Supporting Information

Bjerg et al. 10.1073/pnas.1800367115

SI Methods

Sampling and Incubation. Surface sediment was collected from three locations containing cable bacteria of the genera *Candidatus* Electronema and *Ca*. Electrothrix (1): a freshwater lake at Aarhus University Campus, Denmark (56°09′53″N, 10°12′28″E), a marine salt marsh at Rattekaai, The Netherlands (51°26′21″N, 04°10′11″E), and an intertidal mud flat at Mokbaai, Texel, The Netherlands (53°00′27.1″N, 4°45′05.1″E). Sediments were sieved, homogenized, and incubated at 15 °C with either oxygenated overlying water or an air-exposed sediment surface. The development of cable bacteria was monitored by microscopy and their metabolic activity evaluated by O_2, H_2S, and pH microsensor measurements (2, 3) as previously described (4). When the sediment showed a clear geochemical fingerprint of electrogenic sulfur oxidation (as determined by O_2, H_2S, and pH microsensor profiling) and revealed abundant cable bacteria (as detected by microscopy), sediments were used for transfer to the microscope chamber setups.

Microscope Chamber Setups. Two microscope chamber setups (Fig. S1) were used to examine cable-bacteria filaments. Both setups mimicked the redox gradient conditions that cable bacteria experience in their natural habitat, with a sulfide source (sediment) on one side, and an oxygen source (air) on the other side. In setup A, two wells (diameter 1–4 mm, separation 5 mm) were drilled into 4-mm-thick glass microscopy slides using a diamond drill. One well was filled with the cable-bacteria–enriched sediment, while the other was left open and hence filled with ambient air. Tap water or seawater (depending on the sediment source) was flushed with nitrogen gas and pipetted onto the microscope slide in between the two wells; the wells and interspace were covered by a coverslip (25 × 40 mm), and sealed with Vaseline or silicon grease to prevent evaporation, thus forming a 5-mm-wide, 50–100-μm-high water layer between sediment and air. Cable bacteria reached out of the sediment and moved across the water zone toward the air-filled well within 24 h (Movie S1).

In setup B, glass slabs (obtained by cutting of microscope slides) were glued onto a microscope slide, thus creating a trench (10 × 50 mm) in the middle. This trench was filled with the cable-bacteria–enriched sediment, a large coverslip was mounted on top, and the slide was flooded with nitrogen-gas–flushed water, creating a 5 × 50-mm water-filled interspace between the sediment trench and the edge of the coverslip. The edges of the coverslip were subsequently sealed with nail polish to prevent evaporation but allow oxygen diffusion. As in setup A, cable bacteria reached out of the sediment toward the oxic zone near the edge of the coverslip.

Oxygen and Hydrogen Sulfide Microsensor Measurements. Extralong and thin microelectrodes for O_2 and H_2S were custom-made at Aarhus University, with a tip diameter of less than 50 μm. Microelectrodes were mounted sideways on a motorized micromanipulator, and inserted between the microscope slide and the coverslip of slide setup B. The oxygen concentration was recorded at 100-μm step resolution, from the edge of the coverslip until the sensor was 2 mm beyond the veil of microaerophilic bacteria. For H_2S, a concentration profile was recorded from the edge of the coverslip all the way into the sediment at 200-μm step resolution.

Resonance Raman Microscopy. Most Raman spectra were recorded on a LabRAM HR Evolution confocal Raman microscope (Horiba) equipped with a 500-mW, 532-nm laser, an Andor EM CCD detector adapted to 500–650-nm emissions, 300 grating with a spectral resolution of 2.8 cm^{-1}, and the pinhole set to 250 μm. Additional spectra were recorded on a Renishaw InVia confocal Raman microscope equipped with a Leica light microscope with 50× air objective and 532-nm laser. The output of the laser was set between 4 and 10% of maximum power. Raman spectra were recorded along individual filaments of cable bacteria starting from the sediment and moving toward the air inlet. At each longitudinal position, 2–3 line scans with 10–20 measuring points each were performed across the filament (i.e., perpendicular to its longitudinal axis), with 2.5–20-s exposure time for each measuring point. The ν_{15} (at 750 cm^{-1}) and the ν_{10} vibrational modes (at 1,637 cm^{-1}) were used as measure of cytochrome redox state, and data reported for each filament position are means of the quality-filtered and normalized band intensities (see Data Analysis below) at 750 cm^{-1} and at 1,637 cm^{-1} from multiple line scans (see Data Analysis below).

