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Citation: Viggi, Carolina Cruz, Matturro, Bruna, Frascadore, Emanuela, Insogna, Susanna, Mezzi, Alessio, Kaciulis, Saulius, Sherry, Angela, Mejeha, Obioma K., Head, Ian M., Vaiopoulou, Eleni, Rabaey, Korneel, Rossetti, Simona and Aulenta, Federico (2017) Bridging spatially segregated redox zones with a microbial electrochemical snorkel triggers biogeochemical cycles in oil-contaminated River Tyne (UK) sediments. *Water Research*, 127. pp. 11-21. ISSN 0043-1354

Published by: IWA Publishing

URL: <https://doi.org/10.1016/j.watres.2017.10.002>  
<<https://doi.org/10.1016/j.watres.2017.10.002>>

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Cruz Viggi C, Maturro B, Frascadore E, Insogna S, Mezzi A, Kaciulis S, Sherry A, Mejeha OK, Head IM, Vaiopoulou E, Rabaey K, Rossetti S, Aulenta F.

[Bridging spatially segregated redox zones with a microbial electrochemical snorkel triggers biogeochemical cycles in oil-contaminated River Tyne \(UK\) sediments.](#)

*Water Research* 2017, 127, 11-21.

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**DOI link to article:**

<https://doi.org/10.1016/j.watres.2017.10.002>

**Date deposited:**

13/10/2017

**Embargo release date:**

03 October 2018



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1 **Bridging spatially segregated redox zones with a microbial electrochemical**  
2 **snorkel triggers biogeochemical cycles in oil-contaminated River Tyne (UK)**  
3 **sediments**

4  
5 Carolina Cruz Viggi<sup>1</sup>, Bruna Matturro<sup>1</sup>, Emanuela Frascadore<sup>1</sup>, Susanna Insogna<sup>2</sup>,  
6 Alessio Mezzi<sup>3</sup>, Saulius Kaciulis<sup>3</sup>, Angela Sherry<sup>4</sup>, Obioma K. Mejeha<sup>4</sup>, Ian M. Head<sup>4</sup>, Eleni  
7 Vaiopoulou<sup>5</sup>, Korneel Rabaey<sup>5</sup>, Simona Rossetti<sup>1</sup>, Federico Aulenta<sup>1,\*</sup>

8  
9  
10 <sup>1</sup> Water Research Institute (IRSA), National Research Council (CNR), Italy

11 <sup>2</sup> Department of Chemistry, Sapienza University of Rome, Italy

12 <sup>3</sup> Institute for the Study of Nanostructured Materials (ISMN), National Research  
13 Council (CNR), Italy

14 <sup>4</sup> School of Civil Engineering and Geosciences, Newcastle University, United  
15 Kingdom

16 <sup>5</sup> Center for Microbial Ecology and Technology (CMET), Ghent University,  
17 Belgium

18  
19 \*Corresponding Author

20 E-mail: [Aulenta@irsa.cnr.it](mailto:Aulenta@irsa.cnr.it); Tel: +39-0690672751; Fax: +39-0690672787

21 Address: IRSA-CNR, Via Salaria km 29,300, 00015 Monterotondo (RM), Italy

22

23 **Abstract**

24 Marine sediments represent an important sink for a number of anthropogenic organic  
25 contaminants, including petroleum hydrocarbons following an accidental oil spill.  
26 Degradation of these compounds largely depends on the activity of sedimentary  
27 microbial communities linked to biogeochemical cycles, in which abundant elements  
28 such as iron and sulfur are shuttled between their oxidized and reduced forms. Here we  
29 show that introduction of a small electrically conductive graphite rod (“the  
30 electrochemical snorkel”) into an oil-contaminated River Tyne (UK) sediment, so as to  
31 create an electrochemical connection between the anoxic contaminated sediment and  
32 the oxygenated overlying water, has a large impact on the rate of metabolic reactions  
33 taking place in the bulk sediment. The electrochemical snorkel accelerated sulfate  
34 reduction processes driven by organic contaminant oxidation and suppressed  
35 competitive methane-producing reactions. The application of a comprehensive suite of  
36 chemical, spectroscopic, biomolecular and thermodynamic analyses suggested that the  
37 snorkel served as a scavenger of toxic sulfide via a redox interaction with the iron cycle.  
38 Taken as a whole, the results of this work highlight a new strategy for manipulating  
39 biological processes, such as bioremediation, corrosion, and carbon sequestration,  
40 through the manipulation of the electron flows in contaminated sediments.

41

42 **Keywords:** Contaminated sediments, Iron cycle, Electrochemical snorkel, Oil spill  
43 remediation, Petroleum hydrocarbons, Sulfate reduction, Sulfide scavenging, Sulfur  
44 cycle

45

## 46 **1. Introduction**

47 The release of thousands of tons of petroleum hydrocarbons (PHs) originating from  
48 anthropogenic activity affects the marine environment and causes severe ecological and  
49 economic damage (Bargiela et al., 2015). When an oil spill occurs, a variety of  
50 hydrocarbon removal strategies can be applied to minimize negative environmental  
51 impacts. Biological remediation methods are now widely used due to their lower  
52 environmental impact, adaptability to a range of contamination scenarios, and potential  
53 for full contaminant mineralization (Daghio et al., 2016). In confined hydrocarbon-rich  
54 environments, such as marine sediments, anoxic conditions prevail due to the excess of  
55 organic carbon that leads to rapid depletion of the most thermodynamically favorable  
56 electron acceptors (Bellagamba et al., 2016).

57 In anoxic sedimentary environments, the degradation of organic contaminants is usually  
58 stimulated by the addition of electron acceptors (e.g., oxygen, nitrate or sulfate) to  
59 provide microorganisms with more energetically favorable conditions for hydrocarbon  
60 oxidation (Meckenstock et al., 2004; Spormann and Widdel, 2000). Aerobic  
61 bioremediation is often favored over stimulation of anaerobic/anoxic contaminant  
62 degradation processes due to faster rates of hydrocarbon activation and removal (Viggi  
63 et al., 2015). In this context, a number of different approaches have been proposed to  
64 effectively deliver oxygen to contaminated anoxic sediments in order to accelerate  
65 hydrocarbon biodegradation (Genovese et al., 2014). However, rapid abiotic  
66 consumption of oxygen by reaction with reduced chemical species (e.g.,  $\text{Fe}^{2+}$ ,  $\text{S}^{2-}$ ) and  
67 difficulties in controlling the rate of oxygen release limit the practical application of  
68 these approaches.

69 An alternative approach to overcoming electron acceptor limitation in oil-contaminated  
70 sediments is the use of bioelectrochemical systems (BES) (Lovley and Nevin, 2011;

71 Wang and Ren, 2013). BES employ solid-state electrodes to directly or indirectly  
72 stimulate and control microbial metabolism. Stimulation of microbial activity is driven  
73 by the ability of “electro-active bacteria” (EAB) to exchange electrons with the  
74 electrodes, which can serve as electron acceptors or donors in their energy metabolism  
75 (Borole et al., 2011). Recent, laboratory-scale studies have demonstrated that BES-based  
76 technologies can be successfully applied to remove hydrocarbons from oil-contaminated  
77 sediments (Bellagamba et al., 2016; Daghighi et al., 2016; Morris et al., 2009; Yan and  
78 Reible, 2014).

79 The concept of bioelectrochemical bioremediation to accelerate hydrocarbons  
80 biodegradation in anoxic marine sediment was introduced recently (Viggi et al., 2015).  
81 The system was based on an “Oil-spill Snorkel” consisting of a rod of conductive material  
82 (i.e., an electrode) positioned to create an electrochemical connection between the  
83 anoxic contaminated sediment and the oxic overlying water. In principle, the snorkel  
84 could take advantage of the capability of EAB to anaerobically oxidize hydrocarbons  
85 with a carbon electrode, deployed in the sediment, serving as a respiratory electron  
86 acceptor. The electrons travel from the bottom of the snorkel buried in the sediment  
87 (anode) to the upper part of the snorkel immersed in the overlying oxic water (cathode)  
88 where oxygen is reduced to water, effectively spatially separating the oxidation of the  
89 electron donor (hydrocarbons) from the reduction of the terminal electron acceptor.  
90 Collectively, the results of this preliminary study confirmed the potential of the snorkel  
91 to accelerate biotic and abiotic oxidative reactions taking place within the sediment, as  
92 documented by a 1.7-fold increase in the cumulative oxygen uptake and 1.4-fold  
93 increase in the cumulative CO<sub>2</sub> evolution, in microcosms containing snorkels compared  
94 to snorkel-free controls. Accordingly, the initial rate of petroleum hydrocarbon  
95 biodegradation was also substantially enhanced (Viggi et al., 2015). Despite these

96 promising findings, the fundamental mechanisms underlying the observed enhancement  
97 of hydrocarbon degradation could not be identified and the overall impact of the  
98 microbial electrochemical snorkel on key sediment biogeochemical cycles were not  
99 elucidated. To gain a deeper understanding of these critical factors, further oil-spill  
100 snorkel experiments were conducted using petroleum-contaminated estuarine  
101 sediments from the River Tyne (UK) which had been previously reported to harbor  
102 active sulfate-reducing, and syntrophic methanogenic hydrocarbon-degrading microbial  
103 communities (O'Sullivan et al., 2015; Sherry et al., 2014).

