Transient bottom water oxygenation creates a niche for cable bacteria in long-term anoxic sediments of the Eastern Gotland Basin

# TRANSIENT BOTTOM WATER OXYGENATION CREATES A NICHE FOR CABLE BACTERIA IN LONG-TERM ANOXIC SEDIMENTS OF THE EASTERN GOTLAND BASIN

Ugo Marzocchi[1,2*], Stefano Bonaglia[3], Sebastiaan van de Velde[1,4], Per O.J. Hall[5], Andreas Schramm[6], Nils Risgaard-Petersen[2,6], and Filip J.R. Meysman[4,7]

[1] Department of Chemistry, Research group for Analytical, Environmental and Geo-Chemistry, Vrije Universiteit Brussel, Belgium.

[2] Center for Geomicrobiology, Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus, Denmark.

[3] Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden.

[4] Department of Biology, Universiteit Antwerpen, Antwerpen, Belgium.

[5] Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden.

[6] Center for Electromicrobiology, Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus, Denmark.

[7] Aarhus Institute of Advanced Sciences, Aarhus University, Aarhus, Denmark.

*Correspondence to: Ugo Marzocchi, Center for Electromicrobiology, Section for Microbiology, Department of Bioscience, Aarhus University.

Ny Munkegade 114, 8000-C Aarhus, Denmark.

Email: ugo.marzocchi@bios.au.dk

Phone: +45-87154335



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## ORIGINALITY-SIGNIFICANCE STATEMENT

This study explores the activity, distribution, and diversity of cable bacteria across a natural bottom-water oxygen gradient. Our data show that cable bacteria can live at oxygen concentration < 5 µM, thus extending the oxic range over which cable bacteria can persist towards lower values. To quantify the activity of cable bacteria, we measured electric potential anomalies in the sediment. These data reveal that the impact of cable bacteria on the electric potential can be measured with unprecedented resolution (<30 µV). We also examined the diversity of cable bacteria and showed that up to three species can co-exist at the same site.

## SUMMARY

Cable bacteria have been reported in sediments from marine and freshwater locations, but the environmental factors that regulate their growth in natural settings are not well understood. Most prominently, the physiological limit of cable bacteria in terms of oxygen availability remains poorly constrained. In this study, we investigated the presence, activity, and diversity of cable bacteria in relation to a natural gradient in bottom water oxygenation in a depth transect of the Eastern Gotland Basin (Baltic Sea).

Cable bacteria were identified by FISH at the oxic and transiently oxic sites, but not at the permanently anoxic site. Three species of the candidate genus Electrothrix i.e., marina, aarhusiensis, and communis were found coexisting within one site. The highest filament density (33 m cm-2) was associated with a 6.3 mm wide zone depleted in both oxygen and free sulphide, and the presence of an electric field resulting from the electrogenic sulphur oxidizing metabolism of cable bacteria. However, the measured filament densities and metabolic activities remained low overall, suggesting a limited impact of cable

bacteria at the basin level. The observed bottom water oxygen levels (<5 µM) are the lowest so far reported for cable bacteria, thus expanding their known environmental distribution.

## INTRODUCTION

Cable bacteria are filamentous, multicellular bacteria (*Deltaproteobacteria*, family *Desulfobulbaceae*, Trojan et al. 2016) able to conduct electric currents over centimetre distances in the seafloor, thereby coupling $O_2$ reduction to $H_2S$ oxidation (Pfeffer et al. 2012). In the absence of $O_2$ in the overlying water, $NO_3^-$ and $NO_2^-$ can act as alternative electron acceptors (Marzocchi et al. 2014; Risgaard-Petersen et al. 2014). The metabolism of cable bacteria is generally referred to as electrogenic sulphur oxidation (e-SOx; Malkin et al. 2014). Because of their profound impact on the sediment biogeochemistry, which may even extend to the overlying water, cable bacteria are considered ecosystem engineers (e.g. Seitaj et al. 2015). Their activity can generate a centimeters-thick *suboxic* zone devoid of both $O_2$ and $H_2S$. The separation of the two half-redox reactions results in a characteristic pH signature, with a subsurface pH maximum due to the acid-consuming cathodic $O_2$ or $NO_3^-$ reduction and a deeper pH minimum due to acid-forming anodic $H_2S$ oxidation (Nielsen et al. 2010). Such acidification stimulates the dissolution of FeS and Ca- and Mn-carbonates (e.g. Risgaard-Petersen et al. 2012; Rao et al. 2016; Sulu-Gambari et al. 2016a), with the possible co-release of trace metals like As, Co and Mo (Sulu-Gambari et al. 2017; van de Velde et al. 2017). The liberated $Fe^{2+}$, $Ca^{2+}$, and $Mn^{2+}$ re-precipitate at the sediment surface as Fe- and Mn-oxides and carbonates. Moreover, the conversion of iron from FeS to iron oxides can increase the retention of pore-water inorganic phosphate (Rao et al. 2016; Sulu-Gambari et al. 2016b), and delays the release of $H_2S$ from the sediment in seasonally hypoxic/anoxic basins (Seitaj et al. 2015). Finally, the metabolic activity of cable bacteria generates an electric field (Risgaard-Petersen et al. 2012; Risgaard-Petersen et al. 2014), which can influence the transport of ions in the sediment.

These electric fields have been observed using dedicated microsensors as an increase of the electric potential with depth (Damgaard et al. 2014).