Manipulation Experiments. Two manipulation experiments were performed where electron transport was inhibited and the change in cytochrome redox state was recorded by Raman microscopy. First, oxygen was removed from the oxic end of slide setup A by either filling the air inlet with nitrogen-flushed, oxygen-free water or by flushing it directly with a gentle flow of nitrogen gas. Raman spectra were recorded at approximately 500 μm from the sediment and at the midpoint between the sediment and the start of the oxic zone (as marked by a veil of putative microaerophilic bacteria) every 1–3 min over a period of 15–30 min before and after the manipulation. The first 5 min after removing oxygen were excluded to account for the time it took to fully deplete oxygen at the end of the cable bacteria. Oxygen was reintroduced by stopping the flow of nitrogen gas, and the response in cytochrome redox state was immediately recorded at midpoint only.

Second, a laser microdissection microscope, LMD7000 (Leica), was used to make two cuts 10 μm apart in the cable-bacteria filament, approximately 1,000 μm from the sediment. The cut was microscopically inspected to confirm that the filament sections had separated. Raman spectra were recorded at approximately 500 μm from the sediment, directly before and about 5 min after the cut (the time it took to move the chamber between laser microdissection and Raman microscope).

In both experiments, cable-bacteria filaments, which were only connected to the sediment but did not reach the oxic zone, were used as controls.

Data Analysis.

Preprocessing. Raman spectra were preprocessed (5) by background correction with a sensitive nonlinear iterative peak-clipping algorithm with optimized parameters (smoothing = true, iteration = 70, window = 15).

Quality filtering. Because cable-bacteria filaments are constantly moving, and measuring a cross-section takes >1 min, it was necessary to use the C-H region of the spectra (2,800–3,000 cm^{-1}) as indicator for a cable-bacterium hit. The maximum peak height between 2,800 and 3,000 cm^{-1} was divided by the median baseline from 2,800 to 2,850 cm^{-1}. All ratios from an entire filament dataset were then plotted as histogram for each filament, and datasets with a bimodal distribution of ratios were discarded, as this indicated that multiple cable-bacteria filaments or additional bacteria had been recorded in that dataset. Of the remaining datasets, only spectra with a ratio >3.5 were kept as "high-quality

spectra," while those with a ratio <3.5 were discarded as hits outside the cable bacteria. After the filtering, 3,430 high-quality spectra remained of a total of 8,729 recorded spectra.

Normalization. For the line-scan measurements along cable bacteria and the two manipulation experiments, the band intensity at 750 cm^{-1} was normalized by subtracting the median of the baseline from 735 to 740 cm^{-1} and from 760 to 765 cm^{-1}, to enable comparison within an individual cable bacterium. A similar approach was used for normalization of all other bands (1,130, 1,315, 1,497, 1,588, and 1,637 cm^{-1}). Means were calculated for each individual, normalized line scan, and subsequently the mean for each position or treatment was calculated from these means. SDs were calculated from all individual data points for a given position.

In the two manipulation experiments, where comparison between different filaments was necessary, the band intensity at 750 cm^{-1} before the manipulation were normalized to 1, and any response to the manipulation is given as fold change relative to that value.

Statistical Analysis. All data for which statistical analysis was performed were first tested for deviation from a normal distribution using a Shapiro–Wilk test of normality.

The normalized intensity change between the reduced and the oxidized filament ends was tested for being greater than 0 using a one-sided *t* test. The observed change after oxygen and laser-cutting manipulations in the normalized intensity was tested for being different from zero using a two-sided *t* test (for normal data) or Wilcoxon sign test (for nonnormal data).