104

105

## 106 **2. Materials and Methods**

### 107 *2.1 Experimental setup*

108 The experiments described below are hereafter referred to as oil-spill snorkel  
109 experiments. The estuarine sediments used in this study were obtained from the River  
110 Tyne (UK). Sediment samples were subject to four treatments; 1) oil-supplemented  
111 sediment containing 3 graphite rods, termed "snorkel", 2) sediment with no oil  
112 amendment containing 3 graphite rods, termed "no oil snorkel", 3) oil-supplemented  
113 sediment without graphite rods, termed "control", and 4) sediment with no oil  
114 amendment or graphite rods, termed "no oil control". The oil containing treatments  
115 were amended with Danish Underground Consortium (DUC) light crude oil to a final  
116 concentration of approximately 20 g/kg. To prepare oil contaminated sediment, the  
117 sediment was divided into 4 equal portions; one part was thoroughly mixed with oil  
118 previously dissolved in hexane. The hexane was evaporated by air-drying the sediment.  
119 Finally, the air-dried contaminated sediment was mixed, under nitrogen, with the  
120 remaining three parts of the sediment. This procedure has been used in order to achieve

121 a homogenous contamination of the sediment while preserving a large fraction of the  
122 sediment microbial communities (Viggi et al., 2015).  
123 Microcosms containing oil-supplemented sediment or unamended sediment were  
124 prepared in 100-mL glass cylinders. Each cylinder contained (starting from the bottom)  
125 ~90 grams of sediment, a layer of Norit® granular activated carbon (10 g, serving as  
126 high surface area oxygen reduction catalyst) (Zhang et al., 2009) and 25-30 mL of  
127 synthetic brackish medium (Sea Salts, Sigma Aldrich, diluted 1:4). For the “snorkel” and  
128 “no oil snorkel” treatments, three graphite rods (the snorkels) were inserted vertically  
129 through the layers of different materials to create an electrochemical connection  
130 between the anoxic sediment and the oxygenated overlaying water. Replicate  
131 microcosms were prepared for each treatment and were sacrificially sampled after  
132 incubation for 0, 118, 175, 286 and 466 days.  
133 The microcosms were statically incubated in the dark at  $20 \pm 1$  °C. After 0, 118, 175, 286  
134 and 466 days of incubation, one cylinder from each treatment was sacrificed,  
135 hydrocarbons were extracted from the sediment and analyzed by GC-MS; the aqueous  
136 phase (overlying water) was analyzed by ion chromatography (IC) for quantification of  
137 anions and the gas phase (i.e., gas pockets accumulating within the sediment) was  
138 analyzed by GC-TCD for quantification of methane.

139

## 140 2.2 Analytical methods

141 Methane in the gas pockets was quantified by gas chromatography (GC). Gas samples  
142 (50 µL) were taken from voids in the sediment that occurred in some treatments using a  
143 gas-tight syringe (Hamilton, Reno, NV, USA) equipped with an 8-cm long needle. Gas  
144 samples were analyzed with a Perkin-Elmer Auto System gas chromatograph [stationary  
145 phase: stainless-steel column packed with 60/80 Carboxen™ 1000 molecular sieve

146 (Supelco, USA); carrier gas: N<sub>2</sub> at 20 mL/min; oven temperature: 150 °C; injector  
147 temperature: 200 °C; thermal conductivity detector (TCD) temperature: 200 °C].  
148 Anions in aqueous phase samples were quantified by ion chromatography (IC). Samples  
149 were filtered (0.22 µm pore size) and injected into a Dionex DX-100 system (Dionex  
150 Corp., Sunnyvale, CA) ion chromatograph (column: IonPac AS14; eluent: sodium  
151 carbonate 3.5 mM / sodium bicarbonate 1.0 mM solution). Quantification of total  
152 petroleum hydrocarbons (TPH) in sediment samples was performed by GC-MS. In brief,  
153 sediment samples were air dried and extracted with a Dionex ASE 200 (Dionex Corp.,  
154 Sunnyvale, CA) using an acetone:hexane (1:1 v/v) mixture at 100 °C and a system  
155 pressure of 1500 psi. The solvent extract was evaporated under a stream of nitrogen  
156 and re-dissolved in 10 mL of an *n*-heptane containing *n*-dodecane (*n*-C<sub>12</sub>) and *n*-  
157 tetracontane (*n*-C<sub>40</sub>), each at 10 mg/L, as markers for the GC analysis. To purify the  
158 hydrocarbon extract (i.e., remove polar compounds), it was percolated through a solid  
159 phase extraction cartridge filled with Florisil® and anhydrous sodium sulfate  
160 (Chromabond® Na<sub>2</sub>SO<sub>4</sub>/Florisil®, 6 mL polypropylene columns, 2g/2g). A sample (1 µL)  
161 of the purified hydrocarbon extract was then injected (in pulsed split-less mode) into a  
162 GC-MS (Perkin Elmer Clarus 680/600; column: HP-5 MS (Agilent) 30 m, ID 0.25 mm,  
163 0.25 µm film thickness; carrier gas: helium at 1 mL/min; injector temperature: 280 °C;  
164 oven temperature program: initial Temp 40 °C, 18°C/min to 250°C, 10 °C/min to 280 °C,  
165 hold for 17 min; MS-scan 30-600, 2-32 min). The TPH amount was determined by  
166 summing the unresolved and resolved components eluted from the GC capillary column  
167 between the retention times of *n*-C<sub>12</sub> and of *n*-C<sub>40</sub>, using solutions of diesel motor oil and  
168 diesel mineral oil in hexane as calibration standards (concentration range 0.15–2 g/L).

169

### 170 2.3 *Microbial Community Analysis with Next Generation Sequencing*

171 Thirty 16S rRNA amplicon libraries were generated representing communities from the  
172 sediment used to prepare the microcosms prior to incubation, which were either treated  
173 with oil or left untreated (Time 0). The microbial communities were also characterized  
174 in samples from sediments from the snorkel, control, no oil snorkel, and no oil control  
175 treatments following 175 and 286 days of incubation (n=3 for all). The microbial  
176 community analysis was conducted as follows.

177

### 178 2.3.1 DNA extraction

179 Extractions were performed in triplicate from sediment samples (~400 mg) using a  
180 PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., USA) with a ribolyser  
181 (FastPrep-24, MP Biomedicals, USA). Procedural blanks were performed to ensure  
182 extracts remained contamination-free throughout the extraction procedure. DNA  
183 extractions were carried out according to the manufacturer's instructions with the  
184 following minor modifications: the ribolyser was used at a speed of 6 m/s for 40 s for  
185 homogenisation and cell lysis. DNA extracts were stored at -20°C until further use.

186

### 187 2.3.2 PCR amplification 16S rRNA genes and PCR product purification

188 The variable V4/V5 region of the 16S rRNA gene was amplified using the degenerate  
189 primers, 515F (GTG-NCA-GCM-GCC-GCG-GTA-A) and 926R (CCG-YCA-ATT-YMT-TTR-  
190 AGT-TT) (Quince *et al.*, 2011). In silico testing of the primer set against the SILVA SSU  
191 128 Ref NR database using TestPrime 1.0 (Klindworth *et al.*, 2013) returned values of  
192 88.2%, 85.8% and 85.7% coverage against the Bacteria, Archaea and Eukarya domains  
193 respectively. DNA extracts were diluted (1/10) to reduce the levels of inhibitory  
194 contaminants to prevent PCR inhibition (Head, 1999). PCR reactions contained NH<sub>4</sub>  
195 buffer (50mM), 0.5 mM dNTPs (Invitrogen), 500 nM each primer (ThermoScientific), 2.5

196 U of *Taq* polymerase (Bioline), and 1  $\mu$ l template DNA in a 20- $\mu$ l volume. PCR conditions  
197 were initial denaturation (94°C, 4 min) followed by 30 cycles (95°C, 1 min; 55°C, 0.45  
198 min; 72°C, 1 min) and a final extension (72°C, 10 min) in a thermal cycler (Techne TC-  
199 512, Bibby Scientific Limited). PCR products were analyzed by gel electrophoresis with  
200 1% (w/v) agarose gel in 1 x TAE buffer (Tris-acetate-EDTA buffer; 40 mM Tris, 20 mM  
201 acetic acid, 1 mM EDTA, pH 8.3). Electrophoresis was performed at 100 V for 45 min.  
202 Gels were stained with ethidium bromide and visualized with a BioSpectrum Imaging  
203 System with VisionWorks LS software (UVP, Cambridge, UK). PCR products were  
204 purified with Agencourt AMPure XP PCR purification kit (Beckman Coulter).

205

### 206 *2.3.3 DNA quantification and Ion Torrent DNA sequencing*

207 Purified DNA was quantified on a Qubit 2.0 Fluorometer (Invitrogen) with a Qubit  
208 dsDNA high sensitivity assay kit (Invitrogen). The final concentration of DNA was  
209 adjusted to 100 pM, and equimolar concentrations of DNA from all samples were pooled.  
210 The pooled amplicon library was sequenced on an Ion Torrent Personal Genome  
211 Machine (Life Technologies). Briefly, the library was diluted (26 pM) and emulsion PCR  
212 performed on a OneTouch2 instrument with an Ion PGM Template OT2 400 kit  
213 according to the manufacturer's instructions (Life Technologies). Beads with bound  
214 template DNA were purified on a OneTouch ES system (Life Technologies). Following  
215 enrichment, the beads were loaded onto a PGM 316 chip and sequenced in accordance  
216 with the manufacturer's instructions.

