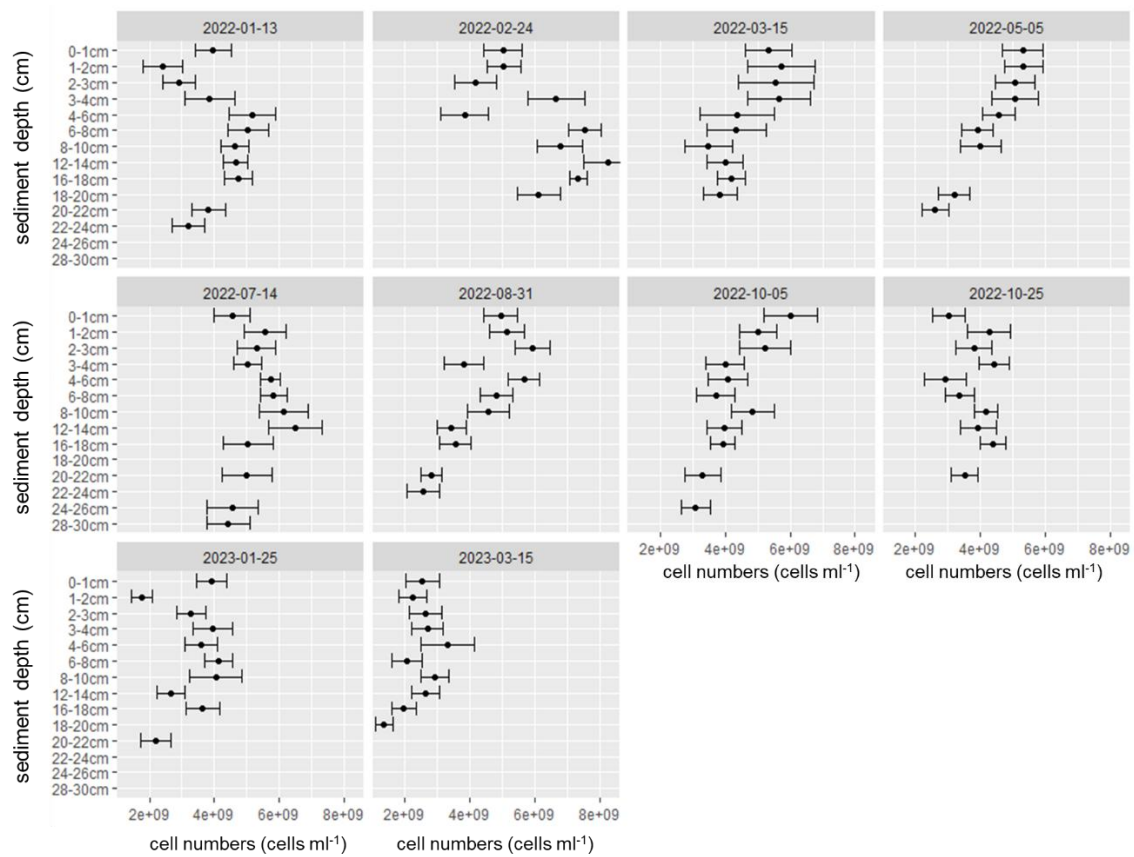


Figure S10A. Bacterial community composition based on RNA-derived 16S rRNA gene amplicons. The relative abundance of the 30 most abundant bacterial families are shown and classified by putative metabolism: potential sulfide-oxidizing bacteria (SOB) are shown in blue, sulfate-reducing bacteria (SRB) in orange, aerobic methanotrophs in red, organotrophs (excluding *Desulfobacterota*) in brown, and cyanobacteria in green. All remaining bacterial genera are grouped under "Others" (grey).

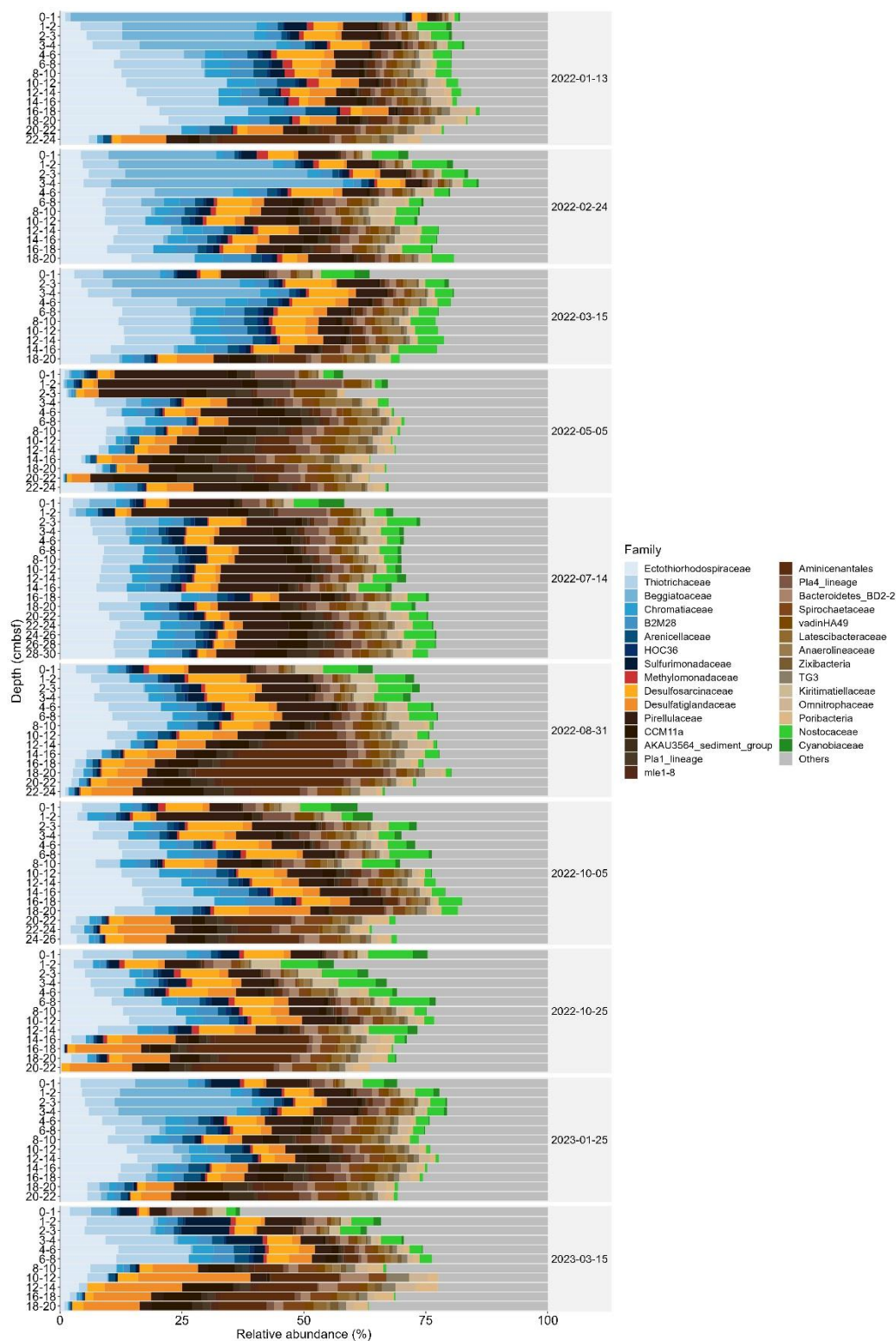


Figure S10B. Community composition based on RNA-derived 16S rRNA gene amplicons from genera of putative SOB.

