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Seafloor chemosynthetic habitats and AOM-influenced sediment microbiome at a cold-water coral site off the Vesterålen coast, northern Norway

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Abstract. Cold seeps associated with cold-water corals have been reported worldwide. Yet, there are still knowledge gaps regarding ecological relationships due to contrasting observations. Here, we report the results from a multidisciplinary study on cold seeps off the Vesterålen coast (northern Norway) hosting coral mounds. We discuss the geochemical results from sediment (carbon-nitrogen systematics, foraminifera) and pore fluids (sulfate, dissolved inorganic carbon, methane) in relation to seafloor habitats (orthomosaics and habitat maps). Microbial mats are the dominant seep-related community, forming white patches of a few ten cm in diameter, mostly distributed along the edges of methane-derived authigenic carbonates and cracks on top of them. Foraminifera tests in the sediment display negative δ^{13}C values down to − 18.5‰, suggesting ongoing authigenic carbonate precipitation. We also report the discovery of a macroscopic white biofilm, observed while slicing a pushcore onboard. Organic matter analyses indicated that the sediment interval hosting this biofilm is associated with a sharp drop in δ^{13}C values, as negative as −43.4‰. Results from 16S rRNA gene analyses on the uppermost 10 cm in the same core showed a significant shift in microbial community. Protebacteria-dominated communities near the seafloor transition to a Halobacterota-dominated composition mainly consisting of ANME-1b anaerobic methanotrophs in correspondence of the biofilm interval. Corals in this area are spatially associated with seafloor chemosynthetic habitats and bubbling, but not vice versa, suggesting that seafloor emissions do not influence coral distribution. Instead, the presence of a methane-charged sediment substrate leading carbonate crust formation and food supply by high-energy currents appears to be a prerequisite for cold-water corals development in this area.

1 Introduction

Reef-building corals inhabiting deep (> 40 m) and cold waters are known as cold-water corals (CWCs). CWCs have been found at all continental margins, at water depths down to 3000 m and temperatures below 5 °C (Freiwald, 2011). They include a variety of scleractinian corals which do not rely on photosynthesis to produce energy, but feed on plankton and other organic particles transported by currents (Hovland, 2008; Roberts et al., 2009). CWCs create a three-dimensional framework spanning tens of meters to kilometers in lateral extent and up to 40 m in thickness (Freiwald, 2011; Sanna et al., 2023). It provides protective habitats for fish and other invertebrates, as well as a hard substrate for the settlement of other sessile organisms, i.e., foraminifera, hydrozoans, octocorals, serpulids, molluscs, bryozoans, brachiopods, and sponges (Freiwald, 2011). CWCs also represent nursery grounds and reproduction environments for commercial fish species (Husebø et al., 2002). Therefore, CWCs play a key role in deep-sea biodiversity and ecosystem functioning. Among them, *Desmophyllum pertusum* (known as *Lophelia*

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pertusa) is the most widespread and dispersed representative, very common in the North Atlantic (Roberts et al., 2009) and along the Norwegian coasts (Fosså et al., 2002; Sanna et al., 2023; Sundahl et al., 2020).

Ocean acidification (OA) poses a potential threat to all coral reefs by reducing their growth rate (Kline et al., 2019) and inducing dissolution of their aragonitic skeletons (Eyre et al., 2018; Wisshak et al., 2012). However, compared to shallow-water tropical corals, around 70 % of CWCs will be exposed to corrosive aragonite-undersaturated conditions in the course of this century, thus resulting in a dramatic loss of habitats and biodiversity (Bernardino et al., 2023; Hennige et al., 2014; Howes et al., 2015; Turley et al., 2007). Reduction of CWC ecosystems brings societal and economic implications, offering new opportunities in pharmaceutical, medical and food research (Beaumont et al., 2007; Foley et al., 2010; Hilmi et al., 2013).

Seafloor methane emissions, known as cold seeps, can contribute to ocean acidification when methane (CH_4) is oxidized to carbon dioxide (CO_2) in the water column (Biastoch et al., 2011; Garcia-Tigreros et al., 2021; Hauri et al., 2024) following Eq. (1):

$$CH_4 + O_2 \rightarrow CO_2 + 2H_2O \quad (1)$$

These seeps are widespread along continental margins (Phrampus et al., 2020) and their association with CWCs has been reported worldwide, i.e., from offshore New Zealand (Liebetrau et al., 2010), Gulf of Cadiz (Pirlet et al., 2012; Rincon-Tomas et al., 2019), Gulf of Mexico (Schroeder, 2002), South China Sea (Xu et al., 2019) and offshore Norway (Ferré et al., 2024; Hovland, 1990, p.199; Hovland and Thomsen, 1997, p.199). The causative link between the occurrence of CWCs and cold seeps has long been debated in the case of *Desmophyllum pertusum*(Hovland, 1990, p.199; Hovland and Thomsen, 1997). It is known that besides adequate environmental conditions (temperature, water current~food supply), CWCs require a hard substrate for colonization and growth of a large colony (Roberts et al., 2009). The substrate must be large and stable enough to resist strong currents. From that perspective, cold seeps are optimal areas as they are often associated with extensive carbonate pavements and/or mounds for their settling and growth. During methane migration through the sediment, microbial communities consisting of methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB) (Boetius et al., 2000; Boetius and Wenzhöfer, 2013) carry out the anaerobic oxidation of methane (AOM) which is a globally widespread biofilter of this greenhouse gas (Egger et al., 2018; Lapham et al., 2024). AOM occurs in a relatively narrow zone within the sedimentary column, located at variable depths beneath the seafloor ranging from a few cm to several hundred meters, mainly depending on the magnitude of methane fluxes (Egger et al., 2018). Below the seafloor, sulfate is the quantitatively dominant electron acceptor available for microbes to oxidize methane and sulfate-driven AOM releases bisulfide (HS^-) and dissolved inorganic carbon in the form of bicarbonate (HCO_3^-) following Eq. (2):

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \quad (2)$$

AOM-related sulfide fluxes (mainly in the form of H_2S) toward the seafloor sustain chemosynthetic habitats, including chemosymbiotic mussels, clams, tubeworms, and microbial mats (Argentino et al., 2022b; Fischer et al., 2012; Sahling et al., 2002; Sen et al., 2018b). The protracted release of bicarbonate via AOM into pore water leads to an increase in alkalinity, which eventually triggers the precipitation of authigenic carbonates (Argentino et al., 2022a; Blumenberg et al., 2015; Crémière et al., 2016; Karaca et al., 2010). Methane-derived authigenic carbonates (MDAC) range from microscopic concretions to kilometers-wide and several-meters-high deposits becoming exposed at the seafloor by winnowing and erosion (Argentino et al., 2019; Judd et al., 2020; Judd and Hovland, 2007, p.200; Tseng et al., 2023). MDAC outcrops are ideal places for the settling and growth of CWCs which benefit from optimal exposure to bottom currents rich in nutrients (Rincon-Tomas et al., 2019). More recent studies suggested that CWCs can gain benefits not only from the presence of hard substrate (Deng et al., 2019; Rincon-Tomas et al., 2019; Xu et al., 2019) but also from chemosynthetically-derived food (Osman et al., 2023; Sert et al., 2025). Other studies found that the major food source in deep-sea corals is photosyntheticallyderived food, even near cold seeps (Becker et al., 2009). These contrasting observations indicate that there are still knowledge gaps regarding the ecological relationships between seeps and CWCs.

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In 2022, a CWC-seep system offshore Vesterålen, Northern Norway, became the subject of the multidisciplinary project EMAN7 - *Environmental impact of Methane seepage and sub-seabed characterization at LoVe-Node 7* – aiming to investigate the interplay between CWCs and methane emissions. Within EMAN7 project, in 2022 we conducted an expedition onboard RV Kronprins Haakon (CAGE22-3, Ferré *et al.*, 2022) during which we also performed Remotely-Operated Vehicle (ROV) seafloor imagery acquisitions and targeted sediment samplings near *Desmophyllum pertusum* coral mounds. In this study we combined the information obtained from seafloor orthomosaics and habitat maps with the geochemistry (pore fluids, organic matter) of shallow sediment cores to understand how methane dynamics regulate the type and distribution of benthic habitats and subsurface carbon cycling. Moreover, we used 16S rRNA gene sequencing to explore downcore shifts microbial diversity and representative taxonomy through a push core showing the presence of a macroscopic white biofilm at 10 cm depth. Our results provide a comprehensive overview of the biogeochemical context in which the Vesterålen CWCs have developed, that will serve both understanding of their current ecology and monitoring of the system response to future climate-induced environmental perturbations.

