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Abundance and Biogeochemical Impact of Cable Bacteria in Baltic Sea Sediments

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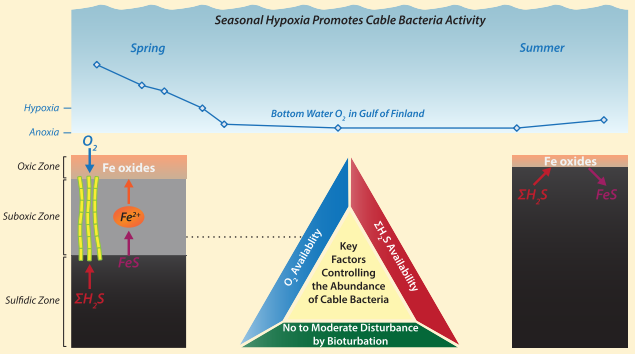
Supporting Information

ABSTRACT: Oxygen depletion in coastal waters may lead to release of toxic sulfide from sediments. Cable bacteria can limit sulfide release by promoting iron oxide formation in sediments. Currently, it is unknown how widespread this phenomenon is. Here, we assess the abundance, activity, and biogeochemical impact of cable bacteria at 12 Baltic Sea sites. Cable bacteria were mostly absent in sediments overlain by anoxic and sulfidic bottom waters, emphasizing their dependence on oxygen or nitrate as electron acceptors. At sites that were temporarily reoxygenated, cable bacterial densities were low. At seasonally hypoxic sites, cable bacterial densities correlated linearly with the supply of sulfide. The highest densities were observed at Gulf of Finland sites with high rates of sulfate reduction. Microelectrode profiles of sulfide, oxygen, and pH indicated low or no in situ cable bacteria activity at all sites. Reactivation occurred within 5 days upon incubation of an intact sediment core from the Gulf of Finland with aerated overlying water. We found no relationship between cable bacterial densities and macrofaunal abundances, salinity, or sediment organic carbon. Our geochemical data suggest that cable bacteria promote conversion of iron monosulfides to iron oxides in the Gulf of Finland in spring, possibly explaining why bottom waters in this highly eutrophic region rarely contain sulfide in summer.



INTRODUCTION

Coastal areas with low oxygen (O_2) in bottom waters are expanding worldwide because of human activities.[1,2] Hypoxia (O_2 < 63 μM), anoxia (O_2 = 0 μM), and the presence of toxic hydrogen sulfide (H_2S), termed euxinia, can lead to loss of marine life and the development of "dead zones".[1] Iron (Fe) oxides and manganese (Mn) oxides in surface sediments can delay the development of bottom water euxinia, preventing the release of H_2S from the sediment.[3,4]

Recently, a group of multicellular filamentous bacteria was discovered,[5] which can promote the formation of Fe oxides and Mn oxides and can efficiently remove H_2S from surface sediments.[6−8] These so-called cable bacteria belong to the *Desulfobulbaceae* family and link the oxidation of free H_2S in deeper sediment horizons to the reduction of O_2 or nitrate (NO_3) by conducting electrons over centimeter scale distances.[5,9] The metabolic activity of cable bacteria results in the development of a suboxic zone (i.e., where O_2 and H_2S are absent) and a unique pH profile,[10] characterized by a rise in pH near the sediment−water interface and a strong acidification in the suboxic zone (of up to ∼2 pH units).[5,11] This so-called "fingerprint for cable bacteria" can be used as an indicator for their activity.[5,11,12] The strong acidification of the pore water can lead to the dissolution of iron monosulfide (FeS)[6,8] and calcium (Ca), Fe and Mn carbonates.[7] When the Fe^{2+} and Mn^{2+} released from these minerals diffuse upward to the oxic zone, Fe oxides and Mn oxides may form.[7,8] Cable bacteria can fundamentally alter the biogeochemistry of coastal systems, as shown in a recent study for a seasonally hypoxic coastal marine basin where the Fe oxides formed through their activity in spring prevented the release of H_2S from the sediment during peak hypoxia in summer.[6,13] At present, it is unknown whether this role of cable bacteria as "ecosystem

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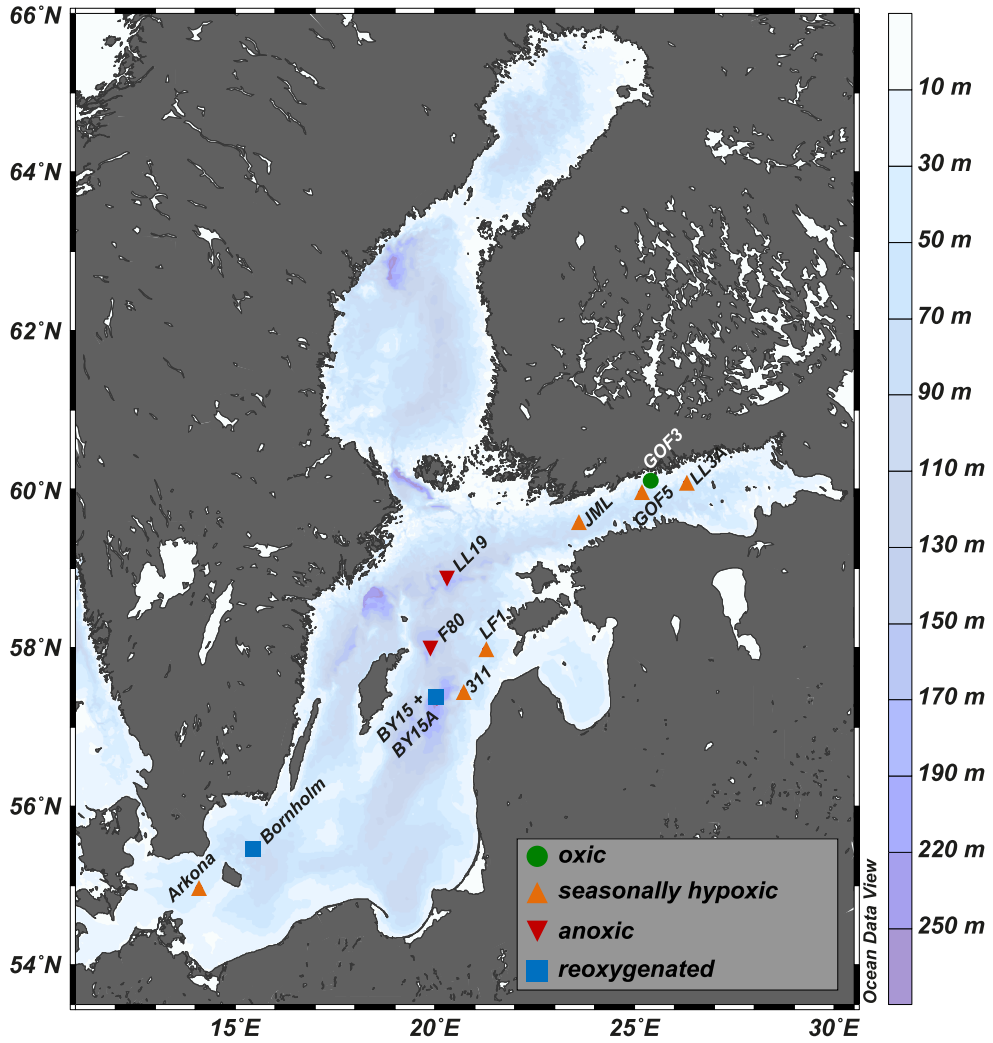

