

Cable bacteria promote DNRA through iron sulfide dissolution

Kessler, Adam J.; Wawryk, Michaela; Marzocchi, Ugo; Roberts, Keryn L.; Wong, Wei Wen; Risgaard-Petersen, Nils; Meysman, Filip J.R.; Glud, Ronnie N.; Cook, Perran L.M.

DOI

[10.1002/lno.11110](https://doi.org/10.1002/lno.11110)

Publication date

2019

Document Version

Accepted author manuscript

Published in

Limnology and Oceanography

Citation (APA)

Kessler, A. J., Wawryk, M., Marzocchi, U., Roberts, K. L., Wong, W. W., Risgaard-Petersen, N., Meysman, F. J. R., Glud, R. N., & Cook, P. L. M. (2019). Cable bacteria promote DNRA through iron sulfide dissolution. *Limnology and Oceanography*, 64(3), 1228-1238. <https://doi.org/10.1002/lno.11110>

Important note

To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

1 **Cable bacteria promote DNRA through iron sulphide dissolution**

2 Adam J. Kessler^{1,2*}, Michaela Wawryk², Ugo Marzocchi^{3,4}, Keryn L. Roberts², Wei Wen Wong², Nils
3 Risgaard-Petersen⁴, Filip J. R. Meysman^{5,6}, Ronnie N. Glud^{7,8}, Perran L. M. Cook²

4

5 ¹ School of Earth, Atmosphere & Environment, Monash University, Victoria, Australia

6 ² Water Studies Centre, School of Chemistry, Monash University, Victoria, Australia

7 ³ Department of Analytical, Environmental and Geo-Chemistry, Free University of Brussels (VUB),
8 Brussels, Belgium

9 ⁴ Center for Electromicrobiology and Section for Microbiology, Aarhus University, Aarhus, Denmark

10 ⁵ Department of Biology, University of Antwerp, Wilrijk, Belgium

11 ⁶ Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft,
12 The Netherlands

13 ⁷ Nordcee, Department of Biology, University of Southern Denmark, Odense, Denmark

14 ⁸ Department of Ocean and Environmental Sciences, Tokyo University of Marine Science and
15 Technology, Tokyo, Japan

16

17 *Corresponding author: adam.kessler@monash.edu

18

19 *"This is the peer reviewed version of the following article: Kessler, A. J. et al. Cable*
20 *bacteria promote DNRA through iron sulfide dissolution. Limnol. Oceanogr. 64, 1228-*
21 *1238, doi:10.1002/lno.11110 (2019)., which has been published in final form at*
22 *<https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lno.11110>. This article may be*
23 *used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use*
24 *of Self-Archived Versions."*

25

26 Key words: cable bacteria; nitrogen; denitrification; DNRA

27

28

29 **Abstract**

30 Cable bacteria represent a newly discovered group of filamentous microorganisms, which are
31 capable of spatially separating the oxidative and reductive half-reactions of their sulphide-oxidising
32 metabolisms over centimetre distances. We investigated three ways that cable bacteria might
33 interact with the nitrogen (N) cycle: (1) by reducing nitrate through denitrification or dissimilatory
34 nitrate reduction to ammonium (DNRA) within their cathodic cells; (2) by nitrifying ammonium
35 within their anodic cells; and (3) by indirectly affecting denitrification and/or DNRA by changing the
36 Fe^{2+} concentration in the surrounding sediment. We performed ^{15}N labelling laboratory experiments
37 to measure these three processes using cable bacteria containing sediments from the Yarra River,
38 Australia, and from Vilhelmsborg Sø, Denmark. Our results revealed that in the targeted systems
39 cable bacteria themselves did not perform significant rates of denitrification, DNRA or nitrification.
40 However, cable bacteria exhibited an important indirect effect, whereby they increased the Fe^{2+} pool
41 through iron sulphide dissolution. This elevated availability of Fe^{2+} significantly increased DNRA and
42 in some cases decreased denitrification. Thus, cable bacteria presence may affect the relative
43 importance of DNRA in sediments and thus the extent by which bioavailable nitrogen is lost from the
44 system.

45

46 Introduction

47 The recent discovery of electric currents linking spatial separated biogeochemical processes (Nielsen
48 et al. 2010) and cable bacteria (Pfeffer et al. 2012) has set a new paradigm for sedimentary
49 biogeochemistry (Nielsen and Risgaard-Petersen 2015; Nielsen et al. 2010). Briefly, cable bacteria
50 are members of the family *Desulfobulbaceae*, which is composed of a range of sulphur oxidising and
51 reducing bacteria. The predominant metabolism of cable bacteria is chemotrophic sulphide
52 oxidation, but these organisms separate the oxidation and reduction half-reactions by conducting
53 electrons along their long, filamentous bodies (up to 3 cm) (Meysman 2017), as represented in Fig 1.