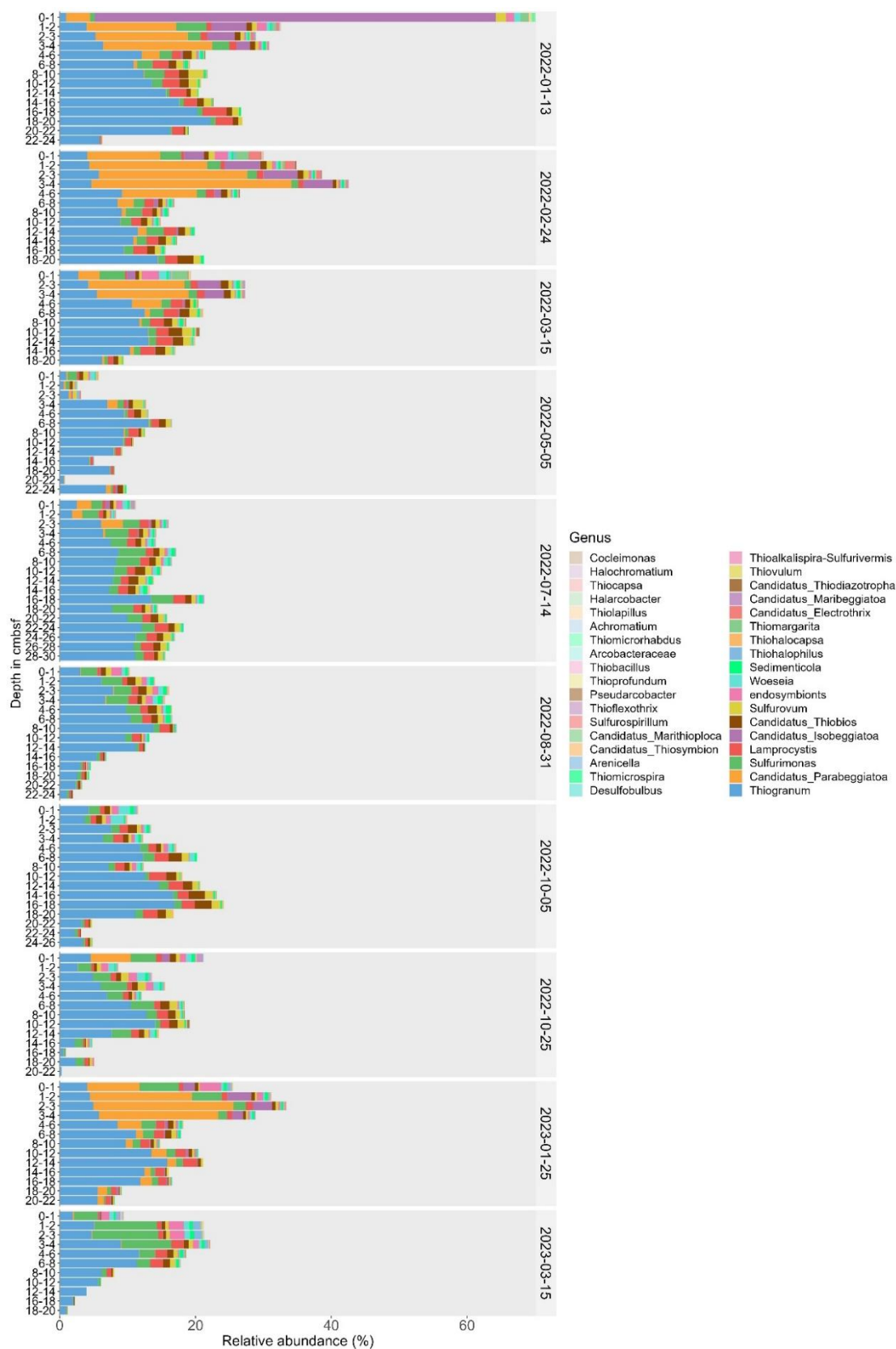


Figure S10C. Archaeal community composition based on RNA-derived 16S rRNA gene amplicons. The relative abundance of archaeal taxa is shown for the 30 most abundant archaeal families. All remaining archaeal genera are grouped under "Others" (grey).