All calculations for data and statistical analysis were performed using the statistical software program R (6).

Quantifying the Voltage Drop Along Individual Cable Bacteria. The recorded gradients in cytochrome redox status of single filaments through the suboxic zone allow for calculation of the voltage gradient, assuming that the cytochromes are in equilibrium with the electron conductor in this zone. The local cytochrome redox potential is determined by the Nernst equation for single electron transfer:

$$\mathrm{E} = E^0 - \frac{RT}{F}\ln\frac{[\mathrm{Red}]}{[\mathrm{Ox}]}, \qquad \textbf{[S1]}$$

where E^o is the midpotential, Red and Ox the numbers of reduced and oxidized cytochromes, and R, T, and F denote the gas constant, the absolute temperature, and the Faraday constant, respectively. The voltage difference between two sites in the suboxic zone, near sediment (a) and near the oxic zone (b), is then

$$\mathrm{E} = E^b - E^a = \left(E^0 - \frac{RT}{F}\ln\frac{[\mathrm{Red}]^b}{[\mathrm{Ox}]^b}\right) - \left(E^0 - \frac{RT}{F}\ln\frac{[\mathrm{Red}]^a}{[\mathrm{Ox}]^a}\right). \qquad \textbf{[S2]}$$

This condenses into

$$\mathrm{E} = \frac{RT}{F}\ln\left(\frac{[\mathrm{Red}]^a}{[\mathrm{Red}]^b}\Big/\frac{[\mathrm{Ox}]^a}{[\mathrm{Ox}]^b}\right). \qquad \textbf{[S3]}$$

Assuming that

$$\frac{[\mathrm{Red}]^a}{[\mathrm{Red}]^b} = \frac{[\mathrm{band\ 750}]^a}{[\mathrm{band\ 750}]^b}, \qquad \textbf{[S4]}$$

and

$$\frac{[\mathrm{Ox}]^a}{[\mathrm{Ox}]^b} = \frac{[\mathrm{band\ 1{,}637}]^a}{[\mathrm{band\ 1{,}637}]^b}, \qquad \textbf{[S5]}$$

the voltage difference can be calculated as

$$\mathrm{E} = \frac{RT}{F}\ln\left(\frac{\frac{[\mathrm{band\ 750}]^a}{[\mathrm{band\ 750}]^b}}{\frac{[\mathrm{band\ 1{,}637}]^a}{[\mathrm{band\ 1{,}637}]^b}}\right). \qquad \textbf{[S6]}$$

The same equation holds when band 750 is replaced with any of the other reduction bands (1,130, 1,315, 1,497, and 1,588, cm^{-1}). Therefore, for the final calculation, we apply the ratio of all reduction bands for every filament (Dataset S1). For the analyzed 2.1–4.3-mm-long suboxic segments of six large cable-bacteria filaments (Rattekaai, The Netherlands), the average voltage difference, normalized to the length of the suboxic zone, was 14.6 mV mm^{-1} ± 4.1 (SD).

This result was confirmed by another measure of $[\mathrm{Ox}]^a/[\mathrm{Ox}]^b$ than the relatively uncertain $1{,}637^a/1{,}637^b$ ratio (Eq. **S5**): The increase in band intensities near the sulfidic zone after laser cutting (Fig. 3) was assumed to represent maximum electron saturation of the cytochromes, and thus represents the situation where all cytochromes are reduced. Therefore, [Ox], being the unsaturated cytochromes, can be expressed as

$$[\mathrm{Ox}] = [\mathrm{Red}]^{\max} - [\mathrm{Red}], \qquad \textbf{[S7]}$$

where $[\mathrm{Red}]^{\max}$ represents the total concentration of cytochromes.