2 Study area

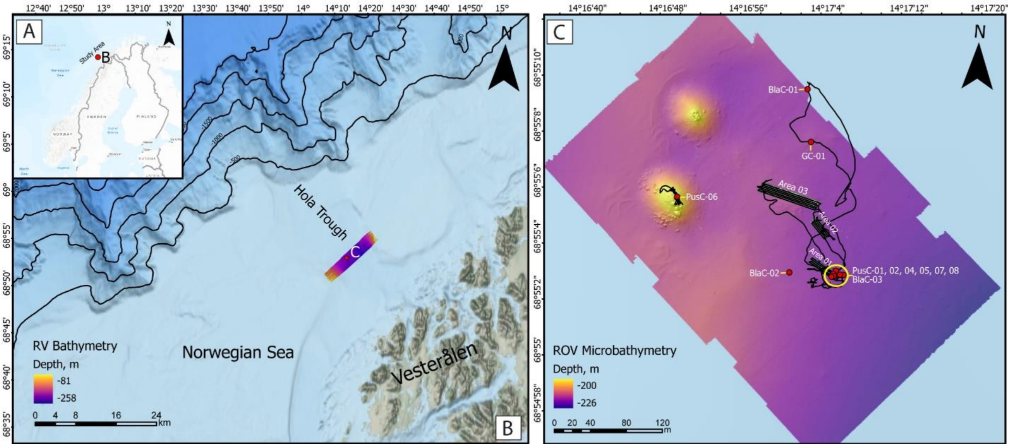

Figure 1. A) Geographical context of the study area located offshore Northern Norway. B) Bathymetric map of the Hola Through and Norwegian shelf off the Vesterålen islands. Ship-based multibeam bathymetry is shown with Viridis color palette accessible to people with color vision deficiencies. C) High-resolution ROV microbathymetry (Ferré et al., 2022, 2024) showing the sediment coring locations (red dots) and the three mosaic areas of Fig. 2. PusC-06 was excluded from analysis due to disturbance caused by reworking during sampling. Two CWC mounds marked by higher topography (yellow color) appear on the top-left corner. The ROV track is also displayed.

The targeted cold seeps investigated here are located on the Norwegian continental shelf at ~ 68.9 °N and ~ 14.3 °E (Fig. 1A), 15.5 km from the Vesterålen coast (Fig. 1B). This area is geomorphologically associated with the Hola Trough, an elongated ~12 km-wide depression carved by ice during Pleistocene glaciations (Ottesen et al., 2005). Water depths at the seep site range between 200 m and 230 m, and the seafloor is wiped by strong bottom currents up to 1 m s^{-1} (Ferré et al., 2024). CTD measurements conducted in 2023 during the cruise CAGE22-3 indicated water temperatures of 6.7 °C near the seafloor increasing to 8 °C at the sea surface, with salinity levels of 34-35 psu (Ferré et al., 2024). The seepage systems have been known since 2008 (Chand et al., 2008) and have been the focus of studies on hydrocarbon composition (Sauer et al., 2015) and seepage paleo-reconstructions (Crémière et al., 2016). The origin of the gas showed a predominantly thermogenic component, including methane with $\delta^{13}C_{CH4}$ values between −60‰ and −52‰ and δD_{CH4} between −225‰ and −191‰, associated with higher alkanes (C_{2+})(Sauer et al., 2015). This gas migrates from late Jurassic to early Cretaceous source rocks and has been seeping since at least ~11 ka (Crémière et al., 2016), producing extensive methane-derived carbonate (MDAC) pavements. The Hola Trough is of great biological interest due to the presence of more than 300 *Desmophyllum pertusum* reefs distributed over an area of almost 50 km^2 with individual reefs having heights up to 17 m and widths > 300 m in the longest direction (NNW-SSE; Bøe *et al.*, 2016). In the investigated seepage area (Fig. 2C) two coral mounds appear on the left side of the microbathymetry. The ROV survey performed during CAGE22-3 (Ferré et al., 2022) enabled high-resolution seafloor mosaicking, habitat mapping, and video-guided coring at specific microhabitats. These efforts aimed to support biogeochemical investigations focused on reconstructing methane dynamics and understanding its influence on seafloor ecosystems near CWCs.

3 Methods

3.1 Seafloor ROV video acquisition and Structure from Motion processing

Seafloor imagery was collected in 2022 during the CAGE22-3 cruise aboard R/V *Kronprins Haakon* (Ferré et al., 2022). During the second ROV dive of the cruise, three sub-areas (Area 01, Area 02, Area 03) were surveyed using high-resolution video acquisition for photogrammetric mosaicking (Fig.1-C). These sites are characterised by extensive carbonate crusts interspersed with microbial mats. They were selected based on intense seep activity previously identified through water column analyses using R/V-mounted multibeam echosounder (MBES) data. This analysis enabled the detection of acoustic flares, indicative of gas seepage from the seafloor, as well as the mapping of their spatial distribution in relation to bathymetric features.

ROV Ægir 6000, a 150 Hp work-class vehicle equipped with various samplers and sensors and operated by the Norwegian Marine Robotics Laboratory (NORMAR) at the University of Bergen (UiB), was deployed with an EM 2024 Kongsberg MBES mounted on it and a dedicated photogrammetry sledge.

The MBES was used to create a high-resolution microbathymetric map of the surveyed region. Mounted on the ROV, this MBES captures detailed topographic data at sub-meter to decimeter scales, offering essential context for interpreting seafloor features in the imagery and improving the spatial precision of photogrammetric outputs.

The photogrammetry sledge included a downward-facing Imenco® Spinner II Shark HD zoom camera mounted perpendicular to the seafloor. Illumination was provided by two high-output strobes (>2500 W) and a pair of deep-sea power lasers spaced 14 cm apart, ensuring consistent lighting and enabling scale calibration. ROV video acquisition, with the downward-facing camera, was conducted along parallel transects spaced 2 m apart, with the ROV maintaining a steady speed of 0.3 knots and an altitude of 2.5 m. This configuration resulted in a field of view slightly greater than 2 m and ensured sufficient image overlap for photogrammetric reconstruction. These acquisition parameters were validated in previous field campaigns (Fallati et al., 2023; Panieri et al., 2024). In total, four ~35 m transects were collected in Area 01, five ~30 m transects in Area 02, and six ~60 m transects in Area 03.

ROV positioning for both MBES and photogrammetric surveys was achieved using the HIPAP 501 USBL (Ultra Short Baseline) system, ensuring high-precision localisation. These positional data were later integrated into the geospatial processing workflow to scale and georeference the resulting models accurately.

ROV videos were processed by automatically extracting one frame per second using VLC Media Player's Scene Video Filter, producing high-definition (1920 × 1080 px) PNG images sorted by survey area. These images were imported into Agisoft Metashape Professional 2.0 (Agisoft, 2018) and processed following a standard Structure from Motion (SfM) workflow (Fallati et al., 2020; Lim et al., 2020; Montes-Herrera et al., 2024; Price et al., 2019). After camera alignment, dense point clouds were generated to produce ultra-high-resolution digital elevation models (DEMs) and orthomosaics. The spacing between the laser pointers served as a ground-truth scale bar for each model.

To georeference the models, USBL-derived coordinates and timestamps were linked to selected video frames, primarily those at the ends and centres of each transect. These data points were further cross-validated against geomorphic features visible in both the ROV-derived microbathymetry and the SfM-generated DEMs. This multisource integration enabled the accurate spatial placement of the models within the WGS 84 / UTM Zone 33N coordinate system.

Substrate semi-automatic classification was performed using Object-Based Image Analysis (OBIA) in the eCognition Developer (Trimble) environment. This approach segments the orthomosaics into meaningful image objects based on spectral, textural, and spatial characteristics (Benz et al., 2004; Blaschke, 2010; Hay and Castilla, 2008; Hossain and Chen, 2019), allowing for the supervised classification of distinct substrate classes, such as methane-derived authigenic carbonate crust (MDAC), fine sediments mixed with gravels and pebbles, microbial mats, boulders, and benthic organisms.

3.2 Sediment coring

Six push cores, three blade cores, and one gravity core (Fig. 1C were collected during the expedition CAGE22-3 (Ferré et al., 2022). . The blade corer consisted of a 32 cm-long frame with a rectangular base of 25 x 10 cm. It was pushed into the seabed using the ROV manipulator arm and a locking system was activated to avoid sample loss. This technique is especially suitable for coarse-grained sediments. We collected three blade cores CAGE22-3-KH-01-BlaC-01, CAGE22-3-KH-01-BlaC-02, and CAGE22-3-KH-01-BlaC-03, hereafter named BlaC-01, BlaC-02 and BlaC-03, respectively. BlaC-01 was collected as a reference core for geochemical interpretations from a seafloor spot barren of chemosynthetic fauna (Ferré et al., 2022). BlaC-02 and BlaC-03 were collected from microbial mats. We also conducted push coring using a 60 cm-long cylindrical tube (8 cm inner diameter) made of fiberglass. The push corer was pushed into the sediment by the ROV during samplings and enabled us to obtain longer cores. We collected six push cores: CAGE22-3-KH-01-PusC-01, CAGE22-3-KH-01-PusC-02, CAGE22-3-KH-01-PusC-04, CAGE22-3-KH-01-PusC-05, CAGE22-3-KH-01-PusC-07 and CAGE22-3-KH-01-PusC-08, hereafter named PusC-01, PusC-02, PusC-04, PusC-05, PusC-07 and PusC-08, respectively and all collected from microbial mats. We also report the data from gravity corer CAGE22-3-KH-01-GC-01 (GC-01), which was collected using a 6-m-long iron barrel hosting a PVC liner with an inner diameter of 10 cm. On deck, the cores were split into 1-m-long sections (or shorter depending on the recovery), capped, labelled, and stored at 4 °C.

