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The effect of oxygen availability on long-distance electron transport in marine sediments

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ABSTRACT

Cable bacteria are long, multicellular, filamentous bacteria that can conduct electrons over centimeter distances in marine and freshwater sediments. Recent studies indicate that cable bacteria are widely present in many coastal environments, where they exert a major influence on the biogeochemistry of the sediment. Their energy metabolism is based on the aerobic oxidation of sulfide, and hence to better understand their natural occurrence and distribution, we examined the growth and activity of cable bacteria in relation to bottom water oxygenation. To this end, we conducted laboratory sediment incubations at four different O\textsubscript{2} levels in the overlying water (10, 20, 40, and 100 % air saturation). The abundance of cable bacteria was determined by fluorescence in situ hybridisation, while their activity was quantified via microsensor profiling and geochemical pore water analysis. Cable bacteria did not develop in the 10% O\textsubscript{2} incubation, but were present and active in all higher O\textsubscript{2} levels. These data show that microbial long-distance electron transport is not limited to fully oxic environment, but can occur under a wide range of bottom water oxygen concentrations. However, the growth rate was notably slower at lower oxygen concentrations, suggesting a reduced metabolic activity of the population when the O\textsubscript{2} supply becomes restricted. Finally, in response to lower O\textsubscript{2} levels, cable bacteria filaments appear to partially crawl out of the sediment and extend into the overlying water, likely to enhance their oxygen supply.
Introduction

In 2010, close inspection of the geochemistry of marine sediments unveiled the existence of electrical currents in the top centimeters (Nielsen et al. 2010). Subsequently, it was discovered that these electrical currents were mediated by “cable bacteria”, which take advantage of long-distance electron transport (LDET) for their sulfur oxidizing metabolism (Pfeffer et al. 2012). Similar to trees on land, which have evolved transport vessels to connect two spatially separated metabolic resources (i.e. nutrients and water from the ground and light from the sky), cable bacteria bridge the distance between two key resources for their energy metabolism (i.e. oxygen and sulfide) by means of electron transport. The bottom cells of the cable filament receive electrons from the oxidation of sulfide, which is abundant in the deeper anoxic zone of the sediment, being the end product from sulfate reduction, which can be responsible for 50 - 90% of the total carbon mineralization in coastal sediments (Jørgensen 1982; Kristensen et al. 2008). The electrons derived from sulfide oxidation are transported from cell to cell to the oxic zone, where they are used in the reduction of oxygen (the overall process is called electrogenic sulfur oxidation or e-SOx). The flow of electrons through the cable bacteria is accompanied by a counter-directional flow of cations through the sediment pore water (Risgaard-Petersen et al. 2015), and so the sediment is effectively transformed into a biogeobattery with an anode (sulfide oxidation reaction) and a cathode (oxygen reduction reaction).

Electrogenic sulfur oxidation has recently been found in a wide variety of habitats in marine and freshwater environments across the globe (Malkin et al. 2014; Risgaard-Petersen et al. 2015; Burdorf et al. 2017). Productive coastal sites, characterized by high organic matter loading and a (periodic) absence of bioturbation seem to be a particularly suitable habitat for cable bacteria. More specifically, e-SOx activity has been demonstrated to occur in intertidal flats (Malkin et al. 2014), bivalve reefs (Malkin et al. 2017; Burdorf et al. 2017), mangrove...
sediments (Burdorf et al. 2016), seasonally oxygen depleted marine basins (Sayama 2011; Malkin et al. 2014; Seitaj et al. 2015), salt marshes (Malkin et al. 2014) and fjord sediments (Burdorf et al. 2017). The distribution of LDET in freshwater sediments is less well characterized, but Risgaard-Petersen et al. (2015) demonstrated the process in a small river, and Müller et al. (2016) reported the presence of cable bacteria in a polluted aquifer.

Given the widespread distribution of long-distance electron transport in marine sediments (Burdorf et al. 2017), and its large effect on the sediment geochemistry (Risgaard-Petersen et al. 2012; Meysman et al. 2015), it is important to unravel the environmental controls that constrain its distribution within in the seafloor. At present however, the metabolism, ecological niche and basic life history traits of the cable bacteria that perform LDET are not well characterized. Foremost, a suitable supply of the electron donor (sulfide) and the electron acceptor (oxygen or nitrate) appear to be key requirements (Nielsen et al. 2010; Marzocchi et al. 2014; Malkin et al. 2014; Meysman et al. 2015). Furthermore, Malkin et al. (2014) speculated that an additional environmental control on the natural distribution of cable bacteria could be the presence of bioturbating fauna. However, the mechanism underlying this control is not yet clear, and cable bacteria have been reported to occur in bioturbated sediments (Burdorf et al., 2017).

To better understand the distribution of LDET in the present ocean as well as on geological timescales, this study examines the influence of the oxygen on the population growth of cable bacteria. To this end, the development of cable bacteria was tracked in long-term sediment incubations in which the overlying water was poised at different oxygen levels. We enumerated the density of cable bacteria by a molecular staining and microscopy, and quantified the metabolic activity through microsensor profiling and geochemical pore water analysis.
Material and Methods

Sediment collection and preparation

Sediment was collected in February 2013 in Lake Grevelingen, a seasonally hypoxic saline water body in the Rhine-Meuse-Scheldt delta in the south-west of The Netherlands. Previous studies have shown that cable bacteria are yearly recurrent within the surface sediments of Lake Grevelingen (Seitaj et al. 2015; Burdorf et al. 2017) reaching high densities in winter and spring within the deeper parts of the lake (> 15 m water depth) (Seitaj et al. 2015).

Sediment was retrieved using a single core gravity corer (UWITEC, Austria) at the Den Osse S1 site (51° 44’ 46.3” N 3° 52’ 45.1” E, water depth 23 m). The top layer of sediment (∼ 10 cm) was sliced off from sediment cores, and transferred into two plastic containers (volume: 28 L), which were filled to the rim and capped with gas-tight lids. The sediment was subsequently stored at 6 ºC for 13 days. The sediment was cohesive and fine-grained (medium grain size: 16 µm; porosity 0.88), and rich in organic matter (organic carbon content: 3.0% of dry weight sediment) and carbonate minerals (CaCO$_3$ content: 22% of dry weight sediment). Sediment properties are summarized in Table 1.

Right before the start of the experiment, the sediment was prepared for incubation as described in Burdorf et al. (2017). First, the sediment was sieved over a 1 mm mesh to remove fauna and small debris (i.e. shells or rocks that could damage the electrodes during microsensor profiling). After sieving, the sediment was homogenized and packed into plastic core liners (inner diameter: 12.5 cm, core liner height: 10 cm, sediment height: 10 cm). Homogenization ensured that starting conditions were similar in all incubated sediment cores.

Sediment cores were finally transferred to a custom-built incubation set-up (as described below).
Sediment incubations

Sediment cores were incubated at four different oxygen levels (resp. 10, 20, 40, 100% O$_2$ air saturation, corresponding to 25, 51, 102, and 255 µmol L$^{-1}$ O$_2$ at a salinity of 28 and temperature of 18 °C). Sediment incubations lasted over a period of three months (109 days). For each O$_2$ treatment, a separate incubation set-up was constructed, which was composed of two cylindrical incubation chambers through which seawater recirculated in a closed-circuit (Fig.1). The first chamber functioned as the sediment incubation chamber (diameter: 28 cm, total volume ~15 L) and held two replicate sediment cores submerged under water. The second chamber (diameter: 13.2 cm, volume ~4 L) functioned as the water reservoir (Fig.1). Recirculation of seawater was driven by a water pump (Eheim, 1250 universal pump, Germany), which was positioned in the water reservoir. Water was pumped from the water reservoir via gas-tight tubing to the sediment incubation chamber (inlet located at about ¾ of the chamber height), and returned to the water reservoir through a connection in the top of the sediment incubation chamber. In the water reservoir, the oxygen level was continuously monitored with an oxygen sensor (Hamilton VisiFerm DO Arc 120 optode, USA) in order to maintain the oxygen concentration of the recirculating water at a stable, predefined level. When the oxygen level dropped below 2.5% of the desired O$_2$ concentration, a switch opened the gas connection of an air-pump (Easy-load L/S, Masterflex, USA). Similarly, when oxygen levels exceeded 2.5% over the desired O$_2$ concentration, the connection to a gas bottle (N$_2$ with 400 ppm CO$_2$) was opened, bubbling the water reservoir until oxygen levels dropped again within the desired range. The four incubation set-ups (one for each O$_2$ treatment) were installed in a climate-regulated room, which was held at 18°C. To minimize the availability of nitrate as an alternative electron-acceptor in electrogenic sulphur oxidation (Marzocchi et al., 2014), low nutrient sea water (NO$_3$ concentration < 0.1 µmol L$^{-1}$) was used as the recirculating water in all set-ups.
The metabolic activity of cable bacteria was assessed via high-resolution depth profiles of pH, H$_2$S and O$_2$ (Nielsen et al. 2010; Meysman et al. 2015). Depth profiles were recorded using commercial amperometric and potentiometric micro-electrodes and a motorized micromanipulator (Unisense A.S. Denmark, tip sizes pH: 200 µm, H$_2$S 100 µm, O$_2$: 50 µm). Sensors were calibrated by standard calibration procedures as described previously (Malkin et al. 2014: H$_2$S: 3 to 5 point standard curve using Na$_2$S standards; O$_2$: 2 point calibration using 100% in air bubbled seawater and the anoxic zone of the sediment; pH: 3 NBS standards and TRIS buffer). The pH data are reported on the total pH scale and $\Sigma$H$_2$S = [H$_2$S] + [HS$^-$] was calculated from H$_2$S and pH values recorded at the same depth using the R package AquaEnv (Hofmann et al. 2010).

