RESEARCH ARTICLE

The rhizosphere of aquatic plants is a habitat for cable bacteria

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One sentence summary: Cable bacteria are ubiquitous and active in the rhizosphere of aquatic plants.

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ABSTRACT

Cable bacteria belonging to the family *Desulfobulbaceae* couple sulfide oxidation and oxygen reduction by long-distance electron transfer over centimeter distances in marine and freshwater sediments. In such habitats, aquatic plants can release oxygen into the rhizosphere. Hence, the rhizosphere constitutes an ideal habitat for cable bacteria, which have been reported on seagrass roots recently. Here, we employ experimental approaches to investigate activity, abundance, and spatial orientation of cable bacteria next to the roots of the freshwater plant *Littorella uniflora*. Fluorescence *in situ* hybridization (FISH), in combination with oxygen-sensitive planar optodes, demonstrated that cable bacteria densities are enriched at the oxic–anoxic transition zone next to roots compared to the bulk sediment in the same depth. Scanning electron microscopy showed cable bacteria along root hairs. Electric potential measurements showed a lateral electric field over centimeters from the roots, indicating cable bacteria activity. In addition, FISH revealed that cable bacteria were present in the rhizosphere of *Oryza sativa* (rice), *Lobelia cardinalis* and *Salicornia europaea*. Hence, the interaction of cable bacteria with aquatic plants of different growth forms and habitats indicates that the plant root–cable bacteria interaction might be a common property of aquatic plant rhizospheres.

Keywords: cable bacteria; filamentous bacteria; aquatic plants; rhizosphere; oxygen distribution; electric field

INTRODUCTION

In water-saturated, organic-rich sediments, oxygen from the water column does not diffuse beyond the uppermost millimeters, whereas deeper sediments often contain millimolar concentrations of sulfide. Aquatic plants adapt to survive in such anoxic sediments by an extensive system of gas spaces supplying oxygen from photosynthesis to the roots (Armstrong *et al.* 1996). Moreover, aquatic plants can release oxygen into the rhizosphere—the zone surrounding the roots—producing geochemical gradients that are subject to daily fluctuations as photosynthesis ceases during night and the oxygenated zone diminishes (Lenzewski *et al.* 2018). In the oxygenated zone, abiotic and biotic sulfide oxidation play an essential role in protecting the roots against phytotoxic sulfide (Sand-Jensen, Prahl and

Stokholm 1982; Jensen, Kühl and Priemé 2007; Lamers *et al*. 2013; Brodersen *et al*. 2018).

Sulfide-oxidizing cable bacteria are filamentous, multicellular members of the family *Desulfobulbaceae* (Pfeffer *et al*. 2012), which live in marine and freshwater sediments and may extend several centimeters from the oxic surface into the sulfidic sediment. By means of internal electron conduction, they couple the oxidation of sulfide at their deep, anodic end to the reduction of molecular oxygen or nitrate at the cathodic end on the sediment surface (Pfeffer *et al*. 2012; Marzocchi *et al*. 2014). The unique morphology and metabolism of cable bacteria can be used to determine their identity and activity in sediments. Strings underneath the outer membrane of cable bacteria run along the length of the filaments, which give rise to their resilient structure and characteristic ridges (Jiang *et al*. 2018). Moreover, the transport of charges by cable bacteria causes an electric field, which can be measured as an increase of electric potential with depth (Risgaard-Petersen *et al*. 2014). The electric field drives an ion current in the pore water, completing the electric circuit and maintaining a charge balance. The activity of cable bacteria strongly impacts the geochemistry of the sediment (Risgaard-Petersen *et al*. 2012), which leads to characteristic geochemical fingerprints: a pH maximum in the oxic zone due to the cathodic oxygen reduction and a decreased pH in the sulfidic zone due to the anodic sulfide oxidation. The lower pH in deeper layers likely promotes iron-sulfide dissolution and, hence, replenishes the electron donor sulfide. An additional source of sulfide replenishment to sustain cable bacteria metabolism can be sulfide diffusion from deeper sediment layers or local sulfate reduction (Nielsen and Risgaard-Petersen 2015). The spatial separation of the two redox reactions causes a suboxic zone of several centimeters that is devoid of measurable sulfide and oxygen (van de Velde *et al*. 2016). Consequently, cable bacteria have a competitive advantage over unicellular sulfide oxidizers, which can thrive only where the gradients of sulfide and oxygen meet. Furthermore, the large span of the cable bacteria makes them tolerant to fluctuating gradients of sulfide and oxygen, and, if needed, they can move to new locations by gliding motility (Bjerg *et al*. 2016). Cable bacteria have a global distribution in marine and freshwater sediments (Trojan *et al*. 2016; Burdorf *et al*. 2017), including the surface of oxygen-releasing seagrass roots, where they have been proposed to protect the plant against toxic sulfide (Martin *et al*. 2018).

This study aimed at testing not only the presence but also the activity and spatial arrangement of cable bacteria around oxygen-releasing roots. We hypothesize that cable bacteria actively perform sulfide oxidation in the rhizosphere of marine and freshwater aquatic plants extending to centimeters away from the oxygen-releasing roots. Thus, these filamentous bacteria conserve energy from oxidation of the electron donor sulfide, produced by sulfate-reducing bacteria in bulk sediments, and reduction of the electron acceptor oxygen, released from the plant roots. Employing microscopic techniques, we investigated the activity and localization of cable bacteria in the rhizosphere of *Littorella uniflora*, which is a small, evergreen, freshwater plant known to release oxygen from the roots (Christensen, Revsbech and Sand-Jensen 1994). Furthermore, we studied the presence of cable bacteria in the rhizosphere of rice, *Lobelia cardinalis* and *Salicornia europaea*, to assess whether the relationship between cable bacteria and aquatic plants could be a common phenomenon.

