Cable bacteria generate a firewall against euxinia in seasonally hypoxic basins


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Seasonal oxygen depletion (hypoxia) in coastal bottom waters can lead to the release and persistence of free sulfide (euxinia), which is highly detrimental to marine life. Although coastal hypoxia is relatively common, reports of euxinia are less frequent, which suggests that certain environmental controls can delay the onset of euxinia. However, these controls and their prevalence are poorly understood. Here we present field observations from a seasonally hypoxic marine basin (Grevelingen, The Netherlands), which suggest that the activity of cable bacteria, a recently discovered group of sulfur-oxidizing microorganisms inducing long-distance electron transport, can delay the onset of euxinia in coastal waters. Our results reveal a remarkable seasonal succession of sulfur cycling pathways, which was observed over multiple years. Cable bacteria dominate the sediment geochemistry in winter, whereas, after the summer hypoxia, Beggiatoaceae mats colonize the sediment. The specific electrogenic metabolism of cable bacteria generates a large buffer of sedimentary iron oxides before the onset of summer hypoxia, which captures free sulfide in the surface sediment, thus likely preventing the development of bottom water euxinia. As cable bacteria are present in many seasonally hypoxic systems, this euxinia-preventing firewall mechanism could be widely active, and may explain why euxinia is relatively infrequently observed in the coastal ocean.

Significance

Seasonal hypoxia is increasing in coastal areas worldwide, as more nutrients are delivered to the coastal ocean and water temperatures are rising due to climate change. Hypoxia reaches a particularly harmful stage when sulfide, which is highly toxic for marine life, is released to the bottom water. Here, we document a natural microbial mechanism that counteracts the release of free sulfide, thus preventing the most adverse stage of seasonal hypoxia. Electricity-generating cable bacteria produce a large pool of oxidized sedimentary iron minerals, which efficiently bind free sulfide. As cable bacteria are likely abundant in many seasonally hypoxic basins worldwide, their “firewall” mechanism may be widespread.


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Results and Discussion
Response of Pore Water Geochemistry to Seasonal Oxygenation. The sediment geochemistry, sediment fauna, and sedimentary microbial communities were surveyed in Marine Lake Grevelingen (MLG, The Netherlands), a coastal water body (salinity ~30) with restricted water exchange with the open North Sea. Over the last decade, MLG has experienced a regular pattern of summer stratification and bottom water oxygen depletion (Fig. S1A), which was also observed in monthly sampling campaigns performed throughout 2012 (Fig. S1B). Bottom water oxygen concentrations (Winkler method; Fig. L4) were near air saturation in winter and early spring, started to decline in April at the onset of stratification, became hypoxic (O₂ < 63 μM) by the end of May, and declined below the detection limit in August (O₂ <1 μM; anoxia). In September, the overturning of the water column resulted in a reoxygenation of the bottom water.

Due to sediment focusing, the deeper basins in MLG experience a strong accumulation (~2 cm y⁻¹) of dark, organic-rich, fine-grained sediment (14). Free sulfide ([H₂S] + [HS⁻]) accumulates in the pore water to high levels (~2 mM at 10 cm depth; Fig. S2), suggesting that intensive organic mineralization takes place in the surface sediment and that sulfate reduction is the major mineralization pathway (estimated to be ~30 mmol S·m⁻²·d⁻¹ in August; Supporting Information). The burial of pyrite and iron sulfides only scavenges ~39% of the sulfide production (Supporting Information), and bottom water concentrations of nitrate (the alternative electron acceptor) are generally low in MLG (Fig. S1B). Accordingly, oxygen appears to be the main electron acceptor for the oxidation of the large amount of free sulfide that is produced by sulfate reduction (Supporting Information).