Microsensor profiling was done at seven consecutive time points during the experiment (2, 16, 25, 39, 52, 69 and 109 days after the start of the incubation). At each time point, two replicate profiles per sensor were recorded in each of the two replicate sediment cores in all four O$_2$ treatments. To enable microsensor profiling, the water circulation was temporarily stopped, and the lid of the incubation chamber was taken off. A custom-built sensor holder enabled to record the depth profiles of pH, H$_2$S and O$_2$ simultaneously (to minimize profiling time and therefore the intrusion of oxygen in the incubation chamber). Although microsensor depth profiling always took less than 1.5 hours per treatment, it involved a small but unavoidable disturbance of the oxygen conditions in the set-ups (max. ~30% O$_2$ increase). Set-ups returned within 6 hours to their target oxygen levels after profiling was completed.

The microsensor data was used to calculate two proxies for the metabolic activity of cable bacteria following the procedure as detailed in Burdorf et al. (2016). A first proxy is the extent of the suboxic zone $\Delta$L, defined as the zone between the oxygen penetration depth
(OPD, depth at which $[O_2] < 1 \ \mu\text{mol}\ \text{L}^{-1}$) and the sulfide appearance depth (SAD, depth at which $[H_2S] > 1 \ \mu\text{mol}\ \text{L}^{-1}$). The suboxic zone reveals the depth horizon over which of cable bacteria actively remove both oxygen and sulfide by e-SOx (Meysman et al. 2015). As described in Schauer et al. (2014) the suboxic zone $\Delta L$ also closely tracks the depth distribution of the filament density during the temporal development of a cable bacteria population. The second proxy of cable bacteria activity is the magnitude of the observed pH excursions in the pore water. The metabolic activity of cable bacteria typically generates large gradients in the pH of the pore water, and to this end, the quantity $\Delta p\text{H}$ is defined as the difference between the maximum pH in the oxic zone and the minimum pH in the suboxic zone. We note that the amplitude of the observed pH excursions is dependent on the acid-base buffering capacity of sediment (e.g. CaCO$_3$ precipitation and dissolution can modulate the observed pH excursions). Therefore, it is difficult to attribute $\Delta p\text{H}$ to a single factor across different sediments. Yet, when using the same sediment (as is done here), the $\Delta p\text{H}$ provides a good proxy to compare cable bacteria activity between different treatments and time points.

**Pore water sampling**

The pore water geochemistry was examined in detail at one time point (39 days after the start of the incubation). Pore water was retrieved and analyzed for Fe$^{2+}$, Mn$^{2+}$, Ca$^{2+}$, NH$_4^+$, SO$_4^{2-}$ and $A_T$ (total alkalinity). To this end, small plastic core-liners (inner diameter 36 mm) were pushed carefully into the center of each of the two sediment cores in all incubation setups (total: 8 subcores). These subcores were carefully expelled and immediately transferred to an anaerobic glove box (Coy Lab Products, USA; N$_2$ atmosphere with 3-5% H$_2$ and 390 µatm CO$_2$). Each subcore was sectioned at 0.5 cm intervals up to 4 cm and at 1 cm intervals up to 10 cm, thus resulting in a maximum of 15 sediment slices per subcore. Each sediment slice was deposited into a 50 mL polypropylene centrifuge tube (TTP, Switzerland) closed off inside the glove box, and transferred to a centrifuge outside the glove box (Sigma 3-18KS,
Sigma Laborzentrifugen GmbH, Germany). After centrifugation (3000 rpm, 10 minutes), the centrifuge tubes were transferred back inside the glove box, and the supernatant was filtered through a 0.45 µm cellulose filter (Millex-HA Filter, Merck Millipore, USA) and distributed over the sample containers for the different analyses.

Subsamples (50 µL of filtered pore water) for cation analysis (Ca$^{2+}$, Fe$^{2+}$, Mn$^{2+}$) were fixed immediately using 2 µL HNO$_3$ (65%, suprapure, Merck, USA) in a 15 mL polypropylene centrifuge tube (TTP, Switzerland) and stored at 4ºC until analysis. Prior to analysis, the samples were diluted 50 times using a mixture of artificial seawater (salinity: 35), 2% HNO$_3$ and an internal standard (Ytterbium at 0.2 mg L$^{-1}$). Total concentrations of dissolved cations were subsequently determined using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICAP 6600 ThermoFisher, USA).

Subsamples for NH$_4^+$ analyses (200 µL of filtered pore water) were diluted 25 times with low nutrient seawater ([NH$_4^+$] < 1 µM) in a 6 mL plastic vial (Pico Prias Vial, Perkin Elmer, USA) and stored at 4ºC for < 48 hours before analysis. The samples were then analyzed using standard colorimetric analysis on a SEAL QuAAtro segmented flow analyzer (Aminot et al. 2009).

Subsamples for SO$_4^{2-}$ analysis (250 µL of filtered pore water) were fixed with 1 mL of a 10 mM ZnAc solution in a 2 mL microcentrifuge tube and stored at -20 ºC. The SO$_4^{2-}$ was determined by ion chromatography using a Na$_2$CO$_3$ (3.5 mM) and NaHCO$_3$ (1.0 mM) buffer as eluent on a Dionex AS14 analytical column (Thermo Scientific) equipped with conductivity detection (ED40 electrochemical detector LC-02).

Subsamples (350 µL) for alkalinity (A$_T$) analysis were collected in 350 µL glass vials and poisoned with 3.5 µL saturated HgCl$_2$ solution. The A$_T$ determination was based on the analysis of Dissolved Inorganic Carbon (DIC) after equilibration with an ambient atmosphere. To this end, the pore water was exposed for sufficiently long time in the N$_2$/CO$_2$ glove box, so
that CO₂ and H₂S could evade until equilibrium was reached. Subsequently, DIC was
analyzed using a Segmented Flow Analyzer (San++ SKALAR) according to the method of
Stoll et al. (2001). The carbonate equilibrium in seawater can be fully determined from two
variables at steady-state (Dickson et al. 2007). In our case, the DIC was measured, while the
pCO₂ in the atmosphere was known (pCO₂ = 390 µatm). Based on this information, we
calculated the A₇ value at each depth using the standard acid-base relations implemented in
the AquaEnv package (input parameters: temperature, salinity, DIC, pCO₂, total NH₄⁺
concentration as measured, total borate concentration as estimated from salinity) in the R
diffuse oxygen uptake rate in the sediment was calculated from the oxygen depth profiles
using Fick’s first law: \( J_{O_2} = \phi \frac{D_{O_2}}{1 - 2 \ln(\phi)} \frac{d[O_2]}{dx} \). Here, the diffusive oxygen flux \( J_{O_2} \) is
calculated using the slope of the oxygen concentration depth profile \( \frac{d[O_2]}{dx} \) and the
molecular diffusion coefficient \( D_{O_2} \) corrected for tortuosity \( (1 - 2 \ln(\phi)) \) and the mean
porosity over the oxic zone \( (\phi = 0.88, \text{Table 1}) \). The molecular diffusion coefficient was
calculated in R using the marelac package (Soetaert et al. 2010a) taking into account the
temperature (18 °C) and salinity (28) of the incubations. Similarly the upward flux of \( \Sigma H_2S \)
was calculated using the slope of the \( \Sigma H_2S \) depth profile using Fick’s first law at the base of
the suboxic zone using the diffusion coefficient for H₂S. Finally upward and downward fluxes
were estimated from the iron pore water profiles using a similar approach and the molecular
diffusion coefficient for ferrous iron (Fe\(^{2+}\)).