MATERIALS AND METHODS

Plants and growth conditions

The freshwater plants *Li . uniflora* and *Lo. cardinalis* (Zoo Zajac, Duisburg, Germany) were grown in a 20-L tank filled with tap water and a layer of quartz sand. Light was supplied in 11 h photoperiods by LED lamps (Classic LED Aquarium Light; Nicrew, Shenzhen City, China).

The halophyte *S. europaea* was sampled from Aggersund, Denmark (57° 0'1.08‘N, 9°17'19.10‘E) using a sediment corer. Plants were rinsed in seawater before measurements.

Rice seeds (*Oryza sativa*) were soaked in water for 24 h and grown in drained soil pots before planting in bowls with a sieved, nutrient-rich and reduced sediment from the banks of river Giber Å, Denmark. The sediment was covered with 1-cm tap water. Plants received daylight, were kept at room temperature and grown for more than 3 months. Subsequently, the rice plants were transferred into a sediment that had been kept anoxic for more than 2 months, and were illuminated by LED lamps (Reflector PAR38; Megaman, Hong Kong, China) for 4 weeks until sampling.

Rhizo-sandwich

The rhizo-sandwich set-up was used to visualize oxygen dynamics by planar optode measurements, determine electric fields by electric potential profiling, and sample in precise spots for scanning electron microscopy (SEM) and quantification of cable bacteria with fluorescence *in situ* hybridization (FISH). The sediment was retrieved from an exposed mud flat in the Giber Å stream system at Vilhemsborg, Denmark (56° 4'3.78‘N, 10°11'9.04'E) at a temperature of 5°C and conductivity of the pore water of 651 μS/cm. There was no odor of H_2S, but the blackish color indicated the presence of iron sulfides. Geochemical characteristics of comparable sediments from stream banks 500 m upstream were described previously (Risgaard-Petersen *et al*. 2015). The sediment was sieved with a mesh size of 0.5 mm and stored in an anaerobic chamber at room temperature for a minimum of 2 weeks to minimize bioturbation during the experiment.

To construct the rhizo-sandwich (Fig. S1A, Supporting Information), microscope slides were glued with silicone sealant (734 Clear; Dow Corning, Midland, Michigan, USA) to the edges of a glass plate (24.5 × 14 cm^2) leaving one side open. A planar optode foil was attached to the inside of the glass plate (Brodersen *et al*. 2017). The sediment was spread out and roots of *Li. uniflora* were placed on the sediment before closing with a second glass plate, clamping and tightening with modeling clay. The sides were covered with an opaque plastic foil and incubated at room temperature in a continuously mixed tap water tank (58 × 29 × 30 cm^3). The rhizo-sandwiches were illuminated on a 10:14 h light–dark cycle by an LED lamp (Reflector PAR38; Megaman, Hong Kong, China) and 300 μmol photons/m^2 reached the canopy of the plants.