Nevertheless, microsensor profiling revealed that O₂ and H₂S were almost never in direct contact in the pore water, as a well-developed suboxic zone was present throughout most of the year (Fig. 1B), i.e., a distinct sediment horizon where neither O₂ nor H₂S were present in detectable concentrations. This suboxic zone was widest in the first part of the year (annual maximum of 17.6 ± 4.6 mm in April), and its width decreased in a stepwise fashion by ~50% in late spring (May, 8.9 ± 2.1; June, 7.0 ± 5.2 mm). When the oxygen saturation of the bottom water dropped below 11% (corresponding to 29 μmol·L⁻¹; July and August, Fig. L4), the H₂S front started to move toward the sediment surface (Fig. 1B). However, free sulfide remained undetectable in bottom water samples collected at 2 m above the sediment surface during the stratified season (H₂S < 0.2 μM; June, August). We additionally determined the [H₂S] concentration in the overlying ~10 cm water of retrieved sediment cores, which confirmed that euxinia did not occur. After the overturning of the basin, the oxygen penetration depth (OPD) was small (0.7 ± 0.1 mm), indicative of strong oxygen uptake caused by reoxidation of reduced compounds. September was also the only month when the pore water depth profiles O₂ and H₂S showed an overlap (Fig. 1B), allowing the direct aerobic oxidation of sulfide (H₂S + O₂ → SO₄²⁻ + 2H⁺). A small suboxic zone reappeared in October (2.2 ± 0.9 mm) and gradually expanded (12.2 ± 1.6 mm in December).

Mechanisms of Suboxic Zone Formation. To elucidate the underlying mechanisms of sulfide oxidation and suboxic zone formation, we developed a pH typology for three known mechanisms of aerobic sulfide oxidation (Fig. S3 and Supporting Information): (i) the e-SOx metabolism of the recently discovered cable bacteria (12, 13, 16); (ii) the cycling of iron between reduced and oxidized mineral forms, which is crucially dependent on solid-phase mixing (17, 18); and (iii) the respiratory metabolism of nitrate-accumulating Beggiatoaceae (19–22). Each of these three pathways is associated with a characteristic pH depth profile (Fig. 2, Fig. S3, and Supporting Information), which reveals when these mechanisms dominate the pore water geochemistry and are responsible for the formation of the suboxic zone (Fig. 1C). This pH typology predicted that e-SOx by cable bacteria was dominant from January to April, whereas metal cycling dominated in May and June, and respiration of nitrate-accumulating Beggiatoaceae created the suboxic zone in fall, after the ventilation and reoxygenation of the bottom water (Fig. 1C).

This temporal succession of sulfur oxidation pathways as predicted by the pH typology analysis was confirmed by direct microscopic observation of microbial and macrofaunal communities. Cable bacteria were enumerated on a seasonal basis by Fluorescence In Situ Hybridization (FISH), which revealed that cable bacteria were abundant in March and May (filament density 402–480 m⁻²•cm⁻²; biovolume 1.8–2.5 mm²·cm⁻³), but remained below the detection limit in August and November (Fig. 1D). When present, cable bacteria were found throughout the upper 40 mm of the sediment, with the maximum density near the sediment surface, high densities in the suboxic zone, and low densities declining into the sulfidic zone (Fig. 24, March). The filament diameter did not differ significantly (т test; n = 15, P = 0.29) between March (1.3 ± 0.3 μm) and May (1.2 ± 0.3 μm), suggesting that cable bacteria populations were phenotypically similar.

Quantitative microscopic enumeration of Beggiatoaceae was conducted each month (Fig. 1D), and showed low densities in spring (biovolume B: 0.02–0.05 mm³·cm⁻³), when only a few filaments were found dispersed throughout the upper 2 cm of sediment, and a
virtual absence from May to August \((B \leq 0.001 \text{ m}^2 \cdot \text{cm}^{-2})\). In September, a population of thin Beggiatoaceae filaments (diameter \(d: 2.4 \mu\text{m}\); mean filament length \(L: 70 \mu\text{m}\); \(B: 0.08 \text{ mm}^2 \cdot \text{cm}^{-2}\)) was found concentrated right at the \(O_2\)–H\(_2\)S interface, which likely catalyzed the direct aerobic oxidation of free sulfide. Laboratory studies have estimated that sulfide oxidation at the oxic–anoxic interface by Beggiatoaceae may be up to 3 times faster than the autocatalytic aerobic chemical oxidation of sulfide (23–25), thus enabling an efficient competition with the abiotic pathway. From October onward, the biovolume of Beggiatoaceae filaments drastically increased \((B: 2.2–7.5 \text{ mm}^2 \cdot \text{cm}^{-2})\), and the depth distribution of the Beggiatoaceae closely tracked the progressive widening of the suboxic zone (Fig. 2C). In addition, the filament diameter and length increased significantly compared with September \((t\) test; \(n = 672, P < 0.001\), regarding both length and diameter), suggesting that a different population of Beggiatoaceae was active in late fall that had a metabolism based on intracellular nitrate respiration. Bacterial cell-lysing experiments confirmed that the large Beggiatoaceae filaments observed at the field site were storing nitrate into intracellular vacuoles (Fig. S4 and Supporting Information).