Cable bacteria have now been reported from an increasing number of sites (Burdorf et al. 2017). These observations are largely derived from indirect observations, such as *ex situ* sediment enrichments or from 16S rRNA sequence databases. To date, only a few studies have documented the natural abundance of cable bacteria in coastal (Malkin et al. 2014; Seitaj et al. 2015; Burdorf et al. 2016; van de Velde et al. 2016; Malkin et al. 2017) and freshwater (Risgaard-Petersen et al. 2015) sediments. Consequently, little is known about the environmental constraints of cable bacteria, especially in terms of electron acceptor and donor availability. Moreover, brackish environments have so far been underexplored, while studies on the diversity and potential co-occurrence of different cable bacteria genotypes are entirely lacking.

High pore water concentrations of $H_2S$ and the absence of bioturbation are characteristic features of marine sediments exposed to anoxic bottom waters. Upon bottom water re-oxygenation, such sediments appear to offer a particularly suitable habitat for cable bacteria (Burdorf et al. 2017). For example, high densities and activity of cable bacteria are consistently found in Lake Grevelingen, a seasonally hypoxic coastal water body in the Netherlands, after the re-appearance of $O_2$ in the bottom water in fall (Seitaj et al. 2015). Yet, it remains unknown whether a similar population dynamic is also occurring in other marine systems, or whether cable bacteria still develop when re-oxygenation events are of lower intensity and have return times longer than seasonal.

This study focuses on the ecology of cable bacteria in the sediment of the Baltic Sea, which is the second largest (373 000 $km^2$) brackish water body in the world. Below the halocline (at approx. 80 m water depth), the water column experiences long periods of anoxia due to sustained thermohaline stratification combined with high respiration, as supported by inputs of nutrients and

organic matter from the catchment area (HELCOM 2009). Inflows of salty, oxygenated water from the North Sea can transiently re-establish oxic conditions in the bottom water of the deeper basins of the Baltic Sea. These events, named Major Baltic Inflows (MBI) are sporadic, with an approximate return time of 10 years (Mohrholz et al. 2015). The Eastern Gotland Basin (EGB) is by far the largest sub-basin of the Baltic Sea with an area of ~90 000 $km^2$ (maximum depth 249 m, average depth 75 m), and is the basin that is most affected by water column hypoxia and anoxia in the Baltic Sea (Carstensen et al. 2014). In December 2014, a MBI occurred in the EGB, interrupting a decade of bottom water stagnation. However, $O_2$ was detected in the bottom water only since February 2015, after complete depletion of $H_2S$ (Naumann et al. 2016). Since then, other secondary inflows have kept the bottom water oxygenated (at least partially and intermittently) until the middle of 2017.

In this study, we investigated the natural distribution, diversity, and activity of cable bacteria in EGB sediments in relation to the temporary oxygenation induced by $O_2$ inflow events. During two successive cruises held in April 2016 and 2017, we collected intact sediment cores from EGB sites that experienced recent bottom water oxygenation, as well as from permanently oxic and permanently anoxic control sites. The metabolic activity of cable bacteria was investigated ship-board, within a few hours after sediment retrieval, by application of microsensor depth profiling. Additionally, fluorescence *in situ* hybridization (FISH) was applied to explore cable bacteria abundance and diversity.

## RESULTS

### Bottom water oxygenation in April 2016 and sediment appearance

Station locations and bottom water conditions (i.e., salinity, temperature, $O_2$, and $NO_x^-$ concentration) are reported in Figure 1 and Table 1. In April 2016, station A (permanently oxic)

was located within the surface mixed layer (Fig. S1), and the bottom water showed $O_2$ levels close to air saturation (330-350 µM), low salinity (7.3), and low temperature (3.8°C). Station D (permanently anoxic) was located below the pycnocline (Fig. S1), and showed higher salinity (12.6) and higher temperature (6.6°C), and almost no detectable $O_2$ (0 - 3 µM). At the deeper stations (E and F, long-term anoxic bottom water that becomes transiently hypoxic during MBI), bottom water $O_2$ levels were 10 – 15 µM at station E and to 20 – 25 µM at station F, as a result of the MBI that had started in spring 2015 (Fig. S2).

Sediment from station A showed two distinct layers with orange-yellow sand overlying the gray clay (Fig. S3). Three specimens of the burrowing isopod *Saduria entomon* and a few *Marenzelleria sp.* burrows were identified on the sediment surface in the box corer (surface area 0.78 $m^{-2}$). Sediment from stations D, E, and F was very similar in appearance, and showed a clear lamination with an alternation of brown, grey, and black bands, and no signs of bioturbation activity (Fig. S3).