**Fluorescent in situ Hybridization (FISH)**

We used Fluorescent in situ Hybridization (FISH) to quantify the abundance of cable bacteria
as a function of depth into the sediment. One sediment subcore was taken per oxygen
treatment (total 4 cores) after 39 days of incubation and sliced at 0.5 cm intervals up to 5 cm
depth (10 slices per core). Each sediment slice was homogenized and 0.5 mL of sediment was
mixed with 0.5 mL 99% ethanol and preserved in -20°C. We followed the FISH protocol
described in Schauer et al. (2014) and the quantification procedure as specified in Seitaj et al.
(2015). In short, subsamples of 100 µl were transferred in 500 µl of a 1:1 mixture of
PBS/ethanol, and subsequently, 10 µl of this mixture was filtered through a polycarbonate
membrane filter (type GTTP, pore size 0.2 µm, Millipore, USA). Hybridization of the FISH-
probe (DSB706 at 35% formamide concentration) was performed according to previously
published FISH protocols (Pernthaler et al. 2001, 2002). Samples were counterstained with
DAPI and subsequently visualized with an epifluorescence microscope (Zeiss Axioplan,
Germany) at 100x magnification. To quantify the cable bacteria, the filters were separated
into discrete virtual fields-of-view. Each field-of-view, or field, is the area of the filter as seen
through the microscope at 100x magnification (or 105 x 141 µm). In each field, the length of
and diameter of all observed cable bacteria filaments are determined. In total 200 fields per
sample were analyzed. In all treatments, cable bacteria abundances are reported as filament
length per volumetric unit (i.e., meter of filament per cm\(^3\) of bulk sediment). Cable bacteria
abundances were also integrated over depth and expressed per unit area of sediment surface
(i.e., meter of filament per cm\(^2\) of sediment).
Biogeochemical model analysis

A simplified reactive transport model was constructed to estimate the mineralization rate of organic matter by fitting numerical model solutions to the estimated $A_T$ and $NH_4^+$ concentration depth profiles. In this model, we assumed that sulfate reduction is the dominant pathway of organic matter mineralization.

Furthermore, we ignore the removal of ammonium through nitrification and anammox, and we ignore carbonate dissolution/precipitation and cable bacteria metabolism in the case of $A_T$.

If $R_{\text{min}}$ is the mineralization rate of organic matter and $\gamma_{CN}$ is the C/N ratio, the associated mass balance equations for $A_T$ (eq. 1.2) and $NH_4^+$ (eq. 1.3) become:

$$
(0.1) \quad CH_2O + \frac{1}{2} SO_4^{2-} \rightarrow HCO_3^- + \frac{1}{2} HS^- + \frac{1}{2} H^+ 
$$

$$
(0.2) \quad \frac{\partial}{\partial x} \left[ \phi D_A \frac{\partial A_T}{\partial x} \right] + R_{\text{min}} = 0
$$

$$
(0.3) \quad \frac{\partial}{\partial x} \left[ \phi D_{NH_4} \frac{\partial [NH_4^+]}{\partial x} \right] + \frac{1}{\gamma_{CN}} R_{\text{min}} = 0
$$

The underlying assumptions are that the sediment is in steady-state, molecular diffusion is the only important transport process, the porosity $\phi$ is constant with depth, and that mineralization rate $R_{\text{min}}$ is also constant with depth. The diffusion coefficients ($D_A$, $D_{NH_4}$) were calculated as a function of the temperature ($18^\circ C$) and salinity (28) of the sediment incubation using the R package marelac (Soetaert et al. 2010a), assuming the diffusion coefficient of bicarbonate is representative for $A_T$. 

As boundary conditions, the model is constrained at the sediment-water interface by fixed concentrations in the overlying water ($A_T$: 4.2 mmol L$^{-1}$ and NH$_4^+$: 0.1 mmol L$^{-1}$) and a no gradient condition at the lower boundary of the sediment domain (10 cm).

The numerical solution for the two partial differential equations (eq. 1.2 and 1.3) was implemented in R following the approach outlined in Soetaert and Meysman (2012). In short, the spatial derivatives of the partial differential equations were expanded as a finite difference grid (200 equidistant layers over the sediment domain of 10 cm). After discretization, the resulting set of ordinary differential equations was integrated using the stiff equation solver *vode* implemented in R through the *deSolve* package (Soetaert et al. 2010b).

The goal of this modelling exercise was to find the mineralization rate which best fit the measured concentration depth profiles of NH$_4^+$ and $A_T$. In a first step, the 10% $O_2$ treatment was analyzed (which did not show e-SOx activity – see discussion below). The mineralization rate $R_{\text{min}}$ was determined from the best fit to the measured NH$_4^+$ depth profile data, while $\gamma_{CN}$ was determined from the best fit to the $A_T$ depth profile (adopting the $R_{\text{min}}$ from the NH$_4^+$ profile). In the remaining treatments (which did show e-SOx activity), we followed a slightly different procedure. This was because cable bacteria have been shown to impact the alkalinity of the pore water, but not the ammonium concentrations (Meysman et al. 2015; Rao et al. 2016; van de Velde et al. 2016). $R_{\text{min}}$ was first calibrated based on the measured ammonium profiles, and subsequently, $R_{\text{min}}$ was calibrated a second time using the alkalinity depth profiles (while using the $\gamma_{CN} = 6.4$ from the 10% $O_2$ treatment).

The quality of the model fit to the data was quantified by the sum of squared errors (eq. 1.4) following Berg et al. (1998).
where $N$ is the number of points in the measured concentration depth profile, $C_i$ is the measured concentration of the solute at a given sediment horizon, and $\bar{C}_i$ is the model predicted concentration (averaged over the same depth layer where the concentration was measured). All further statistical analyses were conducted in the R software.

**Results**

**Microsensor depth profiles**

Initially (i.e. after 2 days of incubation), a similar set of microsensor depth profiles (pH, $H_2S$ and $O_2$) was recorded in all treatments (Fig.2). In all treatments, $\Sigma H_2S$ concentrations increased steeply right below the oxygen penetration depth. The slope of the $\Sigma H_2S$ concentration profile was similar, suggesting high and similar rates of sulfate reduction in all treatments. The oxygen penetration depth (OPD) into the sediment was generally small and increased with increased oxygen concentration in the overlying water. Oxygen penetrated up to $3 \pm 0.2$ mm in the 100% $O_2$ treatment, but only extended $0.4 \pm 0.04$ mm depth in the 10% treatment. The difference between the pH of the overlying water and the asymptotic pH at depth in the sediment decreased with decreasing $O_2$ availability.

Starting from roughly similar initial conditions, the geochemistry subsequently developed in different ways in the four treatments. Electrogenic sulfur oxidation (e-SOx) imposes a characteristic fingerprint upon the pore water in surface sediments (Meysman et al. 2015). From day 16 onwards, this fingerprint was discernable in the 100% $O_2$ treatment, as indicated by a pH maximum ($pH_{max} = 8.35 \pm 0.04$) in the oxic zone of the sediment, the presence of a suboxic zone ($\Delta L = 2.6 \pm 1.5$ mm), and the development of acidic conditions in
the lower part of the suboxic zone (pHmin = 6.7). In the 40% O2 treatment, a similar set of microsensor depth profiles was recorded, though after 39 days of incubation. Although less pronounced than in the 40% and 100% treatments, the characteristic fingerprint of e-SOx was also detected in the 20% O2 treatment after 39 days. Even though the pH maximum in the oxic zone remained small or was absent, the development of a pronounced suboxic zone (ΔL = 6.9 ± 3.4 mm) as well as the acidification of the suboxic zone points towards cable bacteria activity - see discussion in Burdorf et al. (2016); van de Velde et al. (2016) on the absence of pH maxima in electrogenic sediments. In contrast, in the 10% O2 treatment, no suboxic zone developed and no notable pH excursions were detected over the 109 days of the experiment.

The pH profile remained constant with depth at each time point.

The differential development of e-SOx in the various treatments is illustrated by the magnitude of the suboxic zone (ΔL), the amplitude of the pH excursion (ΔpH) and the dissolved oxygen uptake rate (Fig.3). Overall, the temporal evolution of ΔpH was similar in the three treatments that revealed cable bacteria activity (100%, 40% and 20%), and showed two consecutive phases: ΔpH initially increased to a maximum value, after which it stabilized throughout the rest of the incubation. However, the timing of these two phases varied markedly between treatments. In the 100% O2 treatment, the maximum in ΔpH was reached earliest (at day 25) and attained the highest value (ΔpH = 1.9 ± 0.05), while in the 40% (ΔpH = 1.2 ± 0.04 at day 39) and 20% (ΔpH = 0.7 ± 0.08 at day 52) treatments, the pH excursion developed slower and remained smaller. The temporal development of the suboxic ΔL zone also differed between treatments, and was congruent with the temporal evolution of the pH excursion. In the 100% treatment, a fast increase to ΔL = 12 ± 5 mm was noted after 25 days, followed by a small decrease (ΔL = 6.0 ± 0.9 mm at day 52) and a subsequent increase to a maximum of ΔL = 16 ± 4 mm at the end of the experiment. Strikingly, the largest suboxic zone was obtained in the 40% treatment. Here, the suboxic zone first rapidly increased (day
39; ΔL = 14 ± 1 mm), followed by a period of more gradual increase (day 69; ΔL = 16 ± 4 mm) to reach a maximum at the end of the incubation (ΔL = 24 ± 3 mm). In the 20% treatment, the largest extent of the suboxic zone was measured after 52 days (ΔL = 9.5 ± 3 mm), after which it decreased again to 5.0 ± 0.5 mm. Overall, the dissolved oxygen uptake (DOU, Fig 3c) was higher in the treatments with elevated oxygen levels. In all treatments the highest DOU was obtained at the start of the experiment (day 0), which is likely due to a transient increase in the O₂ consumption after sediment homogenization, where a reduced layer at the surface is progressively oxidized. In the 100% treatment the DOU remains high during the initial period of ΔpH increase (from 31 ± 4 mmol m⁻² d⁻¹ at day 0 to 30 ± 0.5 at day 25; Fig.3a). After that, the DOU decreases to attain a minimum value of 12 ± 2 mmol m⁻² d⁻¹ at day 109. The DOU in the 40% treatment also decreases over time, while that in 10%, and 20% treatments remains relatively constant throughout the time series. Note that the sediments showed a very shallow oxygen penetration (as low as 200 µm in the 10% treatment), and so the O₂ depth profiles contain only a limited number of datapoints, which increases the uncertainty on the calculated diffusive oxygen uptake (DOU).