Figure 1. Map of the Baltic Sea showing the locations of the 12 sites. Our sites are classified based on the typical bottom water redox conditions between 2014 and 2016, which are indicated as follows: oxic (green); seasonally hypoxic (orange); anoxic (red); reoxygenated (blue).

engineers" can be generalized to other coastal zones experiencing seasonal hypoxia.

Cable bacteria are tolerant to a wide range of salinities and temperatures, have been observed in a variety of sediments, can occur in bioturbated systems, and may be widespread in the seafloor.[11,14] Their ecological niche has been suggested to be primarily determined by the availability of H_2S as an electron donor, either as FeS or dissolved H_2S, and O_2 or NO_3 as an electron acceptor.[8,14] However, other factors must also be at play because cable bacteria do not always establish when these conditions are met. Cable bacteria can co-occur with other sulfur oxidizing bacteria, such as *Beggiatoaceae*, suggesting competition for the same ecological niche.[6,13] Unlike cable bacteria, *Beggiatoaceae* are not capable of dissolving FeS, implying that their only source of H_2S is from sulfate reduction. However, *Beggiatoaceae* are likely better adapted to low bottom water O_2 than cable bacteria.[15,16]

Since field investigations of cable bacteria are relatively scarce it remains unclear how widespread their occurrence and how prevalent their role in delaying bottom water euxinia is. Here, we present field data on the abundance of cable bacteria and the geochemical characteristics of 12 sites from contrasting depositional environments in the Baltic Sea, as sampled in May and June 2016. We found that cable bacteria are most abundant in sediments of seasonally hypoxic sites with high rates of sulfate reduction. We infer that cable bacteria activity may prevent the development of bottom water euxinia in the highly eutrophic Gulf of Finland in summer.

MATERIALS AND METHODS

Study Area. The brackish Baltic Sea is naturally susceptible to bottom water hypoxia and anoxia due to vertical stratification and limited horizontal water exchange with the adjacent North Sea.[17] Extensive nutrient loading due to human activities and global warming has led to a massive expansion of the areal extent of O_2-depleted bottom water, from ~10 000 km^2 before 1950 to more than 60 000 km^2 in recent years.[18,19]

This study focuses on 12 sites in the Baltic Sea, with varying bottom water redox conditions (Figure 1). These sites are divided into four categories based on their bottom water O_2 characteristics (Table 1): (1) oxic, (2) seasonally hypoxic, (3) anoxic, and (4) reoxygenated. The classification and bottom water O_2 ranges were determined based on HELCOM monitoring data for the period January 2014 to December 2016 (Supporting Information 1.1; Table S1). Three of our sites (Bornholm, BY15, and BY15A) were subject to bottom water reoxygenation during our sampling campaign (Table 1), as a result of two major Baltic inflows that occurred in 2014

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Table 1. Characteristics of the Sites Sampled in May and June 2016

site	bottom water conditions	water depth (m)	latitude and longitude	bottom water O_2 (μM)	O_2 penetration depth (mm)	$\sum H_2S$ appearance depth (mm)	suboxic zone width (mm)	avg. C_{org} in upper 2 cm (wt %)	visual observations: cable bacteria	visual observations: *Beggiatoaceae*
GOF3	*oxic*	55	60°4.43′ 25°19.42′	80	2	27	25	5.8	−	+
Arkona	*seasonally hypoxic*	47	54°58.50′ 14°05.94′	135	3	95[a]	92	5.9	−	+
LF1	*seasonally hypoxic*	67	57°58.95′ 21°16.84′	48	1	11	10	2.9	−	+
311	*seasonally hypoxic*	65	57°26.49′ 20°43.49′	5	0	2	2	1.0	−	+
GOF5	*seasonally hypoxic*	65	59°57.10′ 25°11.02′	10	1	9	8	8.0	+	+
LL3A	*seasonally hypoxic*	60	60°4.43′ 26.18.30′	5	0	6	6	8.0	+	+
JML	*seasonally hypoxic*	80	59°34.92′ 23°37.50′	0	0	0	0	5.9	−	+
LL19	*anoxic*	173	58°52.84′ 20°18.65′	0	0	4	4	9.0	−	+
F80	*anoxic*	194	58°00.00′ 19°53.80′	0	0	0	0	13.2	−	+
Bornholm	*reoxygenated*	87	55°28.09′ 15°28.63′	27	2	31	29	5.4	+	+
BY15	*reoxygenated*	237	57°19.20′ 20°3.00′	10	0	0	0	11.5	−	+
BY15A	*reoxygenated*	200	57°25.51′ 20°1.53′	15	2	6	4	N/A	+	+

[a]Deduced from the pore water profile of the sectioned core; N/A, not available.

and 2015.[17,20,21] During such major Baltic inflows, large amounts of saline O_2-rich North Sea water enter the Baltic Sea and subsequently ventilate the deeper basins.[17]