Introducing this in Eqs. **S4** and **S5** leads to the solution

$$\frac{[\mathrm{Ox}]^a}{[\mathrm{Ox}]^b} = \frac{[\mathrm{band\ 750}]^{\max} - [\mathrm{band\ 750}]^a}{[\mathrm{band\ 750}]^{\max} - [\mathrm{band\ 750}]^b}. \qquad \textbf{[S8]}$$

As before, the band 750 can be replaced with 1,130-, 1,315-, 1,497-, and 1,588-cm^{-1} bands. For the 10 laser-cut Rattekaai cable-bacteria filaments (Fig. 3), the ratio $\mathrm{Ox}^a/\mathrm{Ox}^b$ became 0.62 ± 0.04 SD (±0.08 SD with $\mathrm{Red}^{\max}$ estimate), i.e., not different from the $1{,}637^a/1{,}637^b$ ratio (0.49 ± 0.16 SD); see Dataset S1 for data and calculations. Using this value for the above calculation, and normalizing for length, the voltage gradients were 12.3 mV mm^{-1} ± 3.8 (SD).

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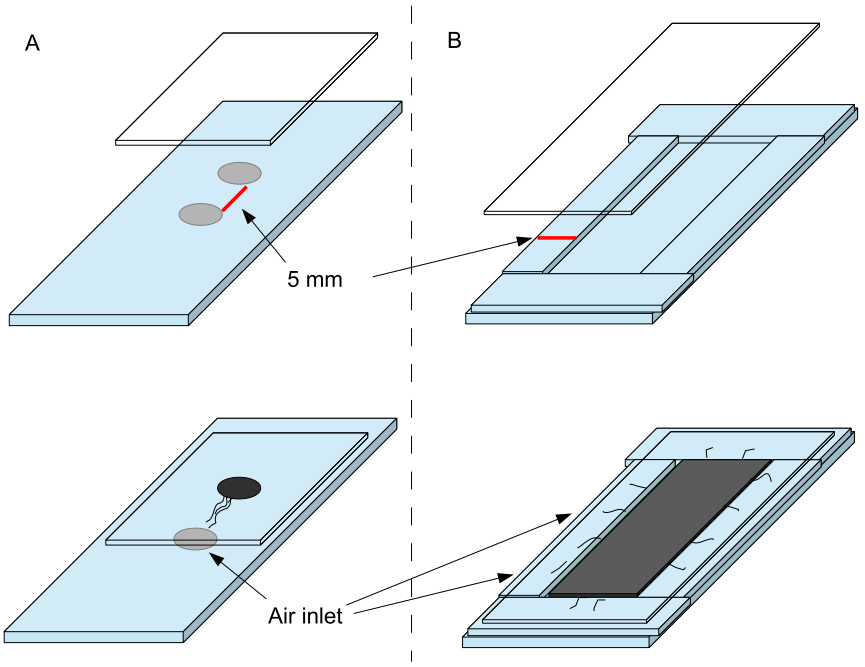


Fig. S1. (*A*) Microscope setup used for gradients and oxygen manipulations. (*Top*) Thick microscope slide with two wells (light gray); red bar shows the 5-mm interspace between wells. (*Bottom*) Filled slide setup with sediment in one well (dark gray), from which cable bacteria reach toward the other well (light gray, air inlet). Availability of oxygen can be manipulated at the open air inlet (marked by an arrow). (*B*) Microscope slide setup used for gradients and laser cuts. (*Top*) Microscope slide with long trench in the center; red bar shows the 5-mm space from trench to edge of slide. (*Bottom*) Filled slide setup with sediment (dark gray) in the trench, from which cable bacteria reach out toward the edge of the slide, where oxygen is available.