**$O_2$, pH, $\Sigma H_2S$, and Electric Potential sediment depth profiles in April 2016**

At station A, the oxygen penetration depth (OPD) was 5.0 ± 0.4 mm (mean ± standard deviation, n = 3) (Fig. 2). Total hydrogen sulphide concentration ($\Sigma H_2S = [H_2S] + [HS^-] + [S^{2-}]$) remained below detection in the top 10-15 mm of sediment, and only increased to 2 µM at 15-20 mm depth. The pH was 8.1 at the sediment-water interface, decreased with depth and stabilized around pH~7 below 5 mm depth. At station D, $\Sigma H_2S$ increased right below the sediment-water interface, and accumulated to 150 µM at ~5 mm depth (though with variability between replicate profiles). The $O_2$ in the overlying water as measured by microsensor profiling was 6.5 µM and the OPD was 0.8 ± 0.4 mm (n = 5) resulting in a zone with $O_2$ and $\Sigma H_2S$ being simultaneously present. At station E, the OPD was 1.0 ± 0.5 (n = 5) mm and $\Sigma H_2S$ was detected (> 1.0 µM) at 7.3 ± 2.3 (n =

3) mm, resulting in a suboxic zone of 6.3 mm. At station F, $\Sigma H_2S$ appeared at 0.8 ± 0.6 (n = 7) mm depth, while the OPD was 2.1 ± 0.3 (n = 5) mm. All six replicate pH microsensor profiles at station F showed a maximum within the first 2.2 mm of the sediment (see Fig. S4 for close up of pH maxima). The maximum pH increase ranged between 0.02 and 0.09 pH units as compared to bottom water values. Oxygen concentrations in the overlying water as recorded by microsensor depth profiling were slightly higher than the *in situ* level reported in Table 1. This discrepancy was likely due to $O_2$ contamination during the *ex situ* incubation procedure.

In 2016, sediment electric potential (EP) profiles were only measured at station A (due to technical problems at other stations). Here, with the overlying water at air saturation, EP gradually increased with depth from the sediment surface (Fig. 3A). The maximum electric potential (100 µV) was measured at 17.6 mm depth. The residual potential (i.e. the EP signal not associated with cable bacteria-mediated long distance electron transfer, LDET) was measured by making the overlying water anoxic and showed a similar trend with depth, though with a lower range (0 - 76 µV; Fig. 3A). The LDET-associated EP (i.e., the difference between profiles measured with oxic and anoxic overlying water) increased within the first 5 mm and remained relatively constant (i.e. 29 ± 0.6 µV; mean ± SD, n = 33) in the depth interval 5 – 18 mm. In a different core, the reversibility of the procedure was tested. To this end, the overlying water of the sediment was made anoxic for a period of 1 hour. Immediately after the re-establishment of oxic conditions in the overlying water, we observed a rapid recovery of the EP down to 1.9 cm into the sediment (Fig. 3B). In this second core, a slightly larger difference between oxic and anoxic potential (50 ± 10 µV) was measured in the depth interval 13.2 – 18.4 mm. The current density as estimated from the electric field and the sediment conductivity was 0.09 mA $m^{-2}$.

**Cable bacteria density and diversity in April 2016**

In 2016 at station A, cable bacteria were detected in the depth range 0 – 25 mm (Fig. 2 A). The total filament density (as determined by FISH probe DSB706) ranged between below detection limit (i.e. < 1.9 m $cm^{-3}$) and 11 m $cm^{-3}$, with no clear depth trend. Depth integrated abundance was 17 m $cm^{-2}$. In the top sediment layer, total cable bacteria density was 6.4 ± 2.6 m $cm^{-3}$ (mean ± SD; n = 3). No cable bacteria were detected at the anoxic station D (Fig. 2 D), while the highest density of cable bacteria filaments was measured at station E. Here, densities peaked in the 0 – 5 mm depth interval (27 ± 16 m $cm^{-3}$), whilst smaller densities were measured down to 15 mm depth (Fig. 2 E). Depth integrated abundance was 27 m $cm^{-2}$. At station F, cable bacteria were detected in the 0 to 15 mm depth interval (Fig. 2 F). The maximum density (12 ± 5 m $cm^{-3}$) was measured in the top 5 mm. Depth integrated abundance was 7.1 m $cm^{-2}$.

The application of species-specific FISH probes revealed the presence of three species belonging to the genus *Ca.* Electrothrix in the EGB sediment. *Ca.* E. aarhusiensis was the most abundant species, accounting for 32% and 52% of all cable bacteria at stations A and F, respectively, and dominating the cable bacteria population at station E (78%), where all three species were present. *Ca.* E. communis accounted for a substantial fraction of the population at both stations A (68%) and F (48%), but at station E accounted only for the 14% of all cable bacteria. *Ca.* E. marina was only detected at station E, and only in low numbers (8% of all cable bacteria). None of the two species belonging to the genus *Ca.* Electronema were found at the investigated stations.

**Bottom water oxygenation in April 2017**

In 2017, the surface mixed layer at station A was compressed compared to 2016 and the pycnocline intercepted the sediment (Fig. S1). Bottom water $O_2$ ranged between 120 and 140 µM over the 11 hour lander deployment, temperature was 5.1°C and salinity was 8.9 (Table 1). Small

but ecologically relevant changes were detected in the $O_2$ levels of the bottom water at stations D and F compared to April 2016. At station D, bottom water shifted from anoxic to hypoxic, while at station F the bottom water changed from hypoxic to anoxic (Table 1). At station D, bottom water $O_2$ was 7 – 9 µM. This increase in $O_2$ concentration compared to 2016 was likely caused by a secondary, shallower, inflow event, which increased the $O_2$ levels between 60 and 160 m depth (Fig. S1). In contrast, at both stations E and F, the bottom water $O_2$ levels were lower compared to 2016 (decreasing to 0 – 5 µM and to 0 µM, respectively) (Table 1).