217

### 218 *2.3.4 Data analysis*

219 Raw sequence reads were retrieved using the Torrent Suite Software V4.0 (Life  
220 Technologies). Sequence reads with a modal length of 428 bp were analyzed in QIIME

221 (Caporaso *et al.*, 2010a). Sequences were assigned to samples based on their unique  
222 barcodes and simultaneously filtered to remove reads with no corresponding barcode,  
223 reads without the correct primer sequence and poor quality reads (those with a quality  
224 value of < 20 were discarded). Operational taxonomic unit (OTU) classifications were  
225 performed using UClust (Edgar, 2010), with an OTU threshold defined at 97% sequence  
226 identity. OTUs were first clustered open reference against the Greengenes 16S rRNA  
227 core alignment (DeSantis *et al.*, 2006) and then clustered de novo. Taxonomy was  
228 assigned using RDP Classifier (Wang *et al.*, 2007) and sequences aligned using PyNAST  
229 (Caporaso *et al.*, 2010b). Chimeric sequences were identified with ChimeraSlayer (Haas  
230 *et al.*, 2011) and removed before subsequent analysis. The average number of reads in  
231 individual binned libraries after filtering was 25,868 with a range from 11,214 to  
232 139,159 reads. Libraries were rarefied to 11,214 reads for comparative analysis. Core  
233 diversity analysis was subsequently performed in QIIME v1.8 to provide a comparative  
234 analysis of the microbial communities between samples. BIOM table data, consisting of  
235 OTU counts per sample, were imported into Microsoft Excel to determine the average  
236 percentage relative abundance (n=3 for all treatments, except T0 where n=6). Phylum  
237 and class level comparisons were performed at a level of  $\geq 0.2\%$  relative abundance.  
238 Abundant taxa for further investigation were determined as those sequences with  $\geq 1\%$   
239 relative abundance. The six amplicon libraries generated from the initial sediments  
240 (Time 0) with and without oil were very similar (Pearson correlation at the genus level  
241  $R^2 \geq 0.957$ ,  $p \leq 0.01$ ), these were therefore treated as replicate samples giving n=6 for  
242 the Time 0 samples. Sequences have been deposited in the NCBI's Short Read Archive  
243 (SRA) under BioProject PRJNA376663.

244

245 2.4 *Catalyzed Reporter Deposition-Fluorescence In Situ Hybridization (CARD-FISH)*  
246 *analysis*

247 Sediment samples and biofilms growing on the surface of the graphite rods (i.e., the  
248 snorkels) were taken for CARD-FISH analysis of the microbial communities. In brief,  
249 approximately 1 g of sediment was fixed in formaldehyde (2% v/v final concentration)  
250 and cells were extracted from sediment particles as described previously (Barra  
251 Caracciolo et al., 2005). The detached cells were filtered through 0.2 µm polycarbonate  
252 filters (Ø 47 mm, Millipore) by gentle vacuum (<0.2 bar) and stored at -20°C until use.  
253 To remove the biomass from the biofilm formed on the graphite rods, the surface of the  
254 electrode was gently scraped with a sterile spatula. The detached biomass was collected  
255 in PBS buffer containing 2% v/v formaldehyde, and filtered as described above. CARD-  
256 FISH was carried out following a previously published protocol using probes targeting  
257 total *Bacteria* (EUB338 I,II,III), and *Deltaproteobacteria* (DELTA495a,b,c) (Fazi et al.,  
258 2008).

259 Probes, labeled with horseradish peroxidase (HRP), were purchased from BIOMERS  
260 (<http://www.biomers.net>). Probe sequences and hybridization conditions were as  
261 reported in probeBase (<http://www.microbial-ecology.net/probebase>). DAPI (4',6-  
262 diamidino-2-phenylindole) staining was performed to determine total cell numbers,  
263 from which the relative abundances of each targeted bacterial population was  
264 calculated. Total cell counts were performed at the end of the CARD-FISH hybridization  
265 procedure by mounting the samples in Vectashield Mounting Medium with DAPI (Vector  
266 Labs, Italy). At least 20 randomly selected microscopic fields for each sample were  
267 analyzed to enumerate the cells by microscopic analysis. Slides were examined by  
268 epifluorescence microscopy (Olympus, BX51) and the images were captured with an

269 Olympus F-View CCD camera and images were processed with Cell<sup>F</sup> software  
270 (Olympus, Germany).

271

## 272 2.5 *geneCARD-FISH assay*

273 GeneCARD-FISH assays were conducted on small sections of filter (see section 2.3)  
274 according to a previously described protocol (Matturro et al., 2016), as detailed in the  
275 following paragraphs.

276

### 277 2.5.1 *Polynucleotide probe design and synthesis*

278 Polynucleotide probes targeting the gene encoding the alpha subunit of alkylsuccinate  
279 synthase (*assA*) were synthesized using a PCR DIG Probe Synthesis Kit (Roche, Italy)  
280 following the manufacturer's instructions. A cloned *assA* gene, obtained from a  
281 hydrocarbon-degrading sulfate-reducing enrichment culture (Aitken et al., 2013), was  
282 used as DNA template. Primers *assA2F* and *assA2R* (Aitken et al., 2013) were used for  
283 PCR amplification. PCR Dig-labeled amplicons were purified with QIAquick PCR  
284 Purification Kit (Qiagen, Italy) and run on a 2% agarose gel to check the incorporation of  
285 DIG molecules. The concentration of purified DIG-labeled polynucleotide probe was  
286 quantified (NanoDrop ND-2000, Italy) and aliquots (10 ng/ $\mu$ L) were stored at - 20°C and  
287 employed during the hybridization step of the geneCARD-FISH assay.

288

### 289 2.5.2 *Hybridization*

290 Before hybridization, cells were pretreated with lysozyme and proteinase K as  
291 previously described (Matturro et al., 2016). Filters were transferred to 100  $\mu$ L of pre-  
292 hybridization buffer HB-I containing the following compounds: 0.25 mg/mL of thymus  
293 DNA, yeast RNA 0.25 mg/mL, formamide 50%, Saline Sodium Citrate SCC 5X, dextran

294 sulfate 10%, SDS 0.1%, EDTA 20 mM, blocking reagent 1% (molecular biology reagents,  
295 Sigma Aldrich, Italy) and incubated at 46°C for 1.5 h. Filters were then transferred to a  
296 fresh tube containing 100 µL of HB-I and DIG-labeled polynucleotide probe (final  
297 concentration 0.5 ng/µL) and then incubated in a thermocycler with the following  
298 cycles: 70°C for 25 min (DNA denaturation) and 46°C overnight (Dig-labeled probe  
299 hybridization). After hybridization, filters were immersed in washing buffer WB-I (SSC  
300 10X, SDS 0.1%) for 30 min at 48°C and then transferred into washing buffer WB-II (SSC  
301 0.1X, SDS 0.1%) for 90 min at 48°C.

302

### 303 *2.5.3 Immunochemical probe detection*

304 Filters were incubated in an antibody buffer AB-I for 45 min at room temperature.  
305 Buffer AB-I consisted of a Western Blocking reagent solution 1% (Sigma Aldrich, Italy)  
306 in phosphate buffered saline, 1X PBS (145 mM NaCl, 1.4 mM NaH<sub>2</sub>PO<sub>4</sub>, 8 mM Na<sub>2</sub>HPO<sub>4</sub>,  
307 pH 7.4). Filters were then immersed into an AB-I buffer solution containing the  
308 antibody anti-DIG (Anti-Digoxigenin-POD Fab fragments, Sigma Aldrich, Italy) to a final  
309 concentration of 0.3U/mL for 90 min at room temperature. After incubation, filters were  
310 washed for 10 min in fresh AB-I buffer at room temperature. Filters were then washed  
311 10X PBS solution for 20 min at room temperature.

312

### 313 *2.5.4 Fluorescent signal amplification*

314 After the immunochemical reaction, filters were placed in the substrate mix with dye-  
315 tyramide for 15 min at 37°C and then washed in 1X PBS for 30 minutes at room  
316 temperature, in water for 1 min and finally in 96% ethanol for 1 min. The filters were  
317 air-dried, placed on a microscope slide and stained with DAPI (4,6-diamidino-2-  
318 phenylindole) to determine total cell numbers in the samples.

319

#### 320 *2.5.5 Microscopy analysis*

321 Hybridized cells carrying the *assA* gene were visualized by epifluorescence microscopy  
322 (Olympus, BX51) and quantified by counting fluorescent cells (at least 100 cells per  
323 grid) on random grids on filter sections. Quantitative data were expressed as cell  
324 numbers per g dry weight of sediment.

325

#### 326 *2.6 X-Ray Photoelectron Spectroscopy of graphite electrodes*

327 The surface chemical composition of the graphite rods was determined by using X-ray  
328 photoelectron spectroscopy (XPS). XPS measurements were performed in a VG Escalab  
329 MkII spectrometer (VG Scientific Ltd, East Grinstead, UK) with a 5-channeltron detection  
330 system and an unmonochromatized radiation source of Al K $\alpha$  (1486.6 eV). The  
331 electrostatic lenses were operated in the selected-area mode A3x12, providing  
332 photoelectron collection from the sample area with a diameter of about 3 mm. The  
333 binding energy (BE) scale was calibrated by using the C1s peak of graphite at BE = 284.6  
334 eV. All the spectra were acquired at the pass energy of 50 eV. Spectroscopic data were  
335 processed using Avantage v.5 software, using a peak-fitting routine with Shirley  
336 background and Scofield sensitivity factors for elemental quantification.