The pH typology analysis predicted that suboxic zone in May and June was no longer formed by cable bacteria but that metal cycling caused the separation of \(O_2\) and H\(_2\)S horizons in the upper first centimeter of the sediment (Fig. 2B). This coincided with a sharp rise in the abundance and diversity of the macrofauna in the surface sediment (Fig. 1D), suggesting that bioturbation could provide the sediment mixing needed to sustain the metal cycling. An alternative explanation would be that mixing via sediment resuspension is specifically intense during May and June, which is unlikely, however, as meteorological conditions at the field site are typically calm in early summer. With the onset of hypoxia in late June, the macrofauna vanished abruptly, and the sediment remained devoid of macrofauna until December, when recolonization started, although population densities remained low throughout winter. Upon recolonization in spring, the fauna was dominated by small polychaetes and juvenile bivalves, which only have a shallow burrowing depth, consistent with the limited suboxic zone of 7–9 mm observed in May and June. Because fauna are highly sensitive to free sulfide (9–11), the deep removal of sulfide by cable bacteria in early spring may have promoted faunal recolonization.

**Microbial Competition for Reduced Sulfur Compounds.** In MLG, we observed that cable bacteria were dominant throughout spring, whereas sulfur oxidation was largely carried out by nitrate-accumulating Beggiatoaceae throughout fall. This pattern was not only observed in 2012, when detailed monthly sampling was conducted, but was confirmed by seasonal surveys over the period 2011–2015 (Fig. 3 and Supporting Information). Combining microsensor profiling and pH-signature analysis, we found that the geochemical signature of cable bacteria is significantly more present in spring, whereas the activity of Beggiatoaceae is more likely encountered in fall (Fig. 3). For example, in spring 2015, all sampled sediment sites below 15 m water depth showed the cable bacteria signature, whereas, in fall 2011 and 2014, all sediment revealed the geochemical signature of nitrate accumulating Beggiatoaceae. This implies that two distinct types of filamentous S-oxidizing bacteria were competing for the same geochemical niche, but that each type was competitively successful during a distinct period of the year.

The development of Beggiatoaceae after summer suggests a better survival of the anoxic period, which could be due to the use of nitrate as an alternative electron acceptor to oxygen. Although both cable bacteria (26) and Beggiatoaceae (19–22) can use nitrate for respiration, cable bacteria reach lower population densities when nitrate is the sole electron acceptor (26). Moreover, the nitrate concentration in the bottom water was low \((< 1.7 \mu\text{M})\) during summer (Fig. S1B), and thus, nitrate reduction was likely...
insignificant in sustaining microbial metabolism during anoxia. However, nitrate accumulation before anoxia could have played a role. Presently, there are no indications that cable bacteria can accumulate electron acceptors, whereas Beggiatoaceae can store nitrate in intracellular vacuoles (19–22, 25), which can be used as an electron acceptor reservoir to survive the summer period of low bottom water oxygenation (25).

Our seasonal surveys indicate that cable bacteria replace the Beggiatoaceae population in winter, yet the reasons for this population switch are not fully understood. One intriguing question is how cable bacteria can “invade” a sediment where a suboxic zone is already established by Beggiatoaceae, as recent laboratory experiments show that cable bacteria filaments progressively extend downward from an initial overlap of oxygen and sulfide near the sediment–water interface (27–29). A preexisting suboxic zone thus poses a barrier for sediment colonization by cable bacteria. Future research should hence clarify the drivers and controls of what appears to be a yearly recurrent, and hence predictable, switch in the cable bacteria population in late spring (30).