Sediment and Pore Water Sampling. Sediment cores (inner diameter 10 cm) were retrieved at 12 sites in the Baltic Sea (Figure 1) using a multicorer (Oktopus GmbH, Germany) during an expedition with R/V *Pelagia* in May and June 2016 (Table 1). From one core per site, two bottom water samples were retrieved from the overlying water. Subsequently, one core was sectioned under nitrogen for sediment and pore water collection (0.5 cm depth resolution for the first 2 cm, 1 cm resolution for 2−10 cm). For each sediment slice interval, a split was made: a subsample was placed in a preweighed glass vial for porosity determination, and another subsample was transferred to 50 mL centrifuge tubes. Subsequently, pore water was extracted by centrifugation (15 min at 4500 rpm), filtered through 0.45 μm nylon filters, and subsampled under nitrogen. Directly after centrifugation, samples for sulfide analysis (0.5 mL) were transferred into glass vials filled with a nitrogen-purged 8 M NaOH solution (1.5 mL). Both H_2S and ammonium (NH_4^+) concentrations were determined using a QuAAtro (Bran+Luebbe) gas segmented continuous flow analyzer onboard ship.[22,23] Pore water sulfate (SO_4^{2-}) was determined using ion chromatography (IC).

High-resolution depth profiles of pH, O_2, and H_2S were retrieved from intact sediment cores within 2 h of retrieval using microelectrodes (50 or 100 μm tip diameter) operated with a motorized micromanipulator (Unisense A.S., Denmark). Calibrations of pH, O_2, and H_2S electrodes were performed as described in Sulu-Gambari et al.[13] Total H_2S ($\Sigma H_2S = H_2S + HS^- + S^{2-}$) was calculated based on Jeroschewski et al.[24]

Sulfate reduction rates (SRR) were measured, at all sites except BY15A, by extracting 5 mL of wet sediment sample from predrilled and taped cores directly upon retrieval using cut off syringes following Egger et al.[25] Within 4 h of core retrieval, 20 μL of carrier free $^{35}SO_4^{2-}$ (74−91 kBq) was injected in these syringes. The sediment was incubated for 20−24 h in the dark under a nitrogen atmosphere, after which it was transferred to 50 mL centrifuge tubes containing 20 mL of deoxygenated 20% zinc acetate to precipitate dissolved $\sum H_2S$ and inhibit biological activity.[26,27] Upon analysis, samples were rinsed twice using deoxygenated bottom water (10 mL) and centrifuged for removal of unreacted $^{35}SO_4^{2-}$.[25] The reduced S was extracted with an acidic chrome chloride solution (48 h) via the passive diffusion method.[28] SSR rates integrated over the entire depth of the core were calculated by comparing the activity (decays per minute) of the radiolabeled total reduced inorganic sulfur (a_{TRIS}) to the total SO_4^{2-} (a_{TOT}) radiotracer as described in Kallmeyer et al.:[27]

$$SRR = [SO_4^{2-}]\cdot\phi\cdot\frac{a_{TRIS}}{a_{TOT}}\cdot\frac{1}{t}\cdot 1.06 \quad (1)$$

where ϕ represents the porosity; t is the incubation time (days), and the correction factor 1.06 represents the expected isotopic fractionation.[27,29]

Diffusive Fluxes. Diffusive fluxes of ΣH_2S and NH_4^+ across the sediment−water interface, upward fluxes of ΣH_2S into the suboxic zone, and downward fluxes of SO_4^{2-} in the upper part of the sediment were determined based on the linear pore water gradient using Fick's first law:[30]

$$J = -\phi D_s\cdot\frac{dC}{dz} \quad (2)$$

where J is the diffusive flux (mmol m^{-2} day^{-1}); ϕ represents the sediment porosity; D_s stands for the sediment diffusion coefficient; C is the concentration of ΣH_2S, NH_4^+, or SO_4^{2-} (mM); and z represents the sediment depth (m). The diffusion coefficient was calculated as a function of pressure, salinity, and

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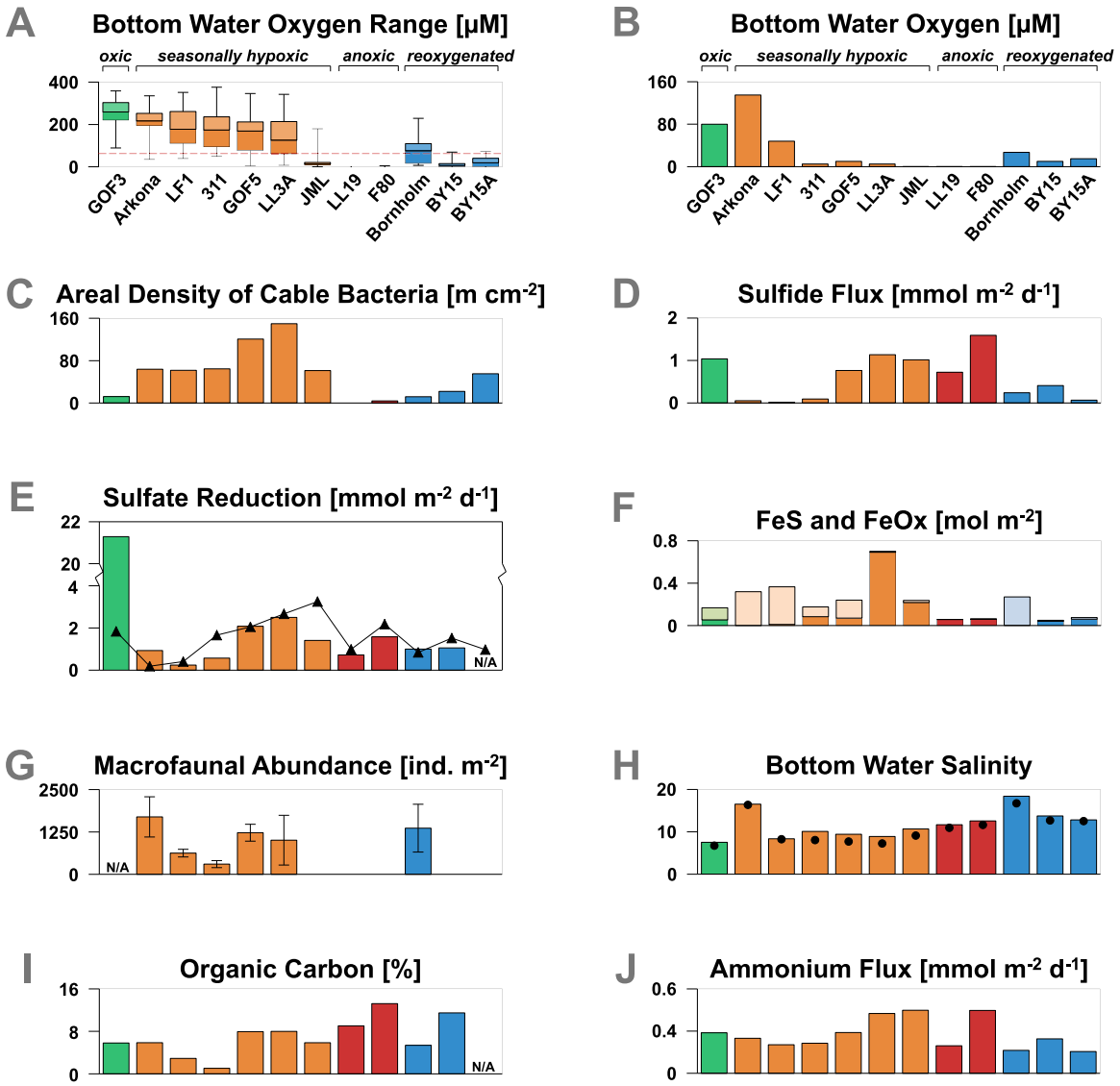