337

338

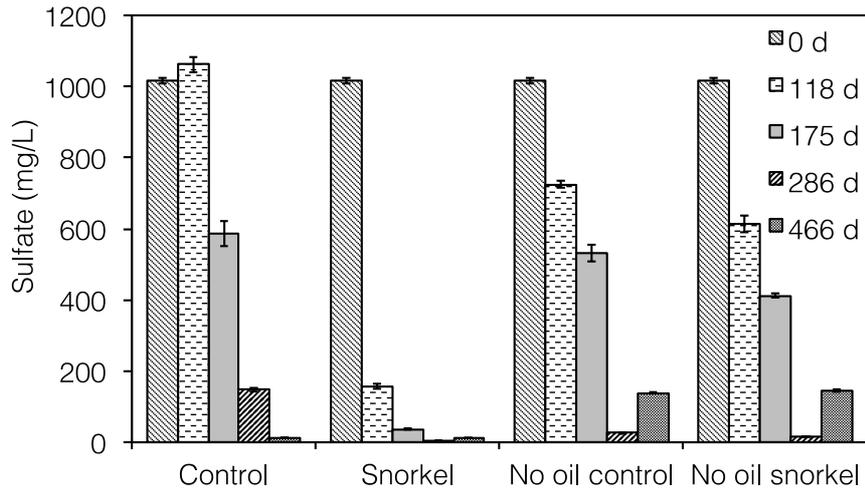
### 339 **3. Results and discussion**

#### 340 *3.1 Sulfate reduction*

341 Sulfate is one the most abundant electron acceptors in anoxic marine and brackish  
342 sediments and typically plays a critical role in the oxidation of organic matter in pristine  
343 and contaminated sediments. Sulfate concentration in the snorkel treatments and

344 control microcosms was monitored following incubation for 0, 118, 175, 286, and 466  
345 days (Figure 1). In the oil-supplemented control with no snorkel (“control”), sulfate  
346 concentration remained nearly constant during the initial 118 days of incubation  
347 (Figure 1); thereafter, sulfate gradually decreased over time and was almost completely  
348 depleted by day 466 (Figure 1). By contrast, significantly greater initial sulfate reduction  
349 rates were observed in the oil-supplemented snorkel-containing sediments (two-tailed  
350 t-test,  $p < 0.005$ ), with over 85% of the initial sulfate (i.e., approximately 1,000 mg/L)  
351 consumed by day 118 (Figure 1). Notably, sulfate reduction was observed also in  
352 microcosms that were not supplemented with oil, being most likely fuelled by the  
353 indigenous organic carbon in the River Tyne sediment. Also in this case, the snorkel  
354 accelerated (though to a lower extent) the removal of sulfate, with no-oil snorkel  
355 treatments displaying a 35% greater cumulative sulfate removal compared to the no-oil  
356 controls by day 118 ( $p = 0.06$ ), and a 25% greater cumulative removal by day 286  
357 ( $p = 0.005$ ) (Figure 1). Notably, by comparing sulfate concentrations in the control and in  
358 the no oil control experiments, it is apparent that, in the absence of the snorkel, the  
359 presence of oil slightly inhibited the activity of sulfate reducing microorganisms in the  
360 River Tyne sediments (Figure 1).

361 A recent study (Daghio et al., 2016), suggested that an electrode potentiostatically  
362 poised at an oxidative value of +300 mV vs. SHE, was capable of indirectly stimulating  
363 the sulfate-driven anaerobic oxidation of toluene, through the electrochemical  
364 scavenging of hydrogen sulfide, a toxic end product of dissimilatory sulfate reduction  
365 (Reis et al., 1992). This hypothesis was supported by the accumulation of elemental  
366 sulfur on the surface of the poised carbon-based electrode.



367

368 **Figure 1.** Concentration of sulfate in the snorkel experiments and controls throughout  
 369 the experimental period. Error bars represent the standard error of replicate samples.

370

371

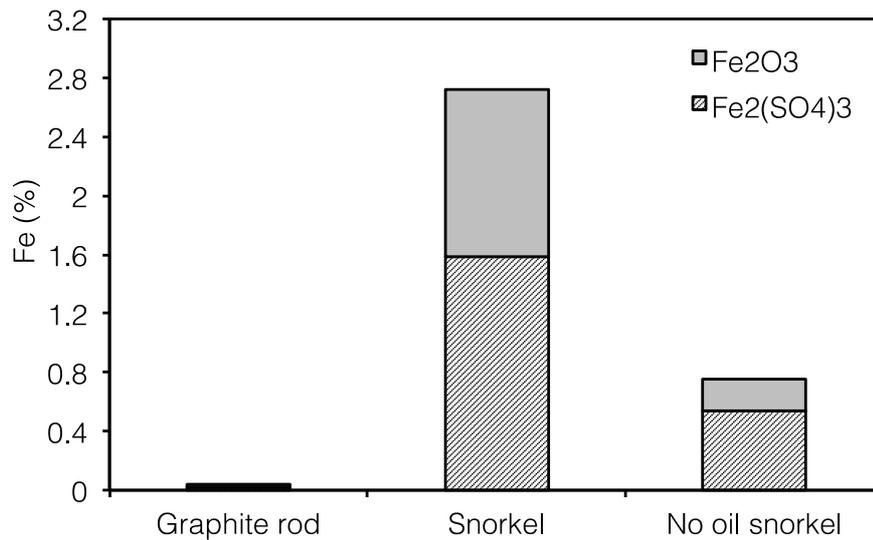
### 372 3.2 XPS analysis of graphite electrodes

373 To test the assumption that the enhancement of sulfate reduction may be due to the  
 374 electrochemical scavenging of sulfide, at the end of the study (day 466), the surface of  
 375 the graphite rods (from the snorkel and the no oil snorkel treatments) were analyzed via  
 376 XPS. For comparative purposes, an identical pristine graphite rod was also analyzed.

377 Interestingly, XPS analyses did not reveal the presence of elemental sulfur in any of the  
 378 snorkel treatments. However, substantial amounts of oxidized iron species, namely  
 379  $\text{Fe}_2(\text{SO}_4)_3$  and  $\text{Fe}_2\text{O}_3$ , were detected on the surface of the snorkels (Figure 2). Notably,  
 380 when the rods were extracted from the sediments, prior to being analyzed by XPS  
 381 analysis, they appeared to be covered by reddish precipitates, providing a further  
 382 qualitative indication of the occurrence of oxidized iron species.

383 Importantly, the abundance of  $\text{Fe}^{3+}$  species in the oil-supplemented snorkel (2.8 atomic  
 384 %) was 4-fold higher than in the no-oil snorkel (0.7 atom%) (Figure 2), raising the

385 intriguing possibility that the accumulation of iron precipitates was correlated with the  
386 rate and extent of observed sulfate reduction. Accumulation of oxidized iron species on  
387 the surface of a conductive snorkel deployed in crude oil marine sediment was also  
388 observed in a previous oil-spill snorkel experiment (Viggi et al., 2015).



389  
390 **Figure 2.** Percentage of Fe species on the surface of oil-supplemented and no oil snorkel  
391 experiments, and in untreated graphite rods.

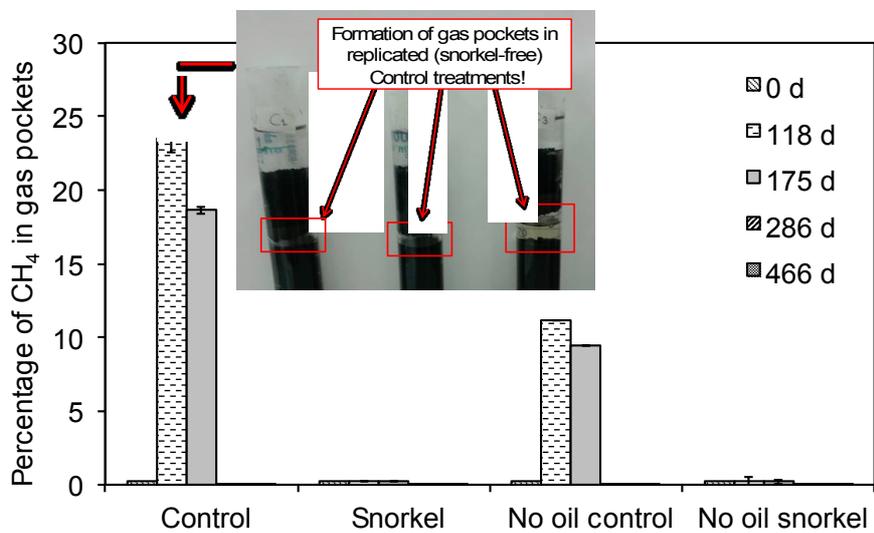
392

393

### 394 3.3 Gas production

395 The presence of snorkels markedly affected gas production and methanogenic activity in  
396 River Tyne sediments. Indeed, gas pockets containing methane (up to 25% vol/vol)  
397 accumulated within the sediment in control microcosms, which did not contain the  
398 snorkel (Figure 3). By contrast, negligible gas production occurred in oil-supplemented  
399 and no-oil snorkel treatments. Although the total cumulative volume of gas produced in  
400 each treatment could not be precisely determined, the higher percentage of methane  
401 observed on days 118 and 175 in the gas pockets occurring in oil-supplemented controls

402 relative to the no-oil controls, provides an indication that at least some of the produced  
403 methane might derive from degradation of oil constituents, with the remainder likely  
404 deriving from the degradation of the organic matter already present in the sediment. In  
405 fact, methanogenic crude oil-degrading capacity has already been reported in the River  
406 Tyne sediments (Gray et al., 2011; Jones et al., 2008). In both oil-supplemented and no-  
407 oil controls, gas production peaked on day 118 and became nearly undetectable by day  
408 286 (Figure 3).