**$O_2$, pH, $\Sigma H_2S$, and EP sediment depth profiles in April 2017**

At station A, $O_2$ concentration in the overlying water was 189 µM and the OPD was 4.0 ± 0.04 mm (n = 4) (Fig. 4 A). As in 2016, $O_2$ concentrations in the overlying water recorded during microsensor profiling exceeded the *in situ* values reported in Table 1. As noted above, this discrepancy was likely due to $O_2$ infiltration during the *ex situ* incubation procedure. pH decreased from 7.7 in the overlying water level to 7.1 – 7.2 at approx. 5 mm depth. One replicate out of three showed a pH maximum (7.8) at the depth of 1.2 mm. At station D, the $O_2$ concentration in the overlying water as recorded by microsensor profiling was 25 µM and the OPD was 1.3 ± 0.5 mm (n = 4) (Fig. 4 D). In all three replicates, pH slightly increased with depth, reaching a subsurface maximum of 7.7 at a depth of 2.4 mm (overlying water pH: 7.6). Deeper in the sediment, pH values decreased and stabilized at 7.2. Free sulphide became detectable ($\Sigma H_2S$ >1 µM) at 0.8 mm depth. At station E, $O_2$ in the overlying water was 17 µM, and the OPD was 1.2 ± 0.6 mm (n = 3) (Fig. 4 E). The pH peaked (7.8) at 1.2 mm and a pH minimum (7.2) was located near 8 mm depth. The $\Sigma H_2S$ concentration remained below detection level to a depth of 5.4 mm. At station F, the OPD was 1.2 ± 0.2 (n = 4) mm (Fig. 4 F). All three pH replicates showed small peaks (+ 0.07 pH units as compared

to overlying water level) at the depth of 3.2 mm. The sediment became sulphidic below 0.8 mm depth.

In 2017, the electric potential (EP) was recorded at three station (D, E, and F). At station D, the EP increased with depth and stabilized at 8 mm to about 0.7 mV (Fig. 5, left panel). The residual EP profile was measured after pulling a tungsten wire across the sediment section to interrupt LDET. After the cut, the EP profile at station D showed a slight decrease in the top 10 mm (-0.23 ± 0.11 mV; mean ± SD; n = 23). At station E, an electric field was measured before the cut to a depth of 12.5 mm, where the EP reached 0.8 mV (Fig. 5, central panel). After the cut, the field almost completely collapsed. The estimated current density was 81 mA $m^{-2}$. At station F, the EP profiles measured before and after the cut had a similar convex shape with maxima of 0.3 mV at 9 mm depth. Hence, there was no indication of any residual EP signal connected to LDET (Figure 5, right panel).

**Cable bacteria occurrence and density in April 2017**

At station A, cable bacteria were detected within the depth interval 5 – 25 mm (Fig. 4, panel A), though with low total filament densities (as estimated using the general DSB706 probe), which ranged between from below detection limit (i.e. <1.9 m $cm^{-3}$) up to 5.3 m $cm^{-3}$, and showed no clear depth trend. Depth integrated abundance was 5.7 m $cm^{-2}$. At station D, cable bacteria were detected only in the top 0 –5 mm depth interval (density = 2.6 m $cm^{-3}$) (Fig. 4, panel D), and the depth integrated abundance was 1.3 m $cm^{-2}$. The highest density of cable bacteria (42 m $cm^{-3}$) was measured at station E in the 0 – 5 mm depth interval (Fig. 4, panel E). Densities were 4.5 and 22 m $cm^{-3}$ in the 5 – 10 and 10 – 15 mm intervals, respectively. Depth integrated abundance was 33 m $cm^{-2}$. At station F, cable bacteria density was 2.3 $cm^{-3}$ in the 5 - 10 and 10 -15 mm depth intervals (Fig. 4, panel F). Depth integrated abundance was 2.3 m $cm^{-2}$.

## DISCUSSION

### Cable bacteria in the sediment of the EGB basin

Our data demonstrate the *in situ* presence and activity of cable bacteria in brackish sediments (salinity 7-14) exposed to low $O_2$ concentrations. In 2016, the detection of cable bacteria at stations E and F, but not at the long-term anoxic station D, indicates the ability of these organisms to colonize sulphide-rich sediment upon re-oxygenation events such as those induced by an MBI in 2014/2015. In 2017, the low bottom water $O_2$ concentration recorded at station E was sufficient to maintain cable bacteria at densities similar to the ones observed in 2016, suggesting that the population was in a stationary phase. The *in situ* $O_2$ level recorded at station E in 2017 (≤5 µM) is the lowest so far reported for cable bacteria. Previously, cable bacteria have been reported at bottom water $O_2$ concentration as low as 100 µM (Seitaj et al. 2015), while laboratory sediment incubations have shown a lower $O_2$ limit of 50 µM (Burdorf et al. 2018). Our observation extends the range of bottom water $O_2$ concentrations that allow for the persistence of cable bacteria to the low end of the hypoxic interval.

In the absence of $O_2$, cable bacteria can use $NO_3^-$ as an electron acceptor (Marzocchi et al. 2014). However, their growth under anoxic $NO_3^-$ amended conditions has been shown to be slower (Marzocchi et al. 2014), indicating that $O_2$ is the favoured electron acceptor. Thus, as long as $O_2$ is available, $NO_3^-$ levels are not expected to play an important role in the distribution of cable bacteria. From the present dataset, we cannot determine to what extent $NO_3^-$ availability alone has influenced cable bacteria growth. At station A, the $O_2$ concentration was 35-fold higher than that of $NO_3^-$ and thus likely, $NO_3^-$ was not important. At the other stations, we cannot exclude that $NO_3^-$ is co-used with $O_2$, however this is not possible to ascertain. As $NO_3^-$ levels varied along with $O_2$ levels at

stations D, E, and F (indicating that $NO_3^-$ is linked with nitrification activity), it is impossible to decouple the effects of the two electron acceptors.