Root-sandwich

In contrast to the rhizo-sandwich, which includes the entire below-ground biomass and sediment, the root-sandwich contains only single plant roots. The root-sandwich maintained the rhizosphere spatially intact while sampling and preparing for FISH analysis.
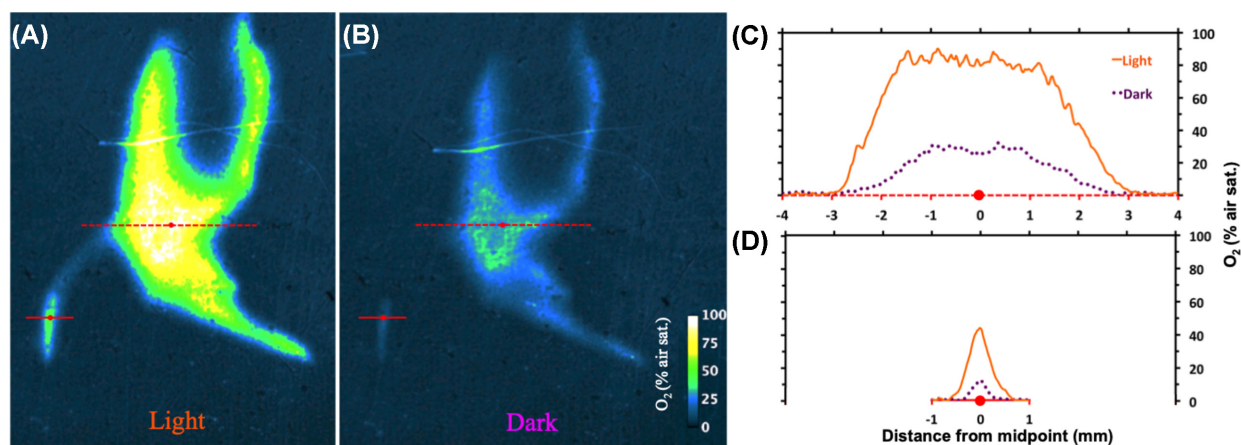
Figure 1. Oxygen dynamics in the rhizosphere of *Li. uniflora* between light (**A**) and dark (**B**) conditions. (**C**) Concentration profile crossing multiple roots (red dotted line, 8 mm). (**D**) Concentration profile crossing a single root (straight red line, 2 mm).
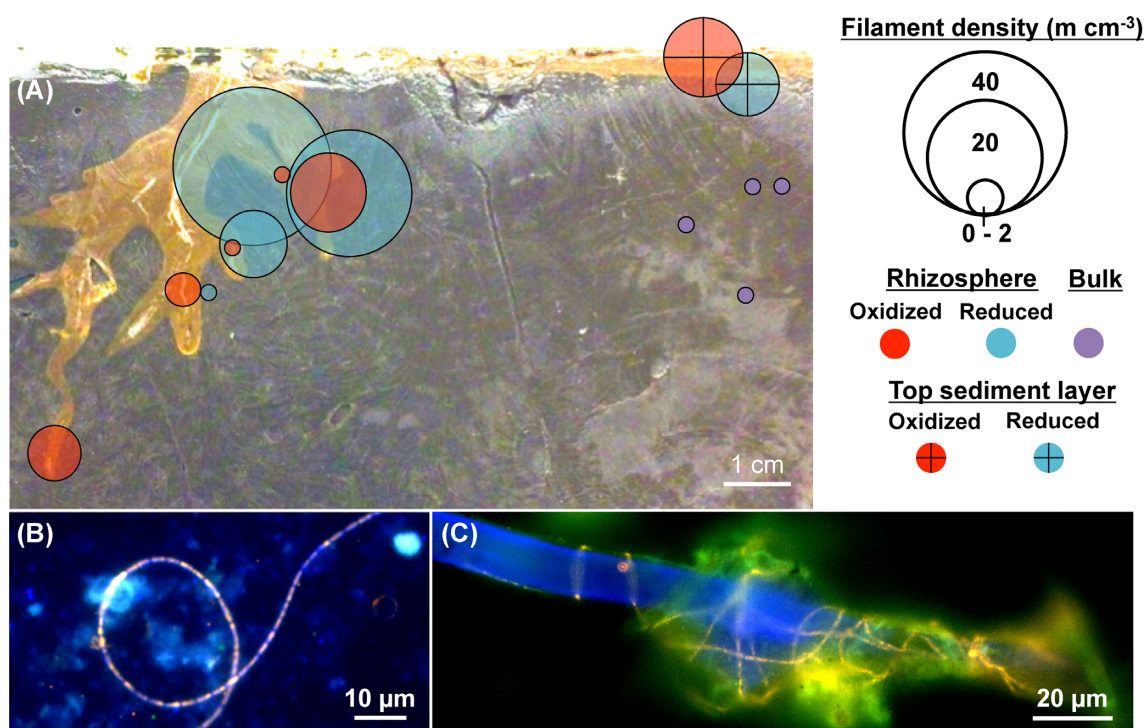

Figure 2. FISH images and distribution of cable bacteria in the rhizo-sandwich of *Li. uniflora*. (**A**) Differentiation between oxidized (red) and reduced (blue) rhizospheres, oxidized (crossed red) and reduced (crossed blue) top sediment layers and bulk sediments (purple). The redox status was estimated from the shading of sediment (bright = oxidized, dark = reduced). The circle midpoints correspond to the sampling location and the circle areas to the cable bacteria density expressed in m/cm^3. (**B** and **C**) FISH micrographs of cable filaments alone and intertwined with a root hair found in the reduced rhizospheres. Micrographs from DAPI staining (blue) were superimposed with FISH images hybridized with probe DSB706 specific for *Desulfobulbaceae* labeled with Cy3 (red) and probe EUB-MIX targeting most bacteria labeled with Atto-488 (green).

Roots of *Li. uniflora* and *Lo. cardinalis* were aligned on a microscope slide (75 × 26 mm) with cover slips as spacers (Figs S1B and S2A, Supporting Information). A suspension of 10 mM iron sulfide (Müller *et al.* 2016) and the iron-reducing culture 1MN, containing groundwater cable bacteria belonging to the family *Desulfobulbaceae* up to 95% of the microbial community (Marozava *et al.* 2018), was added before closing with a second microscope slide and clamping with hair clips. Plants were placed on top of the sediment and sand was filled up to the leaf sheaths, covering both roots and microscope slides. The jar was filled with a 6-cm sediment overlaid with 4 cm of water phase fully submerging the plants. To further stimulate the microbial community, the sediment was inoculated with the culture 1MN by using a syringe. The plants were illuminated on a 10:14 h light–dark cycle by a fluorescent tube (Flora Sun; Zoo Med, USA).

After 13 days, the root-sandwiches containing a single root were removed, wrapped in an aluminum foil, and shock-frozen in liquid nitrogen. A frozen sandwich was placed with the flat side on a laboratory bench so that the lower slide melted while the upper remained frozen. The frozen side containing the root was pulled away, frozen again, covered with pre-cooled glutaraldehyde (final concentration ∼ 2.5%) avoiding rapid thawing

and dried at 50°C for 3 h to prepare for FISH analysis. A root-sandwich containing a dissected root (no contact through plant tissue to the water column) was taken as a control.

The *Desulfobulbaceae*-enriched culture 1MN was cultivated in 50 mL freshwater mineral medium (Widdel and Bak 1992) in 125-mL serum bottles sealed with butyl rubber stoppers under anoxic conditions. The culture was grown with initial concentrations of 30 mM S^0, 30 mM $Fe(OH)_3$ and 0.7 mM HS^-, with 30 mM bicarbonate buffer at pH 6.5. Quartz sand (400 mL, grain size 0.0036–1 mm, porosity 0.2) was washed thrice with tap water, amended with 3 $\mu mol/cm^3$ FeS and filled into a 2-L glass jar.