**Impact of Cable Bacteria on Geochemistry.** Cable bacteria and nitrate-accumulating Beggiatoaceae are both capable of efficient sulfide oxidation leading to the creation of a wide suboxic zone. Hence, with regard to biogeochemical cycling in seasonally hypoxic basins, one could ask to what extent it matters whether one or the other is the dominant sulfur oxidizing microbial population? Solid-phase data collected at the field site reveal a notable difference in the iron mineral phases between spring and fall (Fig. 4A), and suggest that cable bacteria induce a strong seasonal iron cycling. In March, when cable bacteria were active, a strong depletion of Acid Volatile Sulfides (AVS, interpreted to be mostly iron monosulfides, FeS) occurred in the suboxic zone (11.9 ± 7.3 μmol S·g⁻¹ over the first 3.3 cm; Fig. 4A), compared with high values in November (117.0 ± 26.4 μmol S·g⁻¹ over the first 3.3 cm; Fig. 4A), when nitrate-accumulating Beggiatoaceae were abundant. Exactly the opposite trend was seen in the extractable iron (hydr)oxides (FeOOH), which showed a much higher accumulation in spring (163 ± 52 μmol Fe·g⁻¹; Fig. 4A) than in fall (99 ± 27 μmol Fe·g⁻¹). Together with our microsensor and microscopy data, these solid-phase data suggest the following seasonal iron cycle (Fig. 4B): (i) conversion of FeS to FeOOH in spring by cable bacteria; (ii) the downward mixing of the FeOOH-rich surface sediment layer in late spring by the newly colonizing fauna, thus enhancing the observed S oxidation by metal oxide reduction; (iii) the conversion of FeOOH back to FeS when free sulfide rises to the sediment–water interface during the summer hypoxia period; and (iv) the persistence of an FeS pool in the suboxic zone during fall when nitrate-accumulating bacteria are active. Alternative mechanisms, such as winter resuspension events or a strong sedimentation of allochthonous iron (hydr)oxides in winter, cannot suitably explain the observed conversion of FeS to FeOOH in spring (Supporting Information). Accordingly, we conclude that observed seasonal iron cycling is principally driven by the FeOOH-forming activity of cable bacteria in spring, and this iron cycle would not occur if nitrate-accumulating Beggiatoaceae were dominant throughout the year. Our study therefore demonstrates that not only external environmental factors, such as bottom water oxygen availability, are driving the sedimentary iron and sulfur cycling in seasonally hypoxic basins but that the intrinsic population dynamics of the microbial community, and particularly rapid shifts in sulfur oxidizers, can be equally important.

The metabolic activity of cable bacteria exerts a profound impact on the sediment geochemistry through its effect on pore water pH (15). The electrogenic metabolism induces a spatial uncoupling of sulfide oxidation and oxygen reduction (Fig. 2A), and, accordingly, the production and consumption of protons occur widely segregated in space. The establishment of acidic conditions within the deeper suboxic zone promotes the dissolution of iron sulfides, which provides an extra H₂S supply to the cable bacteria in addition to sulfate reduction (16), and the oxidation of this extra H₂S generates more protons, thus establishing a positive feedback (15). In laboratory experiments, it has been shown that FeS dissolution provides up to 40–94% of the sulfide for e-SOx (15, 16), and can completely exhaust the sedimentary FeS pool over a period of weeks (27), while generating a surface enrichment of FeOOH. The observed FeS depletion in the top 4 cm in March shows that cable bacteria also induce strong FeS dissolution in the field, and we speculate that the depletion of the sedimentary FeS stock (Fig. 4A) may have limited the electron donor supply, causing the demise of the cable bacteria population in late spring (30).
Cable Bacteria in Seasonally Hypoxic Systems and Euxinia. Conventionally, the formation of sulfidic bottom waters is considered to be closely linked to the exhaustion of energetically favorable electron acceptors in the water column, such as oxygen and nitrate (1). Once these electron acceptors are depleted in the bottom water, the oxidation of sulfide in the surface sediment layer is halted, thus enabling a release of sulfide to the overlying water. However, laboratory sediment incubations (31, 32) have previously shown that the disappearance of oxygen and nitrate (anoxia) is not necessarily synchronous with the appearance of free sulfide (euxinia). In sediments that contain a large pool of reactive iron (hydr)oxides, the onset of anoxia, this pool can act as a firewall against the release of free sulfide from the sediment, thus delaying the onset of euxinia (31, 32). Iron (hydr)oxides have a high binding capacity for free sulfide (33); therefore, the efflux of free sulfide only starts after exhaustion of the iron (hydr)oxide pool, which can delay euxinia by several weeks, depending on the size of the initial reactive iron pool (31, 32).