Densities of cable bacteria measured in the EGB sediment (1.3 – 33 m $cm^{-2}$) are within the range of *in situ* values reported from freshwater (40 m $cm^{-2}$, Risgaard-Petersen et al. 2015), mangroves (77 m $cm^{-2}$, Burdorf et al. 2016), and marine bioturbated sediments (0.15 – 56 m $cm^{-2}$, Malkin et al. 2017). They are however substantially lower as compared to other coastal sites with cohesive sediments, i.e., the enclosed seasonal hypoxic water body of Lake Grevelingen (402 – 480 m $cm^{-2}$, Seitaj et al. 2015), a subtidal mud accumulation site (168 – 352 m $cm^{-2}$, van de Velde et al. 2016), and a mussel bed (58 – 1038 m $cm^{-2}$, Malkin et al. 2017). Notably, in all these sites, bottom water $O_2$ level was between 100 and 350 µM, whereas with the exception of station A, all stations investigated here had $O_2$ levels ranging between zero and 30 µM, and $NO_3^-$ ranging between zero and 12 µM (Tab. 1). Cable bacteria activity has been shown to decrease in response to decreasing $O_2$ concentrations (Burdorf et al. 2018). It is thus plausible that the relatively low densities found at stations D, E, and F reflect an electron acceptor limitation, and that higher densities might be present during conditions of higher $O_2$ availability (e.g. in the earlier phase of the MBI).

In April 2017, the bottom water at station D showed a slightly higher oxygenation compared to the year before, which exceeded the $O_2$ levels at station E; this stimulated cable bacteria growth, although the abundance was very low. At the low $O_2$ concentrations observed at station D ($\leq$ 3 µM in 2016 and $\leq$ 9 µM in 2017), cable bacteria might have a long lag phase in their development, or alternatively, cable bacteria can only persist at low $O_2$ after having first established a population at much higher $O_2$ levels.

At station A, the cable bacteria density was low in spite of the high bottom water $O_2$ concentrations. Similarly, the current density induced by cable bacteria activity (0.09 mA $m^{-2}$) was two to three orders of magnitude lower compared to the two so far reported *in situ* estimates of

LDET rates based on EP microprofiles, i.e., 14 mA $m^{-2}$ (in freshwater sediment, Risgaard-Petersen et al. 2015) and 412 mA $m^{-2}$ (in marine sediment, Burdorf et al. 2017). Moreover, the current density at station A is also two orders of magnitude lower compared to other *in situ* estimates of LDET rates based on pH and alkalinity profiles, i.e., 4.6 – 30 mA $m^{-2}$ (Malkin et al. 2014) and 25 – 32 mA $m^{-2}$ (van de Velde et al. 2016). Low filament densities and low current densities hence suggest an environmental constraint to the development of the cable bacteria population. At station A, stable $O_2$ and salinity levels allow for benthic organisms to steadily colonize and rework the sediment (HELCOM 1996). Sediment reworking as induced by the lugworm *Arenicola marina* has been previously proposed to reduce (Malkin et al. 2014), but not fully impede (Burdorf et al. 2017) cable bacteria activity. Similarly, infaunal activity may limit the development of highly dense cable bacteria population in the shallower sections of the EGB. Continuous sediment mixing as induced by the active predator *S. entomon* (Ejdung and Bonsdorff 1992) found at station A is expected to exert a higher disturbance on cable bacteria development compared to *A. marina* that can inhabit the same burrow for months (Wells 1949).

FISH analysis revealed the co-existence of up to three species at the same location. Such diversity associated with a relatively low total cable bacterial density indicates a non-exclusive competition between cable bacteria species, even under $O_2$ limiting conditions. Alternatively, the transitional nature of the oxic niche in the deep sediment of the EGB might have prevented the onset of exclusive dominances. While the co-existence of *Ca.* E. marina and *Ca.* E. communis was previously reported from sediment enrichment cultures (Trojan et al. 2016), in our study *Ca.* E. aarhusiensis was always present and seemed even to dominate in the low-oxygen sites. Although this distribution may indicate a particular adaptation of *Ca.* E. aarhusiensis to low oxygen conditions, the high level of uncertainty associated with the individual FISH counts (Figure 2) prevents us from further speculate on the relative importance and ecological niche of the various

cable bacteria species. The detection of all three species of the genus *Ca.* Electrothrix (typically found in marine environments) in the EGB sediment with salinity ranging between 7.3 and 13.6 confirms its wide salinity tolerance as suggested by Trojan et al. (2016). The absence of the genus *Ca.* Electronema, which was reported from freshwater sites within the Baltic Sea catchment area (Risgaard-Petersen et al. 2015; Trojan et al. 2016), further indicates a more restricted salinity tolerance of this genus.