Planar optode measurements

A fluorescence imaging-based planar optode system (Larsen *et al*. 2011; Brodersen *et al*. 2017) was used to visualize the oxygen dynamics in the rhizosphere of *Li. uniflora* and *S. europaea*. An amount of 1.5 mg of platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorphenyl)-porphyrin (PtTFPP; Frontier Scientific, Logan, Utah, USA), 1.5 mg of Macrolex fluorescence yellow (Lanxess, Köln, Germany) as well as 100 mg of polystyrene (Sigma-Aldrich, Taufkirchen, Germany) were dissolved in 1 g of chloroform and knife-coated (~12 μm thickness) on a dust-free PET foil (Puetz Folien, Taunusstein, Germany). On top of that an optical isolation layer, consisting of 100 mg of carbon black (Kremer Pigmente, Aichstetten, Germany) in silicone (ELASTOSIL E4, Wacker, München, Germany), was coated (Larsen *et al*. 2011). The foil was attached with tap water to the rhizo-sandwich slide (*Li. uniflora*) or a transparent acrylic chamber (10 × 10 × 29 cm³) (*S. europaea*) and a vinyl electrical tape (Super 33+; Scotch, Minnesota, USA) was used to seal the edges. Roots of *S. europaea* were held close to the planar optode with 5-mm-wide plankton net stripes while the seawater and sediment from the sampling site was carefully added. The water column above the sediment was approximately 2 cm, submersing most of the biomass. The set-up was equilibrated for 10 h in the dark and 1 h in the light before optode measurements.

The planar optode was excited with an LED lamp (i-led, ILH-GD01-DEBL-SC201; r-s components, Copenhagen, Denmark) powered by a USB-controlled LED driver unit (http://imaging.fish-n-chips.de). Images were taken with an SLR camera (EOS 1300D; Canon, Tokio, Japan) equipped with a macro objective (100mm f/2.8 AT-X M100 AF Pro D; Tokina, Tokio, Japan) and a 530-nm-long pass filter (52 mm x 2 mm). LED excitation and image acquisition were controlled and synchronized with the software look@RGB (http://imaging.fish-n-chips.de). Pieces of the used sensor foils were calibrated in similar tanks with tap water and seawater from the sampling site by bubbling with air and N_2 (Na_2SO_3 was added to obtain fully anoxic conditions). O_2 concentrations within the tank were simultaneously monitored with a calibrated oxygen meter (FireStingGO2 equipped with a robust sensor probe; Pyroscience, Aachen, Germany) and expressed as % air saturation.

Image processing was performed with ImageJ (https://imagej.nih.gov/ij/) using the plugin Ratio Plus (https://imagej.nih.gov/ij/plugins/ratio-plus.html). The image resolution was 40 pixels/mm. Due to drifts throughout the prolonged incubation of the sample (>2 weeks), it was necessary to correct the images. This was achieved by assuming that the bulk sediment was anoxic at all times. Hence, the baseline of the concentration profiles was corrected by subtracting the lowest value in the reduced zone.
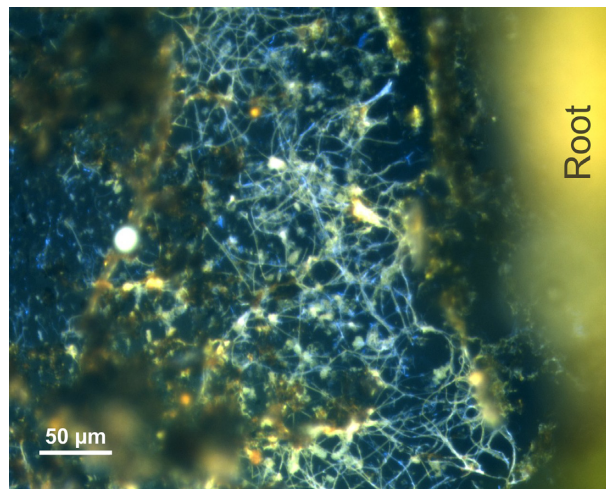

Figure 3. Overlay of micrographs from DAPI and FISH images of cable bacteria next to a root of *Lo. cardinalis* in the root-sandwich. Probe DSB706 specific for *Desulfobulbaceae* labeled with Cy3 (red) and probe FlidDSB194 specific for groundwater cable bacteria labeled with 6-Fam (green) were chosen for hybridization.

Optode imaging of the rhizosphere of *Li. uniflora* was conducted continuously over one light–dark shift in 20 min intervals and over one dark–light shift in 10 min intervals.

FISH analysis

The entire rhizo-sandwich, including one intact plant of *Li. uniflora*, was shock-frozen in liquid nitrogen. Upon thawing, one glass plate could be removed while the sediment remained frozen to the other before it thawed completely. Oxidation of the sediment led to the formation of visible bright shaded zones millimetres in width and thus allowing for sampling in defined areas. Samples were taken from the oxidized rhizosphere and top sediment layer (bright) and the reduced rhizosphere and top sediment layer (dark) 0–5 mm distant from the oxidized zone. Furthermore, samples of bulk sediment were taken, defined as >4 cm away from the oxidized rhizosphere and >2 cm from the water–sediment interface. Sediment samples (15–80 μL) were fixed in 96% ethanol and stored at 20°C.