54 The many biogeochemical implications of long distance electron transport are yet to be fully
55 understood, but in addition to directly influencing the cycling of sulphur (S), cable bacteria also
56 indirectly affect the cycling of other elements. The high production of protons in the deep, anodic
57 part of the sediment results in a pH minimum, as low as < 6.5 (Malkin et al. 2014; Nielsen et al. 2010;
58 Risgaard-Petersen et al. 2012). Such acidification of the pore water stimulates the dissolution of iron
59 sulphide (FeS) and carbonate minerals in the sediment (Risgaard-Petersen et al. 2012), which
60 strongly alters the availability of Fe, Ca, Mn, and P at depth (Rao et al. 2016; Sulu-Gambari et al.
61 2016b; Van De Velde et al. 2016). These dissolved constituents are then free to diffuse to the
62 surface, where oxygen availability and high pH (as induced by the cathodic reaction) favour the
63 precipitation of Fe- and Mn-oxides, Fe-phosphates, and Ca(Mg)-carbonates (Seitaj et al. 2015; Sulu-
64 Gambari et al. 2016a; Sulu-Gambari et al. 2016b). As a result of long distance electron transfer, cable
65 bacteria generate electric fields, which can be measured as an increase in electric potential with
66 depth (Damgaard et al. 2014). This electrogenic sulphur oxidation (e-SOx) is potentially widespread,
67 with cable bacteria discovered in marine systems across the globe (Burdorf et al. 2017; Burdorf et al.
68 2016; Malkin et al. 2014), and more recently in aquifers (Muller et al. 2016) and freshwater
69 sediments (Risgaard-Petersen et al. 2015).

70 Nitrogen (N) is an important nutrient in aquatic environments, and understanding nitrate reduction
71 pathways is an important part of managing ever-increasing global loads (Conley et al. 2009).
72 Denitrification is an important N-removing process, whereby nitrate is reduced to N₂ gas. In
73 competition to denitrification is dissimilatory nitrate reduction to ammonium (DNRA), which retains
74 N in the system. Thus, the balance of denitrification and DNRA can be an important control on
75 whether a system is net N removing or recycling (An and Gardner 2002; Dunn et al. 2013; Gardner et
76 al. 2006; Giblin et al. 2013; Kessler et al. 2018; Roberts et al. 2014). This is of great significance in
77 estuaries in particular, as denitrification-dominated estuaries may remediate high nitrate
78 concentrations, while DNRA-dominated estuaries are likely to pass large bioavailable nitrogen loads
79 to coastal waters and embayments. Nitrification, the oxidation of ammonium to nitrate, can
80 enhance N removal if coupled with denitrification. Nitrification is usually considered an aerobic
81 process, but anoxic nitrification processes are known involving Mn and possibly Fe oxides (Hulth et
82 al. 1999; Mortimer et al. 2004).

83 It is not yet known how cable bacteria control and influence the N cycle. Marzocchi et al. (2014)
84 showed that nitrate can be used as alternative cathodic electron acceptor in the absence of oxygen.
85 Whether the cable bacteria perform denitrification or DNRA is yet unclear, but recent work suggests
86 that cable bacteria can reduce both nitrate and nitrite, but not N₂O (Risgaard - Petersen et al. 2014).
87 However, cable bacteria have not yet been observed *in situ* in a high-nitrate, anoxic environment,
88 and it is not known whether cathodic nitrate-reduction occurs in a sub-oxic zone even in the
89 presence of oxygen. If so, denitrification or DNRA directly as the cable bacteria's cathodic half-

90 reaction may be important processes (Fig 1a). We hypothesize that these direct reactions by cable
91 bacteria may contribute significantly to sediment nitrogen cycling.

92 Furthermore, little is known about indirect effects of cable bacteria on N cycling. A recent study
93 showed that increased Fe^{2+} concentration promotes DNRA over denitrification in estuarine
94 sediments (Roberts et al. 2014) and freshwater lake sediments (Robertson et al. 2016; Robertson
95 and Thamdrup 2017). As cable bacteria can increase pore water Fe^{2+} through acidity generation and
96 dissolution of FeS , we hypothesize that cable bacteria can promote DNRA by other members of the
97 microbial community (Fig 1b).