**Impact on sediment biogeochemistry**

In both years, the higher densities of cable bacteria at station E were associated with the onset of a 6.3 – 6.8 mm wide zone devoid of both $O_2$ and $H_2S$, which hence suggests that the cable bacteria population had a noticeable impact on the sediment biogeochemistry at station E. This is confirmed by two other types of evidence for the occurrence of electrogenic sulphur oxidation (e-SOx) by cable bacteria. First, in 2017, the pH depth profile showed the typical pH excursions (subsurface maximum and minimum) that are associated with the spatial segregation of redox half-reactions during e-SOx. Secondly, the establishment of an electric field further indicates the presence of electric currents running over the same depth interval.

At station F in both 2016 and 2017 as well as at station D in 2017, cable bacteria density was lower and no suboxic zone was detected. However, small pH maxima (pH increased on average by 0.05 units from the water column value) were systematically measured at the transition between the oxic and the sulphidic zones. Although such subsurface pH maxima have been considered a hallmark of e-SOx, we cannot unequivocally attribute these pH maxima to cable bacteria activity, as a pH increase of this magnitude could also be induced by other processes. For example, Rao et al. (2016) attributed small pH maxima to alkalinity generation during the incomplete (non-electrogenic) oxidation of $H_2S$ to elemental sulphur (i.e. $HS^- + ½O_2 + H^+ = S^0 + H_2O$). At station D

in 2017, the higher EP signal measured in the intact core in the top 0.7 mm compared to the cut core might indicate LDET; however, the large variability associated with these EP measurements prevents us from conclusively attribute the observed EP difference to cable bacteria activity. At station F, the close overlap between EP profiles measured before and after the cut indicated that LDET was not active. The convex shape of the profiles has thus to be attributed to diffusion potentials, probably linked to the variations in bottom water salinity associated with the MBI that have occurred since June 2014 (Fig. S2). Low filament densities and the lack of a clear geochemical fingerprint (as recorded by $O_2$, $H_2S$, pH and EP profiling) suggest a negligible impact of the cable bacteria on the sediment geochemistry at stations D and F at the time of measurement.

Previous field studies in a seasonal hypoxic coastal basin (Lake Grevelingen, The Netherlands) have shown that electrogenic sulphur oxidation by cable bacteria has significant indirect impacts on Fe, Mn, and P cycling (Seitaj et al. 2015; Sulu-Gambari et al. 2016a; Sulu-Gambari et al. 2016b). Yet, an important difference is the lower cable bacteria densities (1.3 - 33 m $cm^{-2}$) at our sites as compared to those reported from Lake Grevelingen (i.e. 402 - 480 m $cm^{-2}$). Thus with the exception of station E, both our data on abundance (FISH) and activity (microsensor profiling) indicate no major role for LDET. Thus overall, the impact of e-SOx on the elemental cycling in the EGB appears not to be strong.

**Summary and implications**

In this study, we showed that cable bacteria can take advantage of transient hypoxic niches as induced by Major Baltic Inflow in the deep Eastern Gotland Basin sediment. This finding indicates that cable bacteria can tolerate a low electron acceptor ($O_2$ and $NO_3^-$) availability, which expands our understanding of the conditions suitable for cable bacteria occurrence. Environments with low $O_2$ and $NO_3^-$ availability could be analogous to the ones that favoured the evolution of this

lineage within the *Desulfobulbaceae* from typical anaerobic sulphate reducers to (micro)aerophilic electrogenic sulphide oxidizers.

In addition, our data report the *in situ* diversity and natural abundance of cable bacteria in brackish environments. In spite of a generally low cable bacteria density, three different species co-existed in the EGB sediment. At these intermediate salinities, only marine species were detected while freshwater species were absent, suggesting a niche partitioning between the two candidate genera.

Overall, only one site in the EGB showed microprofiles compatible with cable bacteria activity, this was observed in both years. Low densities and the lack of typical geochemical imprints at the other sites suggest a negligible impact of cable bacteria activity on the sedimentary elemental cycling at the basin level.

## EXPERIMENTAL PROCEDURES

### Sediment sampling and incubations

Two research cruises were conducted in April 2016 and April 2017 on board of R/V Skagerak. Each time, sediment and bottom water samples were collected at four stations along a depth gradient in the East Gotland Basin (Fig. 1). Temperature, salinity and oxygen depth profiles in the water column were obtained by CTD casting (SBE911, SeaBird Electronics, USA). Bottom water $O_2$ concentrations were additionally measured by an $O_2$ sensor (AADI 3830. AANDERAA, Norway) connected to a lander frame and standing at 0.1 - 0.3 m from the seafloor.

At each station, bottom water (3 - 5 m above the seafloor) was sampled via a Rosette sampler carrying 10 L Niskin bottles. Sediment was sampled via a modified box corer (W x D x H : 28 cm x 28 cm x 50 cm) that allowed the retrieval of soft sediment with minimal disturbance (Blomqvist et al. 2015). On deck, the sediment in the box core was subsampled with PVC core

liners (length: 30 cm, inner diameter: 6.0 cm), and subsequently sediment cores were transferred into an aquarium containing bottom water at *in situ* temperature. Oxygen was maintained at *in situ* levels by flushing the aquarium water with a mixture of air and $N_2/CO_2$ ($[CO_2]$ = 400 ppm). The $O_2$ concentration and temperature of the aquarium water was continuously monitored by an oxygen optode (AADI 3830, AANDERAA, Norway) connected to a computer, thus controlling the gas supply and oxygenation. In 2016, this procedure resulted in a shift of the overlying water pH from the *in situ* value of 7.5 – 7.7 to a pH value of 8.0. To overcome this issue, the flushing of the aquarium was not applied in 2017. This procedure allowed to maintain pH close to the *in situ* level, but resulted in a slight increase in the $O_2$ concentration. Such an increase, however, was considered of no influence for the purpose of the study. At each station, within a few hours of sediment retrieval, 3 - 4 replicate cores were depth profiled with microsensors. One of these profiled cores was then sectioned for quantification of the cable bacteria density by fluorescence *in situ* hybridization (FISH).