### Cable bacteria abundances and morphotype

The integrated abundance of the cable bacteria over depth was similar in the three treatments that developed e-SOx (430 – 530 m cm⁻³, Table 2). No cable bacteria filaments were detected in the sediment samples from the 10% treatment (Table 2), matching with the absence of the e-SOx geochemical fingerprint. The depth distribution of cable bacteria (Fig.4) was similar in the 100% and 20% treatments, with the highest abundance in the top (oxic) sediment layer (100%; 523 m cm⁻³ and 20%; 576 m cm⁻³) and a decreasing abundance with depth. In contrast, the 40% treatment showed a more even depth distribution of cable bacteria, with similar filament densities in the first 15 mm of the sediment (280 – 320 m cm⁻³). In all
treatments, a sharp decrease in filament densities occurred when passing from the suboxic zone into the deeper sulfidic (<10% of the total filament length per area was present below the SAD). The diameter of each cable bacteria filament was recorded and provided a mean diameter of 0.98 ± 0.32 µm (filament fragments inspected n = 351). No observable differences in filament width were found between the three oxygen conditions (ANOVA; F = 2.12; df = 2; p = 0.12), yet cable bacteria in the oxic zone were significantly thinner than the cable bacteria in the anoxic zone (0.80 ± 0.26 µm, vs 1.02 ± 0.34 µm; Welch t-test; t = 6.79; df = 314.7; p < 0.001).

**Pore water geochemistry**

Concentration depth profiles of pore water constituents (Fe^{2+}, Mn^{2+}, Ca^{2+}, SO_{4}^{2-}, A_{T} and NH_{4}^{+}) were determined after 39 days of incubation in all treatments. The two replicate cores analyzed in each treatment gave similar depth profiles (Fig.5).

The pore water inventory of dissolved iron (Fe^{2+}) decreased with decreasing O_{2} availability (Fig.5a; Table 2). A clear enrichment of pore water iron was observed in the suboxic zone of the sediment in the 40% and 100% O_{2} treatments, with a subsurface Fe^{2+} peak at 20 mm. The peak concentration of Fe^{2+} was highest in the fully oxic treatment (0.31 mmol L^{-1}). In the 20% treatment, dissolved iron was only detected in the top 5 mm (max [Fe^{2+}]; 0.05 mmol L^{-1}), while in the 10% treatment, dissolved iron remained below the detection limit at all depths.

Dissolved manganese (Mn^{2+}) concentrations also showed a clear trend between treatments (Fig.5b), with a decreasing inventory of Mn^{2+} with decreasing oxygen availability (Table 2). In the 100% O_{2} treatment, Mn^{2+} reached the highest concentration in the upper sediment layer and a small secondary Mn^{2+} peak was situated at 2 cm deep (present in both
replicate cores). In contrast, in the three other treatments, the Mn$^{2+}$ profile showed one subsurface peak, which was located between 1–2 cm deep in the sediment.

All Ca$^{2+}$ profiles showed a subsurface maximum (Fig.5c), indicating CaCO$_3$ dissolution within the suboxic zone, and the pore water inventory of Ca$^{2+}$ decreased with decreasing O$_2$ availability (Table 2). In the 100% and 40% treatments, a strong accumulation of Ca$^{2+}$ was observed in the first 30 mm of the core, with slightly higher concentrations in the fully oxygenated cores (max. [Ca$^{2+}$]: 20.5 mmol L$^{-1}$) compared to the 40% O$_2$ core (max. [Ca$^{2+}$]: 18 mmol L$^{-1}$). Only a small enrichment was measured in the 20% O$_2$ treatment (max. [Ca$^{2+}$]: 14.5 mmol L$^{-1}$) compared to the 10% treatment (max. [Ca$^{2+}$]: 12.3 mmol L$^{-1}$).

The sulfate (SO$_4^{2-}$) depth profiles (Fig.5d) were highly similar in all treatments and decreased from 27-30 mmol L$^{-1}$ in the top of the sediment to 10 – 12 mmol L$^{-1}$ at the bottom of the sediment cores. Alkalinity increased from 5 mmol L$^{-1}$ in the top of the sediment to 25 mmol L$^{-1}$ deeper in the sediment with no real difference between treatments (Fig.5e). The ammonium concentrations (Fig.5f) were also similar in all treatments and steadily increased from 0.1 mmol L$^{-1}$ near the sediment-water interface to ~1.5 mmol L$^{-1}$ at depth.

**Mineralization rates**

To estimate the mineralization rate in the incubated sediment cores, we implemented a simplified reactive transport model, which provided satisfactory fits to the alkalinity (A$_T$) and ammonium (NH$_4^+$) depth profiles in all treatments (Figure 6 and Table 3). The C:N ratio was estimated at 6.4 (fitted as described in section 2.5), which is close to the expected Redfield C:N ratio (6.6). It is slightly lower than the C:N ratio previously measured in bulk organic matter from Lake Grevelingen sediments, (7.2 in situ (Seitaj et al. 2017) and 9.0 in laboratory setup (Rao et al. 2016), which suggests preferential mineralization of nitrogen compared to carbon.
The model-estimated mineralization rate based on the alkalinity pore water profiles (~34 – 39 mmol C m\(^{-2}\) d\(^{-1}\)) fits well the values earlier reported values for sediments from the field site (~28 mmol C m\(^{-2}\) d\(^{-1}\) at 16°C, Rao et al. (2016)). A slightly higher mineralization rate is obtained for the fully oxic treatment (39 mmol C m\(^{-2}\) d\(^{-1}\)), but this remains close to the values computed for the other treatments (37 mmol C m\(^{-2}\) d\(^{-1}\) in the 40% O\(_2\) treatment, 37.6 mmol C m\(^{-2}\) d\(^{-1}\) in the 20% O\(_2\) treatment and 37.5 mmol C m\(^{-2}\) d\(^{-1}\) in the 10% O\(_2\) treatment). Similarly, the modelled ammonification rate is slightly higher in 100% O\(_2\) treatment (6.0 mmol N m\(^{-2}\) d\(^{-1}\)) compared to the other treatments (between 5.3 – 5.5 mmol N m\(^{-2}\) d\(^{-1}\)).

Mineralization rates were estimated by a second, independent method (based on alkalinity changes in the overlying water) and these were fully consistent with the values obtained by model fitting. Total alkalinity in the reservoir water was approx. ~2.0 mM at the start of the closed incubations. On Day 39, the alkalinity concentration in all treatments had similarly increased to a value of ~4.2 mM (as inferred from the concentration at the sediment-water interface in the depth profile). In a closed system with a recirculation water volume of ~15L, and which contains two sediment cores with a diameter of 12 cm, this alkalinity increase translates into an average alkalinity efflux of 38 mmol C m\(^{-2}\) d\(^{-1}\) from the sediment to the overlying water. Assuming the sediment cores are in steady state, alkalinity efflux forms a proxy for the alkalinity production, further substantiating that our estimates for mineralization rate \(R_{\text{min}}\) obtained by model fitting are accurate.