The rhizosphere of *S. europaea* was sampled as a sediment core containing an entire plant, drained and cut into slices. The bowl containing rice plants and the sediment was drained. Rice plants and the sediment were carefully lifted out and separated with gloved hands, leading to the emergence of roots partly covered with the sediment. The adhering sediment on the roots was taken off from the roots with a spatula tip, fixed in 96% ethanol and stored at −20°C.

Sediment samples were homogenized by mild ultrasound 3 × 20 s at intervals of 10 s (SonopulsHD 2070; Bandelin, Berlin, Germany). Aliquots were diluted 10-fold in 0.1% sodium pyrophosphate and 1% agarose was added in a ratio of 1:50 [v/v]. FISH was performed as described by Pernthaler *et al*. (2001) with the specific oligonucleotide probes DSB706 (Loy *et al*. 2002) and FliDSB194 (Müller *et al*. 2016) (Table S1, Supplementary Information) at a formamide concentration of 35% for the detection of filamentous *Desulfobulbaceae*. Samples were covered with Citifluor (Citifluor, Hatfield, Pennsylvania, USA), Vecta Shield (VWR, Søborg, Denmark) and counterstained with 1 mg/mL 4′,6-diamidin-2-phenylindol (DAPI). Rhizo-sandwiches, *S. europaea*, and rice samples were additionally hybridized with the oligonucleotide probes EUB-MIX (Amann *et al*. 1990; Daims *et al*. 1999).

The negative control probe NON-EUB (Manz *et al.* 1992) was run along with each analysis. For the determination of the density of cable bacteria (m/cm^3), the length of all positively stained filaments consisting of at least four cells was measured using the imaging software NIS-Elements (Version 4.50; Nikon Instruments Inc., NY, USA).

Electron microscopy

A root fragment of *Li. uniflora* from the rhizo-sandwich was washed in tap water, air dried on a silicon waver and analyzed by high-vacuum (13 pA) SEM (Versa 3D DualBeam; FEI, Hillsboro, Oregon, USA) operated in 5-kV mode.

Electric potential profiling

Electrical potentials were measured with a self-made microelectrode (Damgaard, Copenhagen K, Denmark; Risgaard-Petersen and Nielsen 2014), custom made millivoltmeter (Aarhus University, Aarhus C, Denmark) and 16-bit A/D converter (ADC-216; Unisense A/S, Aarhus N, Denmark). On days 16 and 23 of the incubation period, a rhizo-sandwich was transferred to a smaller water tank ($36 \times 15 \times 37$ cm^3). The measurement was carried out at 15°C after acclimatization overnight and a prolonged light phase of several hours. A micromanipulator (Unisense, Aarhus N, Denmark) was used to profile in step sizes of 100 μm on day 16 and 50 μm on day 23 controlled with the software SensorTracePro (Unisense, Aarhus N, Denmark). The electrical potential microelectrode was manually adjusted in a horizontal motion to measure depth profiles in different distances away from the root (Fig. S3A, Supporting Information). Electric potential depth profiles were measured in a randomized order from bottom (32 mm depth) to top (5 mm above sediment) against a general-purpose reference electrode (REF201 Red Rod electrode; Radiometer Analytical, Copenhagen, Denmark). Profiles were recorded after shutting down the illumination and the water pump. Data processing included the discarding of data points below 30.5 mm to ensure the same depth of all profiles for plotting and the subtraction of the mean water-phase electric potential from all data points of each profile. Extracting the normalized data points of certain depths from all profiles on a respective day allowed plotting of the normalized electric potential as a function of distance away from the root.

RESULTS

Oxygen dynamics in the rhizosphere

The rhizo-sandwich (Fig. S1, Supporting Information) was a set-up designed to investigate oxygen release from the roots. Parts of the rhizosphere of *Li. uniflora* were oxygenated throughout light and dark phases. O_2 concentrations reached up to 90% saturation, where several roots were close together and up to 44% at a single root (Fig. 1). In the case of the root bundle, O_2 concentrations decreased from 90% saturation to anoxic conditions over 2.6 mm. The gradient for a single root decreased from 40% saturation to anoxic conditions within a distance of 0.6 mm. After 1 h in the dark, concentrations dropped to steady-state conditions of 12% and 30% O_2 air saturation for the single root and the denser root bundle, respectively. After 40 min illumination, the maximal steady-state oxygen concentrations were reached again. Control samples of the root-sandwich revealed that the oxidation of the rhizosphere, indicated by red iron-hydroxide

precipitations, solely originated from oxygen release of intact roots (Fig. S2B,C, Supporting Information).

Furthermore, planar optode imaging showed that the rhizosphere of *S. europaea* was oxygenated with concentrations up to 68% air saturation during light exposure (Fig. S5A, Supporting Information).

Distribution of cable bacteria in the rhizosphere

The rhizo-sandwich further allowed precise sampling of the rhizosphere of *Li. uniflora*. Cable bacteria were enriched above the background density in bulk sediments in the reduced zone near the top (7 m/cm^3) and the roots (reduced rhizosphere) (2–39 m/cm^3) as well as in the oxidized sediment at the top (11 m/cm^3) and rhizosphere (2–10 m/cm^3) (Fig. 2, Fig. S4, Supporting Information). Cable bacteria in the rhizosphere were observed as single filaments and as aggregates of multiple filaments, intertwined with root hairs (Fig. 2B and C). In contrast, the root-sandwich allowed us to determine the undisturbed spatial arrangement of cable filaments in the rhizosphere as FISH analysis was carried out directly on the microscope slide of the root-sandwiches. Cable bacteria were found next to roots of *Lo. cardinalis* (Fig. 3) and *Li. uniflora* (Fig. S2D, Supporting Information). Interestingly, cable filaments were patchily distributed rather than forming a continuous pattern, showing multiple hotspots along the root.