Figure 2. (A) Range in bottom water O_2 (μM) for 2014−2016 based on the HELCOM database where available. The red dashed line (located at 63 μM) indicates the hypoxic boundary. The solid line between the boxes is the median, whereas the boxes represent the lower and upper quartiles. The error bars indicate the minimum and maximum O_2 levels. (B) Bottom water O_2 concentrations (μM) in May/June 2016 derived from microelectrode profiles near the sediment−water interface. (C) Areal density of cable bacteria (m cm^{-2}). (D) Upward flux of sulfide toward the sediment−water interface/suboxic zone (mmol m^{-2} day^{-1}). (E) Sulfate reduction rates (mmol m^{-2} day^{-1}; black triangles represent the downward flux of sulfate in mmol m^{-2} day^{-1}). (F) Sedimentary FeS (AVS) and labile Fe(III) oxides (mmol m^{-2}; integrated over top 2 cm; the dark and light colors represent FeS and labile Fe(III) oxides respectively). (G) Macrofaunal abundance (ind. m^{-2}). The error bars represent the standard deviation. (H) Bottom water salinity. The black dots represent the bottom water salinity prior to the major Baltic inflows. (I) Sediment organic carbon (%; averaged over top 2 cm). (J) Upward flux of ammonium (mmol m^{-2} day^{-1}) toward the sediment−water interface. The study sites are classified based on bottom water redox conditions as described in Figure 1.

temperature using the R package *marelac*[31] and corrected for the ambient tortuosity.[32]

Macrofaunal Density. A benthic lander system equipped with three chambers, with a surface area of 144 cm^2 per chamber, was utilized to collect sediment samples for the determination of macrofauna.[33] The first ∼12 cm of the sediment was collected by the chambers, which was subsequently sieved (5 mm mesh size). Macrofauna were retrieved and stored in 4% formaldehyde in plastic jars until determination of the species and their abundance.[34] At site GOF3, no macrofauna samples were collected. For the determination of ash free dry mass (AFDM), macrofaunal specimens were first dried, weighed, and then ashed. The AFDM was calculated based on the difference of the dry weight and the weight of the remaining ash.

Identification of Sulfur Oxidizing Bacteria and Cable Bacteria Quantification. To identify which sulfur oxidizing bacteria were present (provisionally determined based on thickness and length of filaments), surface sediments were analyzed onboard using a stereomicroscope. *Beggiatoaceae* filaments were distinguished from cable bacteria filaments based on their greater thickness and prominent sulfur inclusions. The abundance of *Beggiatoaceae* was not enumerated. For the quantification of cable bacteria, sediment cores were sectioned at all sites (0.5 cm depth resolution for the first 2.5 cm). Each sediment slice was homogenized, and then 0.5 mL of sediment was mixed with 0.5 mL of 99% ethanol and

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stored at −20 °C. Fluorescence in situ hybridization (FISH) was used for microscopic identification and quantification of cable bacteria filaments, as described in Seitaj et al.[6] Aliquots of 100 μL were transferred in a 1:1 mixture of PBS/ethanol (500 μL). Subsequently, 10 μL of this mixture was filtered through a polycarbonate membrane (type GTTP, pore size 0.2 μm, Millipore, USA). Cable bacteria filaments were detected with a *Desulfobulbaceae*-specific oligonucleotide probe (DSB706; 5′-ACC CGT ATT CCT CCC GAT-3′) after counter staining with DAPI (1 μg/mL) under an epifluorescence microscope (Zeiss Axioplan, Germany) at 100× magnification. The abundance of cable bacteria was determined by determining the length and diameter of all observed cable bacteria in a field (105 × 141 μm) on the filter at 100× magnification (200 fields per sample). The cable bacteria abundances are reported either as filament length per volumetric unit (m cm^{-3}) or integrated over depth expressed per unit area of sediment surface (m cm^{-2}).

Incubation Experiment. The overlying water in two intact sediment cores from site GOF5 and BY15A was continuously aerated onboard to see whether a high concentration of O_2 in the bottom water would induce the metabolic activity of cable bacteria. After ∼5 days the two cores were subjected to high-resolution depth profiling.