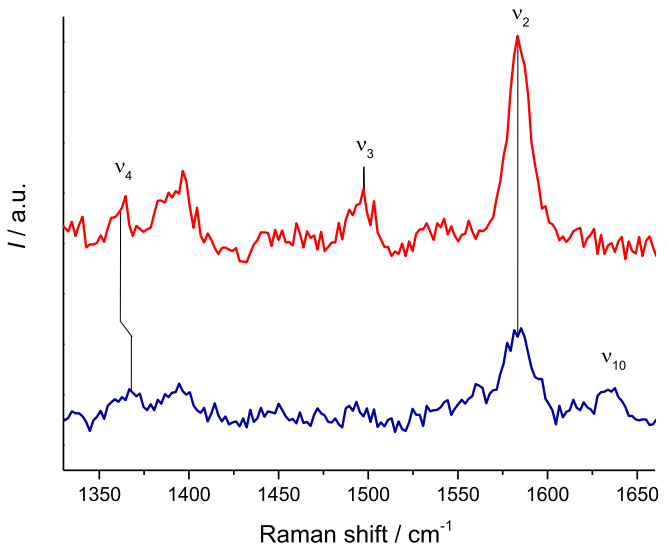


Fig. S2. Raman spectra from a >4-μm-thick individual cable-bacterium filament from a marine salt marsh (Rattekaai, The Netherlands) near sediment (red) and near oxygen (blue). An enlargement of the area between 1,330 and 1,660 cm^{-1} of the same spectrum displayed in Fig. 1*D* is shown.

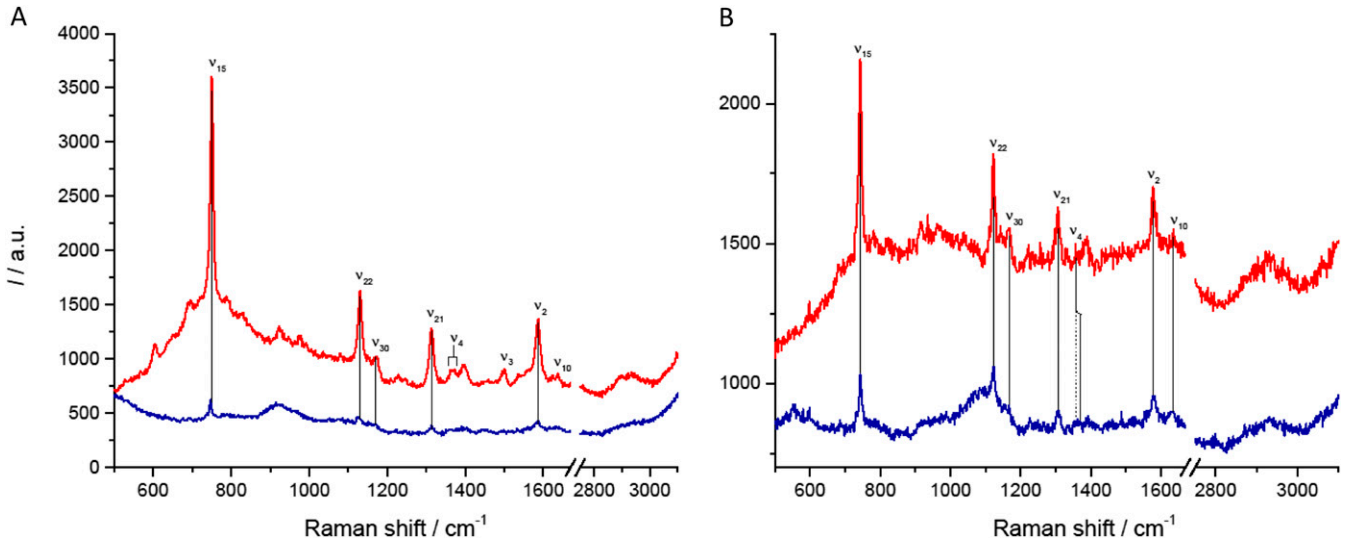


Fig. S3. (*A*) Raman spectra from a thin, individual cable-bacterium filament from freshwater sediment (Aarhus, Denmark) near sediment (red) and near oxygen (blue). (*B*) Raman spectra from an individual cable-bacterium filament from intertidal mud flat (Mokbaai, Texel, The Netherlands) near sediment (red) and near oxygen (blue).