3.3 Pore fluid samplings and analyses (CH_4, SO_4^{2-}, DIC, $\delta^{13}C_{DIC}$)

Pore water extractions were conducted in a cold room (4 °C) immediately after coring operations. We sampled every 2 cm using rhizon soil moisture samplers (5 cm-long filter; 0.15 µm mesh) and 10 mL sterile plastic syringes. We split the pore water samples into two aliquots: one aliquot for dissolved inorganic carbon (DIC) analyses was transferred into 2 mL glass scintillation vials to which we added 10 µL of $HgCl_2$ saturated solution. DIC samples were then stored in the dark at 4 °C; the other aliquot for sulfate analysis was transferred into 5 mL Eppendorf tubes and stored at −20 °C. DIC concentrations and isotopic composition ($\delta^{13}C$) were measured on a Thermo Fisher Scientific MAT 253 IRMS coupled to a Gasbench II at the Stable Isotope Laboratory (SIL) of the Department of Geosciences, UiT. Isotopic values were normalized to Vienna Pee Dee Belemnite standard (VPDB) using three in-house calcite reference materials covering a $\delta^{13}C$ range from −48.95‰ to 1.96‰. DIC concentration was calculated by comparing the IRMS peak areas for the samples with calibration curves made from two $NaHCO_3(aq)$ stock solutions. Repeatability precision (1s) of $\delta^{13}C$ and DIC concentration based on five duplicate samples was better than 0.23‰ and 1.2 mM, respectively. The sulfate concentration was measured via ion chromatography at TosLab in Tromsø (NO). Accuracy and precision were estimated based on repeated measurements of certified materials. The measured values agreed within the uncertainty of the certified value and are commonly associated with a precision of 15% (relative standard error RSE). Bulk sediment samples were collected from the bottom of push cores and from depths ranging from 5 to

15 cm in blade cores using a cut-off syringe. Around 5 mL of sediment were transferred to 20 mL serum vials containing two glass beads and 5 mL of 1 M NaOH. The vials were immediately closed with a rubber septum and aluminum crimp seal and stored upside-down at 4 °C. Methane concentration was measured on a Thermo Fisher Scientific GC Trace 1310 gas chromatograph at the SIL laboratory.

3.4 Benthic foraminifera isolation and isotope analyses ($\delta^{13}C$, $\delta^{18}O$)

Push core PusC-08 and blade core BlaC-01 were sliced onboard every cm. For each sample, we weighed around 50 g of dry sediment and wet-sieved it through 63 µm and 125 µm sieves. We dried the residue at 50 ˚C and hand-picked benthic foraminifera with identification at genus or species level. Picking was conducted from the fraction >125 µm using a stereomicroscope. From each aliquot we selected a number of individuals ranging from 5 to 15 for isotope analysis. The dominant species in most of the samples were *Cibicidoides lobatulus*, *Cassidulina spp*. and *Trifarina spp*. Some of the samples were barren of these target foraminifera, thus resulting in a total of 41 and 13 samples for PusC-08 and BlaC-01, respectively. Isotope analyses were conducted at the SIL laboratory using a Thermo Fisher Scientific MAT 253 IRMS with a Gasbench II. Precision on two replicate reference materials ($n = 6$) was better than 0.1‰ for both carbon and oxygen. All isotope results were normalized to Vienna Pee Dee Belemnite standard (VPDB) using three in-house reference materials.

3.5 Sediment chemical analyses (TOC, TN_d, $\delta^{13}C_{org}$, $\delta^{15}N_d$)

We conducted geochemical investigations on the core PusC-08 containing a biofilm described below and shown in Fig 5A, and reference core BlaC-01 and analysed a total of 45 sediment samples from PusC-08 and 15 samples from BlaC-01 collected every cm down the cores. Prior to the analyses, the samples were treated with 6 N HCl to remove the carbonate component following the protocol reported in Argentino *et al.* (2023). Decarbonated material was analyzed using a Thermo Fisher Scientific MAT 253 Isotope Ratio Mass Spectrometer (IRMS) coupled to a Flash HT Plus Elemental Analyzer hosted at the SIL laboratory. The $\delta^{13}C$ and $\delta^{15}N$ values were obtained by normalization to international standards, Vienna Pee Dee Belemnite, VPDB ($\delta^{13}C$) and Air-N_2 ($\delta^{15}N$), using three in-house reference materials. Repeatability precision (1s) of four duplicate samples measured in the same analytical session was better than 1.7‰, 0.12 %, 0.66‰, and 0.011 % for $\delta^{13}C_{org}$, TOC, $\delta^{15}N_d$, TN_d (total nitrogen in the decarbonated material), respectively. The C/N atomic ratio was calculated using the atomic mass weighted ratio of TOC and TN as $C/N = (TOC/12.011)/(TN/14.007)$. The precision (1s) on C/N values was better than 0.65.

3.6 DNA extraction and sequencing

Sediment samples for DNA analyses were collected from PusC-08 immediately after core recovery using sterile spoons and stored frozen at −80 °C until processing. Samples were collected every cm in the interval 0-10 cm. For DNA extraction, 0.2-0.5 g of sediments was extracted using the DNeasy Power Soil Kit (Qiagen, Carlsbad, CA) and stored at −80 °C. DNA quality was measured by a NanoDrop Spectrophotometer and a Qubit Fluorometer (Invitrogen). The V4 region of the 16S SSU rRNA gene was amplified following the Earth Microbiome Project (EMP) (Gilbert et al., 2014) 16S Standard191Illumina library preparation protocol, using the forward-barcoded 515f (5’-3’192GTGTYCAGCMGCCGCGGTAA) and 806r (5’-3’ GGACTACNVGGGTWTCTAAT). The quality of PCR products was assessed using 1 % agarose gel electrophoresis and GelRed staining, and three replicates per sample were chosen for sequencing. Illumina MiSeq sequencing was performed at the Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory (Lemont, IL, USA). The amplicon analyses were performed with the QIIME2 environment and using QIIME2 plugins as previously described in Aalto *et al.*(2022). Briefly, Illumina forward and reverse reads and the corresponding barcode files were imported and demultiplexed using the Earth Microbiome Project paired-end flag. All reads were quality filtered, de-replicated, and chimera-checked using all default parameters in DADA2 v2021.2.0 (Callahan et al., 2016). The reads were merged and amplicon sequence variants (ASVs) were determined using DADA2 v2021.2.0. The DADA2 statistic on sequence reads is provided in Data set S1. A 16S from the SILVA v138.1 database was trained using RESCRIPt (Quast et al., 2013; Robeson et al., 2021). The ASVs were classified with the self-trained classifier database (Yilmaz et al., 2014). Sequences are being processed for archiving with the European Nucleotide Archive and will be available under accession PRJEB96327.

3.6.1 Statistical analyses

All ASVs not assigned to the expected kingdom (Archaea and Bacteria) were removed. Downstream analysis was completed in R (R Core Team, 2021), using the `microeco´ v0.11.0 (Liu et al., 2021) and `vegan´ v2.5-7 packages (Oksanen et al., 2001). In particular, the R packages were used to calculate relative organismic abundances, observed alpha diversity, beta diversity using unweighted UniFrac, PERMANOVA, and Pearson correlation.

4 Results

4.1 Orthomosaics and benthic coverage maps

The morphobathymetric map produced from the ROV MBES data revealed a more complex area compared to the relatively level surrounding seafloor. The three orthomosaics (Fig. 2, A1, A2, A3), generated at millimetric resolution, cover a total area of 1,307 m² and generally show a hard seafloor composed of compact patches of MDAC, colonized by numerous benthic organisms, such as sponges, sea anemones, and sea fans. Outside the MDAC areas, the seafloor is characterized by fine sediments mixed with gravels and pebbles, which dominate the mapped area and include coral rubble.

The high resolution of the orthomosaics enabled the detection and mapping of small, subcircular microbial mat patches, mainly found along the edges of the carbonate crusts (Fig. 2, C1, C2, C3). These patches vary in surface area from 0.002 to 0.17 m², with an average of 0.02 m² and an average perimeter of 0.58 m.