**Discussion**

**Growth dynamics of cable bacteria in marine sediments**

By monitoring the imprint of e-SOx on pore water solutes (e.g. pH, H\(_2\)S and O\(_2\)), the metabolic activity of cable bacteria can be tracked non-destructively through time, providing information on their growth and development (Schauer et al. 2014). In the fully oxygenated treatment, we observed substantial e-SOx activity within 16 days (Fig.2), indicating that a
A sizeable and active population of cable bacteria had developed. This confirms observations from previous laboratory enrichment experiments, where the first detectable sign of cable bacteria activity is typically observed after 5-14 days, depending on incubation temperature (Schauer et al. 2014; Vasquez-Cardenas et al. 2015; Rao et al. 2016). These observations confirm that cable bacteria are fast growing, and thus capable of rapidly establishing long-distance electron transport in the surface layer of aquatic sediments (Pfeffer et al. 2012). At the same time, the metabolic activity of cable bacteria has a large impact on the sediment through an increase in oxygen consumption, the depletion of the sulfide in the first centimeters of the sediment, and via intense pore water acidification (>1.5 pH units, e.g. Vasquez-Cardenas et al. 2015; Burdorf et al. 2017; Fig. 2). The fast growth dynamics of cable bacteria allows drastic shifts in the geochemical cycling of aquatic sediments over short time intervals (on the order of days to weeks), thus creating the possibility of rapid transient changes in sediment geochemistry. This has important consequences for the interpretation and analysis of sediment geochemistry data, which is at present largely dominated by a steady-state perspective (Boudreau 1997).

The results obtained here are in line with previous laboratory enrichments, which suggest that the development of cable bacteria population includes four consecutive periods: a lag phase, an exponential growth phase, a steady-state plateau and a final senescence phase (Schauer et al. 2014; Vasquez-Cardenas et al. 2015; Burdorf et al. 2017). The lag phase includes several days without notable signs of e-SOx activity (no suboxic zone is present and no acidification of the subsurface is detected); in the period of exponential growth, e-SOx activity becomes detectable and rapidly increases (the sulfide appearance depth deepens fast, e.g. 1 cm over 3 days, Burdorf et al. (2017) and pH excursions become apparent); in the subsequent period of stabilization, e-SOx activity becomes constant; the expansion of the suboxic zone is halted and the pH profile remain more or less stable; finally, in the period of
decline, e-SOx activity gradually fades (the pH excursions in the pore water become smaller and the sulfide again moves upwards towards the sediment-water interface).

In our experiment, we quantified this temporal pattern by tracking the extent of the suboxic zone $\Delta L$ and the acidification excursion $\Delta pH$ (Fig. 3a-b). Both these quantities are indicators for the intensity of the metabolic activity of cable bacteria. The width of the suboxic zone $\Delta L$ is governed both by the intensity of cathodic oxygen reduction

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O,$$

which decreases the oxygen penetration depth, and by anodic sulfide oxidation

$$H_2S + 4H_2O \rightarrow SO_4^{2-} + 8e^- + 10H^+,$$

which increases the sulfide appearance depth. Similarly, the value of $\Delta pH$ is governed by proton consumption near the sediment-water interface due to cathodic oxygen reduction and proton production at depth resulting from anodic sulfide oxidation.

The treatments which developed an active cable bacteria population (100%, 40% and 20% $O_2$) all proceeded through a similar development pattern, which roughly fits the four-phase pattern described above. All three treatments first showed an exponential growth phase, after which the e-SOx activity peaked and subsequently declined again (Fig. 3a-b). The lag phase was not as clear as in previous studies, likely because the time resolution of microsensor profiling in the initial stage of our experiment was rather course. Similarly, the decline phase as observed here was not as pronounced as in previous studies (the $\Delta pH$ goes through a maximum, and the suboxic zone $\Delta L$ also goes through a maximum, but then increases again in the 100% and 40% treatments). Overall, the growth dynamics of cable bacteria seems to be of a boom-and-bust nature, i.e., a fast development towards maximal activity followed by subsequent decline.

It should however be noted that this growth pattern has always been observed after a disturbance event. In the laboratory enrichment experiments conducted until present, this disturbance event involves an initial “reset” of the geochemistry, either through sediment

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mixing or through sediment asphyxiation, and subsequently re-exposing the sediment to an oxygenated overlying water column. Natural analogues of such a sediment disturbance are likely erosion-resuspension events or the development of bottom water hypoxia. Yet, it is not clear at present how important these disturbance events for the natural distribution of cable bacteria. Currently, only a few studies have documented the temporal dynamics of cable bacteria populations under natural conditions (Seitaj et al. 2015; van de Velde et al. 2016; Malkin et al. 2017), and these studies do not yet enable general conclusions on the natural growth dynamics of cable bacteria. It hence remains unclear whether cable bacteria have an ephemeral and opportunistic lifestyle (i.e. they grow in a boom-bust fashion after a sediment disturbance), or whether than they can establish continuous populations for long time within aquatic sediments.

**Sulfur cycling in electro-active sediments**

Cable bacteria have been shown to thrive in a large number of coastal marine habitats worldwide (Malkin et al. 2014; Burdorf et al. 2017). A common factor of these habitats (i.e. mangrove sediments, intertidal mud flats, bivalve reefs) are the high rates of sulfate reduction, which results in high rates of free sulfide production, and hence a large pool of reduced sulfur compounds in the top centimeters of the sediment (e.g. free sulfide, elemental sulfur, iron sulfides, pyrite). Cable bacteria appear to mainly – or even exclusively - utilize free sulfide (H₂S) as the electron donor in anodic sulfide oxidation (Nielsen and Risgaard-Petersen 2015). Sulfide consumption is thought to take place throughout the suboxic zone, and free sulfide is supplied in three principle ways to the cable bacteria (Meysman et al. 2015; Fig.7). Firstly, free sulfide is produced in deeper sediment layers by sulfate reduction, and generates a diffusive flux upwards to the suboxic zone. This free sulfide is consumed by the cable bacteria at or just below the sulfide appearance depth. Secondly, iron sulfides (FeS) are
dissolving within the suboxic zone, as a result of the acidification induced by anodic sulfide oxidation (here ~ 2 pH units on Day 23 in the 100% O₂ treatment, Fig.3a). Iron sulfide dissolution releases free sulfide to the pore water (FeS + H⁺ → Fe²⁺ + HS⁻), which is then consumed by the cable bacteria. Note this creates a positive feedback loop, where a higher metabolic activity of cable bacteria induces a higher acidity of the pore water, which then stimulates FeS dissolution and free sulfide release, but also again sustains a higher metabolic activity (Meysman et al. 2015). A third source of free sulfide is due to sulfate reduction within the suboxic zone. Because sulfide is depleted below detection limit within the suboxic zone, this gives rise to a cryptic sulfur cycle, where the free sulfide produced by sulfate reduction is immediately scavenged and oxidized by the cable bacteria. Model simulations reveal that cable bacteria must have a high affinity for free sulfide in order to keep the free sulfide concentration to below detection limit (Meysman et al. 2015).

We examined the relative importance of the three supply pathways by establishing a sedimentary budget for sulfur and iron. This mass balance procedure was applied to all four treatments on Day 39. The procedure delineates three biogeochemical zones (oxic zone, suboxic zone and sulfidic zone), and it is assumed that the activity of cable bacteria is restricted to the suboxic zone. Fluxes and reaction rates are shown in Table 3 and Figure 7 (see the caption of Table 3 for the calculation procedure). The sulfur budget reveals that sulfate reduction is the most important source of sulfide to the cable bacteria (Table 3). Most is coming from sulfate reduction in deeper layers (67-82% of the sulfide supply), while a smaller amount (18-20 %) is coming from sulfate reduction within the suboxic zone itself. In the 100% O₂ treatment, FeS dissolution amounts to 2.5 mmol m⁻² d⁻¹, which equals 12% of the total free sulfide supplied to the suboxic zone. In the 40% O₂ treatment, the share of FeS dissolution is even less (5% or 1.0 mmol m⁻² d⁻¹ or), while all sulfide in the 20% treatment stems from sulfate reduction and no dissolution of FeS was observed (Fig.5). Previous studies
attribute a markedly higher share of the sulfide used in e-SOx to FeS-dissolution. During incubation of Baltic sea sediments, recovered in Aarhus Bay, Risgaard-Petersen et al. (2012) found that almost all sulfide used in e-SOx originated from FeS-dissolution (94%). A field study on a subtidal mud bank in Belgium (van de Velde et al. 2016) calculated a smaller share of FeS in sulfide production for e-SOx (37%). Accordingly, the fueling of e-SOx via FeS dissolution seems to be highly variable between environments.