98 In marine microbial fuel cells, a current is generated between a buried anode and cathode in the
99 overlying water, allowing oxidising microbes such as *Desulfobulbus* to oxidise sulphide in the
100 absence of an oxidant (Lowy et al. 2006). The possible occurrence of biologically-mediated anoxic,
101 anodic nitrification is currently debated (He et al. 2009; Qu et al. 2014; Vilajeliu-Pons et al. 2018; Xu
102 et al. 2015). As cable bacteria function analogously to a microbial fuel cell (Tender et al. 2002), we
103 hypothesize that cable bacteria can promote anoxic nitrification (Fig 1c) either directly as part of
104 their metabolism, or via symbiotic microbes using the anode provided by the cable bacteria.

105 In this study, we investigated the three hypothesized cable-bacteria-mediated nitrogen cycling
106 reactions described above, as depicted in Fig 1. First, we measured rates of denitrification and DNRA
107 and compared their relative contribution to nitrate reduction in sediments with and without cable
108 bacteria to address whether cable bacteria could lead to a stimulation of DNRA relative to
109 denitrification. Second, we repeated this experiment in sediment with active cable bacteria and
110 inactivated cable bacteria to address if such a stimulation could be attributed to the ability of cable
111 bacteria to perform DNRA or alternatively to promote DNRA by increasing Fe^{2+} availability. Third, we
112 tested whether cable bacteria can promote anoxic nitrification in oxygen-free environments, by two
113 methods. One method involved addition of $^{15}\text{NH}_4^+$ to the deep, anoxic part of the sediment. If cable
114 bacteria promoted anoxic, anodic nitrification, this would produce $^{15}\text{N-NO}_3^-$, which would
115 subsequently be rapidly reduced to $^{15}\text{N-N}_2$. The other method involved adding different
116 concentrations of $^{15}\text{NO}_3^-$ to the water overlying the sediment, resulting in varying penetration of
117 $^{15}\text{NO}_3^-$ into the sediment. Therefore if anoxic nitrification occurred, higher $^{15}\text{NO}_3^-$ concentrations
118 would result in greater overlap of the zones of anoxic nitrification and $^{15}\text{NO}_3^-$ denitrification, and the
119 measured rate of denitrification of ambient $^{14}\text{NO}_3^-$ would increase with $^{15}\text{NO}_3^-$ concentration.

120

121 **Materials and methods**

122 **Sites and sediment collection**

123 Sediment and water were collected from near to Scotch College (55°32'63.48"E 58°10'85.4"N) in the
124 Yarra River Estuary, Melbourne, Australia. This site is usually located in the salt wedge of the estuary,
125 and is characterised by periodical hypoxia in the bottom waters during low rainfall, combined with
126 aphotic sediments due to the high turbidity of the overlying fresh water layer (Roberts et al. 2012).
127 The site has been used previously for studies of the fate of nitrate during nitrate reduction (Roberts
128 et al. 2012; Roberts et al. 2014) and investigation of the sediment has shown an *in situ* population of
129 cable bacteria (Burdorf et al. 2017).

130 In addition to the experiments with sediment from the Yarra River, the anoxic nitrification
131 experiment was supplemented with a similar experiment using riparian sediment from Vilhelmsborg
132 Sø (56°04'00.9"N 10°11'01.7"E), an artificial freshwater lake near Aarhus, Denmark.

133

134 **Signatures for cable bacteria activity**

135 Cable bacteria development was monitored in the Yarra River experiments by high-resolution pH
136 profiles. A 50 µm tip pH sensor (Unisense) was mounted on a motor-driven micromanipulator and
137 profiles recorded at 50 µm steps near to the surface, and 200 µm steps below 2 mm. A reference
138 electrode (REF201 Red Rod electrode; Radiometer Analytical, Denmark) was kept in the overlying
139 water. Both electrodes were connected to a high-resistance ($> 10^{13} \Omega$) multimeter (Unisense).

140

141 Cable bacteria development was monitored in the Vilhelmsborg Sø experiments by high-resolution
142 Electric Potential (EP) depth profiles, measured with house-built microsensors (Damgaard et al.
143 2014). The sensors were mounted on a motor-driven micromanipulator and profiles were recorded
144 at 400 µm steps. A reference electrode (as above) was used. The EP sensor and the reference
145 electrode were connected to a custom-made voltmeter with high internal resistance $> 10^{14} \Omega$
146 (Aarhus University, Denmark) connected to a 16-bit analog-to-digital converter (AD216, Unisense,
147 Denmark). The EP profiles also served to identify the depth and intensity of anodic activity (Risgaard-
148 Petersen et al. 2014).