Solid-Phase Analysis. Sediment samples from core sectioning were freeze-dried and subsequently ground in an argon-purged glovebox. Solid-phase Fe was fractioned into (1) labile Fe(III) oxides and Fe(II) ($FeS + FeCO_3$), (2) crystalline Fe minerals, (3) magnetite, and (4) pyrite (FeS_2), using a combination of two extraction methods (Poulton and Canfield;[35] Claff et al.[36]) as described by Hermans et al.[21] Solid-phase S was separated into (1) acid volatile sulfur (AVS; FeS) and (2) chromium reducible sulfur (CRS; FeS_2), using the method after Burton et al.[37] as modified by Kraal et al.[38] FeS and FeS_2 were quantified by iodometric titrations.[39] Total solid-phase Mn was determined by ICP-OES, after dissolution using a mixture of HF and HNO_3. Sediment porosity and a sediment density of 2.65 g cm^{-3} were used to convert Fe oxide and FeS contents in mol g^{-1} to mol m^{-2}.[40]

RESULTS AND DISCUSSION

Classification of Sites Based on Bottom Water Oxygen. The range in bottom water O_2 for 2014−2016 varied greatly among the 12 sites (Figure 2A; Table S1). Bottom waters at GOF3 were characterized by high concentrations of O_2 (>150 μM) and were never hypoxic (<63 μM). Five of the seasonally hypoxic sites—Arkona, LF1, 311, GOF5, and LL3A—in contrast, showed a distinct seasonal cycle with high bottom water O_2 most of the year and relatively low O_2 in summer (<63 μM for ∼60−90 days). Site JML was hypoxic most of the time and only briefly became oxic (in June 2015). Bottom waters at sites LL19 and F80 were always anoxic. At the reoxygenated sites, the bottom water O_2 varied between 0 and 150 μM. At the time of sampling, bottom water O_2 was low or absent at all of the seasonally hypoxic sites and the reoxygenated sites, except Arkona and LF1 (Figure 2B).

Abundance of Cable Bacteria in Baltic Sea Sediments. Visual observations of the surface sediment onboard ship by light microscopy revealed the presence of filaments that were likely cable bacteria. These filaments were observed at the seasonally hypoxic sites GOF5 and LL3A and at the reoxygenated sites Bornholm and BY15A, while *Beggiatoaceae* were found at all sites (Table 1). Thick microbial mats of *Beggiatoaceae* were observed only at the reoxygenated sites BY15 and BY15A in the Eastern Gotland basin (Figure S1). A more detailed examination of the surface sediments using FISH revealed that cable bacteria were present at all sites except for LL19 (Figure S2). Cable bacterial abundances, however, varied greatly between sites and also with sediment depth (Figures S2 and S3).

At the oxic site GOF3, the areal density of cable bacteria was low (12 m cm^{-2}; Figure 2C). At four of the seasonally hypoxic sites—Arkona, LF1, 311, and JML—the areal density was 5 times higher (∼60 m cm^{-2}). The seasonally hypoxic sites GOF5 and LL3A, both located in the Gulf of Finland, had the highest abundance of cable bacteria (121 and 150 m cm^{-2}, respectively). Such densities are comparable with sediments with active cable bacteria communities as found, for example, in seasonally hypoxic Lake Grevelingen.[28,41] The two anoxic sites LL19 and F80 had the lowest abundance of cable bacteria (0 and 4 m cm^{-2}), while at the reoxygenated sites—Bornholm, BY15, and BY15A—abundances varied (12 and 55 m cm^{-2}). The low amounts of cable bacteria at discontinuous depths at the reoxygenated sites are likely inactive remnant cells that have not yet decayed (Figure S2). At sites BY15 and BY15A, the abundances of cable bacteria were similar to those observed in reoxygenated Baltic Sea sediments in a recent study.[42]

Evidence for cable bacteria activity was visible in high-resolution depth profiles of pH, O_2, and $\sum H_2S$, at sites GOF5 and LL3A (Figure S4). Sediments at these sites were characterized by the presence of a suboxic zone (Table 1) and a distinct pH peak near the subsurface. However, with a minimum pH of ∼7, only moderate acidification of the pore water in the suboxic zone was observed when compared to the pH of ∼6.5, which is typical for cable bacteria activity.[5,11] At the time of sampling, bottom water O_2 levels were extremely low at these sites (Table 1; 5−10 μM saturation). Limited availability of O_2 typically results in the collapse of the metabolic activity of cable bacteria.[16] The observed pH profiles at site GOF5 and LL3A thus may reflect a residue signal of cable bacteria activity shortly after such a collapse. The depth profiles of pH, O_2, and $\sum H_2S$ at sites BY15 and BY15A, in contrast, resembled the typical pH profiles for activity of *Beggiatoaceae*.[6] This pH signature is characterized by a low pH near the sediment surface as a result of proton formation and a high pH in the deeper sediment horizon caused by proton consumption by $\sum H_2S$ oxidation.[6]

In summary, the highest abundances of cable bacteria were found at the seasonally hypoxic sites, in particular those located in the Gulf of Finland. Furthermore, we found that the presence of cable bacteria does not imply that the characteristic fingerprint for cable bacteria activity is observed in the pore water. However, the pH, O_2, and $\sum H_2S$ fingerprints, for sites with the highest cable bacteria density (GOF5 and LL3A), did show a strong similarity with the typical fingerprint for cable bacteria activity.

Controls on the Abundance and Activity of Cable Bacteria. Metabolic activity of cable bacteria requires bottom water O_2 and availability of $\sum H_2S$.[8] While bottom water O_2 was typically available at the oxic, seasonally hypoxic, and reoxygenated sites (Figure 2A), only the seasonally hypoxic sites had a high abundance of cable bacteria (Figure 2C). Here we assess the relationship between the abundance of cable bacteria at our 12 Baltic Sea sites with $\sum H_2S$ supply from

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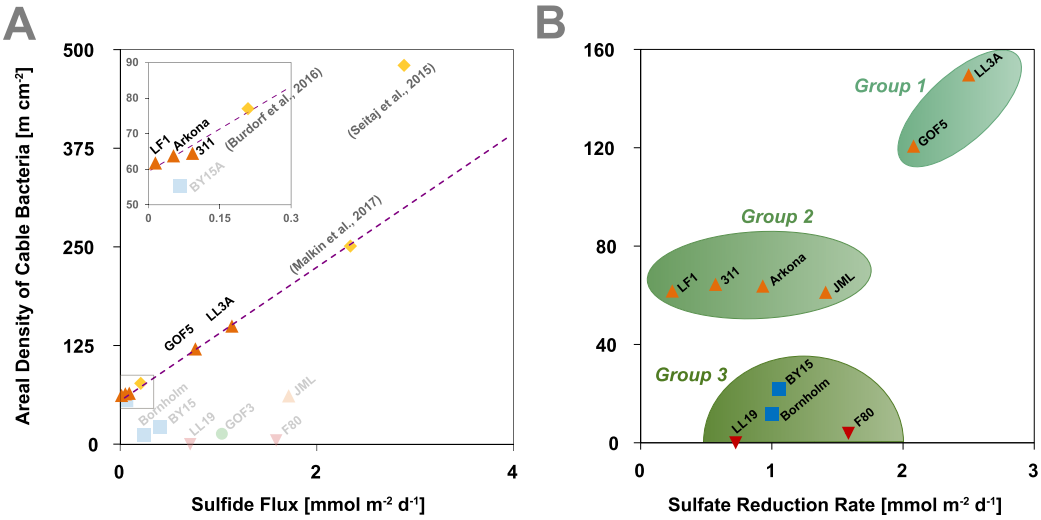