At present, this iron oxide-mediated firewall mechanism has not been demonstrated to occur in seasonally hypoxic basins. Moreover, it is also not expected to occur, as the mechanism requires a yearly buildup of an iron (hydr)oxide pool before the onset of summer anoxia, which is not obvious in seasonally hypoxic environments. Coastal sediments typically accumulate sizeable pools of iron (hydr)oxides only when subjected to strong levels of bioturbation by infauna (34). The ventilation of macrofaunal burrows with oxygen-rich overlying water promotes the oxidation of dissolved ferrous iron (Fe(II)) in the pore water (35), whereas particle reworking enhances the oxidation of deeply buried iron sulfides by transporting them upward to the oxic zone of the sediment (36). However, as shown here, such large and deep-burrowing fauna are typically absent from seasonally hypoxic sediments, because the yearly recurrent oxygen depletion increases mortality and decreases recruitment success (11, 37). For this reason, the sediments of seasonally hypoxic coastal systems are generally thought to have a low buffer capacity toward the release of free sulfide (38).

Although sampling at monthly resolution is not sufficient to accurately document the magnitude of the time lag, we observed that anoxia did not coincide with euxinia. Even when the bottom water was devoid of oxygen in August, and nitrate was fully depleted, no free sulfide (concentrations were below the detection limit of <0.2 μM) was detected in the bottom water. Two potential mechanisms could explain this delay in the formation of euxinia relative to anoxia. The presence of a deep suboxic zone before the onset of bottom water anoxia is one potential mechanism. Before free sulfide can escape the sediment, the suboxic zone has to be transiently replenished with free sulfide, either from local production of sulfide via sulfate reduction or via diffusion of sulfide from deeper sediment horizons. As both cable bacteria and Beggiatoaceae induce a suboxic zone, they both in a suboxic zone. Coastal sediments typically accumulate sizeable pools of iron (hydr)oxides only when subjected to strong levels of bioturbation by infauna (34). The ventilation of macrofaunal burrows with oxygen-rich overlying water promotes the oxidation of dissolved ferrous iron (Fe(II)) in the pore water (35), whereas particle reworking enhances the oxidation of deeply buried iron sulfides by transporting them upward to the oxic zone of the sediment (36). However, as shown here, such large and deep-burrowing fauna are typically absent from seasonally hypoxic sediments, because the yearly recurrent oxygen depletion increases mortality and decreases recruitment success (11, 37). For this reason, the sediments of seasonally hypoxic coastal systems are generally thought to have a low buffer capacity toward the release of free sulfide (38).

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The iron oxide-mediated firewall mechanism is a more effective mechanism for H$_2$S removal, and has been previously shown to delay sulfide effluxes from sediments for a period of weeks (31, 32). Our results show that cable bacteria generated a large pool of reactive iron (hydr)oxides before the onset of summer hypoxia (0.95 mol Fe m$^{-2}$, as calculated from the difference in FeS and FeOOH inventories over 0–4 cm between spring and fall; Supporting Information). Given a depth-integrated sulfide production rate of 30 mmol H$_2$S m$^{-2}$ d$^{-1}$, this iron (hydr)oxide pool could potentially buffer H$_2$S production up to ~36 d. This period is considerably longer than the observed period of anoxia at the study site in 2012 (<20 d), and hence may explain the observed absence of bottom water euxinia in August 2012.

Conclusion

Cable bacteria have only recently been discovered (12, 13), and, hence, little is known about their ecology, life cycle, and natural distribution. Our results demonstrate that cable bacteria can have a major impact on sedimentary biogeochemical cycling in a seasonally hypoxic basin, with potential basin-scale impacts on water column chemistry. In a first report on the occurrence of cable bacteria under natural conditions, it was demonstrated that cable bacteria thrive globally in a wide range of marine sediment habitats, such as coastal mud plains and salt marshes, but that they particularly seem abundant in seasonally hypoxic basins (14). If cable bacteria in other coastal systems follow a similar seasonal cycle to that of MLG, the iron oxide firewall mechanism proposed here could be widely prevalent, and may explain the relatively rare reports of euxinia in coastal systems affected by seasonal hypoxia. However, to accurately document the magnitude and efficiency of this buffer mechanism, a comparison of the sediment geochemistry and microbiology is needed across multiple seasonally hypoxic systems at a higher-than-monthly resolution. Such investigations are crucial, given that seasonal hypoxia in coastal areas is increasing worldwide due to anthropogenic nutrient input and climate change (1).