OBIA-derived benthic coverage maps identify the main seafloor classes in the high-resolution orthomosaics. Area 1 (Fig. 2, A1-B1), covering 312 m², primarily consists of fine sediments with gravels, occupying about 267 m². Microbial mats are located near the carbonate crusts (4.6 m²) in the south-central part of the area, with a total surface area of 0.26 m². In the area, two benthic landers are present, and well visible in the orhomosaic (Fig. 2, A1) as part of the project LoVeOcean, Lofoten-Vesterålen Ocean Observatory (https://loveocean.no/).

Area 2 is smaller (Fig.2, A2-B2), at 258 m², and features a central, slightly elevated subcircular MDAC patch (42.2 m²), surrounded by fine sediments with gravels and pebbles (211 m²). This area also displays a more complex three-dimensional structure and hosts numerous benthic organisms and small microbial mat patches, which together cover 0.22 m².

Area 3 comprises the largest portion of the mapped seafloor (Fig. 2, A3-B3), spanning 738 m². Most of this area is dominated by fine sediments with gravels (437.7 m²), along with extensive, discontinuous MDAC outcrops (282 m²) concentrated in the northwestern section. Small, subcircular bacterial mats are also visible along the rims of the carbonate crusts, with a total surface coverage of 1.65 m².

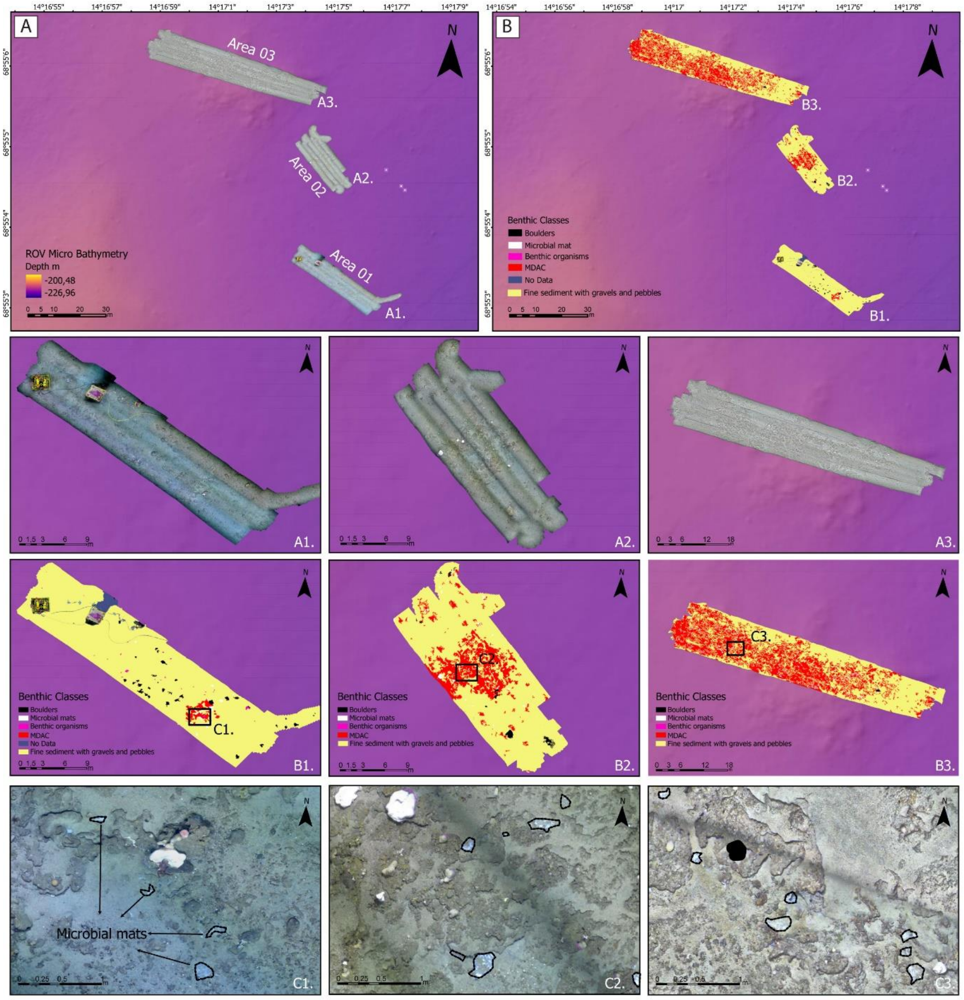

Figure 2. A. Close-up of the microbathymetry area with high-resolution orthomosaics of the three surveyed subareas (Area 1, Area 2, Area 3). B. Benthic coverage maps produced through OBIA showing the seafloor composition and distribution of methane-derived authigenic carbonate crusts (MDAC), fine sediments with gravels and pebbles, benthic organisms, and microbial mat patches. The orthomosaics (A1, A2, A3) and the benthic coverage maps (B1, B2, B3) reveal subcircular microbial mats, primarily located along the margins of the MDAC outcrops (C1, C2, C3).

4.2 Pore water geochemistry and methane concentration

Sulfate concentrations in surface sediments (0-1 cm below the seafloor) varie from a normal seawater composition ~29 mM (Millero, 2005) to as low as 24.3 mM (PusC-01; presence of a microbial mat at the seafloor). Sulfate values < 1 mM are observed in two cores, PusC-02 and PusC-04, at 24 cm and 10 cm, respectively (Fig. 3). Downcore concentration profiles in the investigated cores include concave-up (PusC-01, PusC-04, PusC-08), concave-down (GC-01), quasi-linear (PusC-05, BlaC-01, BlaC-02, BlaC-03) shapes (Fig. 3), and two kink-type profiles (PusC-02 and PusC-07). Dissolved inorganic carbon in surface sediments (0-1 cm) ranges from 2.2 mM (BlaC-01) to 5.1 mM (PusC-01), with overall increasing downcore trends. The steepest gradients occur starting at ~5 cm in PusC-02, between 2-6 cm in PusC-04 and 15-31 cm in PusC-07. The other cores have low or quasi-zero sulfate gradients throughout the sampled depths. DIC isotopic composition ($\delta^{13}C$) in surface sediment ranges from −22.5‰ (PusC-01) to −1.1‰ (BlaC-01). Isotopic values decrease with depth, with the most negative value $\delta^{13}C = -29.8$‰ measured at 6 cm in PusC-04 and at 18 cm in PusC-08, which also corresponds to an inversion in isotopic trends (becoming heavier down the core). Methane concentrations in the push cores range from 1.2 mM (PusC-02) to 3.4 mM (PusC-04), whereas blade cores contain 0.003 mM (BlaC-01; 10 cm depth), 0.010 mM (BlaC-02; 5 cm depth) and 1.1 mM (BlaC-03; 15 cm depth).