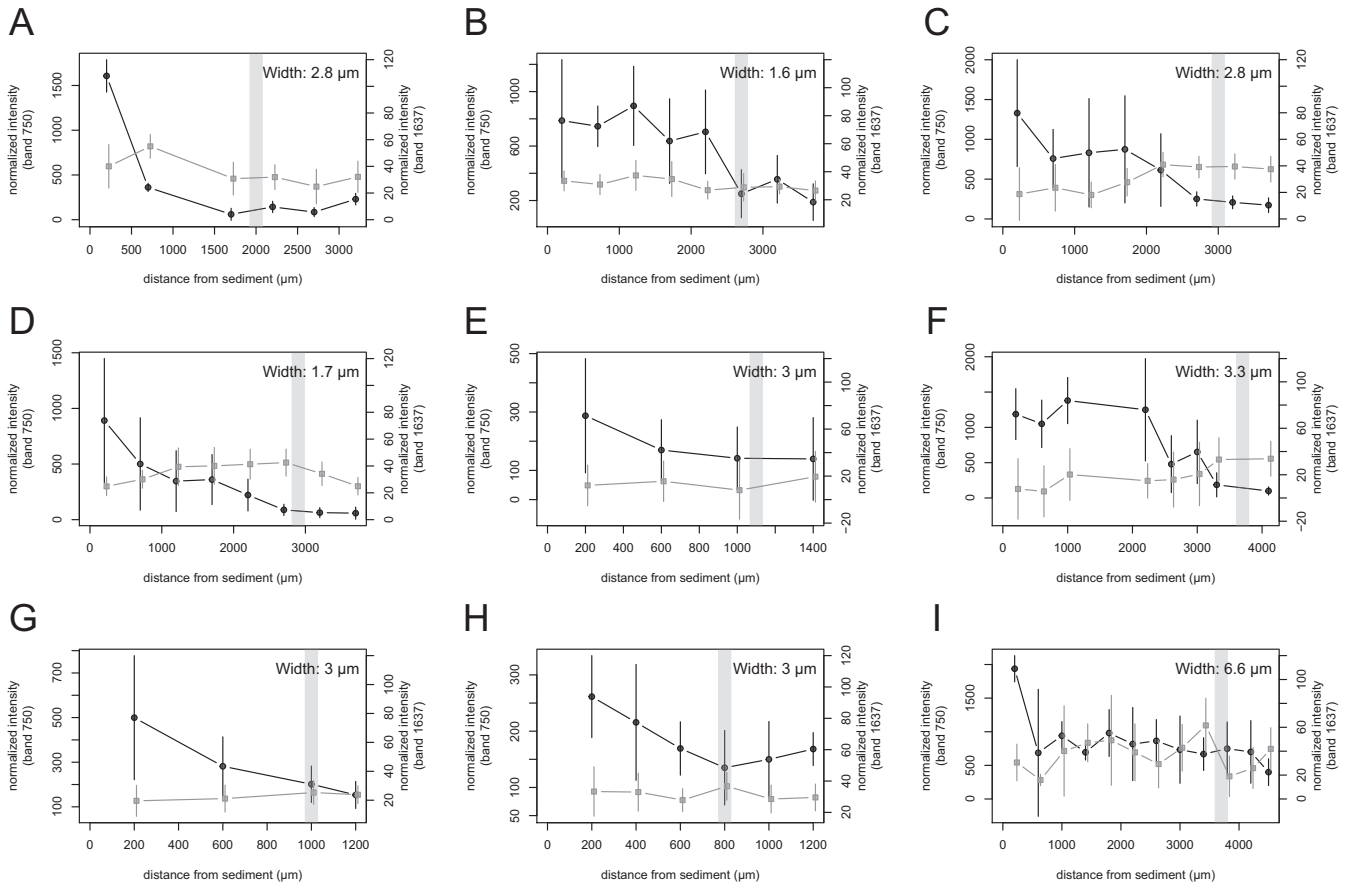


Fig. S4. (Continued)