409  
410 **Figure 3.** Percentage (% vol/vol) of methane in gas pockets formed in snorkel-free  
411 control experiments. Error bars represent the standard error of replicate samples. The  
412 inset photo illustrates the gas pockets seen in snorkel-free sediments.

413  
414  
415  
416 A large number of studies focusing on the anaerobic decomposition of organic matter in  
417 both freshwater and marine environments have stressed the importance of sulfate  
418 reduction and methanogenesis (Kuivila et al., 1989; Lovley and Klug, 1986; Oremland  
419 and Polcin, 1982; Oremland and Taylor, 1978). Thermodynamic and kinetic reasoning

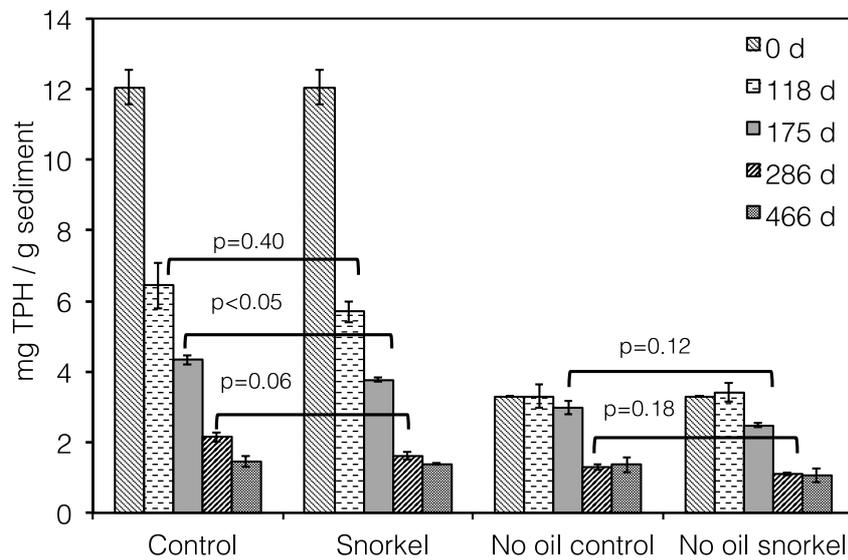
420 suggests that sulfate-reducing bacteria can outcompete methanogens due to the higher  
421 energy yield of sulfate reduction to sulfide driven by H<sub>2</sub> (or acetate) relative to the  
422 corresponding reduction of carbon dioxide to methane (or acetate disproportionation to  
423 methane and carbon dioxide), as well as to the higher growth rate of sulfate reducers on  
424 these substrates, compared to methanogens (Conrad et al., 1986). Here, in microcosms  
425 containing the snorkel, sulfate-reducing bacteria almost completely outcompeted  
426 methanogens. By contrast, in microcosms not containing the snorkel, sulfate reduction  
427 (though at lower rate than in the snorkel experiments) and methanogenesis occurred  
428 simultaneously, suggesting the two metabolic processes are competitive yet not  
429 mutually exclusive (Paulo et al., 2015).

430

#### 431 *3.4 Crude oil biodegradation*

432 In oil-supplemented microcosms, the removal of TPH appeared to be accelerated (up to  
433 30% greater removal by day 175,  $p < 0.05$ ) by the presence of the snorkel (Figure 4).  
434 These findings are consistent with the results of a previous microcosm study carried out  
435 using a different sediment contaminated with a different oil, hence indicating that the  
436 snorkel-induced stimulatory effect on hydrocarbon biodegradation was reproducible in  
437 sediments from distant geographical locations (Viggi et al., 2015).  
438 Consistent with the industrial setting from which the River Tyne sediments were  
439 obtained, low levels of hydrocarbons were detected in the microcosms, which were not  
440 specifically amended with the DUC crude oil (Figure 4). In these microcosms, TPH  
441 concentration decreased over time to a similar extent both in the no oil control and in  
442 the no oil snorkel treatments. No stimulatory effect of the snorkel on removal of  
443 indigenous hydrocarbon contamination was apparent, possibly due to the fact that the  
444 indigenous hydrocarbons in the River Tyne sediment resulted from chronic

445 contamination and were probably less available to the hydrocarbon-degrading  
 446 organisms present, compared to freshly spiked hydrocarbons in the oil-supplemented  
 447 treatments.



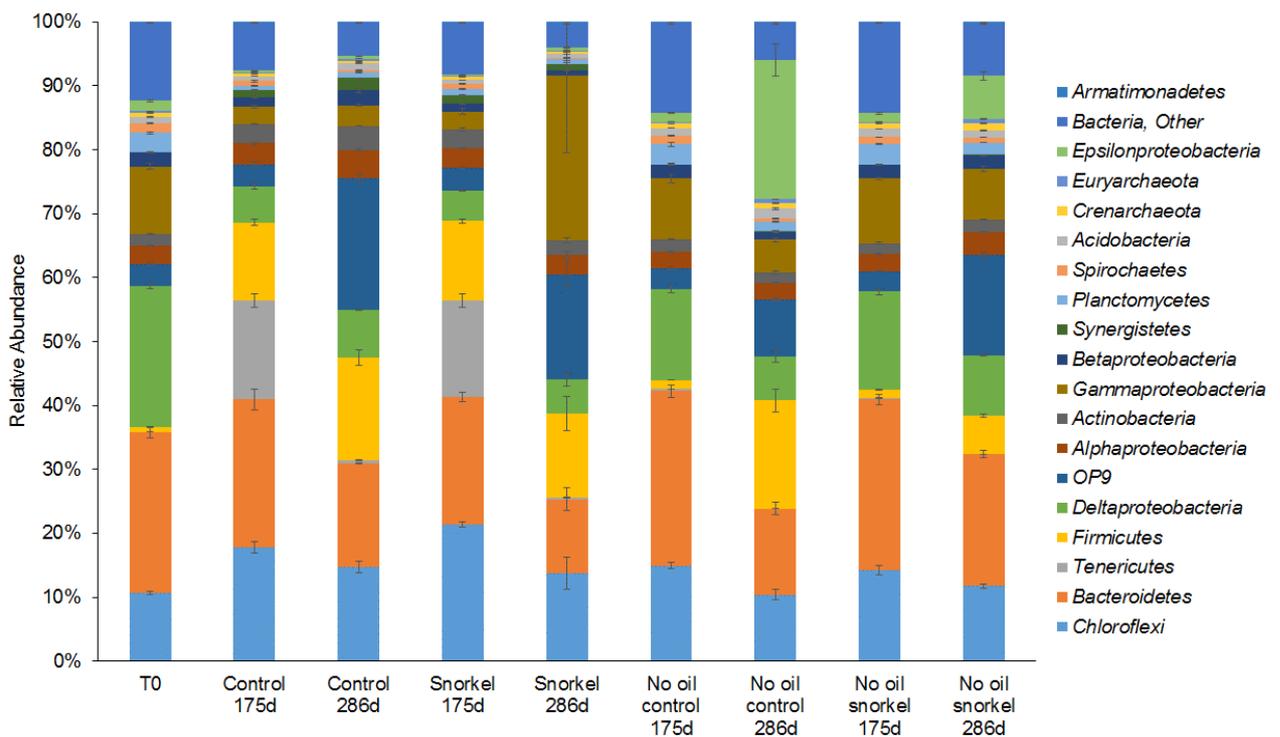
448  
 449 **Figure 4.** Concentration of TPH in the different in snorkel experiments and controls  
 450 throughout the experimental period. Error bars represent the standard error of  
 451 replicate samples.

452  
 453

### 454 3.5 Effect of electrochemical snorkels on sediment microbial communities

455 Thirty 16S rRNA gene amplicon libraries were generated which represented microbial  
 456 communities from the bulk sediment at the start of the experiment (T0) and in the  
 457 snorkel, no oil snorkel, control, and no oil control treatments following 175 and 286  
 458 days of incubation (Figure 5). The microbial composition across the 30 amplicon  
 459 libraries was 98.6% ±0.61 Bacteria (range 97.8-99.5) and 1.4% ±0.61 Archaea (range  
 460 0.5-2.2). At the phylum level, archaeal sequences comprised a small percentage of the  
 461 total microbial communities (*Euryarchaeota* 0.3% ±0.10; *Crenarcheota* 0.6% ±0.17  
 462 relative abundance, Figure 5), and therefore archaeal communities were not

463 investigated further. The most abundant phyla in the snorkel treatment at 175 days  
 464 were *Chloroflexi* (21%), *Bacteroidetes* (20%), *Tenericutes* (15%), *Firmicutes* (13%),  
 465 *Deltaproteobacteria* (5%), OP9 (4%), *Alphaproteobacteria*, *Actinobacteria* and  
 466 *Gammaproteobacteria* (3%), and *Betaproteobacteria*, *Synergistetes* and *Planctomycetes*  
 467 (1%) (Figure 5, Supplemental Figure S1A). Successional changes in the microbial  
 468 communities in the snorkel treatment were detected by day 286 with a decrease in the  
 469 relative abundance of *Tenericutes* (from 15% to 0.3%), and an increase in OP9 (from 4%  
 470 to 16%) and *Gammaproteobacteria* (from 3% to 26%) (Figure 5, Supplemental Figure  
 471 S1B). *Deltaproteobacteria* were detected in high relative abundance in the initial  
 472 sediments (T0, 22%), with a subsequent decrease in abundance in all treatments (Figure  
 473 5).  
 474



475  
 476 **Figure 5.** Phylum level comparison of microbial communities in River Tyne oil spill  
 477 snorkel experiments. The phylum Proteobacteria were further sub-divided into Class.