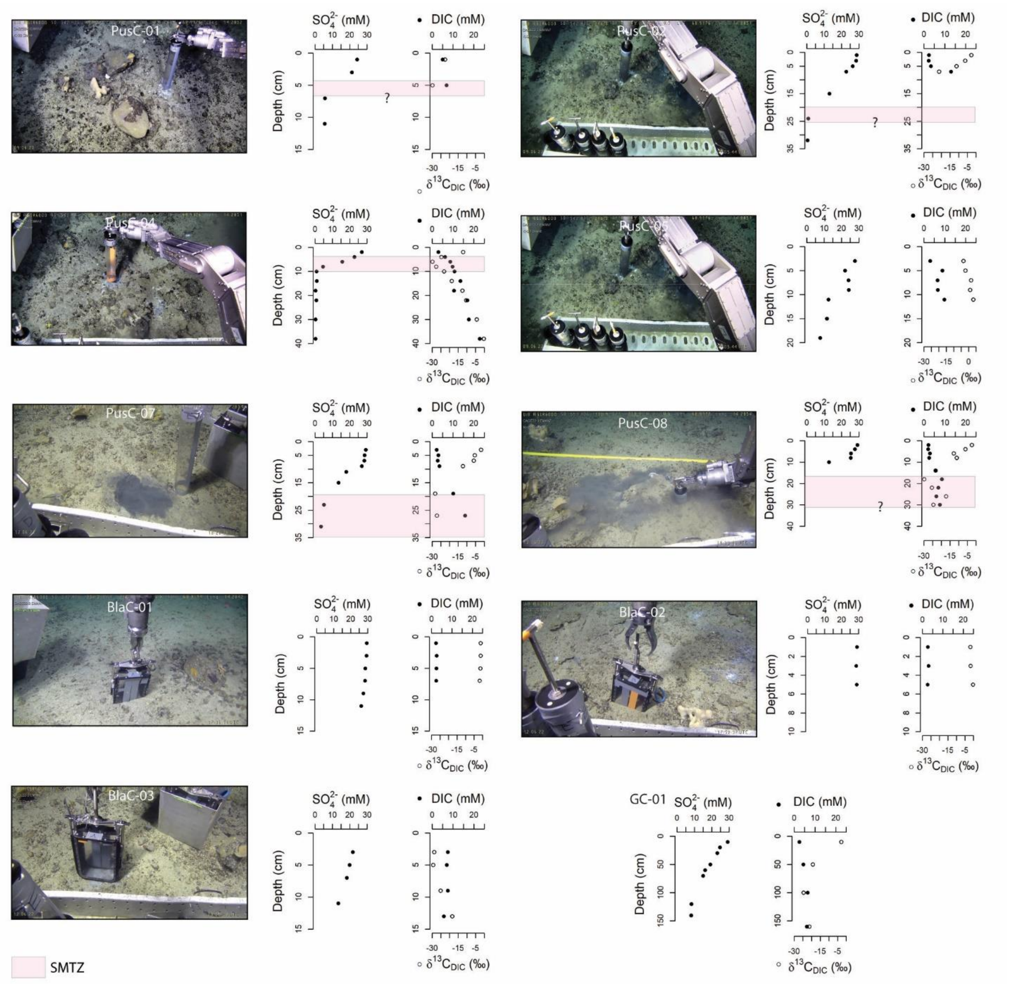

Figure 3. Seafloor images and pore water geochemical profiles of sediment cores collected from the CWC area. Locations in relation to cold water coral mounds is shown on the microbathymetry of Fig. 1C and on the mosaics and habitat maps of Fig. 2. The sulfate-methane transition zone (SMTZ) is marked with pink color and identified in correspondence of a drop in sulfate concentrations and $\delta^{13}C_{DIC}$, and increases in DIC concentrations.

4.3 Foraminifera geochemistry

The carbon isotopic composition $\delta^{13}C$ of *Cibicidoides lobatulus*, *Cassidulina spp*. and *Trifarina spp*. shows a marked difference between reference core BlaC-01 and PusC-08. In BlaC-01, $\delta^{13}C$ ranges from 0.8‰ to 1.9‰, from −1.5‰ to −1.0‰ and from 0.3‰ to 0.9‰, respectively. In push core PusC-08, the same categories of foraminifera show values from − 18.5‰

to 1.7‰, from −15.0‰ to 1.0‰ and from −0.1‰ to 1.0‰. In the blade core, *Cibicidoides lobatulus*, *Cassidulina spp*. and *Trifarina spp*. have $\delta^{18}O$ values within the intervals 2.4-3.5‰, 4.1-4.3‰ and 2.3-2.7‰. In PusC-08, the range of $\delta^{18}O$ values are 2.0-4.1‰, 1.8-4.6‰ and 1.7-2.9‰. BlaC-01 displays rather flat downcore isotope profiles (Fig. 4A), while PusC-08 shows a sharp increase in $\delta^{18}O$ and decrease in $\delta^{13}C$ starting at 20 cm below the seafloor (Fig. 4B).

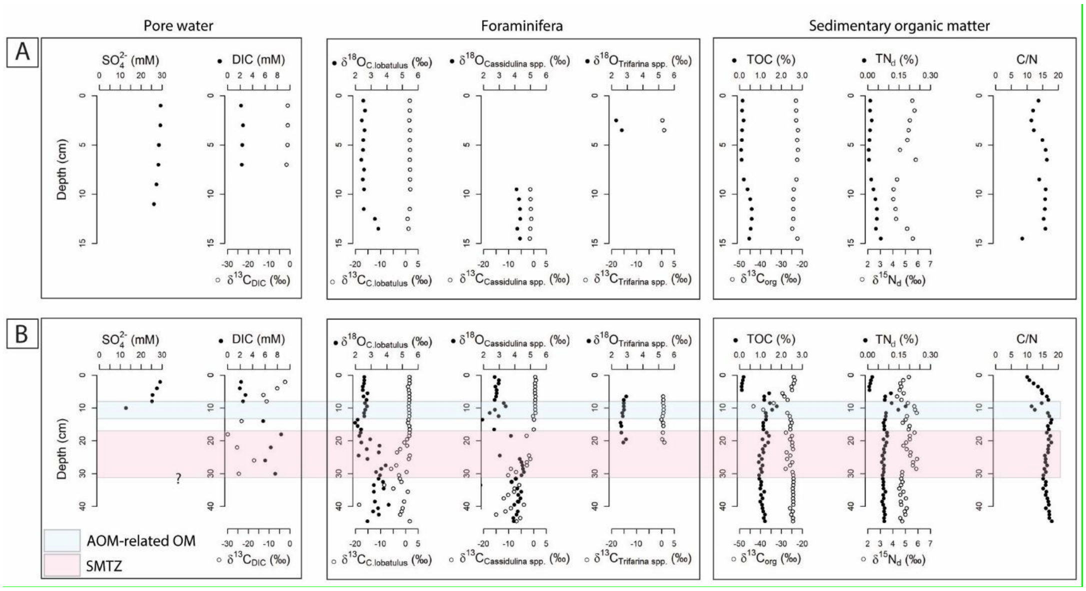

Figure 4. Pore water, foraminifera and sediment geochemical profiles of reference core BlaC-01 (A) and PusC-08 (B). The SMTZ is marked with pink color in PusC-08, whereas it has not been intercepted in BlaC-01. We marked in blue color the interval containing AOM-related organic matter in PusC-08, as determined by carbon-nitrogen interpretations.

4.4 Carbon-nitrogen geochemistry

TOC contents in BlaC-01 range from 0.07 % to 0.58 % and are associated with $\delta^{13}C_{org}$ values from −24.8‰ to −22.1‰. TN_d contents range from 0.01 % to 0.06 %, with isotopic values $\delta^{15}N_d$ between 4.1‰ and 5.8‰. The resulting C/N values range from 8.5 to 16.3. PusC-08 has TOC contents ranging from 0.09 % to 1.78 %, associated with $\delta^{13}C_{org}$ values from −43.4‰ to −23.6‰. TN_d and $\delta^{15}N_d$ varies between 0.01 % and 0.18 %, and between 4.3‰ and 5.9‰. C/N values range from 10.0 to 17.9. A sharp decrease in $\delta^{13}C_{org}$ and C/N values is observed between 5-11 cm which matches positive peaks in TOC, TN_d and $\delta^{15}N_d$ (Fig. 4B). Similar patterns, though less pronounced, are observed at approximately 17 cm, 25 cm, and 40 cm. During onboard sediment slicing, we observed a macroscopic biofilm at a depth of 10 cm (Fig. 5A). The biofilm is white in color and exhibits a soft, slimy texture.

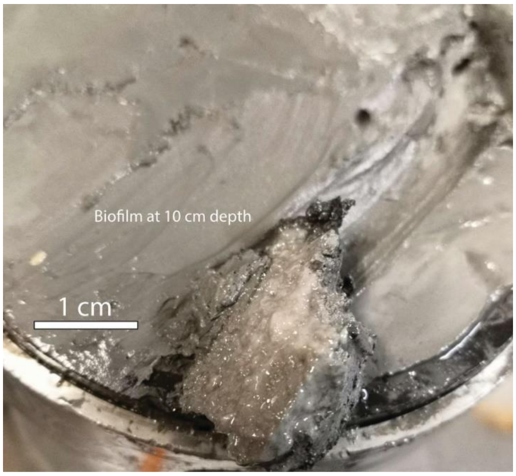

Figure 5. Photograph of a white biofilm encountered at 10 cm while slicing PusC-08. The sediment was sampled every cm in the interval 0-10 cm for microbiological analyses.

4.5 Sediment microbiome

The phylum-level taxonomic composition of 16S rRNA gene–informed ASVs showed a steady, depth-structured shift across the 10-cm sediment column (Fig. 6A). Triplicates from each depth were largely consistent, with the exception of single outlier replicates at 8 and 9 cm in phylum-level abundances. Across depths, relative abundances changed progressively: Proteobacteria exceeded 50% at 1 cm but declined with depth, such that by 8 cm the community was largely composed of other phyla. In contrast, Desulfobacterota and Campylobacterota increased from the shallow to deeper layers. Around 7 cm, the profile shifted again, with Halobacterota increasing sharply below this horizon. Looking at the same microbial community on a family level the aforementioned depth-structured shift was less apparent and steady. However, notable shifts could be documented. Thioglobaceae decreased over the first 3 cm of depth whereas Sulfurovaeae increased moving down to 7 cm. Below 7 cm of sediment depth, a clear cut in the community structure could be observed after which organisms of the ANME families could be detected.

Alpha- and beta-diversity patterns indicate a depth-structured reorganization of the microbiome across the push-core. Alpha diversity (observed ASVs) rose steadily from 1 to 6 cm, consistent with increasing richness/niche diversification in the upper layers, then declined below 6 cm, coincident with the depth at which the taxonomic profile began to turn over. Beta diversity resolved three depth-associated clusters: a cohesive group comprising all samples from 1–6 cm; a clearly separated group comprising 8–10 cm; and 7 cm samples positioned between them, consistent with a transitional assemblage. Together, these patterns point to a stratified microbiome with a boundary around 6–7 cm, above which communities are richer and more similar to one another, and below which communities are less diverse and compositionally distinct.