149

150 **Nitrate reduction experiments**

151 To study the direct and indirect effects of cable bacteria on nitrate reduction, ^{15}N experiments with
152 cores having active cable bacteria ("active-cables") or inactive cable bacteria ("inactivated-cables")
153 and cores without any cable bacteria ("no-cables") were performed.

154 Collected sediment was sieved (0.5 mm), homogenised, and packed into short
155 polymethylmethacrylate (PMMA) core liners (L = 120 mm, ID = 42 mm). Cores were incubated in 10 L
156 of oxygenated site water for approximately 3 weeks (with a maximum of 24 cores per bath). Three
157 weeks was chosen as a time where we expect significant cable bacteria activity based on typical
158 dynamics observed in Yarra River and other sediments (Burdorf et al. 2017). Table 1 shows the
159 details of the nitrate reduction experiments performed. To avoid the development of e-SO_x, the
160 sediment was cut at 2 mm depth every 1-2 days; the frequency of cutting varied over the various
161 experiments and is detailed in Table 1. Cutting is achieved by inserting a fine wire into the sediment
162 at ~ 2 mm depth (just below the depth of oxygen penetration) using two pins, and pulling the wire

163 through the sediment, effectively slicing a surface layer without removing it from the core. This
164 action inhibits the respiration and carbon uptake of the cable bacteria (Pfeffer et al. 2012; Vasquez-
165 Cardenas et al. 2015) and consequently their growth. This treatment is referred to as “no-cables”.
166 Cores with inactivated cable bacteria were prepared by cutting only once immediately (< 1 hour)
167 before the experimental incubation. Therefore, this “inactivated-cables” treatment maintains the
168 same biogeochemical conditions as an uncut core, but excludes the direct influence of the cable
169 bacteria metabolism and e-SOx (Risgaard-Petersen et al. 2015). As shown previously, the Fe^{2+} pool is
170 diminished by diffusion after cable bacteria are inactivated; as diffusion is slow over cm distances,
171 the Fe^{2+} pool in anoxic layers of sediments with inactivated cable bacteria does not change
172 significantly within one hour of inactivation and persists even 33 hours after inactivation (Risgaard-
173 Petersen et al. 2012). To control for any small amount of oxygen entrained by inserting the cutting
174 wire into the sediment, the wire was inserted into each “active-cables” and “inactivated-cables”
175 sediment at every cutting time, but removed without drawing it through the sediment and
176 inactivating the cable bacteria.

177 Cores were transferred to separate, individually stirred PVC tube (L = 240 mm, ID = 50 mm) filled
178 with oxic site water. The overlying water in the PVC tubes were amended to a final concentration of
179 $30 \mu\text{M } ^{15}\text{NO}_3$, and were sealed with a rubber stopper. After several hours (see Table 1), the stopper
180 was gently removed. Samples of the overlying water were collected for $^{15}\text{N-N}_2$ (12 mL in a glass vial
181 (Labco Exetainer)) and $^{15}\text{NH}_4^+$ (6 mL in a polypropylene (PP) centrifuge tube, Falcon), both preserved
182 with $100 \mu\text{L } 50\% \text{ZnCl}_2$. The surface 2 cm of sediment was then extruded, transferred to a beaker
183 containing 2% ZnCl_2 and quickly and gently homogenised, then transferred to 12 mL glass vials for
184 $^{15}\text{N}_2$ analysis. A 6 mL subsample of this slurry was also collected in a PP centrifuge tube for $^{15}\text{NH}_4^+$
185 analysis. For details of this method, see Kessler et al. (2018).

186 $^{15}\text{N-N}_2$ was measured by adding a 4 mL He headspace to the 12 ml glass vials, and analysed using a
187 Sercon isotope ratio mass spectrometer (IRMS). $^{15}\text{NH}_4^+$ was extracted with 1:1 2 M KCl, shaken for 1
188 hours at 120 rpm. The supernatant after centrifuging was transferred to a glass vial, purged with He
189 and the NH_4^+ converted to N_2 with alkaline hyperbromite (Risgaard-Petersen et al. 1995) and
190 measured by IRMS. Denitrification and DNRA were measured as the rate of production of $^{15}\text{N-N}_2$.