478 Error bars represent the standard error of replicate samples (n=3, except T0 where  
479 n=6).

480

### 481 3.5.1 Putative *n*-alkane degraders

482 At a higher resolution within the *Chloroflexi*, the uncultured genera T78 and SHD-231 in  
483 the order *Anaerolineales* were enriched at 175 and 286 days in the snorkel and control  
484 experiments which contained oil, compared to the controls without oil and the initial  
485 sediments (Figure 6A, Supplemental Figure S2). A significant enrichment of the  
486 *Anaerolinea*, predominantly in the oil-amended treatments, suggests a role in crude oil  
487 biodegradation within the sediments. The obligately anaerobic, non-photosynthetic  
488 *Anaerolinea* (Yamada et al., 2006) have previously been implicated in oil biodegradation  
489 coupled to sulfate-reduction in River Tyne sediment microcosms (Sherry et al., 2013a),  
490 in methanogenic oil sands tailings ponds (An et al., 2013), in enrichments cultures from  
491 a low-temperature, sulfidic natural hydrocarbon seep (Savage et al., 2010) and in oil  
492 contaminated mud-flat sediments (Sanni et al., 2015). Specifically, the uncultured genus  
493 T78 have been described as saccharolytic (Yamada et al., 2006) and carbohydrate  
494 utilisers (Miura and Okabe, 2008; Yamada and Sekiguchi, 2009). T78 have been  
495 identified in samples from four biogas plants and six wastewater treatment plants used  
496 as inocula to investigate the degradation of cellulose and straw in batch cultivation tests  
497 (Sun et al., 2016). T78 have also been detected in anaerobic digesters processing sewage  
498 sludge (Ariesyady et al., 2007) and used for co-digestion of whey permeate and cow  
499 manure (Hagen et al., 2014). The increasing occurrence of the presence of members of  
500 the *Anaerolineae* in this study and previous studies suggests they may play a key role in  
501 anaerobic hydrocarbon degradation.

502 Organisms from the *Tenericutes* from within the Class *Mollicutes* were significantly  
503 enriched in oil-amended snorkel and control experiments at 175 days, compared to the  
504 oil-free controls and initial sediments (Figure 6B). The apparent absence of *Mollicutes* in  
505 the initial sediments (Time 0), the significant enrichment in only those experiments  
506 which contain oil at 175 days (where ~4 mg TPH/g sediment remained in both systems)  
507 and the absence of *Mollicutes* in the oil-free controls suggests members of the *Mollicutes*  
508 play a role in the degradation of crude oil compounds. *Mollicutes* have previously been  
509 detected in salt-marsh sediment microcosms treated with Mississippi Canyon Block 252  
510 oil (MC252) from the Deepwater Horizon (DH) spill, where they were described as 'late  
511 responders' to the oil as their relative abundance did not increase until 3 weeks after the  
512 addition of the oil (Hagen et al., 2014). In another Deepwater Horizon study, *Mollicutes*  
513 were found in the gut of oysters taken from oil-contaminated areas (King et al., 2012),  
514 suggesting a tolerance of members of the *Mollicutes* to hydrocarbons. *Tenericutes*  
515 (*Mollicutes*) have also been found as important components of the microbial  
516 communities associated with natural hydrocarbon seeps (Skenner et al., 2016).

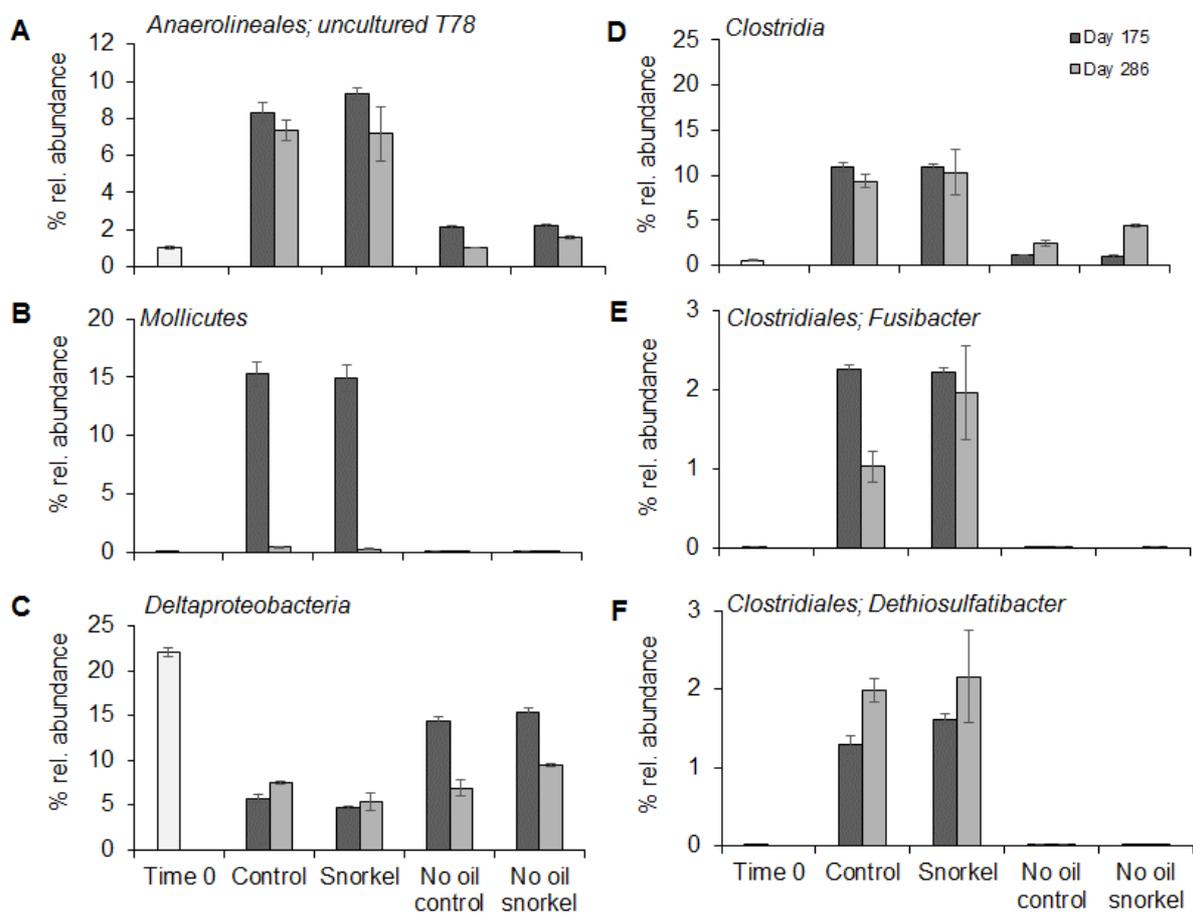
517

### 518 3.5.2 Sulfate-reducing bacteria (SRB) and fermentative Clostridia

519 The relative abundance of *Deltaproteobacteria* was highest in the time 0 sediments  
520 (22% of reads from *Deltaproteobacteria*; Figure 6C), when sulfate concentration was  
521 high ~1000mg/L. By day 175 reads from *Deltaproteobacteria* decreased to around 5%  
522 relative abundance in both treatments containing oil (control and snorkel; Figure 6C)  
523 despite differences in the levels of sulfate at this time point (control ~ 600 mg/L,  
524 snorkel ~ 10 mg/L). In treatments without oil addition the relative abundance of reads  
525 from *Deltaproteobacteria* was greater than 14-15%; Figure 6C). This suggests that some  
526 deltaproteobacterial SRB may be sensitive to the addition of oil as has been shown

527 previously (Koo et al., 2015). Moreover, previous studies of sulfate-driven oil  
528 degradation in River Tyne sediments have indicated that despite oil degradation clearly  
529 being driven by sulfate-reduction, selection for deltaproteobacterial sulfate-reducing  
530 bacteria was modest (Sherry et al., 2013a). It has been suggested that this may be due to  
531 petroleum hydrocarbon degradation in these systems being driven by sulfate-reducers  
532 acting as terminal oxidizers in a syntrophic food chain, rather than being the primary  
533 hydrocarbon oxidizers, and thermodynamic arguments have been proposed in support  
534 of this hypothesis (Head et al., 2014). Further evidence to support the possibility that  
535 sulfate-reducers are terminal oxidizers in a syntrophic food chain is the enrichment of  
536 organisms from the class *Clostridia* within the *Firmicutes* on day 175 and 286 in both oil-  
537 amended treatments (control and snorkel ~10% of reads, Figure 6D) relative to the oil-  
538 free controls and the sediments at time 0 (0.6-4% of reads, Figure 6D). Within the  
539 *Clostridiales*, the genera *Fusibacter* and *Dethiosulfatibacter* were enriched in response to  
540 oil on day 175 and 286 (control and snorkel, Figure 6E and 6F) compared to oil-free  
541 controls and the sediments at time 0. *Fusibacter* spp. have been associated with oil-  
542 producing wells (Ravot et al., 1999), sites contaminated with chlorinated solvents (Lee  
543 et al., 2011), and degradation of PAHs following the Deepwater Horizon spill (Kappell et  
544 al., 2014). Furthermore, *Fusibacter* were shown to be involved in the degradation of  
545 alkanes, crude oil and aromatics to volatile fatty acids (VFAs) in a study investigating  
546 which crude oil components contribute to oil field souring (Hasegawa et al., 2014).  
547 *Fusibacter* prefer anaerobic environments and are thiosulfate-reducers with a  
548 fermentative metabolism that can produce acetate, butyric acid, CO<sub>2</sub>, and H<sub>2</sub> from  
549 carbohydrates (Basso et al., 2009). Similarly, *Dethiosulfatibacter* are able to use  
550 thiosulfate and elemental sulfur as electron acceptors and can produce CO<sub>2</sub>, H<sub>2</sub>, acetate,  
551 and propionate from organic matter (Takii et al., 2007). Interestingly, geochemical data