The absence of transient sulfide accumulation in the pore water and the persistence of a suboxic zone indicates that e-SOx must consumes all the free sulfide that is produced in the sediment. Since sulfate reduction rates are high in our sediments (Table 3), e-SOx rates are also high (17.8 mmol S m$^{-2}$ d$^{-1}$ – 20.9 mmol S m$^{-2}$ d$^{-1}$; Table 3) and substantially exceed the rates obtained in previous studies (3.3 mmol S m$^{-2}$ d$^{-1}$ – 7.3 mmol S m$^{-2}$ d$^{-1}$; Risgaard-Petersen et al. 2012; van de Velde et al. 2016). In theory, the rates of anoxic sulfide oxidation and cathodic oxygen reduction must be coupled through an electron balance. However, the DOU values obtained were far too low to match the estimated sulfide consumption via e-SOx (assuming that all sulfide is oxidized to sulfate). To balance the predicted 18 - 21 mmol m$^{-2}$ d$^{-1}$ of anodic sulfide removal (Table 3), a cathodic O$_2$ uptake rate of 36 - 42 mmol m$^{-2}$ d$^{-1}$ would be needed. The estimated DOU values are however much lower (7 – 16 mmol m$^{-2}$ d$^{-1}$, Table 3). There are a number of ways to explain this. A first possibility is that part of the free sulfide produced by sulfate reduction is not re-oxidized, and hence, some free sulfide diffuses out of the sediment. While this could be occurring in the 10% O$_2$ treatment, it is excluded in all other treatments, because a suboxic zone formed, which implies that all free sulfide in the sediment must be effectively reoxidized. A second possibility is that all sulfide is oxidized, but not all the way up to sulfate, thus producing sulfur compounds with an intermediate oxidation state, like elemental sulfur or thiosulfate. For example, if anodic sulfide oxidation would only produce elemental sulfur, ($H_2S + 4H_2O \rightarrow S^0 + 2e^- + 2H^+$), then the corresponding
DOU range would become 9 – 10.5 mmol m\(^{-2}\) d\(^{-1}\), which matches the estimated range. However, this would imply that substantial amounts of elemental sulfur would accumulate in the sediment. Although no solid phase analysis was performed, we have no indications (e.g. sediment discoloration) for such an accumulation of elemental sulfur. Another option is that the cathodic oxygen reduction is underestimated. This could occur when cable bacteria not only access the oxygen within the sediment core, but that part of their filament sticks out of the sediment to directly consume O\(_2\) in the water column. This way of oxygen consumption would not be reflected in our O\(_2\) depth profiles and DOU values.

To test whether cable bacteria access the oxygen directly in the water column, we set up a new small sediment incubation experiment. To this end, small transparent sediment chambers (height: 50 mm; width: 10mm; depth: 2mm) were constructed (Fig. 8), which were dug into the sediment, level with the sediment-water interface. The chambers were incubated for 16 days with two oxygen levels in the overlying water (i.e. 268 µmol L\(^{-1}\) and 160 µmol L\(^{-1}\), corresponding to 100% and 50% O\(_2\) saturation). At the end of the incubation period, the glass chambers were retrieved from the sediment, washed and inspected by microcopy. This experiment showed numerous cable bacteria filaments sticking out of the sediment in the lower oxygen treatment, while no cables were seen sticking out at the higher oxygen treatment (Fig. 8). Accordingly, at reduced O\(_2\) concentrations, the cable bacteria appear to extend from the sediment, in order to harvest O\(_2\) within the diffusive boundary layer above the sediment. This way, their oxygen consumption would occur above the sediment-water interface, and hence will not be accounted for in the DOU (which is only based become on O\(_2\) data below the sediment-water interface).

**Metabolic activity of cable bacteria and O\(_2\) availability**

Our experiments here provide the first insight into this oxygen dependence of metabolic activity of cable bacteria and O\(_2\) availability. Conspicuously, the time interval over which the cable bacteria...
developed in our sediment incubations was dependent on the oxygen availability. Based on ΔpH (Fig.3a), we observed a shorter exponential growth phase in the fully oxic treatment (i.e. from day 2 to day 25) in contrast to the 20% treatment (Fig.2, Fig.3). The time point of maximum cable bacteria intensity (as defined by the maximum in ΔpH) was reached on Day 25 in the 100% treatment, on Day 39 in the 40% treatment and on Day 52 in the 20% treatment. Similarly, the width of the suboxic zone (Fig.3b) increased to a maximum within 25 days in the 100% treatment (to 1.5 cm in 25 days), while this took 39 days in the 40% treatment and up to 58 days in the 20% treatment.

The population dynamics suggests a slower development in the lower oxygen treatments (Fig.2, Fig.3), while the sulfur and iron budgets also show a lower anodic sulfide oxidation at day 39 (Table 3). Our time series provide various lines of evidence for the idea that the rate of e-SOx decreases when the O₂ levels in the overlying water decreases (Fig.2). Firstly, higher e-SOx rates would cause higher oxygen uptake rates. Despite the relative large uncertainty, the DOU values obtained from our time series experiments show a clear decreasing trend with O₂ availability, with systematically lower DOU values in the 20% and 40% oxygen treatments (Fig.3c). Second, when the metabolic activity of the cable bacteria increases, one expects the associated pH excursions to also increase. Through increased rates of anodic sulfide oxidation, leading to increased proton production at depth, and increased cathodic oxygen reduction, leading to higher proton consumption rates near the sediment interface, one obtains a larger ΔpH excursion. Our data suggest that higher O₂ availability indeed leads to higher ΔpH values (Fig.3a). The highest ΔpH was reached in the 100% (1.9, day 25), followed by the 40% (1.3, day 39). Thirdly, when the metabolic activity of the cable bacteria increases, one expects a higher accumulation of cations in the pore water. This is because the metabolic activity of cable bacteria has a large effect on the geochemistry of the sediment, through its impact on pH (Risgaard-Petersen et al. 2012; Meysman et al. 2015; Rao...
et al. 2016), which is a geochemical “master variable” (Stumm and Morgan 1981). Laboratory experiments (Risgaard-Petersen et al. 2012; Rao et al. 2016), field measurements (Seitaj et al. 2015; van de Velde et al. 2016) and modelling (Meysman et al. 2015) have shown that the acidification induced by e-SOx leads to the dissolution of acid-sensitive minerals, such as FeS and CaCO₃, in the suboxic zone.

On Day 39 of the incubation, the total inventories of remobilized cations Fe²⁺, Ca²⁺ and Mn²⁺ are larger with higher O₂ availability (Table 2), indicating mobilization of Fe²⁺ through the dissolution of FeS (Fig.5a), of Ca²⁺ through the dissolution of CaCO₃ (Fig.5c) and of Mn²⁺ likely associated to FeS and CaCO₃ minerals (van de Velde et al., 2017; Fig.5b). This hence suggests that increased e-SOx activity leads to increased acidification of the suboxic zone, thus stimulating the dissolution of acid-sensitive minerals. However, it should be noted that our pore water analyses only provided a snapshot of the sediment geochemistry at a single time point (i.e. on Day 39 of the incubation). Cable bacteria induce a transient development of the geochemical cycling in the sediment. As noted above, this transient development occurred at different “speeds” in the different treatments (Fig.3). The large remobilization of Fe²⁺, Mn²⁺ and Ca²⁺ are primarily dependent on absolute minimum pH reached in the pore water. In all treatments with cable bacteria, the pH minimum reached similar levels over time (6.1 – 6.3) and would enable a similar dissolution of e.g. CaCO₃ and FeS in all treatments.

In contrast to the higher activity of cable bacteria derived from the geochemistry, the quantification of the cable bacteria at day 39 (Fig.4) shows a similar length density of cable bacteria between the 20%, 40% and 100%. Accordingly, the difference in e-SOx activity at day 39 between treatments is contradicted by the counts of cable bacteria (Fig.4, Table 2). The integrated length density of the cable bacteria (~ 430 – 530 m cm⁻²) did not vary systematically between treatments. The density of cable bacteria is related not only to the
activity of the cable bacteria, but is also a result of the population dynamics. At the time of slicing cable bacteria activity was already decreasing in the 100% treatment, while the cable bacteria activity was still increasing in the 20% and 40% treatment.

Lower oxygen availability thus seems to decrease the activity of the cable bacteria, and delay the effect of e-SOx on the sediment geochemistry. The growth of cable bacteria in a sediment covered with a photosynthetic biofilm (Malkin and Meysman 2015), raises the question whether the opposite also holds true (faster growth, faster acidification with more O₂). In such environments O₂ saturation surpasses 100% and could induce an even faster acidification of the suboxic zone through e-SOx.

**The oxygen limit of electrogenic sulfur oxidation**

The present model on the environmental conditions controlling cable bacteria growth is based on three main assumptions. Firstly, a sufficient amount of a suitable electron acceptor must be present. In our experiments we find that cable bacteria develop when at least 50 µmol L⁻¹ O₂ (20% treatment, Fig.2, Fig.4) is available. Alternatively, cable bacteria can use nitrate as an electron acceptor (tested with >200 µmol L⁻¹ in laboratory setup, Marzocchi et al. 2014), but in this study, the use of nitrate by cable bacteria was minimized by incubating the sediment in low nutrient seawater ([NO₃] < 0.1 µmol L⁻¹). The second requirement for cable bacteria growth is the availability of an electron-donor (H₂S). Finally, cable bacteria activity is proposed to be limited by the physical disturbance of the filament-network by bioturbators (Pfeffer et al. 2012; Malkin et al. 2014; Rao et al. 2014). But recent findings indicate that the control of bioturbation on cable bacteria distribution might be less efficient than previously thought (Malkin et al. 2017; Burdorf et al. 2017).