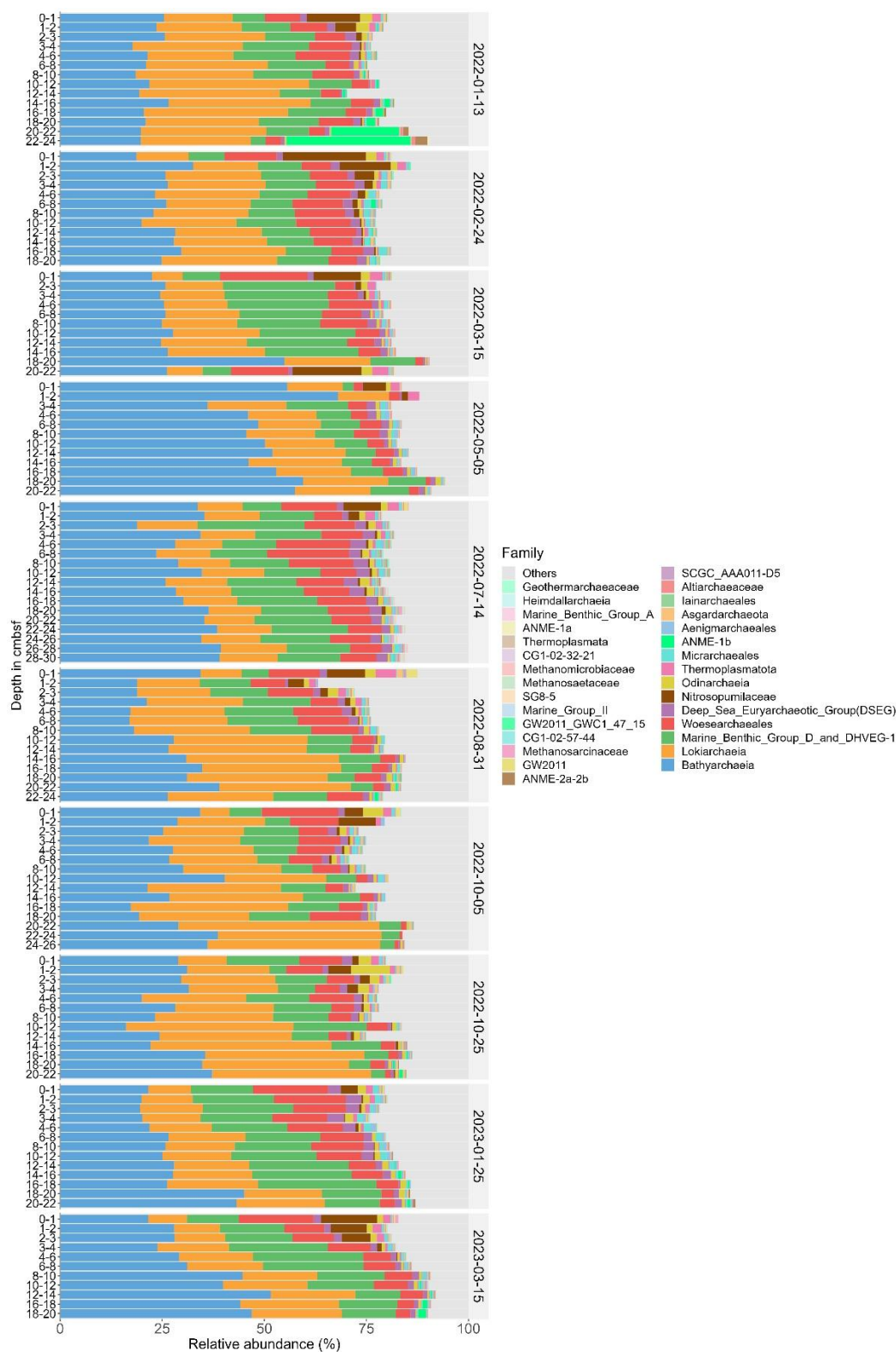


Figure S11. A significant number of reads mapped to eukaryotic contigs, unknown organelle sequences, or contigs that were unclassified, very short, low-depth, or unbinned. The microbial coding sequence (CDS) gene abundances was rCLR transformed, computed robust Aitchison distances, and then projected the high-dimensional structure into 2D using Multi-Dimensional scatter (MDS) plot for both metagenome (A) and metatranscriptome (B) samples. Replicates from each time point clustered closely, indicating temporal consistency. PCA bi-plot reveals a tight coupling between seasonal marine sediment dynamics and microbial functional profiles (C, D). Dimension 1 (>50% variance) separates samples into distinct Spring (Feb/Mar) and Autumn (Oct) clusters. Feb-March samples are defined by vectors for CAZymes and Peptidases, consistent with the degradation of fresh organic matter from algal blooms. Furthermore, strong associations with Iron cycling, Sulfur, and Nitrogen metabolism reflect the community's reliance on alternative electron acceptors during stratification-induced oxygen depletion. In contrast, October samples are driven almost exclusively by O₂ respiration, capturing the metabolic shift following fall re-oxygenation events. Crucially, a temporal disconnect emerges in early October: while the metagenome (DNA) retains an anaerobic-like signature, the metatranscriptome (RNA) shifts strongly toward O₂ respiration. This indicates that the microbial community launches a rapid transcriptional response to re-oxygenation before the genomic community composition fully turns over.

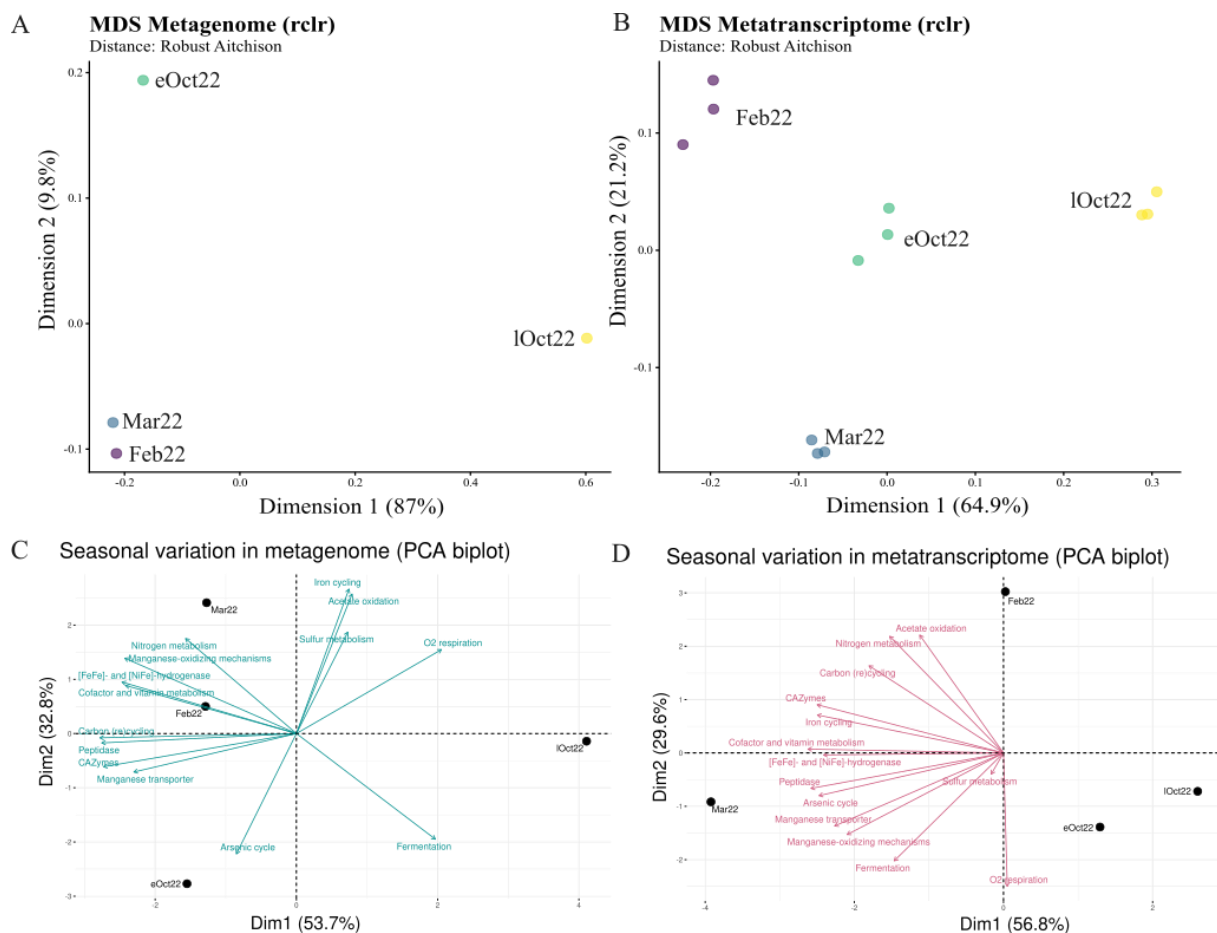


Figure S12. Pathway enrichment analysis using FRY with mixed P-values. Differential expression analysis was followed by pathway enrichment using the FRY method to identify statistically significant KEGG modules. The figure displays enriched KEGG modules, showing their direction of regulation (up- or downregulated) across the compared time points (x-axis). Modules were selected based on FRY-derived mixed P-values, which is a non-directional p-value, testing for any differential expression in the gene set regardless of direction (up or down). A significance threshold of $P \leq 0.05$ was applied across all comparisons. The y-axis shows the names of KEGG modules, grouped according to their respective KEGG pathway categories.