552 suggest that in the presence of crude oil, the snorkel may promote oxidation of sulfide to  
 553 elemental sulfur and/or thiosulfate, potentially explaining the selection of these  
 554 organisms in treatments containing electrochemical snorkels (see section 3.7 below).  
 555 Both *Fusibacter* and *Dethiosulfatibacter* are hydrogen-producing acetogenic bacteria  
 556 that are potentially involved in fermenting components of oil to VFA which may be  
 557 utilized by sulfate-reducing terminal oxidizers.  
 558



559

560 **Figure 6.** Relative abundance (%) of rRNA gene sequences (n=11214) from oil spill  
 561 snorkel experiments at 0, 175 and 286 days. The uncultured genus T78 lineage in the  
 562 order *Anaerolineales* (A), class *Mollicutes* (B), class *Deltaproteobacteria* (C), class  
 563 *Clostridia* (D), genus *Fusibacter* (order *Clostridiales*) (E) and genus *Dethiosulfatibacter*

564 (order *Clostridiales*) (F). Error bars denote standard error (n=3, except Time 0 where  
565 n=6). Note differences in scale.

566

567

568 *3.6 Effect of electrochemical snorkels on abundance of prokaryote cells,*

569 *Deltaproteobacteria and anaerobic alkane-degrading bacteria*

570 Consistent with the effect of electrochemical snorkels on sulfate-reduction rates and

571 hydrocarbon degradation, the snorkel and the supplied oil were found to exert an effect

572 on the overall abundance of prokaryote cells. After 286 days of incubation, in the oil-

573 supplemented snorkel treatment, the abundance of DAPI-stained cells in the bulk

574 sediment was higher (p=0.007) than in the corresponding control ( $4.4 \pm 0.1 \times 10^8$  vs.

575  $2.9 \pm 0.1 \times 10^8$  cells per gram of dry sediment; Figure 7a). This corroborated the

576 suggestion that electrochemical snorkels promoted growth-linked metabolism within

577 the oil-treated sediment (Figure 7a). Notably, the DAPI cell counts in the bulk sediment

578 of no-oil controls ( $3.9 \pm 0.1 \times 10^8$  cells per gram of dry sediment) was substantially higher

579 than in the oil-supplemented controls with no snorkel, clearly indicating that the

580 presence of oil adversely affected microbial activity (Figure 7a).

581 The trend in total cell counts was mirrored by the abundance of *Deltaproteobacteria* in

582 the different treatments. Their higher abundance in the oil-supplemented snorkels with

583 respect to the (oil-supplemented) controls is consistent with the observed positive effect

584 of the snorkel on sulfate-reducing activity and alkane degradation. The trend in the

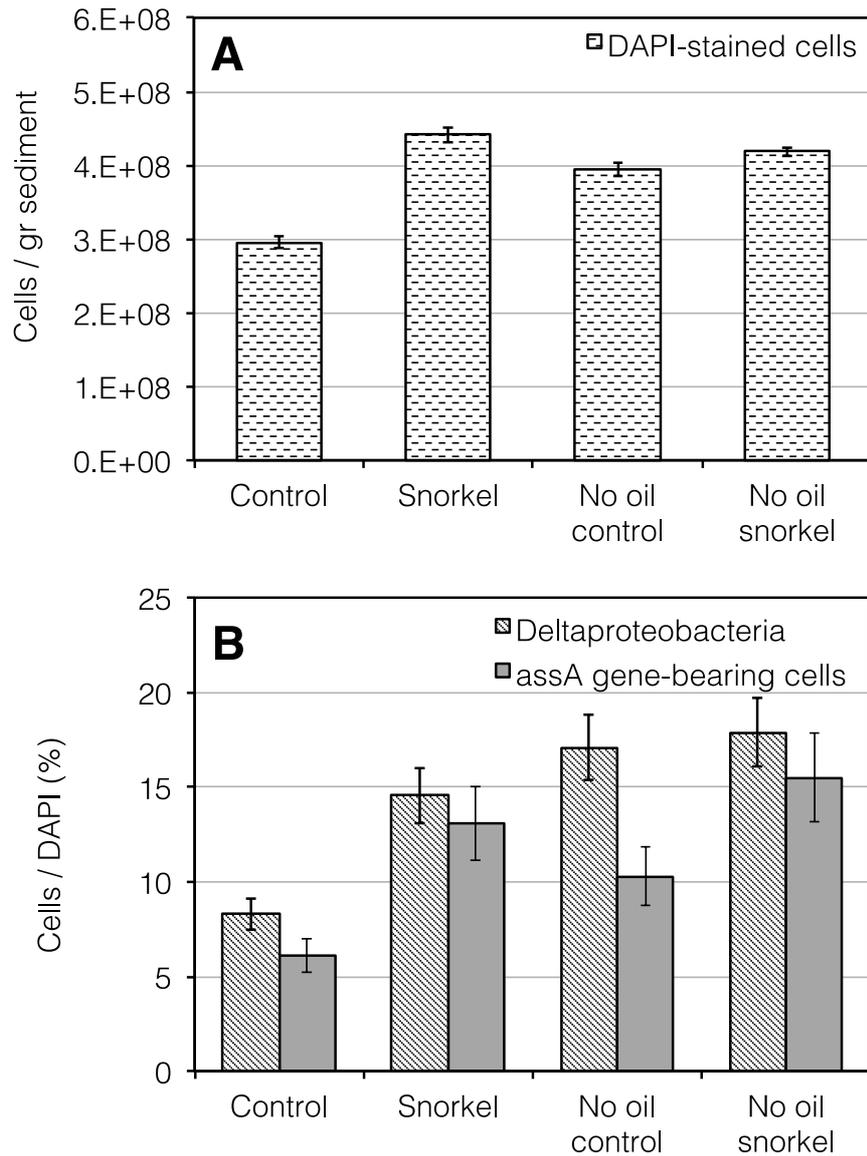
585 *Deltaproteobacteria* was also mirrored in the relative abundance data at 286 days (cf.

586 Figure 7b with Figure 6C).

587 An increasing number of studies have shown that fumarate addition is a key mechanism

588 of alkane activation under anoxic conditions (Callaghan et al., 2010; von Netzer et al.,

589 2013). This reaction is catalyzed by alkylsuccinate synthase. The gene encoding the  
590 alpha subunit of alkylsuccinate synthase *assA*, can serve as a biomarker of anaerobic  
591 alkane degradation. Here, a geneCARD-FISH approach was employed to enumerate the  
592 presence and enrichment of cells carrying the *assA* gene in the different treatments, both  
593 in the bulk sediment and on the surface of the graphite snorkels. Interestingly, after 286  
594 days of incubation, the measured concentration of cells carrying the *assA* gene was  
595 similar to the abundance of *Deltaproteobacteria*, suggesting that sulfate-reducing  
596 bacteria also played a role in direct petroleum hydrocarbon oxidation in River Tyne  
597 sediments (Sherry et al., 2013b). It remains unclear why, in no-oil treatments, the  
598 measured concentration of *Deltaproteobacteria* and cells carrying the *assA* gene was  
599 higher than in the oil-supplemented microcosms. This may result from the fact, that in  
600 the absence of snorkels the presence of oil adversely affected the autochthonous  
601 microbial populations in the sediment, as is evident from the DAPI count data and the  
602 Ion Torrent sequencing data (Figure 6C and 7A). Analysis of the biofilm growing on the  
603 surface of the snorkels indicated that cells carrying the *assA* gene were present at  
604 comparable levels ( $11\pm 2$  vs.  $7\pm 1$  % of total bacteria) in the oil-supplemented snorkels  
605 and the no-oil snorkels, suggesting a minor role for the electrode biofilm in the observed  
606 degradation of hydrocarbons.