191 This nitrate experiment was performed twice, with slight modification. In the first experiment, the
192 whole extruded 2 cm was slurried as above. In the second experiment, the extruded sediment was
193 halved vertically. One half was slurried as above, while the second half was transferred to a 50 mL PP
194 centrifuge tube which was then flushed for > 1 min with Ar to prevent oxidation. These samples
195 were centrifuged and 1 mL of the filtered ($0.22 \mu\text{m}$) supernatant added to 0.5 mL 0.01 M ferrozine
196 and stored in the dark. These samples were analysed for total dissolved iron concentration
197 spectrophotometrically following Stookey (1970), by measuring the intensity of the purple ferrozine
198 complex in an ammonium acetate buffer after addition of hydroxylamine hydrochlorite, with all
199 reagents made as described in (Viollier et al. 2000). Fe(III) is negligible in the filtered pore water
200 (Roberts et al. 2014), and so the total dissolved iron concentration was treated as being Fe^{2+} .

201 ANOVA was used to compare treatments in each experiment using the software R (v 3.2.0) following
202 Crawley (2012). As denitrification and DNRA rates depend on a number of factors (e.g. carbon,
203 temperature) which may vary between experiments, we compare the contribution of DNRA to total
204 nitrate reduction, defined as $\% \text{DNRA} = 100 \times \text{DNRA} / (\text{denitrification} + \text{DNRA})$. This approach is
205 similar to previous work on the relative importance of these processes (Kessler et al. 2018; Roberts
206 et al. 2014).

207

208 **Nitrification experiments**

209 Two types of experiment were performed to measure anoxic nitrification, with each experiment
210 replicated. Table 1 summarises the details and procedures of the experiments performed.

211 *¹⁵NH₄⁺ experiments*

212 To test if cable bacteria can promote nitrification in anoxic sediments, ¹⁵N experiments were
213 performed with sediments from Yarra River, Australia and Vilhelmsborg Sø, Denmark.

214 Cores with treatments “active-cables”, “inactivated-cables” and “no-cables” were prepared,
215 incubated and cut as described for the nitrate reduction experiments, except that the PMMA core
216 liners were replaced with polypropylene tubes (L = 70 mm, ID = 20 m) created by cutting 60 mL
217 syringes (Thermo). 0.1 mL of anoxic, 100 mM ¹⁵NH₄Cl was injected via a hypodermic needle through
218 a port 1.5 cm below the sediment surface into the centre of the tube. Tubes were then transferred
219 to separate, individually stirred 50 mm ID PVC tube filled with oxic site water to prevent cross-
220 contamination. After ≤ 60 min (see Table 1), tubes were removed from the bath and quickly
221 extruded. Two, 1 cm slices were transferred to separate beakers containing 2% ZnCl₂ and quickly and
222 gently homogenised, then transferred to 12 mL glass vials for later analysis of ¹⁵N-N₂.

223 The method varied slightly for the experiments performed with Vilhelmsborg Sø sediments as
224 follows. Sediment was sieved (0.5 mm), poured into a glass aquaria, and incubated with aerated tap
225 water. The overlying water was replaced weekly to avoid accumulation of metabolic products and to
226 replenish nutrients. On the day of sampling, half of the cores were cut at a depth of 3 mm to inhibit
227 cable bacteria activity, and this treatment is referred to as “inactivated-cables” as above. Sediment
228 cores were extracted from the tank and were immediately incubated for 15 min in a water bath with
229 acetylene (10% partial pressure) to inhibit nitrification activity (Berg et al. 1982) in the surface
230 millimetre of sediment, thereby minimizing eventual diffusion of nitrate from the surface sediment
231 layer to the lower layer where the anodic reaction occurred. Cores were subsequently extracted
232 from the bath and 0.1 mL of a 100 mM solution of anoxic ¹⁵NH₄Cl was injected at 1.5 cm depth as
233 above. At each time-point (see Table 1), three cores were processed as follows: each cores was
234 sliced at 3 and 21 mm depth. Sediment from zero to 3 mm depth (surface) and from 3 to 21 mm
235 depth (bottom) was transferred into falcon tubes containing a solution of Allylthiourea (100 μM) to
236 stop nitrification activity (Ginestet et al. 1998 and references therein) and gently stirred to minimize
237 gas exchange with the atmosphere. 3 mm was chosen for the first slice to reflect the expected depth
238 of the anodic cable activity in the cores based on microprofiler measurements (Fig S1) and previous
239 experience with these sediments. These measurements were not possible with the Yarra River
240 sediments. A total depth of 21 mm was chosen to closely match the 20 mm total depth analysed for
241 the Yarra River cores. The solution was then left for a short period (< 2 min) to allow the coarser
242 sediment particles to settle out, before an aliquot of the supernatant was transferred into 6 mL glass
243 vials and fixed with 100 μL ZnCl 50% (w:w) for later ¹⁵N-N₂ analysis.