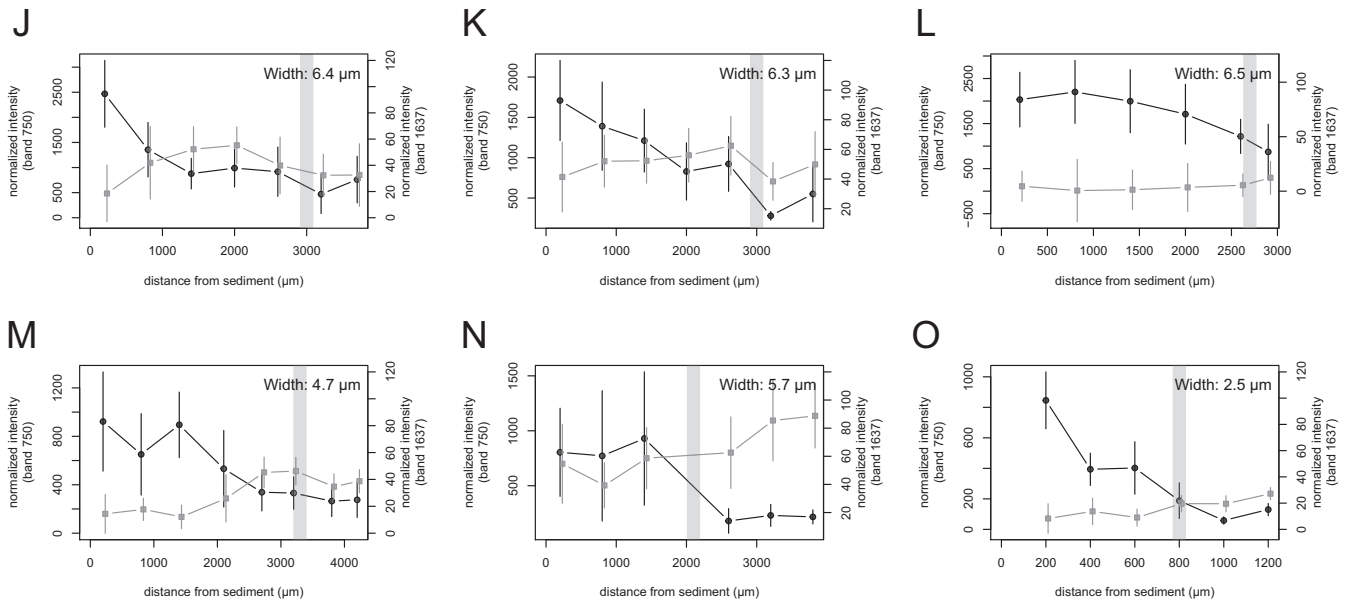


Fig. S4. Cytochrome redox gradient along individual cable-bacteria filaments reaching from sediment toward oxygen. The displayed values (mean ± SD; n = 1–35 spectra per position) are normalized band intensities at 750 cm^{-1} (black dots) and 1,637 cm^{-1} (gray squares). (*A–D*) Cable bacteria from freshwater sediment (Aarhus, Denmark). (*E–H*) Cable bacteria from intertidal mud flat (Mokbaai, Texel, The Netherlands). (*I–O*) Cable bacteria from marine salt marsh (Rattekaai, The Netherlands).