607

608 **Figure 7.** Concentration of total DAPI-stained cells in the bulk sediment of the different

609 experiments (a). Concentration of *Deltaproteobacteria* (as detected by CARD-FISH) and

610 of cells carrying the *assA* gene in the bulk sediment of the different treatments (as

611 detected by geneCARD-FISH) (b). All measurements were carried out on samples taken

612 after 286 days of incubations. Error bars represent the standard error of replicate

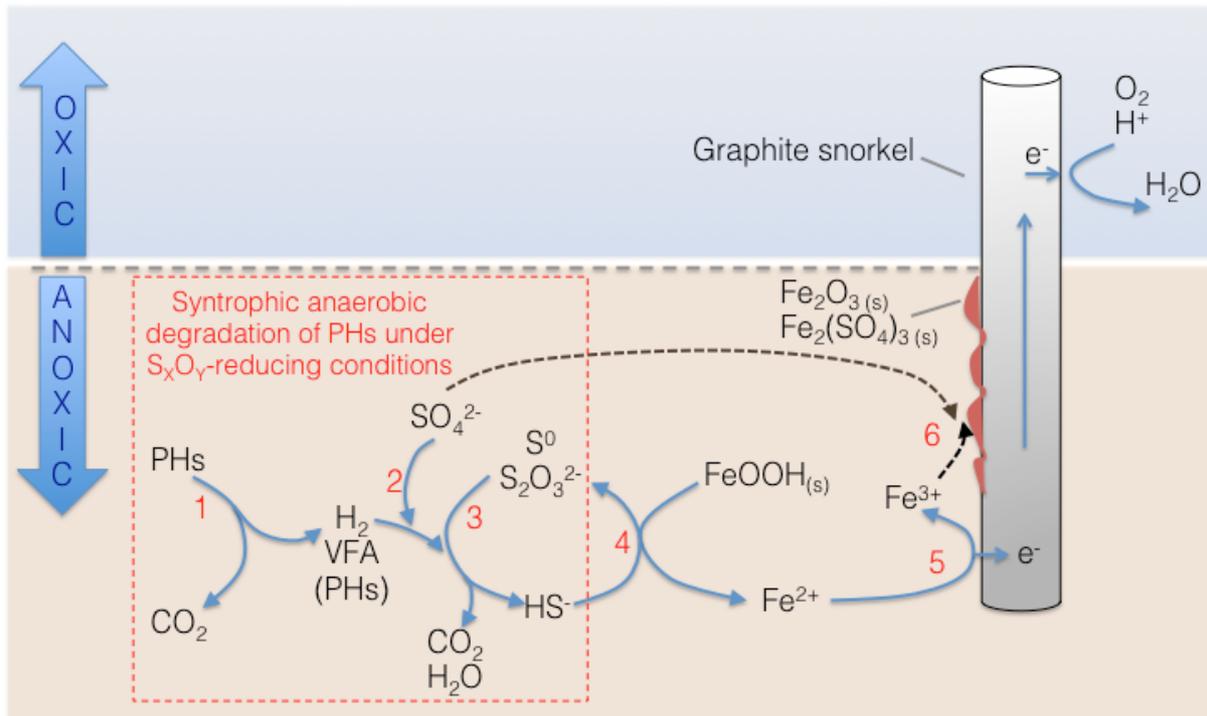
613 samples.

614

615

616 3.7 *Interaction of graphite electrodes and sediment biogeochemical cycles*

617 This study provides evidence that bridging spatially separated redox zones (i.e., the  
618 anoxic sediment with the oxic overlying water) with an electrically conductive snorkel  
619 has a remarkable impact on a number of biogeochemical processes taking place in the  
620 bulk sediment, even at a substantial distance from the surface of the conductive element  
621 (i.e., the snorkel). The most striking finding was the acceleration of sulfate reduction in  
622 the presence of an electrochemical “snorkel”, with this process being apparently coupled  
623 to the formation of oxidized iron species on the surface of the snorkel. Possibly, Fe(III)  
624 species accumulating at the surface of the rod derived from the reductive dissolution of  
625 iron minerals (e.g., Fe(III) (hydr)oxides) in the sediment into Fe(II), driven by  
626 biogenically produced hydrogen sulfide (Aller and Rude, 1988), followed by the  
627 (bio)electrochemical re-oxidation of Fe(II) to Fe(III) at the electrode surface (Figure 8).  
628 It is worth noting, that GC-MS analyses of sediment extracts indicated the presence of  
629 substantial amounts of elemental sulfur in the River Tyne sediments (data not shown),  
630 suggesting that this compound was a likely product of hydrogen sulfide oxidation, and  
631 that this reaction most probably also occurred under “natural” conditions. Interestingly,  
632 thermodynamic calculations indicated that sulfide oxidation to elemental sulfur coupled  
633 to Fe(III) reduction to Fe(II), under conditions relevant to those occurring in the snorkel  
634 experiments (i.e., pH, concentration of reactants and products), is extremely sensitive to  
635 Fe(II) concentration and becomes endergonic at Fe(II) concentrations higher than  $10^{-5}$   
636 M (Figure 9). This provides an indication that, by scavenging Fe(II), the snorkel may  
637 affect hydrogen sulfide accumulation and in turn sulfate reduction.



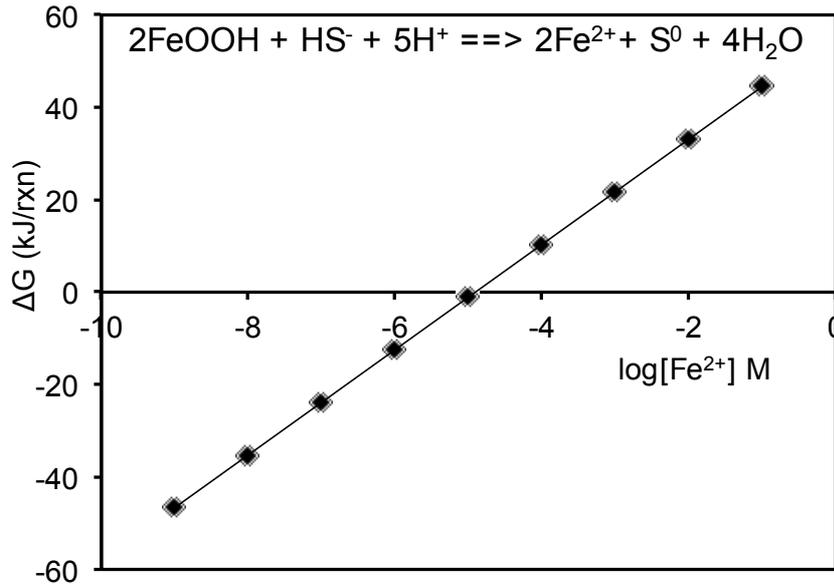
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640 **Figure 8.** Tentative model depicting the effects of an electrochemical snorkel on  
 641 chemical and biological reactions taking place in sediments contaminated by petroleum  
 642 hydrocarbons. Legend: (1) Fermentative degradation of Petroleum Hydrocarbons (PHs),  
 643 tentatively catalyzed by *Clostridia*; (2) Sulfate reduction coupled to oxidation of  
 644 fermentation products or petroleum hydrocarbons, tentatively catalyzed by  
 645 *Deltaproteobacteria*; (3) Sulfur / thiosulfate reduction coupled to oxidation of  
 646 fermentation products; (4) Abiotic reduction of Fe(III)hydroxides coupled to oxidation  
 647 of sulfide to sulfur / thiosulfate; (5) Biotic/abiotic oxidation of  $\text{Fe}^{2+}$  coupled to electrode  
 648 reduction; (6) Precipitation of poorly soluble Fe(III) compounds.

649

650

651



652

653 **Figure 9.** Influence of Fe<sup>2+</sup> concentration on the Gibbs free energy change of Fe(III)  
 654 (hydr)oxide reduction by hydrogen sulfide.

655

656

#### 657 4. Conclusions

658 The results of this study provide an additional line of evidence that a microbial  
 659 electrochemical snorkel (i.e., a single graphite electrode half-buried in anoxic sediments  
 660 and half exposed to oxygenated overlying water) has a remarkable impact on

661 biogeochemical redox processes taking place in the bulk sediment. The most noticeable

662 effect was observed on sulfate reduction, which was substantially accelerated by the

663 presence of the snorkel compared to snorkel-free controls, while methanogenesis was

664 apparently suppressed. Accumulation of oxidized iron species on the electrode surface,

665 along with thermodynamic calculations suggested that the occurrence of a sulfide-

666 driven iron redox cycle was triggered by the presence of the snorkel. Acceleration of

667 sulfate reduction corresponded to a slightly enhanced removal of petroleum

668 hydrocarbons may be facilitated by removal of toxic sulfide linked to reduction of iron

669 oxides in the sediment. This process may enhance overall sulfide oxidation with the  
670 Fe(II) generated from sulfide oxidation being removed by oxidation at the  
671 snorkel/electrode surface, maintaining a low Fe(II) concentration that provides a  
672 thermodynamic driver favoring sulfide oxidation, while replenishing Fe(III) as an  
673 oxidant, sustaining the process over extended time periods.

674

675

### 676 **Acknowledgements**

677 This work was financially supported by the European Commission within the Seventh  
678 Framework Programme under Grant Agreement No. 312139 (“Kill-Spill: Integrated  
679 biotechnological solutions for combating marine oil spills”).

680

### 681 **Author contribution**

682 C.C.V. set up and monitored the oil-spill snorkel experiments. B.M., E.F., and S.R.  
683 performed and analyzed FISH experiments. S.I. developed the GC-MS method and  
684 contributed to the interpretation of chemical data. A.M and S.K performed and analyzed  
685 XRD experiments. A.S. and O.K.M. and I.M.H performed NGS analysis. E.V. and K.R.  
686 contributed the analysis and interpretation of Fe and S biogeochemical cycles. F.A  
687 conceived the experimental plan. All authors contributed to writing of the manuscript.  
688 F.A., S.R., K.R. and I.M.H. were co-Investigators on the Kill-Spill Project.

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