If we apply these conditions – at least 50 µmol L⁻¹ O₂, and sufficient H₂S availability – onto the modern oceans, possible habitats for cable bacteria are present across a vast depth.
range. Sulfate reduction is predominately present on the continental shelf and slope (e.g. >80% of global sulfate reduction is limited to 1000m water depth (Jørgensen and Kasten 2006; Bowles et al. 2014), although local deep-sea “hotspots” exist. These sites in the deep-sea with significantly higher sulfate reduction have a supplemental source of either methane (cold seeps), sulfur (hydrothermal vents) or organic matter (carcasses).

With sufficient sulfide available, the cable bacteria distribution on the continental slope and shelf is most likely to be limited by oxygen availability and bioturbation. Although the majority of the oceans are fully oxic, oxygen depletion is globally increasing (Diaz and Rosenberg 2008). Human-impacted coastal systems, where additional nutrients run-off into the marine waters from land, are increasingly enduring (seasonal) oxygen depletion. Cable bacteria are present and active in these seasonal hypoxic systems, but e-SOx activity has only been measured during periods with high oxygen levels (Seitaj et al. 2015; Burdorf et al. 2017). Apart from coastal regions, oxygen depletion also occurs naturally in productive, typically upwelling region of the oceans. These so-called oxygen minimum zones (abbreviated as OMZ) are persistent features in productive oceanic regions with high upwelling rates. Although there is a large geographical variability to the extent of these oxygen deficient water layers, OMZ waters are usually found between 100 – 1800 m deep in the bathyal zone (Gallo and Levin 2016). Sediments under these oxygen deficient waters are usually organic carbon rich, sulfidic (Middelburg and Levin, 2009) and typically harbor big sulfide oxidizing bacteria like Thioploca and Beggiatoa. Although species of cable bacteria have been described which are able to use nitrate as an alternative electron donor, the high concentrations of these incubations (>200 µmol L⁻¹ NO₃) and the fact that cable bacteria likely cannot accumulate nitrate, leaves the questions open whether this process actually can occur within the OMZ. On the continental rise however, the sediment is covered by waters of varying oxygen concentrations, as it crosses through the upper and lower boundary of the
oxygen minimum zones. Also termed as oxygen limited zones (Gilly et al. 2013), an important oxygen gradient is present in these zones. Here, cable bacteria might benefit from an absence of large bioturbators and just enough oxygen to develop.

The $O_2$ availability can also be used to speculate about a geological timeframe when e-SOx first developed. Although, the scientific debate on oceanic oxygen concentrations is still open (Lyons et al. 2014), the first “whiffs” of oxygen produced by cyanobacteria are placed in the Archeon eon around 3 Ga years ago (Anbar et al. 2007). The extensive presence of reductive materials in the atmosphere as well as in the oceans however prevented substantial oxygen accumulation. The first great oxidation event (GOE) on the border of the Archeon and the Proteozoic eon (2.54 Ga) led to the first accumulation in oxygen to $10^{-4} – 10^{-1}$ of present levels in the atmosphere (Lyons et al. 2014). However oxygen is not hypothesized to have reached the bottoms of the oceanic basins at that time, which are thought to still have been euxinic (sulfidic, Canfield (1998)) or ferruginous (iron-rich). Whether e-SOx could have developed within this timeframe would rely on the spatial heterogeneity in oxygen concentrations. Given the large anoxic past of the earth’s sediment, the electron donor (sulfide) would have been ample available, and the evolution of large bioturbators did not yet start. One could imagine that an intertidal area with a cyanobacteria mat on the sediment could have created the environment needed for cable bacteria development. During the second oxidation event (~0.54 Ga) oxygen rose up to present levels. The sufficient oxygen concentrations throughout the oceans and the absence of bioturbators at the start of the oxidation may have created the ideal conditions for the widespread evolution of e-SOx.

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References


reduction and sulfide oxidation in marine sediment. ISME J. **8**: 1682–1690.
doi:10.1038/ismej.2014.19

Meysman, F. J. R., N. Risgaard-Petersen, S. Y. Malkin, and L. P. Nielsen. 2015. The
geochemical fingerprint of microbial long-distance electron transport in the seafloor.

Müller, H., J. Bosch, C. Griebler, L. R. Damgaard, L. P. Nielsen, T. Lueders, and R. U.
Meckenstock. 2016. Long-distance electron transfer by cable bacteria in aquifer
sediments. ISME J. **10**: 2010–2019. doi:10.1038/ismej.2015.250

Nielsen, L. P., and N. Risgaard-Petersen. 2015. Rethinking sediment biogeochemistry after

Electric currents couple spatially separated biogeochemical processes in marine
sediment. Nature **463**: 1071–1074. doi:10.1038/nature08790

Pernthaler, A., J. Pernthaler, and R. Amann. 2002. Fluorescence In Situ Hybridization and
Catalyzed Reporter Deposition for the Identification of Marine Bacteria. Appl.

hybridization (FISH) with rRNA-targeted oligonucleotide probes. Methods Microbiol.
**30**: 207–226. doi: 10.1016/S0580-9517(01)30046-6

Pfeffer, C., S. Larsen, J. Song, and others. 2012. Filamentous bacteria transport electrons over
centimetre distances. Nature **491**: 218–221. doi:10.1038/nature11586

electrogenic sulfide oxidation on elemental cycling and solute fluxes in coastal

production in intertidal sands intensified by lugworm bioirrigation. Estuar. Coast.
Shelf Sci. **148**: 36–47. doi:10.1016/j.ecss.2014.06.006


Figure captions

Figure 1: The setup of the sediment incubations. Arrows indicate the direction of the water flow. The sediment cores were incubated in a sediment incubation chamber which was connected to a water reservoir. An oxygen optode connected to a PC continuously measured the oxygen concentration within the water reservoir. When the measured oxygen concentration was 2.5% over or below the wanted concentration, the outlet to either the N₂/CO₂ gas (to lower the O₂) or to the air pump (to raise the O₂) was opened.

Figure 2: Microsensor depth profiles of the oxygen treatments over time. Sediments of all treatments were profiled repeatedly over the timespan of the experiment (109 days). For each time point in every treatment one set of representative microprofiles is depicted. No cable bacteria activity was recorded in the 10% treatment.

Figure 3: Activity parameters of the cable bacteria derived from the microsensor depth profiles over time. Each color is one oxygen treatment. (a) Changes in delta pH over time, where delta pH is the difference in pH between the anodic and cathodic zones of cable bacteria growth. (b) Changes in suboxic zone width through time, where suboxic zone is measured as the distance between the oxygen penetration depth and the sulfide appearance depth. (c) Changes in dissolved oxygen uptake rate (DOU) over time.

Figure 4: Cable bacteria length density in the different treatments at day 39 as quantified using fluorescent in situ hybridization. Abundances are given in m of cable bacteria per cm⁻³ of sediment. Dotted line indicates the depth at which sulfide is available in the sediment core.
(or sulfide appearance depth, SAD). Oxygen penetration depth is less than 0.5 cm in all treatments.

**Figure 5:** Pore water solute depth profiles at day 39. for (a) Fe$^{2+}$, (b) Mn$^{2+}$, (c) Ca$^{2+}$, (d) Mg$^{2+}$, (e) Total Alkalinity (TA) and (f) NH$_4^+$. The line is the mean value of two sediment-cores. Each color is one oxygen treatment.

**Figure 6:** Model estimation of the mineralization rate in the sediment cores. A simple reactive transport model was set up to estimate the carbon and nitrogen mineralization rate in each treatment. Nitrogen mineralization is here assumed to be the same as ammonification. The rows are the different oxygen concentration. Points are the measured concentrations and the lines the modelled TA and NH$_4^+$ concentrations.

**Figure 7:** A schematic representation of the iron and sulfur cycling in a sediment with e-SO$_x$ activity. The sediment is divided into three zones: the oxic, suboxic and anoxic zone. Arrows between boxes are fluxes which are quantified and given in Table 3. Cable bacteria activity induces the dissolution of iron-sulfide (box FeS, rate ISD) into H$_2$S and Fe$^{2+}$ in the suboxic zone. Ferrous iron can then diffuse upwards to the oxic zone, leading to oxidation to FeOOH (rate FIO) or downwards to the anoxic zone to re-precipitate as FeS (rate ISP). Total sulfide available for e-SO$_x$ equals the sulfate reduction in the suboxic zone (SRR 1), and the upward flux of sulfide (Fup) formed in the anoxic zone (SRR 2) and the sulfide from FeS dissolution (ISD).

**Figure 8:** Schematic representation of the small transparent sediment chamber that was dug into the sediment to image the sediment-water interface (left). On the right two images which
show cable bacteria sticking out of the sediment at lower $O_2$ concentrations (top) and no cable bacteria sticking out at higher $O_2$ concentrations (bottom).