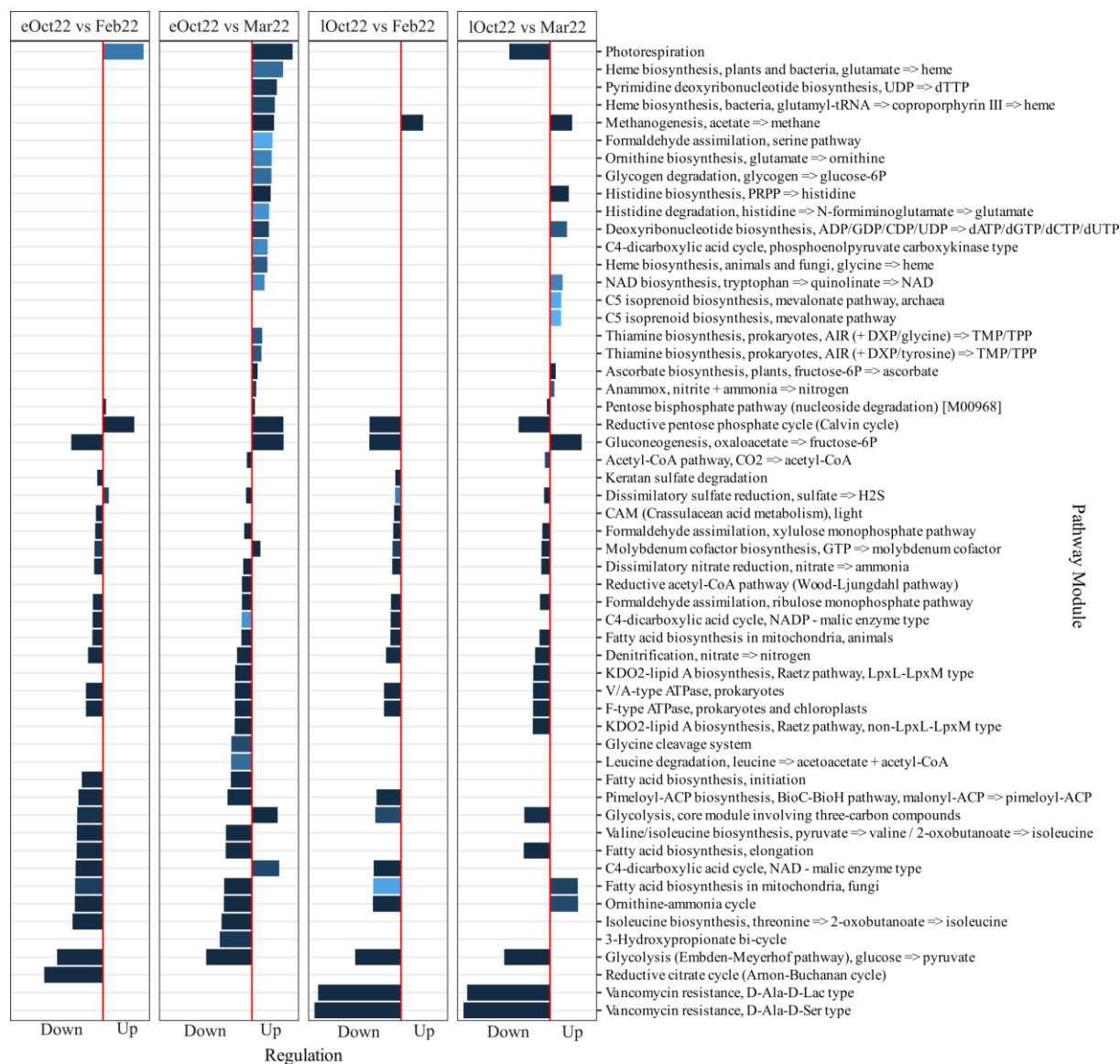


Figure S13AB. PCA bi-plot of CAZymes abundances (A) and pathway abundances of Carbon (re)cycling (B).