Figure 3. (A) Linear correlation between diffusive supply of $\sum H_2S$ (mmol m^{-2} day^{-1}) and areal density of cable bacteria (m cm^{-2}). The toned down sites in the background are omitted from the linear correlation, because other factors, such as insufficient bottom water O_2, controlled the abundance of cable bacteria. (B) Relationship between measured sulfate reduction rates (mmol m^{-2} day^{-1}) and areal density of cable bacteria (m cm^{-2}). GOF3 is omitted from the plot, because of the exceptionally high sulfate reduction rate. The sulfate reduction rate at BY15A is not available. The sample sites are classified based on the bottom water redox conditions as described in Figure 1.

sulfate reduction and dissolution of FeS, macrofaunal abundance, salinity, sediment organic matter, the rate of organic matter degradation, and potential competition of cable bacteria with *Beggiatoaceae*.

Any free $\sum H_2S$ that was not removed chemically or microbially may have diffused into the zone were cable bacteria were active (Figure S4). Diffusive $\sum H_2S$ fluxes into the suboxic or oxic zone (Figures 2D, S5, and S6) varied greatly between sites with highest fluxes at the oxic site (GOF3; 1 mmol m^{-2} day^{-1}), the three seasonally hypoxic sites in the Gulf of Finland (JML, GOF5, LL3A; 0.8−1.1 mmol m^{-2} day^{-1}), and the two anoxic sites (LL19, F80; 0.7−1.6 mmol m^{-2} day^{-1}). Sulfide fluxes were much lower at the other seasonally hypoxic sites (Arkona, LF1, 311; 0.01−0.09 mmol m^{-2} day^{-1}) and the reoxygenated sites (Bornholm, BY15, BY15 A; 0.07−0.4 mmol m^{-2} day^{-1}). Strikingly, the abundance of cable bacteria depends linearly on the diffusive supply of $\sum H_2S$, at all seasonally hypoxic sites, except JML (Figure 3A). Data for sediments populated by cable bacteria, in other systems, such as Lake Grevelingen (March 2012),[6] Wadden Sea (Mussel Reef; June 2013),[41] and a mangrove,[28] follow the same general trend (Figure 3A). In contrast, our anoxic and reoxygenated sites and the seasonally hypoxic site JML do not show such a correlation, likely because O_2 was limiting during most of the year. We conclude that, when sufficient bottom water O_2 is available during a large part of the year, the supply of $\sum H_2S$ acts as the main control on the abundance of cable bacteria.

Sulfate reduction can provide a key supply of sulfide to cable bacteria.[41] We found a large variation in rates at our 12 sites (Figures 2E, S5, and S7), with the highest rate observed at the oxic site GOF3 (21.4 mmol m^{-2} day^{-1}). Of the other sites, the seasonally hypoxic sites GOF5 and LL3A had the highest sulfate reduction rates (2.1 and 2.5 mmol m^{-2} day^{-1}, respectively). Comparison of the sulfate reduction rates and diffusive fluxes for GOF5 and LL3A shows that a significant proportion of the $\sum H_2S$ that was produced diffused to the suboxic or oxic zone. When we plot the areal density of cable bacteria against the sulfate reduction rates, no direct relation can be found. However, three groups can be identified (Figure 3B) which consist of (1) sites with a high areal density of cable bacteria in concert with high sulfate reduction rates and seasonal bottom water O_2 availability; (2) sites with sufficient bottom water O_2 during a large part of the year, but relatively low sulfate reduction rates and $\sum H_2S$ availability; and (3) sites with a low areal density of cable bacteria and low bottom water O_2 availability during the year. At the two seasonally hypoxic sites GOF5 and LL3A in group 1, sulfate reduction rates were high (2.1 and 2.5 mmol m^{-2} day^{-1}, respectively) close to the sediment surface (Figure S5). Such conditions likely allow cable bacteria to access electrons released from $\sum H_2S$ by sulfate reduction close to the oxic zone, stimulating a rapid and dense growth.[41]

Iron monosulfide can also serves as a source of $\sum H_2S$ for cable bacteria, with cable bacteria inducing the dissolution of FeS by pore water acidification. The Fe^{2+} released by dissolution can subsequently be oxidized upon contact with O_2 to form Fe oxides.[8,12] When bottom water O_2 levels are low again, the Fe oxides are converted back to FeS upon contact with $\sum H_2S$.[13] Because of this "pool switching" mechanism and the fact that the sediment FeS concentrations observed in June 2016 represented one time point at the beginning of summer, we consider the sum of the FeS and Fe oxides as a more representative term for the total pool of potentially available FeS for cable bacteria (hereafter FeS + FeOx) at the end of summer. The lowest FeS + FeOx pools were found at both anoxic sites and the two reoxygenated sites BY15 and BY15A (0.05 and 0.08 mol m^{-2}; Figure 2F). The highest FeS + FeOx was found at site LL3A (0.7 mol m^{-2}). Most of the FeS + FeOx (~71%) at site GOF5 consisted of Fe oxides, likely as a result of cable bacteria activity prior to our sampling campaign (Figure 2F). In contrast, at site LL3A, ~99% of the FeS + FeOx consisted of FeS, likely because Fe oxides formed by cable bacteria already underwent conversion to FeS because of the low bottom water O_2 concentrations at the time of sampling. This pool of FeS, if dissolved in spring, for example, in 100 days, is equivalent to a $\sum H_2S$ supply of 7 $mmol^{-2}$ day^{-1}. Given the sulfate reduction rate of 2.5 mmol m^{-2} day^{-1}