Materials and Methods

Sampling. We performed monthly sampling campaigns on the RV Luctor in 2012, in the seasonally hypoxic MLG (39). Investigations took place in the Den Ose basin, a deep gully located in the southwestern part of the lake (maximum water depth 34 m; 51.747°N, 3.890°E), and we examined the water column chemistry and sediment biogeochemical processes. Discrete bottom water samples were collected with a 12-L Niskin bottle to assess the O$_2$ and H$_2$S concentrations. Water samples from the Niskin bottle were collected via gas-tight Tygon tubing. Bottom water oxygen concentrations were measured using an automated Winkler titration procedure with potentiometric end-point detection (Mettler Toledo DL50 Titrator and a platinum redox electrode). Bottom water $2H_2S$ concentrations were determined spectrophotometrically (40). Intact sediment cores (6 cm Ø) were retrieved with a UWITEC gravity corer in triplicates. All cores were inspected on retrieval, and only undisturbed cores were used for measurements. Immediately after collection, sediment cores were transported to a nearby laboratory, where microprofiling was started within 2 h of collection and conducted under climate-controlled conditions (temperature of in situ bottom water).

Microsensor Profiling. Microsensor profiling was performed using commercial microelectrodes (Unisense A.S.) for O$_2$ (25- or 50-μm tip diameter; Unisense), pH (200-μm tip diameter), and H$_2$S (50-μm tip diameter). Oxygen microprofiles were made at 25- to 50-μm resolution, with a two-point calibration made in air-saturated seawater (100% saturation) and at depth in anoxic sediment (0% saturation). For H$_2$S and pH, depth profiles were made at 200-μm resolution in the oxic zone, and 400- or 600-μm resolution below. Calibrations for pH were made with three National Bureau of Standards (NBS) standards and a Tris buffer to correct for salinity effects, and pH is reported on the total scale. For H$_2$S, a five-point calibration was made using Na$_2$S standards. Total free sulfide ($2H_2S + H_2S$) was calculated from H$_2$S based on pH measured at the same depth using the R package AquaEnv (41). The OPD is operationally defined as the depth below which [O$_2$] < 1 μM, and the sulfide appearance depth (SAD) is operationally defined as the depth below which [H$_2$S] > 1 μM. The diffusive oxygen uptake (DOU) was calculated from the oxygen depth profiles as in ref. 14.

Pore Water and Solid-Phase Geochemistry. Pore water was extracted from the sediment using centrifugation (15 min at 4,500 x g) and was filtered (0.45 μm) and subsampled under N$_2$. Centrifuged pore water was subsampled for sulfide, where samples were fixed with zinc acetate and stored at 4°C. For H$_2$S and pH, depth profiles were made at 200-μm resolution in the oxic zone, and 400- or 600-μm resolution below. Calibrations for pH were made with three National Bureau of Standards (NBS) standards and a Tris buffer to correct for salinity effects, and pH is reported on the total scale. For H$_2$S, a five-point calibration was made using Na$_2$S standards. Total free sulfide ($2H_2S + H_2S$) was calculated from H$_2$S based on pH measured at the same depth using the R package AquaEnv (41). The OPD is operationally defined as the depth below which [O$_2$] < 1 μM, and the sulfide appearance depth (SAD) is operationally defined as the depth below which [H$_2$S] > 1 μM. The diffusive oxygen uptake (DOU) was calculated from the oxygen depth profiles as in ref. 14.

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sulfur. AVS and chromium reducible sulfide (CRS) were quantified using iodometric titration. Solid-phase Fe phases were also extracted and separated according to ref. 45. Microscopic identification of cable bacteria was achieved by FISH, using a Desulfobulbus-species-specific oligonucleotide probe (DS8706; 5′-ACC GTT CCT CCC GAT-3′), according to Schauer et al. (27). The depth distribution of cable bacteria was quantified in March, May, August, and November 2012. Cable bacteria biomass per unit of sediment volume (in cubic millimeters per cubic centimeter) was calculated based on measured filament length and diameter, as well as the areal biomass of cable bacteria (in cubic millimeters per square centimeter) by depth integration over all eight sediment layers. For macrofauna analysis, sediment collected from eight cores (for a total surface area of ~0.02 m²) was wet-sieved (mesh size 1.0 mm) on board. Subsequently, macrofauna were carefully handpicked and preserved in 4% (vol/vol) formalin solution stained with rose bengal. All individuals were identified to species level, where possible, using a stereomicroscope (Leica MZ26), and macrofauna abundance (individuals per square meter) was calculated.

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