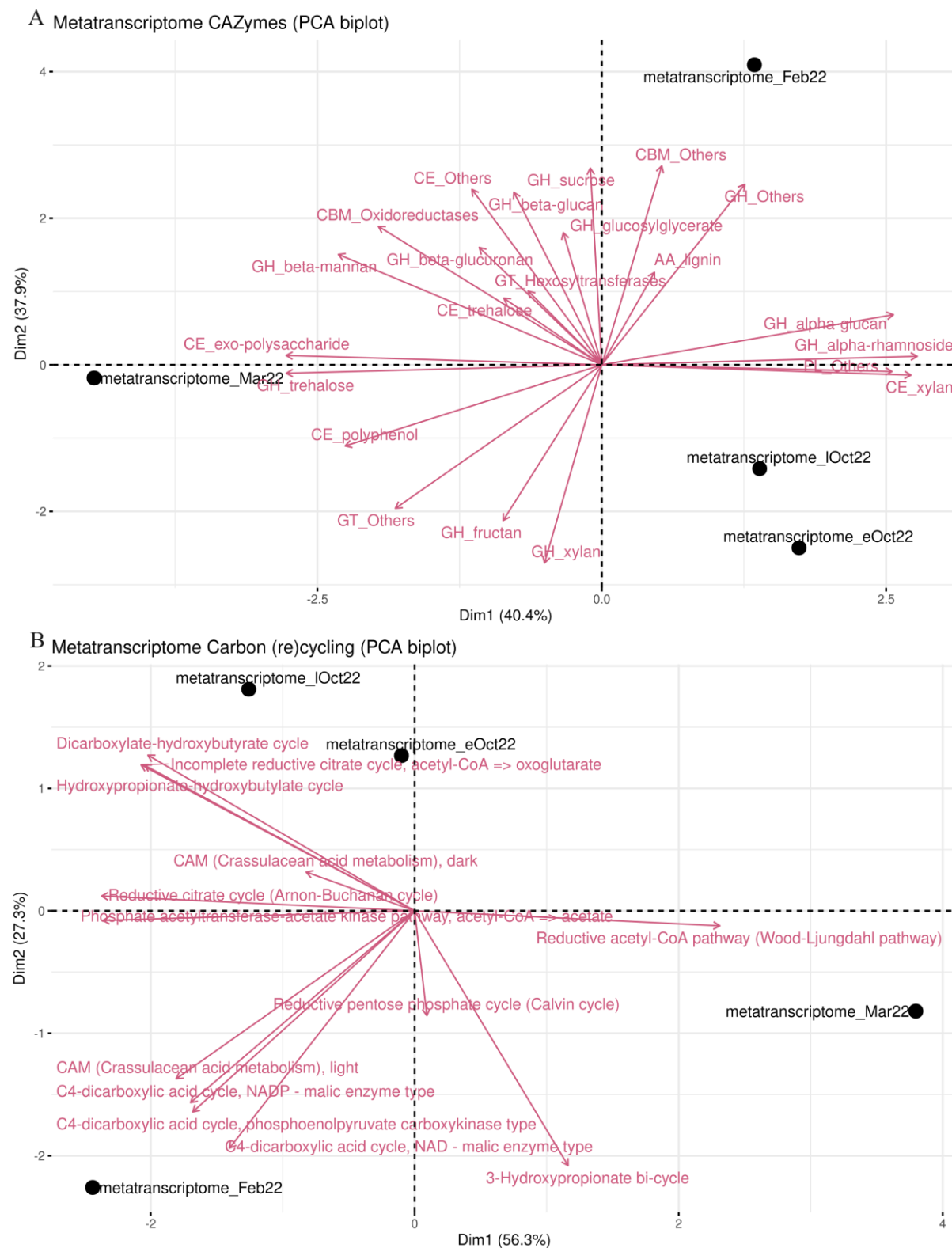


Figure S13CDE. Genes involved in nitrogen metabolism (C), sulfur metabolism (D) and iron cycling (E).

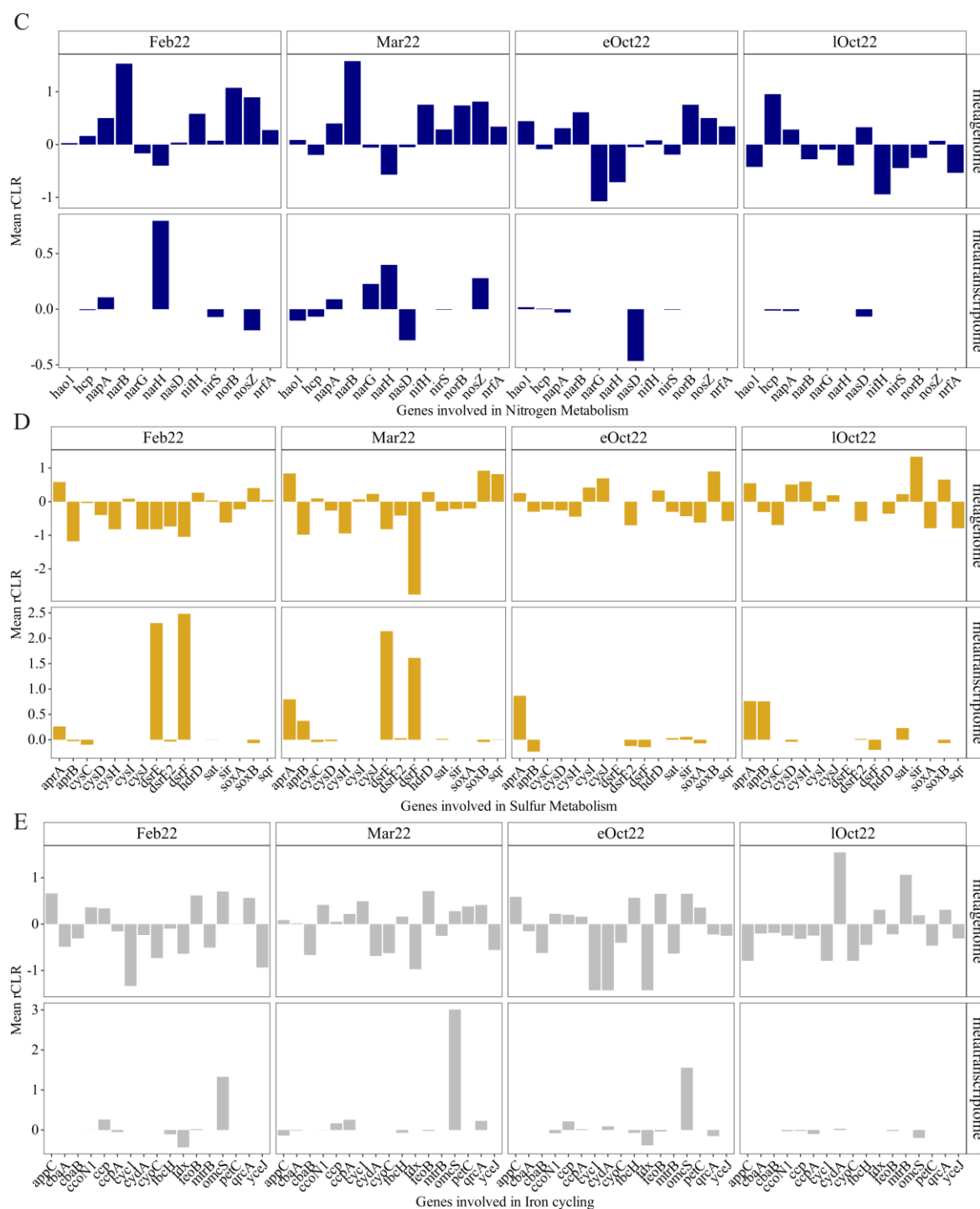


Figure S14. Taxonomic abundances of pathways associated with Carbon (re)cycling (A) acetate oxidation (B), Reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) (C), Nitrogen metabolism (D), Sulfur metabolism (E) and hydrogenase (F). Taxonomic abundance profiles reveal decoupling between genomic potential and transcriptional activity, where metagenomic composition remained remarkably static across seasons while metatranscriptomes fluctuated dynamically. In February/March, carbon (re)cycling transcripts were dominated by *Woeseliales* and *Flavobacteriales*, identifying them as the primary responders degrading fresh algal blooms. Concurrently, the strictly anaerobic *Desulfobacterales* and unclassified Archaea showed a distinct transcriptional expansion in the Reductive acetyl-CoA pathway during March, confirming their activity within anoxic microniches despite oxic water column conditions. By late October, the community metabolism shifted toward acetate oxidation and sulfur cycling, driven overwhelmingly by *Desulfatiglandales*, which appear to be the key taxa responsible for the fall recovery and recycling of accumulated end-products.