Furthermore, SEM images of washed root fragments from the rhizo-sandwich of *Li. uniflora* showed cable filaments being captured and intertwined in root hairs (Fig. 4B and C). The typical distinct ridges running along the filaments (Fig. 4D) confirmed the identity of the filaments as cable bacteria.

Cable filaments in the rhizosphere of *S. europaea* varied in diameter from 0.6 μm up to 2 μm (Fig. S5B, Supporting Information). Cable filaments were also found in the rhizosphere of rice plants (Fig. S5C, Supporting Information).

Electric potential profiling

In order to assess the build-up of an electric field around the roots, electric potential profiles were recorded at different distances from the root of *Li. uniflora* in the rhizo-sandwich. Consistent with the cable bacteria distribution in the rhizosphere, an increase of 1.5 mV over a distance of 21.5 mm was measured on day 16, indicating that cable bacteria are active around the roots (Fig. 5B). On day 23, the electric potential increased by 3.4 mV over a distance of 8 mm from the root (Fig. S3C, Supporting Information). A distinct electric potential minimum was observed as the sensor passed close by the bending root at a depth of 20–26 mm (Figure S3B, Supporting Information). Probably, the sensor tip stroked the root, as it actually hit the root and broke in a subsequent effort to profile 1 mm closer. Furthermore, the electric potential increased with depth in all profiles (Fig. 5C, Fig. S3B, Supporting Information).

DISCUSSION

In our experiments, we found cable bacteria next to the roots of the freshwater plant *Li. uniflora*, where molecular oxygen produced redox gradients (Figs 1 and 2). This oxygen release from roots is in accordance with earlier reports that *Li. uniflora* intensively oxygenates the rhizosphere (Christensen, Revsbech and Sand-Jensen 1994; Kofoed *et al.* 2012). Interestingly, the cable bacteria distribution in the rhizosphere indicated that most of
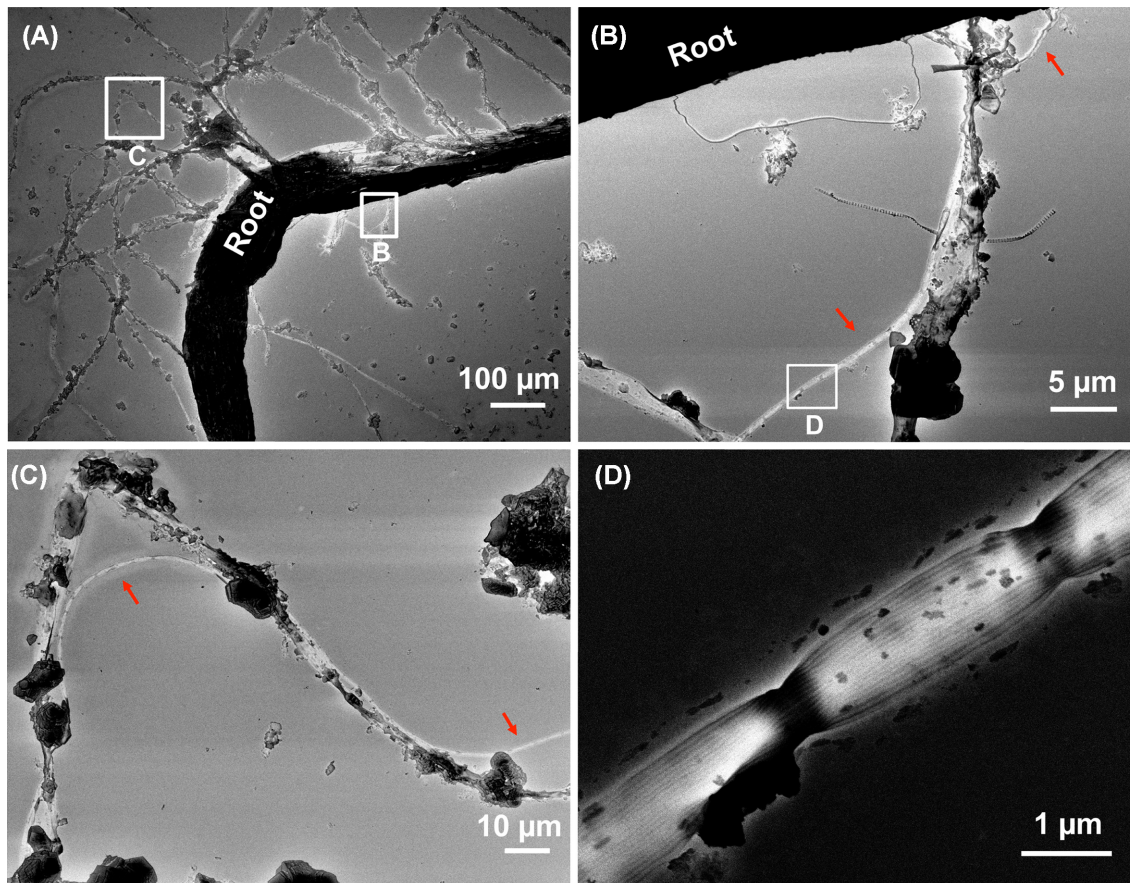
Figure 4. SEM images of cable filaments in close association with a *Li. uniflora* root and root hairs. (**A**) Electron micrograph of a *Li. uniflora* root with root hairs. (**B** and **C**) Magnifications of squares in (A). Red arrows indicate cable filaments. (**D**) Magnification of the square in (B), showing ridges of the cable filament.
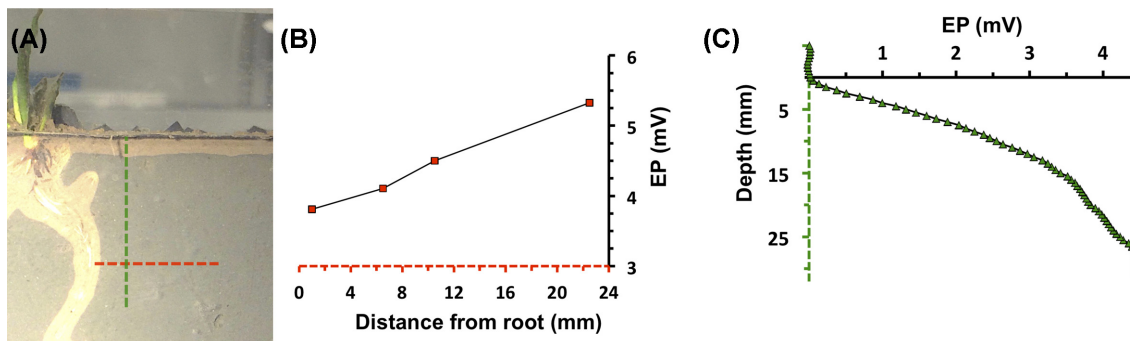