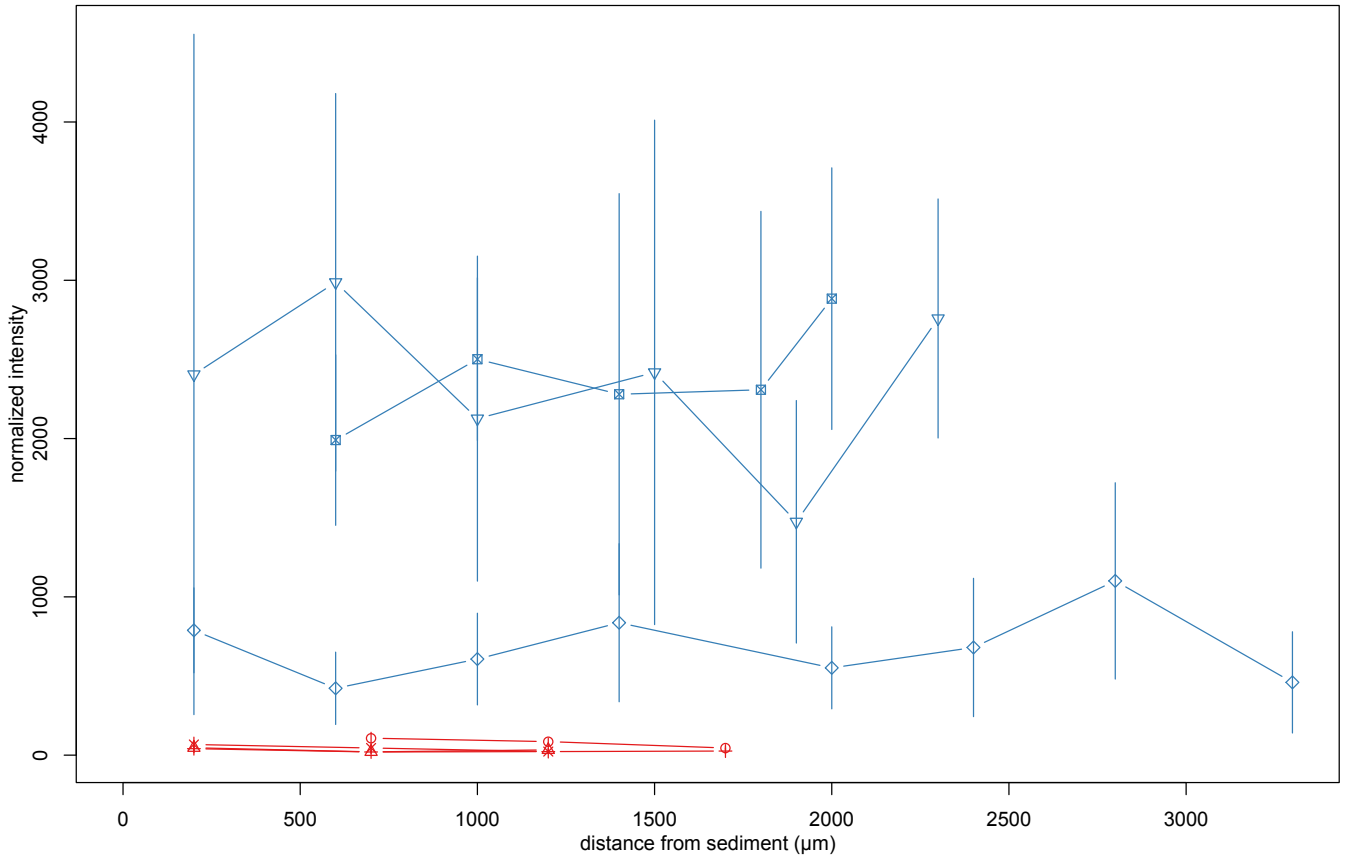
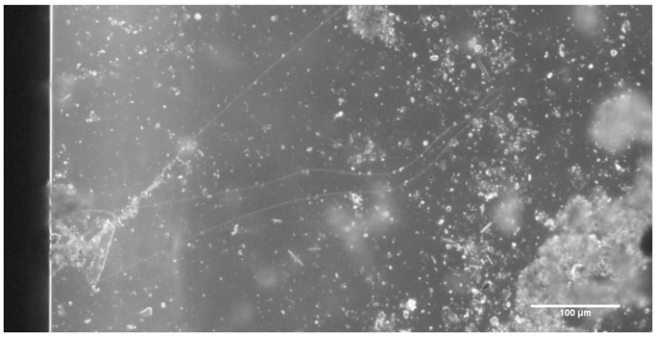


Fig. S5. Cytochrome redox state (mean ± SD of the normalized band 750-cm^{-1} intensities) along control cable bacteria. Red, four filaments in fully oxic water (98 spectra); blue, three filaments, which did not reach the oxic zone (301 spectra).



Movie S1. Time-lapse movie of cable bacteria emerging from sediment (left side) in the microscope chamber setup and gliding toward oxygen (to the far right). The time was compressed from 16 min to 17 s. (Scale bar, 100 µm.)

Movie S1

Dataset S1. Data and calculations to quantify the voltage drop over the length of individual cable-bacteria filaments.

Dataset S1