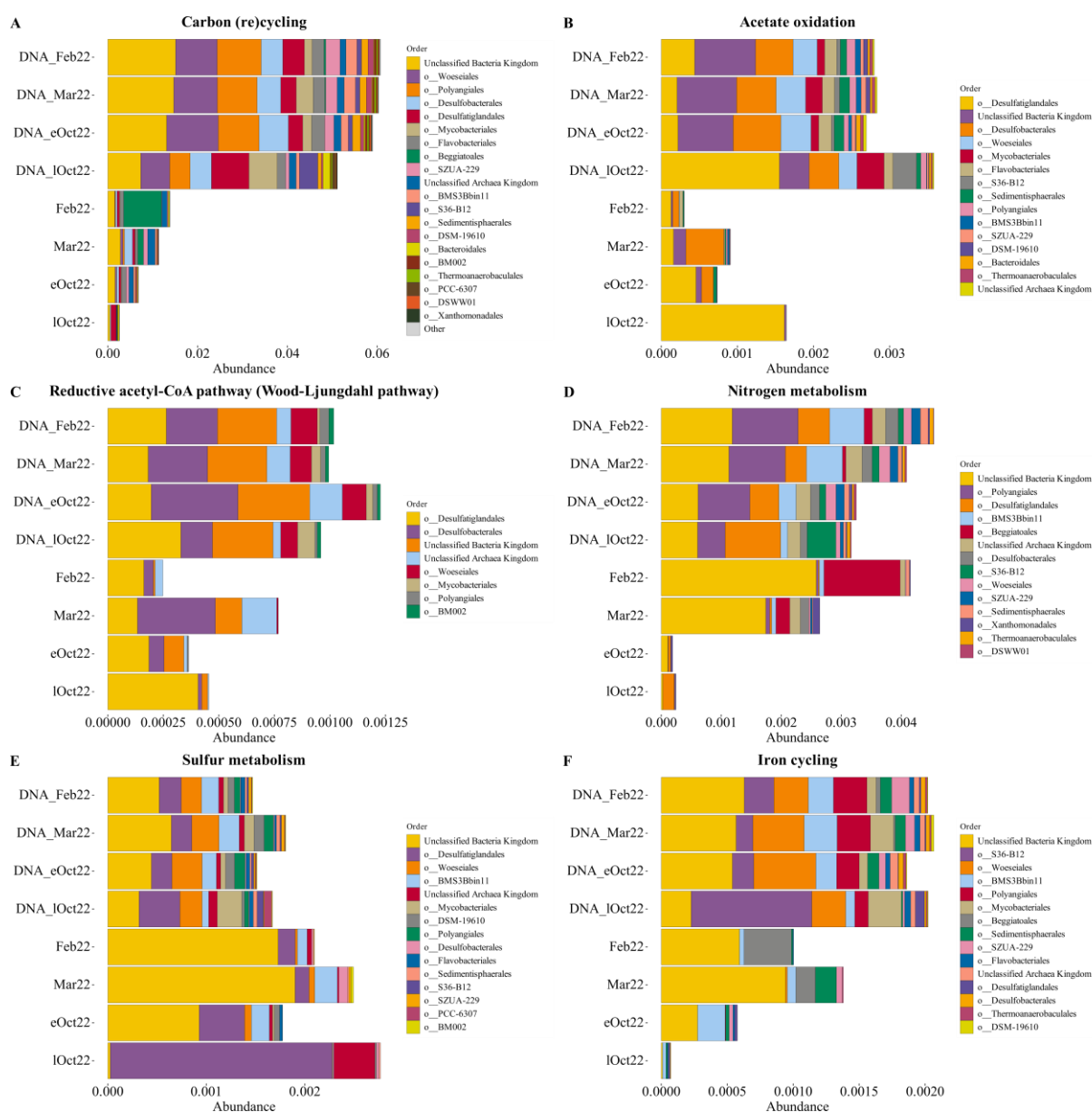


Figure S15. Temperature (A), oxygen (B) and sulfide concentrations (C, D) during benthic chamber incubations with the MiniChamber Lander System on the 29. August 2023. For comparison, sulfide concentrations were measured inside (C) and outside (D) of the benthic chamber, while oxygen and temperature were measured only inside the chamber. The vertical line marks the closing of the benthic chamber and thus the start of the incubation time.

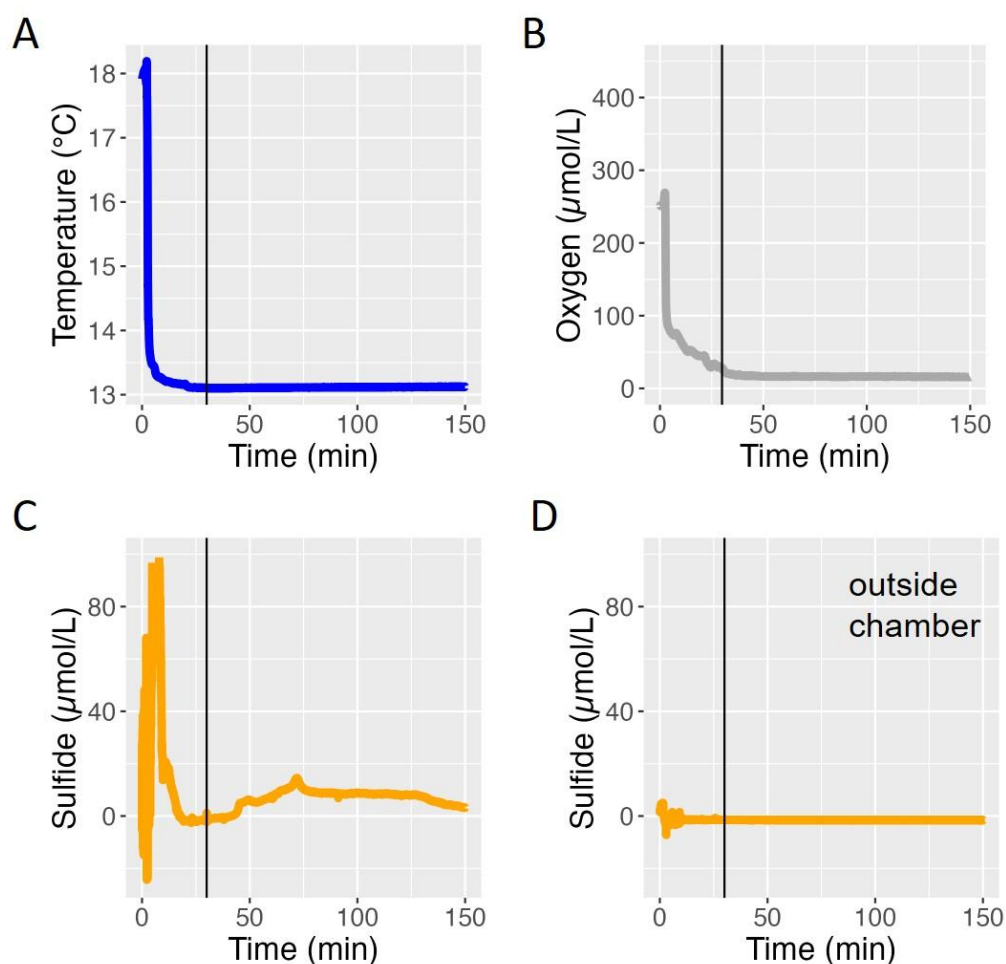
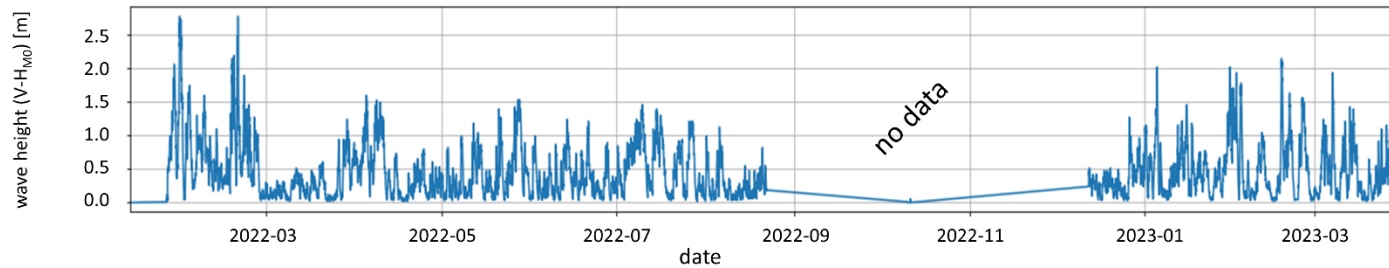
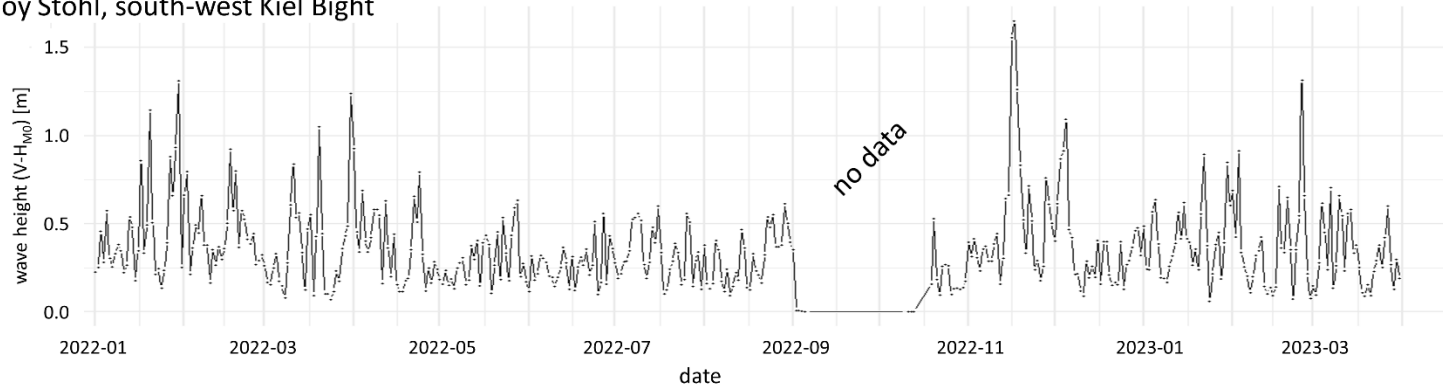