Figure 5. Electric potential (EP) as a function of distance from the root and depth on day 16. (**A**) Picture of the *Li. uniflora* root, which was targeted for electric potential profiling. (**B**) Electric potential at different distances from the root at a depth of 23.5 mm. The red dotted line corresponds to the length of the red dotted line in (A). (**C**) Electric potential depth profile at a distance of 6 mm from the root. The green dotted line corresponds to the length of the green dotted line in (A).

the cells were located in the reduced sediment next to the oxidized rhizosphere. Similar spatial distributions of cable bacteria have been reported for marine sediments where the highest number of cable bacteria cells can be found in the suboxic zone beneath the oxic, top sediment layer (Larsen *et al.* 2015; van de Velde *et al.* 2016). In addition, cable bacteria were present in the rhizosphere of rice, one of the most important food crops, and *S. europaea,* a halophyte growing in coastal areas, where a high input of organic matter into sediments facilitates sulfide production by sulfate-reducing bacteria. The rhizosphere of rice (Larsen, Nielsen and Schramm 2015) and *S. europaea* (Fig. S5A,

Supporting Information) gets oxygenated and therefore fulfills the basic prerequisite for a habitat of cable bacteria.

Cable bacteria were oriented laterally next to the roots of *Lo. cardinalis* (Fig. 3) and *Li. uniflora* (Fig. S2D, Supporting Information), which released oxygen along the entire roots. This spatial orientation of the cable bacteria suggests that they exploited sulfide sources in the bulk sediment. In contrast, Martin *et al.* (2018) found cable bacteria filaments along the surface of seagrass roots, where oxygen release was restricted to the root tips. The predominant source of sulfide for cable bacteria along these seagrass roots could have been sulfate reduction stimulated by
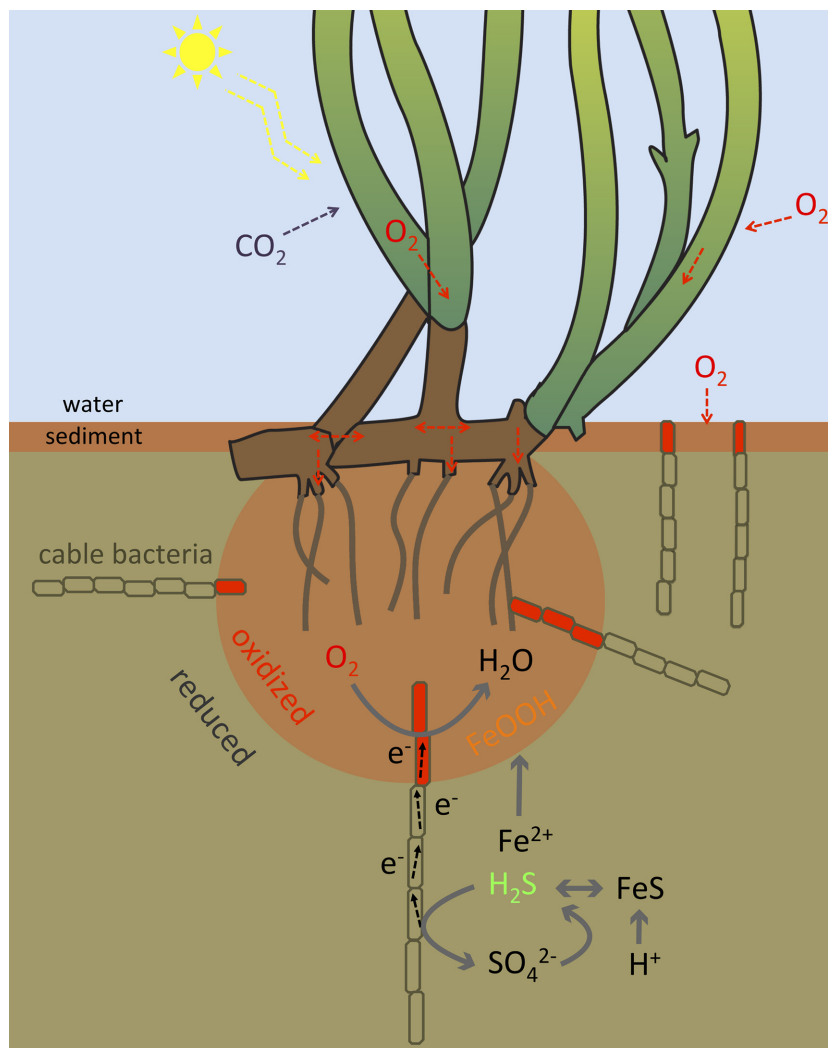
Figure 6. Conceptual diagram of cable bacteria in the rhizosphere. Cable bacteria reduce the oxygen released from the plant roots and oxidize sulfide. The sulfide gets replenished from local sulfate reduction, diffusion from deeper sediment layers and dissolution of iron sulfides promoted by the acidity-producing sulfide oxidation. The dissolved ferrous iron diffuses towards the oxygen-releasing roots, where it precipitates to iron oxide.