**Microsensor measurements**

High-resolution depth profiles of $H_2S$, $O_2$, pH, and the electric potential (EP) were recorded on board R/V Skagerak with micro-electrodes built at Aarhus University (Revsbech and Jorgensen 1986; Revsbech 1989; Jeroschewski et al. 1996; Damgaard et al. 2014). The microsensors were mounted on a motor-driven micromanipulator and depth profiles were recorded at 100 - 400 µm vertical resolution (≥3 depth profiles for each analyte). The sensor tip was manually positioned at the sediment surface, while observing it through a horizontal dissection microscope. Oxygen, $H_2S$ and pH sensors were connected to a 4-channel multimeter (Unisense, Denmark). A reference electrode (REF201 Red Rod electrode; Radiometer Analytical, Denmark) was used for pH and EP measurements. For EP measurements, the sensor and the reference electrode were connected to a

custom-made voltmeter with high internal resistance (>$10^{14}$ Ω) connected to a 16-bit analog-to-digital converter (AD216, Unisense, Denmark).

A two-point calibration was applied for the $O_2$ sensor using the signal in the overlying water in the aquarium (with known air saturation level as measured by the optode) and in the anoxic sediment (0% air saturation). The $H_2S$ sensor was calibrated in an $O_2$-free HCl solution (~100 mL) by stepwise addition of 500 µL of a 10 mM $Na_2S$ stock solution. The exact $H_2S$ concentration at each calibration step was determined after Zn-Acetate fixation using the Cline (1969) method. Total hydrogen sulphide ($\Sigma H_2S = [H_2S] + [HS^-] + [S^{2-}]$) concentrations were calculated at each depth from the measured $H_2S$ and pH values (Jeroschewski et al. 1996). The pH is reported on the NBS scale and the pH sensor was calibrated using 4.0, 7.0, and 10.0 NBS buffers (AVS TITRINORM, VWR Chemicals, Denmark).

The principle of EP sediment profiling and its application to quantify cable bacteria activity has been described in great detail in Damgaard et al. (2014) and in Risgaard-Petersen et al. (2014). Briefly, EP profiles in the sediment can develop from LDET (i.e., spatial segregated redox half-reactions as induced by metabolic activity of cable bacteria) as well as due to concentration gradients of major ions in the pore water (i.e., diffusion potentials that arise by the difference in diffusional speed of ionic species). These two phenomena can be distinguished from each other by perturbation experiments. For instance, the contribution of LDET to the sediment EP can be identified as the rapid drop in EP when LDET is impeded (Risgaard-Petersen et al. 2014; Risgaard-Petersen et al. 2015). This can be achieved by excluding electron acceptors from the overlying water (removal of $O_2$ from the overlying water by $N_2$ flushing), or alternatively by pulling a wire through the core section below the oxic zone and thus physically disrupting the cable bacteria filaments. During the 2016 campaign, EP depth profiles were measured in the presence and absence of $O_2$ in the overlying water at station A. In 2017, EP depth profiles were measured in stations D, E,

and F after pulling a wire through the core section at a depth of 3 - 4 mm. For comparison, both procedures were applied at station E, leading to the same result. The electric field *E* was determined from the LDET-linked EP signal as $-d\psi_{ox}/dx - d\psi_{anox}/dx$ where $d\psi_{ox}/dx$ and $d\psi_{anox}/dx$ are the gradients of EP near the sediment-water interface, in presence and absence of $O_2$ in the overlying water, respectively (or when the cut was applied, before and after the cut, respectively). The current density *J* (the LDET current per unit of sediment area, in mA $m^{-2}$) was calculated from the electric field *E* and the sediment conductivity by means of Ohm's law, $J = \sigma_{sed} * E$ (Risgaard-Petersen et al. 2014). The conductivity of the overlying water $\sigma_W$ was determined as a function of salinity and temperature using the relationship as in (Weyl 1964). Sediment conductivity $\sigma_{sed} = (\phi/\theta^2)*\sigma_W$ was estimated from overlying water conductivity after correction for porosity $\phi$ and tortuosity $\theta^2 = 1-2*\ln(\phi)$ (Damgaard et al. 2014).

**Fluorescence in situ hybridization (FISH)**

One of the cores used for microsensor profiling was sectioned at 5 mm intervals to a depth of 3 cm for quantification of cable bacteria density by FISH. From each depth interval, 0.5 mL of homogenized sediment was transferred into a 2 mL Eppendorf tube and fixed with 0.5 mL of ethanol (99.8 % molecular grade). Samples were kept frozen at -20°C until further analysis. Once in the laboratory, samples were homogenized by mild ultrasonic treatment 3 × 20 s with 10 s between cycles (SonopulsHD 2070; Bandelin) and subsamples of 100 µl were transferred to 900 µl of 0.1% sodium pyrophosphate-buffer. FISH was performed according to previously published protocols (Pernthaler et al. 2001). Cable bacteria were detected with probe DSB706 (Loy et al. 2002), targeting most *Desulfobulbaceae* as previously described (Pfeffer et al. 2012). For each sample, filament length densities (i.e., meters of filament per $cm^3$ of sediment) were estimated using the line-intercept method (Newman 1966) as previously described for cable bacteria (Pfeffer et al.