**Figure 9:** A summary figure of the effect of oxygen availability at day 39 on the dissolved oxygen uptake (DOU), delta pH and $Fe^{2+}$ inventory. Height of the bars indicate the mean, lines the standard deviation.
Figure 1
Figure 2

\[ \begin{array}{cccc}
\text{J 2} & \text{J 16} & \text{J 39} & \text{J 109} \\
\text{O}_2 \text{ and H}_2\text{S} (\mu\text{mol l}^{-1}) & \text{O}_2 \text{ and H}_2\text{S} (\mu\text{mol l}^{-1}) & \text{O}_2 \text{ and H}_2\text{S} (\mu\text{mol l}^{-1}) & \text{O}_2 \text{ and H}_2\text{S} (\mu\text{mol l}^{-1}) \\
\end{array} \]
Figure 3

(a) ΔpH

(b) Suboxic zone (mm)

(c) DOU (mmol m⁻² d⁻¹)

Days since start incubation (J)
Figure 4

The figure shows three histograms comparing length density (m cm$^{-3}$) across different depths (mm). Each histogram represents a different scenario:

- **Left Histogram**: 100% density at depth, indicating a peak value at the surface.
- **Middle Histogram**: 40% density at depth, showing a lower peak value compared to the first.
- **Right Histogram**: 20% density at depth, indicating the lowest value across the three scenarios.

The SAD (Surface Absorption Distance) is marked at the top of each histogram, indicating the depth at which the density values are measured.
Figure 6

Total Alkalinity (mmol l⁻¹)

- Depth (mm):
  - 80
  - 60
  - 40
  - 20
  - 0

- Depth (mm):
  - 80
  - 60
  - 40
  - 20
  - 0

- Depth (mm):
  - 80
  - 60
  - 40
  - 20
  - 0

- Depth (mm):
  - 80
  - 60
  - 40
  - 20
  - 0

NH₄ (mmol l⁻¹)

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35

- Depth (mm):
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35
Figure 7

e-SOx oxygen consumption

Cable bacteria

O$_2$

FeOOH

FIO

FeS

SRR 1

SO$_4^{2-}$

e-SOx

H$_2$S

ISD

F$_{up}$

Fe$_{2+}$

ISP

H$_2$S

SRR 2

SO$_4^{2-}$

H$_2$S

FeS

e-SOx sulfide consumption
Figure 8

$O_2$: 160 \mu\text{mol L}^{-1}$

$O_2$: 268 \mu\text{mol L}^{-1}$
Figure 9
**Table 1:** Overview of the characteristics of the Lake Grevelingen sediment used in this experiment. Values are given as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Lake Grevelingen</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>0.88 ± 0.03</td>
<td><em>This study</em></td>
</tr>
<tr>
<td>Solid phase density</td>
<td>2.6 ± 0.03</td>
<td><em>Rao et al. 2016</em></td>
</tr>
<tr>
<td>Median grain size</td>
<td>16.00 ± 0.01µm</td>
<td><em>This study</em></td>
</tr>
<tr>
<td>Organic C content</td>
<td>3.4 ± 0.2 %</td>
<td><em>Rao et al. 2016</em></td>
</tr>
<tr>
<td>Inorganic C content</td>
<td>2.6 ± 0.2 %</td>
<td><em>Rao et al. 2016</em></td>
</tr>
<tr>
<td>CaCO$_3$</td>
<td>22.0 ± 0.4 %</td>
<td><em>Rao et al. 2016</em></td>
</tr>
<tr>
<td>C:N ratio</td>
<td>6.4 -</td>
<td><em>This study</em></td>
</tr>
</tbody>
</table>
Table 2: Overview of the inventories of dissolved Fe$^{2+}$, Mn$^{2+}$, Ca$^{2+}$ in the pore water and cable bacteria abundance (filament length density) after 39 days of incubation. Inventories of pore water solutes (mmol m$^{-2}$) were calculated as the depth integral of the pore water concentration over the top 8 cm and are displayed as the difference in inventory compared to the 10% O2 treatment. Abundance of cable bacteria (in m of cable bacteria per cm$^{-2}$) is calculated as the depth integral of the filament length density as displayed in Figure 4.

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>10%</th>
<th>20%</th>
<th>40%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$_d$ inventory</td>
<td>mmol m$^{-2}$</td>
<td>0</td>
<td>0.25</td>
<td>1.60</td>
<td>3.02</td>
</tr>
<tr>
<td>Mn$_d$ inventory</td>
<td>mmol m$^{-2}$</td>
<td>0</td>
<td>0.29</td>
<td>0.99</td>
<td>1.77</td>
</tr>
<tr>
<td>Ca$_d$ inventory</td>
<td>mmol m$^{-2}$</td>
<td>0</td>
<td>59</td>
<td>235</td>
<td>240</td>
</tr>
<tr>
<td>Filament length density</td>
<td>m cm$^{-2}$</td>
<td>0</td>
<td>530</td>
<td>502</td>
<td>434</td>
</tr>
</tbody>
</table>
Table 3: Overview of all fluxes and rates calculated in the various oxygen treatments at Day39 of the sediment incubations. The corresponding scheme of elemental cycling schematic is found in Figure 7. [1] The total mineralization rate $R_{\text{min}}$ was obtained from the model fit to the ammonium and alkalinity profiles. [2] The total sulfate reduction was estimated as $\text{SRR} = 0.5 \times R_{\text{min}}$, thus assuming that sulfate reduction is the primary mineralization pathway and that other pathways are quantitatively unimportant. [3] The upward flux of free sulfide from the sulfidic to the suboxic zone ($F_{\text{up}}$) was calculated from the microsensor depth profiles of H2S. [4] The rate of FeS dissolution in the suboxic zone (ISD) was calculated from the $\text{Fe}^{2+}$ depth profiles as the sum of the upward and downward fluxes away from the subsurface $\text{Fe}^{2+}$ maximum. [5] The rate of FeS re-precipitation (ISP) at the upper boundary of the sulfidic zone was calculated as the downward $\text{Fe}^{2+}$ flux away from the subsurface maximum. [6] The rate of ferrous iron oxidation within the oxic zone (FIO) was calculated as the upward $\text{Fe}^{2+}$ flux away from the subsurface maximum, thus assuming that no $\text{Fe}^{2+}$ diffuses out of the sediment. [7] The rate of sulfate reduction in the sulfidic zone was estimated as $\text{SRR}_2 = F_{\text{up}} + \text{ISP}$ [8] The rate of sulfate reduction in the suboxic zone was estimated as the difference between the total rate and that in the sulfidic zone $\text{SRR}_1 = \text{SRR} - \text{SRR}_2$ [9] The rate of electrogenic sulfur oxidation is finally calculated as $\text{eSO}_x = F_{\text{up}} + \text{ISD} + \text{SRR}_1$. [10] The dissolved oxygen uptake (DOU) is calculated using Fick’s first law from the microsensor depth profiles.
<table>
<thead>
<tr>
<th>Process</th>
<th>Unit</th>
<th>10%</th>
<th>20%</th>
<th>40%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C mineralisation</td>
<td>Rmin mmol C m$^{-2}$ d$^{-1}$</td>
<td>35.3</td>
<td>35.6</td>
<td>34.0</td>
<td>38.7</td>
</tr>
<tr>
<td>N mineralisation</td>
<td>Nmin mmol N m$^{-2}$ d$^{-1}$</td>
<td>5.5</td>
<td>5.5</td>
<td>5.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Sulfate reduction</td>
<td>SRR mmol S m$^{-2}$ d$^{-1}$</td>
<td>17.7</td>
<td>17.8</td>
<td>17.0</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>SRR1 mmol S m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>2.01</td>
<td>2.86</td>
<td>3.64</td>
</tr>
<tr>
<td></td>
<td>SRR2 mmol S m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>15.8</td>
<td>14.1</td>
<td>15.8</td>
</tr>
<tr>
<td>Upward H2S flux</td>
<td>Fup mmol S m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>9.2</td>
<td>11.3</td>
<td>12.4</td>
</tr>
<tr>
<td>FeS dissolution</td>
<td>ISD mmol Fe m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.94</td>
<td>2.47</td>
</tr>
<tr>
<td>FeS precipitation</td>
<td>ISP mmol Fe m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.46</td>
<td>0.98</td>
</tr>
<tr>
<td>Ferrous Iron Oxidation</td>
<td>FIO mmol Fe m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.48</td>
<td>1.49</td>
</tr>
<tr>
<td>Electrogenic sulfur oxidation</td>
<td>eSOx mmol S m$^{-2}$ d$^{-1}$</td>
<td>n.d.</td>
<td>11.2</td>
<td>15.1</td>
<td>18.5</td>
</tr>
<tr>
<td>Diffusive Oxygen Uptake</td>
<td>DOU mmol O$_2$ m$^{-2}$ d$^{-1}$</td>
<td>3.5 ± 0.8</td>
<td>6.8 ± 5.1</td>
<td>13.6 ± 2.5</td>
<td>16.2 ± 7.6</td>
</tr>
</tbody>
</table>