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Table 2. Macrofaunal Individuals per Square Meter for Six Taxonomic Classes in May and June 2016

site	*Polychaeta*	*Sipunculida*	*Oligochaeta*	*Bivalvia*	*Malacostraca*	*Insecta*	total
GOF3	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Arkona	1111 ± 393	0	69 ± 62	306 ± 243	139 ± 124	69 ± 76	1694 ± 593
LF1	23 ± 33	139 ± 57	0	463 ± 87	0	0	625 ± 113
311	23 ± 33	81 ± 48	0	185 ± 65	0	12 ± 26	301 ± 104
GOF5	1100 ± 225	12 ± 26	0	58 ± 129	12 ± 26	46 ± 33	1227 ± 249
LL3A	995 ± 741	0	0	0	0	12 ± 26	1007 ± 734
JML	0	0	0	0	0	0	0
LL19	0	0	0	0	0	0	0
F80	0	0	0	0	0	0	0
Bornholm	1278 ± 717	0	0	0	0	83 ± 28	1361 ± 705
BY15	0	0	0	0	0	0	0
BY15A	0	0	0	0	0	0	0

at LL3A, this indicates that sediment FeS could be a major source of $\sum H_2S$ for cable bacteria in this region. In summary, the sites with the highest abundance of cable bacteria were characterized by a large FeS + FeOx pool, relatively high rates of sulfate reduction and upward fluxes of $\sum H_2S$, and large seasonal variations in bottom water O_2 with high concentrations in winter and spring (Figures 2 and 3). We conclude that both sulfate reduction and the dissolution of FeS act as a source of $\sum H_2S$ for cable bacteria in the Gulf of Finland.

Bioturbation can inhibit the development of cable bacteria by damaging the bacterial filaments, causing a disruption of the electrochemical signal.[11] Previously, high numbers of polychaetes belonging to the genus *Marenzelleria* were observed at the oxic site GOF3.[43] Visual observations during slicing of the cores for pore water collection also suggest disturbance by macrofauna at this site. Polychaetes also dominated the macrofaunal community at Arkona, GOF5, LL3A, and Bornholm, whereas bivalves were most abundant at LF1 and 311 (Table 2 and Figures S9–S11). The genera of polychaetes (ind. m^{-2}) differed among these four sites (Figure S10A). The three major polychaetes observed at Arkona were *Scoloplos*, *Bylgides*, and *Terebellides*. At sites GOF5 and LL3A, *Marenzelleria* dominated, whereas *Scoloplos* were the most prevalent polychaetes at Bornholm. In terms of macrofaunal biomass (AFDM), site GOF5 and LL3A had the highest biomass of polychaetes (1.8 and 2.2 g m^{-2}, respectively; Figure S10B). The biomass of polychaetes at Arkona was comparatively low (0.2 g m^{-2}), even though the absolute number of polychaetes at Arkona was similar to that of GOF5 and LL3A (Table 2). Macrofauna were absent at JML, LL19, and F80 (Figure 2G). In general, macrofaunal abundances at our 12 sites were relatively low (<1700 ind. m^{-2}; Figure 2G) when compared to other temperate coastal systems, such as, for example the Western Black Sea shelf (6 000–10 000 ind. m^{-2})[44,45] and the North Sea (2 400–21 000 ind. m^{-2}).[46] The relatively impoverished macrofaunal community in the Baltic Sea is a consequence of its natural constraints by brackish conditions that limit macrofaunal abundance and diversity and the recent human-induced increased prevalence of hypoxia.[47] We did not find a relationship between macrofaunal abundances (expressed as ind. m^{-2} and AFDM g m^{-2}) and cable bacterial abundances (Figure S12A,B), suggesting that at most sites the impoverished macrofaunal communities did not significantly hamper growth of cable bacteria. Interestingly, the areal density of cable bacteria and biomass (AFDM) of polychaetes was highest at sites GOF5 and LL3A (Figure S12C). This further indicates that the polychaetes did not disturb the growth of cable bacteria. However, at our oxic site GOF3, which was characterized by high bottom water O_2 and a high diffusive supply of $\sum H_2S$, the low areal density of cable bacteria was likely due to intense bioturbation by *Marenzelleria*. A similar low areal density of cable bacteria was observed in a recent study at a permanently oxic site in the Eastern Gotland Basin and was also attributed to the presence of *Marenzelleria*.[42]

Cable bacteria can tolerate a wide range of salinities, because they occur in marine, brackish, and fresh water environments.[48] Bottom water salinity varied widely among our 12 sites, from 7.5 to 18.5, but showed no relationship with the abundance of cable bacteria (Figures 2H and S12D). Bottom water salinities at our study sites were slightly higher than under normal circumstances because of recent inflows of saline water from the North Sea (Figure 2H). Organic carbon in the upper 2 cm of the surface sediment varied from 1 to 13 wt % (Table 1), and again, there was no relationship with the abundance of cable bacteria (Figures 2I and S12E). The rate of anaerobic degradation of organic matter, here approximated by the ammonium flux toward the sediment–water interface (Figures 2J and S8) showed the same linear trend with cable bacterial abundances as $\sum H_2S$ at most seasonally hypoxic sites (Figure S12F).

The absence of a correlation between the abundance of cable bacteria, macrofaunal abundances, salinity, and sediment organic carbon (Figures 2 and S12) highlights that in sediments with no to moderate disturbance by bioturbation, the availability of O_2 and $\sum H_2S$ are the key controls that determine the abundance of cable bacteria. While at the oxic site GOF3 both O_2 and $\sum H_2S$ were abundantly present, cable bacteria were likely inhibited by high macrofaunal activity. At the anoxic sites LL19 and F80, there was insufficient O_2. At the reoxygenated sites Bornholm, BY15, and BY15A, O_2 concentrations likely remained too low for cable bacteria. *Beggiatoaceae* were abundantly present as thick mats, and because they are better adapted to such low bottom O_2 conditions, they likely outcompeted the cable bacteria. This is supported by our observation that reoxygenation of the overlying water for sediment from site BY15A in the laboratory did not result in cable bacteria activity (Figure S13A).

The seasonally hypoxic sites provided the best conditions for cable bacteria, with the variation in abundance between sites explained by the diffusive supply of $\sum H_2S$ (Figure 3A). These sites had a high O_2 availability during a major part of the year in concert with a high diffusive supply of $\sum H_2S$ and a large FeS + FeOx pool (Figure 2F). Because the bottom water O_2

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levels at both sites were extremely low at the time of sampling (~5−10 μM; Table 1), cable bacteria were not very active. Strikingly, the activity of cable bacteria in a sediment core retrieved from GOF5 could be stimulated rapidly (within ~5 days) upon reoxygenation of the overlying bottom water (Figure S13B).