2012). Microscopic analyses were carried out on an Axiovert 200M epifluorescence microscope (Carl Zeiss, Göttingen, Germany). The topmost sediment layer, where cable bacteria densities generally were highest, were analysed in triplicate; the subsequent deeper samples were not replicated. In the topmost sediment layer, the five so far known cable bacteria species were identified using species-specific probes as described in (Trojan et al. 2016), i.e. *Ca.* Electrotrhix marina (probe: EXma1271-CY3), *Ca.* Electrothrix aarhusiensis (EXaa430-FAM), *Ca.* Electrothrix communis (EXco1016-CY3), *Ca.* Electronema nielsienii (ENni1437-CY3), and *Ca.* Electronema palustris (ENpa1421-FAM) . Sediment subsamples were treated with either two or one species-specific probe i.e. EXma1271-CY3 + EXaa430-FAM; or EXco1016-FAM + ENpa1421-CY3; or ENni1437-CY3. Subsamples were counterstained with the general Desulfobulbaceae probe DSB706 and the general DNA stain ‘4,6-diamidino-2-phenylindole (DAPI). Species-specific length densities were determined for each probe separately.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.



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**FIGURE LEGENDS**

Table 1. Bottom water temperature, salinity, $O_2$ and $NO_x^-$ ($NO_3^-$ + $NO_2^-$) concentration measured at each station in April 2016 and 2017. Temperature, salinity, and $O_2$ were measured by a CTD unit mounted on a lander. Oxygen ranges show minimum and maximum values measured by the oxygen sensor throughout the deployment (> 11 h). Nitrate + nitrite samples were collected via automated syringes installed onto the lander frame.

Figure 1. Gotland Island and the Eastern Gotland Basin. Sampling stations are indicated as blue dots. Color scale on the right indicates water depth.

Figure 2. Panel A: sediment microprofiles and cable bacteria densities at the four stations in 2016. Oxygen (blue), pH (red), and $H_2S$ (black) lines indicate sediment microprofiles at (from left to right) station A, D, E, and F. Dotted lines indicate single microprofiles, bold lines indicate average microprofiles. Grey bars show total cable bacteria densities integrated over 5 mm depth intervals. In the top 5 mm depth interval, specific probes were applied to quantify the densities of single species. Green bars indicate *Ca.* El, aarhusiensis. Magenta bars indicate *Ca.* El. communis. Orange bars indicate *Ca.* El. marina.

Panel B-C: FISH micrographs of cable bacteria. (B) *Ca.* Electrothrix aarhusiensis hybridized with probe EXco1016-FAM (green). (C) *Ca.* Electrothrix communis, hybridized with probe EXaa430-CY3 (red).

Figure 3. Electric potential (EP) microprofile (mean ± s.e.m., n = 3) in sediment from station A, in 2016. Panel A shows EP distribution measured in sediment with *in situ* overlying water at ambient $O_2$ level (white squares) and immediately after the water was turned anoxic (black squares). To illustrate the immediate reversibility of the process, panel B shows the EP distribution in an additional sediment core where the *in situ* overlying water was first turned anoxic (black squares) and then re-aerated to reach $O_2$ ambient level (white squares).

Figure 4. Sediment microprofiles and cable bacteria densities at the four stations in 2017. Oxygen (blue), pH (red), and $H_2S$ (black) lines indicate sediment microprofiles at (from left to right) station A, D, E, and F. Dotted lines indicate single microprofiles, bold lines indicate average microprofiles. Grey bars show cable bacteria densities integrated over 5 mm depth intervals.

Figure 5. Electric potential (EP) microprofiles (mean ± s.e.m., n = 3) in sediment from stations D, E, and F, in 2017, measured before (white squares) and immediately after the sediment was cut at the depth of 3 - 4 mm (black squares) by pulling a tungsten wire across the core section.

| Coordinates | | | 2016 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Station* | *Longitude* | *Latitude* | *Temp. (°C)* | *Salinity* | *$O_2$ (µM)* | *$NO_x^-$ (µM)* | *Temp. (°C)* | *Salinity* | *$O_2$ (µM)* | *$NO_x^-$ (µM)* |
| *A (60 m)* | 19°04’951 | 57°23’106 | 3.8 | 7.3 | 330 - 350 | 9.7 | 5.1 | 8.9 | 120 - 140 | 6.0 |
| *D (130 m)* | 19°19’414 | 57°19’671 | 6.6 | 12.6 | 0 – 3 | 0.0 | 6.7 | 12.7 | 7 - 9 | 7.8 |
| *E (170 m)* | 19°30’451 | 57°07’518 | 6.8 | 13.0 | 10 - 15 | 8.4 | 6.9 | 13.1 | 0 - 5 | 2.9 |
| *F (210 m)* | 19°48’035 | 57°17’225 | 7.4 | 13.8 | 25 - 30 | 12.4 | 7.3 | 13.5 | 0 | 0.0 |