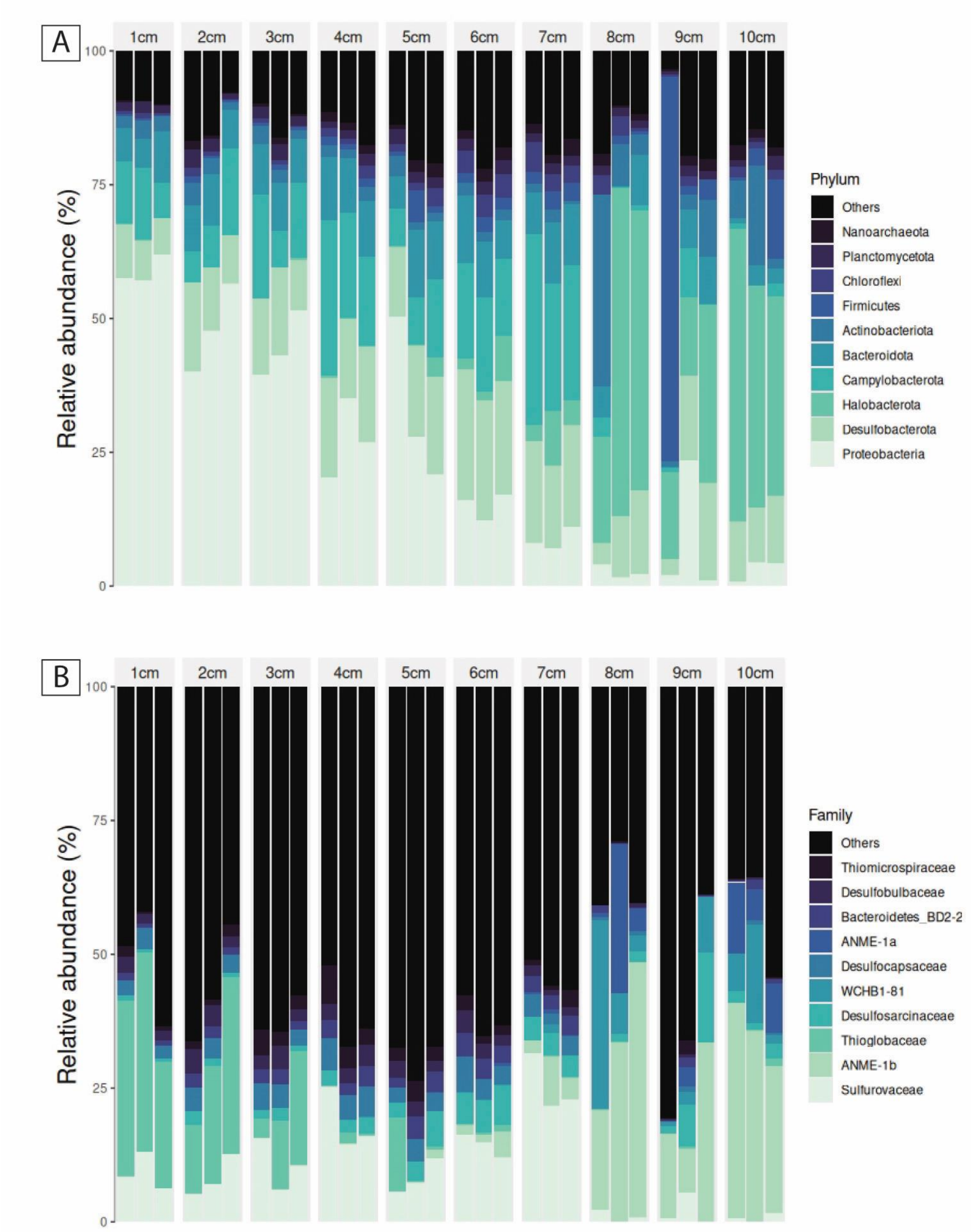

Figure 6. A) Relative abundance of 16S rRNA gene-informed ASVs on phylum level in the first 10 cm of PusC-08. B) Relative 345 abundance of 16S rRNA gene-informed ASVs on family level in the first 10 cm of PusC-08.

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5 Discussion

5.1 Biogeochemistry of CWC-associated cold seep habitats

Type and distribution of seafloor habitats in deep marine settings are tightly linked to organic matter fluxes to the sediment surface and remineralization underneath. Organic matter is an important source of nutrients for benthic fauna and microbes, and regulates subsurface biogeochemical processes including methanogenesis (Whiticar, 2020). Within the sediment, microbes break down the organic matter into inorganic substances via a “cascade” of oxidative reactions (respiration) involving different electron acceptors as a function of free energy gain (Schulz and Zabel, 2000; Wehrmann and Ferdelman, 2014). At or near the seafloor, dissolved oxygen (O_2) is energetically more convenient to microbes, whereas moving deeper into the sediments, respiration involves NO_3^-, Mn(IV) and Fe(III) oxides, and SO_4^{2-}, followed by methane formation (Schulz and Zabel, 2000; Whiticar, 2020). These redox processes determine the so called biogeochemical zonation of the sediments which consists of, moving from the seafloor downward, the oxic zone (oxygen reduction zone-aerobic respiration), the suboxic zone (including nitrate, manganese and iron reduction zones), and the anoxic zone (including sulfate reduction and methanogenic zones)(Schulz and Zabel, 2000; Tostevin, 2019). The SMTZ is an important redox boundary located between the sulfate reduction zone and the methanogenic zone. Chemosynthetic habitats found at cold seeps rely on hydrogen sulfide fluxes from the SMTZ which are maintained by AOM (Foucher et al., 2007; Levin, 2005; Levin et al., 2016, p.201). There is a causative link between magnitude of methane fluxes, depth of the SMTZ, sulfide fluxes and type of chemosynthetic communities at the seafloor (Argentino et al., 2022b; Fischer et al., 2012; Levin, 2005; Levin et al., 2016; Sahling et al., 2002). Hydrogen sulfide in aquatic environments is generally viewed as a toxicant able to interrupt cellular respiration due to its lipid solubility (Bagarinao, 1992; Riesch et al., 2015; Tobler et al., 2016). Therefore, at cold seeps, only organisms that have adapted to varying levels of sulfide exposure are present and their distribution follows subsurface geochemical gradients. Microbial mats are found in correspondence of the most intense seepage areas with shallower SMTZs (~5-10 cm below the seafloor), whereas higher organisms (clams, mussels, tubeworms) in symbiosis with sulfur-oxidizing bacteria colonize lower-seepage intensity areas with deeper SMTZs (~10-50 cm below the seafloor) (e.g. Fischer *et al.*, 2012; Sen *et al.*, 2018; Lee *et al.*, 2019; Argentino *et al.*, 2022b; Barrenechea *et al.*, 2025). We identified the SMTZs in our cores where a quasi-zero sulfate content matched a drop in $\delta^{13}C_{DIC}$ and an increase in DIC concentration, evidence of ongoing AOM. Whenever either sulfate or DIC data were absent, we relied on other parameters that were available. In the study area the SMTZ ranges from ~5 cm (PusC-01, PusC-4) to ~25 cm (PusC-02, PusC-07, PusC-08) (Fig. 3). No SMTZs were intercepted in the other cores. Besides BlaC-01 and gravity core GC-01, all the other cores were collected from microbial mats. However, while the SMTZ depths in PusC-01 and PusC-4 are typical of those expected from microbial mats, the cores outside the mats areas showed somewhat anomalous profiles. The high-resolution seafloor imagery and habitat maps show that the sampled mats form small (< 50 cm) and highly heterogeneous features with reduced thickness giving color shades from white to light grey (Fig. 2). Chemosynthetic mats can rapidly colonize new substrates (e.g. seep sites, vents, wood, whale carcasses) within a few months (Alain et al., 2004; Girard et al., 2020; Guezennec et al., 1998; Kalenitchenko et al., 2016), as well as being rapidly eroded away or consumed by grazers when the substrate is no longer present (Niemann et al., 2013; Seabrook et al., 2019; Sen et al., 2018b; Thurber et al., 2013). It is known that cold seeps can be dynamic environments characterized by spatial and temporal variations in gas seepage distribution (Ferré et al., 2020; Greinert, 2008). A substantial variability in seepage activity between 2018 and 2022 at this site (Ferré et al., 2024) is consistent with the concave-shaped sulfate profiles in the cores and deeper SMTZs (Kasten et al., 2003). The seep carbonate pavements in the CWC area act as a barrier to upward migrating sulfide-rich fluids, controlling the distribution of mats around the edges of carbonate crusts or in apparently carbonate-free seafloor areas (Fig. 2). Strong bottom currents present in this area (Ferré et al., 2022, 2024) might also contribute to the observed patterns, since mats are sensitive to physical disturbances (Cardoso et al., 2019; Noffke, 2010; Pan et al., 2019). Siboglinid tubeworms are common at Arctic cold seeps (Argentino et al., 2022b; Sen et al., 2018b, a) and represent the dominant benthic community at nearby seeps in canyons on the lower continental slope off Lofoten–Vesterålen islands (Sen et al., 2019). The general lack of tubeworms in the investigated seepage site must be related to the sedimentary substrate (Bellec et al., 2024; Sen et al., 2019), i.e. hard carbonate pavements and/or coarse sediment (Fig. 2), since tubeworms require softer sediments to settle (Hilário et al., 2011; Southward et al., 2005). Overall, our multidisciplinary dataset demonstrates that the combination of a methane-charged sediment and a high-energy oceanographic setting favoring steady food supply are prerequisites for CWC development in this area. Moreover, microbial mats and MDACs were frequently observed in direct contact with CWCs (either adjacent to or at their base) during ROV dives (Ferré et al., 2024), although in our orthomosaics and habitat maps (Fig. 2) corals are absent. The CWCs in this area are always associated with chemosynthetic substrates and bubbling, but not vice versa. This suggests that seafloor emissions alone do not play a key role in controlling coral distribution, as they show neither strong positive nor negative effects. Instead, CWCs appear to selectively grow on outcropping hard substrates located at local seafloor topographic reliefs (Ferré et al., 2024).