In summary, this indicates three requirements for a high abundance of cable bacteria: (1) high bottom water O_2 availability during a major part of the year; (2) availability of ΣH_2S; (3) no to moderate disturbance by macrofaunal bioturbation. Such conditions are found at our study sites in the Gulf of Finland. Other factors, such as the availability and degradation of organic matter and bottom water salinity (within the salinity range at our sites, i.e. 7.5−18.5) are of less importance. The strong dependence of cable bacteria on the availability of $\sum H_2S$ is typical for chemoautotrophic sulfur-oxidizing bacteria (e.g., Nelson and Jannasch[49]). However, recent work on cable bacteria indicates that they are likely heterotrophs.[50] Apparently, the requirements of cable bacteria for organic carbon are relatively easily met, allowing the availability of ΣH_2S to become a key control.

Biogeochemical Impact of Cable Bacteria. Cable bacteria activity can strongly impact sedimentary Fe and S cycling,[6,7,13] because the acidification of the pore water (pH ~6.5; Figure S13B)[5,11] can facilitate the dissolution of FeS and promote the formation of Fe oxides.[8,12] The FeS + FeOx pool in the upper part of the sediment (Figure 2F) provides insight into the maximum amount of Fe oxides that can be formed on a seasonal basis. This amount of Fe oxides ultimately controls how much $\sum H_2S$ can be sequestered before free $\sum H_2S$ is released into the water column during bottom water anoxia.

In the Gulf of Finland at site GOF5, the metabolic activity of cable bacteria and associated pore water acidification in spring likely contributed to the formation of the Fe oxides (0.17 mol m^{-2}) observed in the surface sediment in June 2016 (Figures 2F and 4A). The estimated depletion of FeS at GOF5 over the first 2.5 cm is 0.17 mol m^{-2} (Figure 4B). Assuming that all Fe^{2+} released upon dissolution of FeS would precipitate as Fe oxides upon contact with O_2, this would give an increase in Fe oxides of 0.17 mol m^{-2}, which is in line with our observations. The abundant presence of Fe oxides in surface sediments has previously been shown to delay euxinia in seasonally hypoxic Lake Grevelingen.[6] At our site GOF5, the sulfate reduction rate was 2 mmol m^{-2} day^{-1}. This would imply that if the activity of the cable bacteria ceases because of the onset of hypoxia, the Fe oxide layer can temporarily delay the escape of ΣH_2S from the sediment for a period of ~85 days.

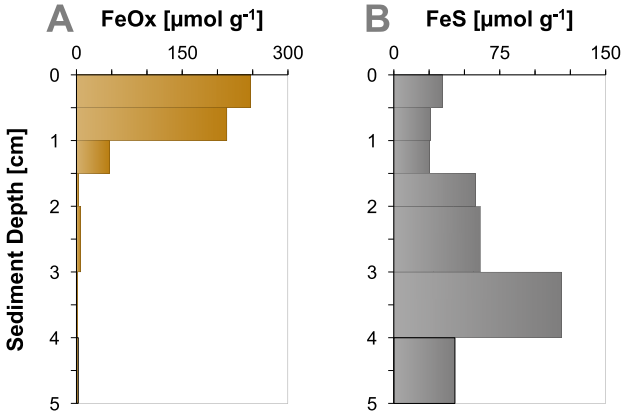

Figure 4. Solid-phase profiles of (A) labile Fe(III) oxides (FeOx) and (B) FeS (AVS) for GOF5 in June 2016.

An alternative mechanism for the development of an Fe oxide layer could be bioirrigation by *Marenzelleria*. These *Marenzellaria* are capable of pumping O_2 into pore waters, thereby enhancing the oxidation of reduced Fe.[51] However, the number of *Marenzellaria* that was observed at GOF5 was relatively moderate (1100 ± 225 ind. m^{-2}; Table 2), and results of a reactive transport model for a similar site suggest that much higher population densities >3000 ind. m^{-2} are required to have a significant effect on the formation of Fe oxides.[51] Bioirrigation by *Marenzelleria* also typically leads to oxidation of the sediment down to a depth of several centimeters.[51] Because we observed Fe oxides only within the top 1 cm of the surface sediment at site GOF5, this confirms that the role of *Marenzelleria* with respect to oxidation of the sediment by bioirrigation was likely negligible. The insignificant impact of *Marenzelleria* on biorrigation is further supported by bromide incubations performed on intact sediment cores retrieved from site LF1 and Arkona (Supporting Information 1.10; Figure S14). These incubations indicate very low rates of bioirrigation at site LF1 and Arkona, despite the presence of polychaetes (Table 2).

Water column monitoring data indicate that the bottom waters in the Gulf of Finland are rarely euxinic (Figure 2A). We suggest that cable bacteria are responsible for the absence of $\sum H_2S$ in the bottom water of the Gulf of Finland in summer, by inducing the formation of strong surface enrichments of Fe oxides in winter and/or spring. The Gulf of Finland is only the second system for which this Fe oxide buffer mechanism has been suggested, after Lake Grevelingen, and the first with a relatively low bottom water salinity (~9−11 versus ~32).[52] The Fe oxide buffer mechanism induced by cable bacteria is likely of importance in many other eutrophic, brackish coastal areas characterized by moderate of disturbance by bioturbation, high bottom water O_2 and a high sediment supply of $\sum H_2S$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b01665.

Bottom water O_2 ranges; visual observation of *Beggiatoaceae*; FISH analysis of cable bacteria filaments; high-resolution depth profiles of pH, O_2, and $\sum H_2S$; depth profiles of sulfate reduction rates and pore water SO_4^{2-}, $\sum H_2S$, and NH_4^+; linear pore water gradients of $\sum H_2S$, SO_4^{2-}, and NH_4^+ used for diffusive fluxes; macrofauna; relationship between controls on cable bacterial abundances; laboratory induced activity of cable bacteria; and bioirrigation (PDF)

Experimental data (XLSX)

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Author Contributions

C.P.S. conceived and designed the study. M.H., W.K.L., S.H.-M., and N.A.G.M.v.H. carried out the fieldwork and the analysis of the samples. M.H. and C.P.S. wrote the manuscript with contributions from all coauthors.

Notes

The authors declare no competing financial interest.

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