Figure S16. Wave heights at two buoys within the Kiel Bight in one-year period from January 2022 to April 2023. Buoy Heiligenhafen ($54^{\circ} 23,79960' N$, $10^{\circ} 55,80000' E$) is situated in the south-east of the Kiel Bight, Buoy Stohl ($54^{\circ} 29,22600' N$, $10^{\circ} 12,07620' E$) in the south-west, both are operated by LKN.SH. The wave height is shown as significant wave height in V-HM0 measured by a DWR. The highest wave heights are measured between December and March reaching above 2.5 m, 1.5 m respectively.

Buoy Heiligenhafen, south-east Kiel Bight



Buoy Stohl, south-west Kiel Bight



Supplementary Data:

Data S1: *GTDB-Tk classification of all the bins after MetaBAT2.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 1).

Data S2: *Read mapping statistics across samples and genomic features. Depicting the number of reads from each sample and their mapping percentages to metagenome-assembled genomes (MAGs) and annotated genes derived from MAG assemblies. Time points are labeled as Feb22 (February 2022), Mar22 (March 2022), eOct22 (early October 2022), and lOct22 (late October 2022). Reads were mapped to all MAGs regardless of taxonomic classification (column: percent reads mapped to MAGs). Additionally, reads were mapped to protein-coding genes (CDS) and non-coding genes (tRNA, rRNA, tmRNA) annotated on contigs/scaffolds assigned to archaea, bacteria, mitochondria, prokaryotes, and unknown categories based on classification by the Tiara tool (column: percent reads mapped to genes).*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 2).

Data S3: *Z-scores of metagenome and metatranscriptome abundances of each GTDB-Tk predicted taxonomy represented in Figure 5A.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 3).

Data S4: *CheckM's assessment of MAG bins. It provides robust estimates of genome completeness and contamination by using collocated sets of genes that are ubiquitous and single-copy within a phylogenetic lineage. The last column of the table is GTDB-TK's classification of the MAG bins merged for convenience.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 4).

Data S5: *Up/downregulation of KEGG modules. FRY output with P.value.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 5).

Data S6: *Differential expression of KEGG modules. FRY output with P.valueMixed.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 6).

Data S7: *Relative gene abundance/expression for all the samples with taxonomy assigned by GTDB-Tk.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 7).

Data S8. *Station numbers and coordinates of the sampling sites of the 15-month time series. Ref KBP refers to the long-term water column time series station at Boknis Eck.*

The complete table is provided in the accompanying Supplementary Data Excel file (Sheet 8).

References

1. Grasshoff K, Kremling K, Ehrhardt M. *Methods of seawater analysis*. Wiley-VCH (1999).
2. Gindorf S, Bange HW, Booge D, Kock A. Seasonal study of the small-scale variability of dissolved methane in the western Kiel Bight and Eckernförde Bay (Baltic Sea) during the European heat wave in 2018. *Biogeosciences* **19**, 4993-5006 (2022).
3. Ivanenkov VN, Lyakhin YI. Determination of total alkalinity in seawater. In: *Methodes of hydrochemical investigations in the ocean* (eds Bordovsky OK, Ivanenkov VN). Nauka Publ. House, (1978).
4. Cline J. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* **14**, 454-458 (1969).
5. Bower CE, Holm-Hansen T. A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 794-798 (1980).
6. Stookey LL. Ferrozine - a new spectrophotometric reagent for iron. *Analytical Chemistry* **42**, 779-781 (1970).
7. Adam N, *et al.* Deltaproteobacterium Strain KaireiS1, a Mesophilic, Hydrogen-Oxidizing and Sulfate-Reducing Bacterium From an Inactive Deep-Sea Hydrothermal Chimney. *Frontiers in Microbiology* **12**, 686276 (2021).
8. Kostka JE, Luther GW. Partitioning and Speciation of Solid-Phase Iron in Salt-Marsh Sediments. *Geochim Cosmochim Ac* **58**, 1701-1710 (1994).