5.2 Geochemical and genetic indications of methanotrophic biomass within the sediments

In this section we interpret the geochemical anomalies measured in the sediment of PusC-08 and the origin of the white biofilm found 10 cm below the seafloor (Fig. 5). Generally, the bulk organic matter composition of marine sediments includes a mixture of marine algae and vascular plants debris from land (Burdige, 2005; Emerson and Hedges, 1988; Meyers, 1994, 1997). The elemental and isotopic signatures of these two categories have been widely used for assessing the contribution of marine versus terrestrial organic inputs in paleoceanography (e.g. Kang *et al.*, 2007; Knies & Martinez, 2009; Hasegawa *et al.*, 2013; Knies, 2022). Marine-derived organic matter is commonly associated with $\delta^{13}C$ values between −23‰ and − 16‰ (Emerson and Hedges, 1988; Meyers, 1994), $\delta^{15}N$ values as high as 7‰ (Wada, 1980), and C/N ratios between 4 and 10 (Meyers, 1994). Terrestrial organic matter from C3 plants which dominate the Arctic regions has lower $\delta^{13}C$ values < −25‰ (Meyers, 1994; Naidu et al., 2000; Rachold and Hubberten, 1999; Ruttenberg and Goñi, 1997), $\delta^{15}N$ values ~0‰ (Kienast et al., 2005), and C/N ratios > 20 (Emerson and Hedges, 1988; Meyers, 1994). These ranges can vary regionally but they hold true for most marine settings, with minor adjustments of the end-member compositions (Argentino et al., 2023; Knies et al., 2007; Knies and Martinez, 2009). In PusC-08, $\delta^{13}C_{org}$ reaches values as negative as −43.4‰, which cannot be explained by a simple mixture of marine and terrestrial organic matter. Similar values in bulk sediment were reported from cold seeps in the SW Barents Sea (−42‰, Argentino *et al.*, 2023b), offshore Pakistan (−42‰, Yoshinaga *et al.*, 2014) and interpreted as indicative of significant contributions of methanotrophic biomass to the organic matter pool. In fact, methanotrophic microbes inhabiting cold seeps incorporate variable amounts of isotopically-fractionated carbon from methane (Kurth et al., 2019; Wegener et al., 2008), which is known to carry negative $\delta^{13}C$ signals, generally < −50‰ (Judd and Hovland, 2007; Milkov and Etiope, 2018; Whiticar, 2020). Therefore, we confidently ascribe the isotopically-depleted $\delta^{13}C$ values in PusC-08 between 5.5 cm and 11.5 cm to higher contents of methanotrophic biomass. The concomitant drop in C/N values in that interval supports this interpretation, since bacterial organic matter has low C/N ratios ~5 (Madigan et al., 2017). We extrapolated the isotopic ($\delta^{13}C_{org}$, $\delta^{15}N_d$) and elemental (atomic C/N) compositions of the methanotrophic end-member via modeling of the respective downcore profiles. We selected the interval 5.5-10.5 cm which hosts steep linear isotopic gradients which we hypothesize to indicate the presence of AOM-related biomass. The slope of the linear fit to the plot $\delta^{13}C_{org}$ x TOC to TOC values (Fig. 7A) provides the $\delta^{13}C_{org}$ composition of the TOC added to the sediment (Coffin et al., 2017) which generates the anomaly seen in the profile. Our model yielded $\delta^{13}C_{org} = -73.7‰$ ($r^2 = 0.96$; n =6). This value agrees well with previous studies on methane seep microbiota (House et al., 2009) and with $\delta^{13}C_{org}$ values as negative as −81‰ measured on methanotrophic-dominated biomass contained in methane-derived carbonates (Feng et al., 2021). The same modeling approach was applied to $\delta^{15}N_d$ (plot $\delta^{15}N_d$ x TN_d to TN_d) and the resulting $\delta^{15}N_d$ value is 6.9‰ ($r^2 = 0.99$; n =6) (Fig. 7B). Argentino *et al.* (2023) found much lower isotopic values in methanotrophic-influenced sediments from Håkon Mosby Mud Volcano. This difference can be interpreted as due to different nitrogen assimilation pathways by AOM-SRB consortia and/or different nitrogen substrates isotopic composition (Dekas et al., 2009, 2014; Gruber and Galloway, 2008). For the calculation of the atomic C/N ratio, we normalized TOC and TN_d by their atomic weights of 12.011 and 14.007, respectively, and plotted their normalized values TN_d to TOC (Fig. 7C). The linear regression equation ($TN_d = 0.1399 \times TOC - 0.008$) was used to calculate the TOC value when the $TN_d = 1$. The resulting value of 7.2 is consistent (within uncertainties of the model) with a microbial end-member (Madigan et al., 2017). We did not conduct carbon-nitrogen analysis on pure biofilm material found at 10 cm but the good fit of the regression models of sediment samples which include the biofilm points to a unique end-member. The biofilm would represent microbial consortia performing AOM which are spread over the 5.5-11.5 cm interval and locally accumulated at 10 cm to form a macroscopic feature.

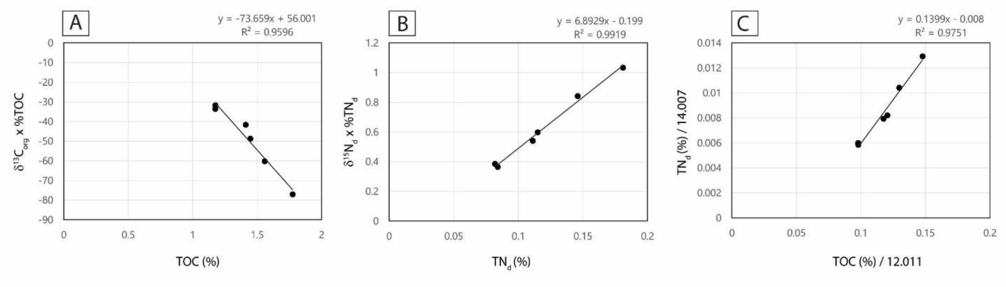

Figure 7. Linear regression models used for the extrapolation of the methanotrophic end-member in the sediment interval 5.5-11.5 cm of PusC.08. A) Plot of measured $\delta^{13}C_{org}$ x TOC concentrations vs. TOC (%); B) Plot of measured $\delta^{15}N_d$ x TN_d concentrations vs. TN_d (%); C) Plot of TN_d vs TOC concentrations normalized for their atomic masses. The slopes of the linear fits are presented in the equation above each plots.