the release of organic compounds from the roots, which possibly promoted a rather longitudinal orientation of the filaments along the surface of the roots.

In order to investigate a potential direct connection of cable bacteria to the root, SEM was performed with a washed root fragment of *Li. uniflora*. Despite earlier reports that *Li. uniflora* lacks root hairs (Søndergaard and Laegaard 1977; Farmer 1985; Beck-Nielsen and Vindbæk Madsen 2001), root hairs were clearly visible in our incubations (Fig. 4). Cable filaments were present along the root hairs and eventually pointing towards the root, suggesting a tight attachment. The SEM pictures suggest a close spatial interaction between the cable bacteria and roots or root hairs, which might be an adaption to small-scale, local redox gradients of oxygen and sulfide. The close interaction with root hairs and a possible direct connection to the root is supported by the observation that cable bacteria were predominantly found in the root hair region of seagrass roots (Martin *et al.* 2018). A direct connection of cable bacteria to surfaces was also shown in bioelectric systems where they were attaching with one end of the filament to an anode, forming a network of pilus-like nanofilaments (Reimers *et al.* 2017).

Upon dark–light shifts, the oxygen front around the roots changed with a speed of 0.3 mm/h, as shown for a single root of *Li. uniflora*. Cable bacteria filaments can grow centimeters in length (Pfeffer *et al.* 2012), which would span this whole fluctuating redox zone in the rhizosphere and allow for continuous sulfide oxidation around the root even though the redox gradients change. However, cable bacteria have a gliding motility of up to 2.9 mm/h and could also adapt to the diurnally moving oxygen gradients by chemotaxis (Bjerg *et al.* 2016).

Taken together, examples such as seagrass (Martin *et al.* 2018), S. europaea, *Li. uniflora, Lo. cardinalis* and rice highlight the capability of cable bacteria to exploit the rhizosphere of aquatic plants in sulfide-rich marine, but also in freshwater sediments, where cable bacteria might be involved in a cryptic sulfur cycle (Risgaard-Petersen *et al.* 2015) (Fig. 6).

Beyond the mere presence, we also showed the activity of cable bacteria in the rhizosphere of *Li. uniflora*. Recent studies have shown that the activity of cable bacteria can be inferred from gradients of electric potential, which was measured by vertically pushing electric potential electrodes into the top sediment layer (Damgaard; Risgaard-Petersen and Nielsen 2014; Nielsen and Risgaard-Petersen 2015). An increase in electric

potential in a horizontal manner at centimeter distances away from the roots was measured, indicating the establishment of an electric field caused by the activity of cable bacteria (Fig. 5B, Fig. S3C, Supporting Information). Hence, cable bacteria conduct electrons from the sulfidic zone to the rhizosphere, most likely by the oxidation of sulfide in the bulk sediment and the reduction of oxygen released from the roots. In addition, the sediments of the rhizo-sandwiches showed changes in electric potentials at the sediment surface (Fig. 5C, Fig. S3B, Supporting Information), indicating the activity of cable bacteria at the water–sediment interface. This implies that the dominant parameters determining the distribution of the cable bacteria in the rhizosphere are the geochemical gradients of sulfide and oxygen, although other plant-derived factors cannot be excluded.

The H_2S oxidation of cable bacteria in the rhizosphere might lead to a protection of the plant root from exposure to the toxic sulfide and lead to a faster oxidation reaction compared to a pure abiotic chemical oxidation of sulfide by oxygen (Luther *et al.* 2011). Furthermore, the activity of cable bacteria strongly impacts the geochemistry of the sediment in the rhizosphere, potentially influencing nutrient cycling (Sulu-Gambari *et al.* 2016) and promoting iron-oxide precipitation (Risgaard-Petersen *et al.* 2012). Iron oxides react with free H_2S by oxidizing H_2S to elemental sulfur or by precipitation of FeS (Seitaj *et al.* 2015). Hence, cable bacteria may contribute to both immediate and long-term protection of roots against toxic, free sulfide.

In conclusion, our data indicate that cable bacteria are ubiquitous and active in the rhizosphere of aquatic plants where they take advantage of the electron acceptor oxygen released from the roots. The electric metabolism of cable bacteria presumably has a strong impact on rhizosphere geochemistry.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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