9. Heron G, Crouzet C, Bourg ACM, Christensen TH. Speciation of Fe(II) and Fe(III) in Contaminated Aquifer Sediments Using Chemical Extraction Techniques. *Environmental Science & Technology* **28**, 1698-1705 (1994).
10. Lewis EL, Perkin RG. The practical salinity scale 1978: conversion of existing data. *Deep Sea Research Part A Oceanographic Research Papers* **28**, 307-328 (1981).
11. Røy H, Weber HS, Tarpgaard IH, Ferdelman TG, Jørgensen BB. Determination of dissimilatory sulfate reduction rates in marine sediment via radioactive ³⁵S tracer. *Limnology and Oceanography: Methods* **12**, 196-211 (2014).
12. Kallmeyer J, Ferdelman TG, Weber A, Fossing H, Jørgensen BB. A cold chromium distillation procedure for radiolabeled sulfide applied to sulfate reduction measurements. *Limnology and Oceanography: Methods* **2**, 171-180 (2004).
13. Jørgensen BB. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. *Geomicrobiology Journal* **1**, 11-27 (1978).
14. Perner M, *et al.* Environmental changes affect the microbial release of hydrogen sulfide and methane from sediments at Boknis Eck (SW Baltic Sea). *Frontiers in Microbiology*, <https://doi.org/10.3389/fmicb.2022.1096062> (2022).
15. Klindworth A, *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research* **41**, e1 (2013).
16. Ding J, Zhang Y, Wang H, Jian H, Leng H, Xiao X. Microbial Community Structure of Deep-sea Hydrothermal Vents on the Ultraslow Spreading Southwest Indian Ridge. *Front Microbiol* **8**, 1012 (2017).

17. Cerqueira T, Pinho D, Froufe H, Santos RS, Bettencourt R, Egas C. Sediment Microbial Diversity of Three Deep-Sea Hydrothermal Vents Southwest of the Azores. *Microb Ecol* **74**, 332-349 (2017).
18. Bokulich NA, *et al.* Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**, 90 (2018).
19. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**, 581-583 (2016).
20. Quast C, *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research* **41**, D590-596 (2013).
21. Pedregosa F, *et al.* Scikit-learn: Machine Learning in Python. *Journal of Machine Learning Research* **12**, 2825-2830 (2011).
22. Kaehler BD, Bokulich NA, McDonald D, Knight R, Caporaso JG, Huttley GA. Species abundance information improves sequence taxonomy classification accuracy. *Nature Communications* **10**, 4643 (2019).
23. Price MN, Dehal PS, Arkin AP. FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS One* **5**, e9490 (2010).
24. Liu C, Cui Y, Li X, Yao M. microeco: an R package for data mining in microbial community ecology. *Fems Microbiol Ecol* **26**, fiae255 (2021).
25. Pasolli E, *et al.* Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. *Cell* **176**, 649-662.e620 (2019).

26. Stewart RD, *et al.* Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nature Communications* **9**, 870 (2018).
27. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. metaSPAdes: a new versatile metagenomic assembler. *Genome Research* **27**, 824-834 (2017).
28. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* **31**, 1674-1676 (2015).
29. Kang DD, *et al.* MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* **7**, e7359 (2019).
30. Olm MR, Brown CT, Brooks B, Banfield JF. dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication. *The ISME Journal* **11**, 2864-2868 (2017).
31. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research* **25**, 1043-1055 (2015).
32. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* **36**, 1925-1927 (2020).
33. Mussig AJ, Chaumeil P-A, Chuvochina M, Rinke C, Parks DH, Hugenholtz P. Putative genome contamination has minimal impact on the GTDB taxonomy. *Microbial Genomics* **10**, (2024).
34. Karlicki M, Antonowicz S, Karnkowska A. Tiara: deep learning-based classification system for eukaryotic sequences. *Bioinformatics* **38**, 344-350 (2022).

35. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068-2069 (2014).
36. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* **14**, 417-419 (2017).
37. Srivastava A, *et al.* Alignment and mapping methodology influence transcript abundance estimation. *Genome Biology* **21**, 239 (2020).
38. Ritchie ME, *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* **43**, e47-e47 (2015).
39. Wu D, Lim E, Vaillant F, Asselin-Labat M-L, Visvader JE, Smyth GK. ROAST: rotation gene set tests for complex microarray experiments. *Bioinformatics* **26**, 2176-2182 (2010).
40. Klingenberg H., P. M. How to normalize metatranscriptomic count data for differential expression analysis. *PeerJ* **5**, e3859 (2017).
41. Alhamdoosh M, *et al.* Combining multiple tools outperforms individual methods in gene set enrichment analyses. *Bioinformatics* **33**, 414-424 (2017).
42. Fuchs G. Alternative Pathways of Carbon Dioxide Fixation: Insights into the Early Evolution of Life? *Annu Rev Microbiol* **65**, 631-658 (2011).
43. Panagiotis S Adam GB, Simonetta Gribaldo. Evolutionary history of carbon monoxide dehydrogenase/acetyl-CoA synthase, one of the oldest enzymatic complexes. *PNAS*, (2018).

44. Longhui Deng DB, Clemens Glombitza, Bo Barker Jørgensen, Hans Røy, Mark Alexander Lever. Drivers of methane-cycling archaeal abundances, community structure, and catabolic pathways in continental margin sediments. *Frontiers Microbiology*, (2025).
45. Suhadolnik MLS, Costa PS, Castro GM, Lobo FP, Nascimento AMA. Comprehensive insights into arsenic- and iron-redox genes, their taxonomy and associated environmental drivers deciphered by a meta-analysis. *Environment International* **146**, 106234 (2021).
46. Wang X, *et al.* Comparative genomic and metabolic analysis of manganese-oxidizing mechanisms in *Celeribacter manganoxidans* DY25T: Its adaptation to the environment of polymetallic nodules. *Genomics* **112**, 2080-2091 (2020).
47. Peters JW, *et al.* [FeFe]- and [NiFe]-hydrogenase diversity, mechanism, and maturation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1853**, 1350-1369 (2015).
48. Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Research* **46**, D624-D632 (2018).
49. Zheng J, Ge Q, Yan Y, Zhang X, Huang L, Yin Y. dbCAN3: automated carbohydrate-active enzyme and substrate annotation. *Nucleic Acids Research* **51**, W115-W121 (2023).
50. Silva-Solar S, Viver T, Wang Y, Orellana LH, Knittel K, Amann R. Acidimicrobiia, the actinomycetota of coastal marine sediments: Abundance, taxonomy and genomic potential. *Systematic and Applied Microbiology* **47**, 126555 (2024).

51. Garber AI, *et al.* Metagenomic Insights Into the Microbial Iron Cycle of Subseafloor Habitats. *Frontiers in Microbiology* **12**, (2021).
52. Cartron ML, Maddocks S, Gillingham P, Craven CJ, Andrews SC. Feo – Transport of Ferrous Iron into Bacteria. *Biometals* **19**, 143-157 (2006).
53. Bradshaw C, *et al.* Physical Disturbance by Bottom Trawling Suspends Particulate Matter and Alters Biogeochemical Processes on and Near the Seafloor. *Frontiers in Marine Science* **8**, (2021).