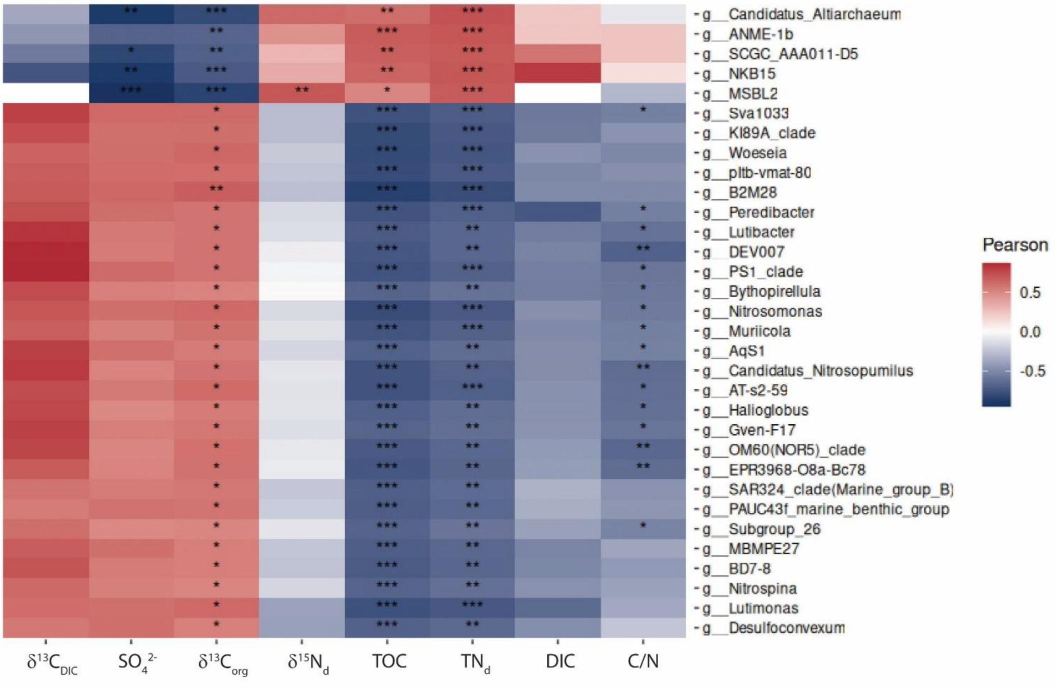

Figure 8. Pearson correlation of environmental parameters and 16S rRNA gene-informed ASVs on genus level. Only genera with at least one high score “***” correlation were selected.

Future metagenomic analyses will be conducted on samples from PusC-08 for identifying the key microbial players and their ecological roles. Still, correlations between biogeochemical parameters from pore waters and carbon-nitrogen s with 16S rRNA ASVs at the genus level reveal a clear division of the microbial community into two distinct groups, driven primarily by TOC, TN_d, SO_4^{2-}, and $\delta^{13}C_{org}$ (Fig. 8). The first group, comprising archaeal taxa, is associated with organic-rich, nitrogen-rich, sulfate-depleted environments and plays a key role in methane oxidation and carbon fixation. In contrast, the second group, consisting largely of bacterial taxa, thrives in sulfate-rich, inorganic carbon-enriched conditions and is involved in sulfate reduction and sulfur cycling, highlighting the complementary roles of these groups in driving key biogeochemical processes. The first group includes the top 5 genera: g__Candidatus_Altiarchaeum, g__ANME-1b, g__SCGC_AAA011-D5, g__MSBL2, and g__NKB15. Among these, g__Candidatus_Altiarchaeum is known to act as a carbon dioxide sink, contributing to carbon fixation(Probst et al., 2014). Similarly, g__SCGC_AAA011-D5 belongs to the Nanoarchaeota archaeon, which is also adapted to anaerobic conditions. g__MSBL2 (classified as Methylocella) is a methanotroph, suggesting its involvement in methane oxidation. Together, these taxa likely contribute to carbon fixation, methane cycling, and organic matter degradation. The negative correlation with $\delta^{13}C$ suggests that these taxa, particularly g__ANME-1b, use isotopically light organic matter, likely incorporating the strongly negative isotopic signature of methane-derived carbon into their biomass, further supporting their potential link to anaerobic methane oxidation (AOM). The second group is more diverse, with many taxa positively correlated with SO_4^{2-} and $\delta^{13}C$, suggesting adaptation to sulfate-rich environments and involvement in sulfur cycling, likely including sulfate-reducing bacteria (SRB). Other genera showing strong inverse correlations with sulfate concentration in pore water and with $\delta^{13}C_{org}$ correspond to sulfate-reducing *Deltaproteobacteria* which assimilates bicarbonate released by organoclastic sulfate reduction in marine sediments, i.e. MSBL2 (Vuillemin et al., 2022). The positive correlation with $\delta^{13}C$ indicates a preference for isotopically heavier carbon sources, potentially linked to sulfur-based metabolic pathways. Other correlations relate to genera which are found in “normal” marine sediments unaffected by seepage.

Macroscopic biofilms similar to the one found in our study have been reported by Briggs et al. (2011) from methane seeps at Hydrate Ridge (offshore of Oregon, USA), northern Cascadia Margin (offshore of Vancouver Island, Canada), and the Indian Ocean (offshore India), and by Gründger et al. (2019) from gas hydrate pingoes in the NW Barents Sea. In those studies, the biofilms consist of ANME-SRB consortia performing AOM (Briggs et al., 2011; Gründger et al., 2019), and the reported isotopic values for the pure biofilm masses yielded $\delta^{13}C$ as negative as −43‰ (Briggs et al., 2011). We speculate that in our case the modelled biofilm isotopic composition has lighter $\delta^{13}C$ due to relatively larger incorporation of methane-derived carbon (Kurth et al., 2019; Wegener et al., 2008). These biofilms, including the one in our study, are always associated with shallow SMTZs at cold seeps. What leads to the accumulation of macroscopic masses is the occurrence of pockets, cracks or fractures within the sediment, which provide high accommodation space (Briggs et al., 2011; Gründger et al., 2019). In PusC-08 we observed a network of microfractures in the core but no clear correlation with biofilm position was noticed during sediment slicing. The biofilm is located slightly above the SMTZ zone defined using pore water profiles (Fig. 3), which suggests that there is methane bypassing the SMTZ and being locally consumed within fractures (Briggs et al., 2011). The lack of indication of paleo-SMTZs in geochemical profiles of foraminifera (Fig. 4B) enable us to exclude that the biofilm could represent past conditions with higher methane fluxes, but rather suggests ongoing activity. In summary, cold seeps offshore Vesterålen islands revealed a unique Arctic environment where CWCs coexist with seafloor chemosynthetic communities and local sedimentary hotspots of methane oxidation.

6 Conclusions

Methane seepage in the Hola area off the coast of Vesterålen (northern Norway) is known for its peculiar association with *Desmophyllum pertusum* cold water corals, but only recently it was possible to explore the distribution of seafloor ecosystems and to conduct microhabitat-specific samplings for biogeochemical investigations. Using ROV imagery collected in 2022 during expedition CAGE22-3, we generated orthomosaics and habit maps for three smaller areas in proximity to the coral mounds. A striking feature of this site is the presence of widespread MDAC pavements, partially exposed at the seafloor. White microbial mats forming small patches (a few tens of cm) thrive along the edges of exposed carbonate crusts and on their fractures. We collected ROV-guided pushcores and blade cores from mats (whenever sediment cover permitted) and background areas to compare their subsurface biogeochemistry and link it to seafloor observations. Sulfate-methane transition zones as shallow as ~5 cm and an increase in DIC carrying a ^{13}C-depleted methane signature are measured beneath the mats, indicating high methane fluxes and intense AOM rates. A pushcore from a mat revealed the presence of a buried macroscopic white biofilm at 10 cm. Organic matter (TOC, δ^{13}C, TN$_d$, δ^{15}N) and 16S rRNA gene analyses on this core provide evidence for the accumulation of methanotrophic biomass dominated by ANME anaerobic methane-oxidizing archaea leading to bulk sediment δ^{13}C values as negative as −43.4‰. Secondary overgrowth of MDAC on foraminiferal tests was identified by bulk δ^{13}C values as low as − 18.5‰. These anomalies match the modern SMTZ interval with pore waters showing the highest DIC concentration and lightest DIC isotopic composition (δ^{13}C = −29.8‰), thus suggesting ongoing carbonate precipitation. Corals in this area are spatially linked to chemosynthetic substrates and bubbling, though the reverse is not true, indicating that seafloor emissions do not influence directly the local coral distribution. Nonetheless, the presence of seep-related carbonate outcrops and high-energy currents (major suppliers of food source for CWCs), remains the strongest prerequisite for the development of cold-water corals, which in this specific area seem to settle preferentially on elevated seafloor reliefs.

Data availability

Microbathymetry and orthomosaics from the EMAN7 Expedition 2022 are openly available on Dataverse.NO at https://doi.org/10.18710/1FCVAE. Geochemistry data are currently accessible to reviewers during the review process and will be made publicly available upon publication. DNA data have been submitted to the European Nucleotide Archive and will be available under accession PRJEB96327.

Author contributions

G.P. and C.A. conceived the study and conducted the sediment and pore fluid samplings onboard, C.A. wrote the initial manuscript with specific inputs from S.B., H.C.B and I.B. for the microbiological section, J.G.C. for the introduction on corals, and L.F. for image processing. C.A., L.F. and S.B. produced the figures and tables. All the authors contributed to the discussion and improvement of the paper since the early draft.

Competing interests

The authors declare that they have no conflict of interest.

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