244 *¹⁵NO₃⁻ experiment*

245 An alternative experiment to the ¹⁵NH₄⁺ experiments was used to measure anoxic nitrification. In this
246 experiment different concentrations of ¹⁵NO₃⁻ were added to the water overlying the sediment. A
247 full explanation of the rationale for this experiment is included in the discussion section.

248 Cores were prepared, incubated and cut as described for the nitrate reduction experiments using
249 Yarra River sediment. Sediment was transferred to individual 50 mm ID PVC tube amended with
250 different concentrations of Na¹⁵NO₃ (see Table 1). PVC tubes were sealed with a rubber stopper and

251 stirred for 3 hours, after which the surface 3 cm of sediment was extruded into a beaker containing
252 30 mL 2% ZnCl₂ and quickly and gently homogenised, then transferred to 12 mL glass vials. Two
253 experiments were performed to span a large range of nitrate concentrations (see Table 1). D₁₄ is
254 defined as the rate of denitrification of ambient ¹⁴NO₃⁻, and was calculated as $D_{14} = D_{15} \times p_{29} / (2 \times p_{30})$
255 following Nielsen (1992), where $D_{15} = p_{29} + 2 \times p_{30}$ is the rate of accumulation of ¹⁵N-N₂ and p₂₉ and
256 p₃₀ are the rates of accumulation of ²⁹N₂ (¹⁴N¹⁵N) and ³⁰N₂ (¹⁵N¹⁵N) respectively.

257

258 **Results**

259 **Nitrate reduction experiments**

260 In the first nitrate reduction experiment (Fig 2a), the no-cables treatment showed appreciably lower
261 rates of DNRA ($2.3 \mu\text{mol m}^{-2} \text{h}^{-1}$) than the active-cables treatment ($12.8 \mu\text{mol m}^{-2} \text{h}^{-1}$, $p < 0.005$). No
262 difference was observed in denitrification rate ($p = 0.1$), resulting in a much greater contribution of
263 DNRA in the active-cables treatment (%DNRA = 45 %) compared with the without-cables (%DNRA =
264 10 %). The second nitrate reduction experiment (Fig 2b) showed similar results, with similar rates of
265 denitrification between treatments ($p = 0.2$) and slightly higher DNRA rates in the active-cables and
266 inactivated-cables treatments leading to a significantly higher %DNRA in these treatments (5.0 % and
267 4.4 %) compared with the no-cables sediment (%DNRA = 2.3 %, $p = 0.03$). The active-cables and
268 inactivated-cables treatments showed no significant differences in denitrification rate ($p = 0.9$),
269 DNRA rate ($p = 0.8$) or %DNRA ($p = 0.6$). Notably, while the DNRA rates were similar in the two
270 experiments, denitrification rates were approximately an order of magnitude higher in the second
271 experiment (Fig 2b), resulting the much smaller values of %DNRA. The highest denitrification rate
272 observed ($300 \mu\text{mol m}^{-2} \text{h}^{-1}$) would represent a decrease of $< 20\%$ in the added $^{15}\text{NO}_3^-$ concentration
273 over the experimental incubation.

274 Fig 3a shows that pore water average Fe^{2+} was significantly enhanced in the active-cables and
275 inactivated-cables treatments compared with the no-cables control ($p < 0.005$). The contribution of
276 DNRA to nitrate reduction (%DNRA) was only weakly correlated with pore water Fe^{2+} (Fig 3b, $p =$
277 0.2), and similarly neither the rates of denitrification ($p = 0.1$, Fig S2a) nor DNRA alone correlated
278 strongly with Fe^{2+} ($p = 0.8$, Fig S2b).

279 **Nitrification experiments**

280 Fig 4a shows production of $^{15}\text{N-N}_2$ in the $^{15}\text{NH}_4^+$ nitrification experiments. In all three experiments, a
281 small amount of $^{15}\text{N-N}_2$ ($\leq 1 \mu\text{mol m}^{-2} \text{h}^{-1}$) was measured in the deep layer of the active-cables cores,
282 but was not significantly different compared to the controls ($p > 0.05$). Rates measured in the
283 surface layer of sediment were approximately 5-10 times higher than rates in the bottom layer in the
284 Yarra River experiments. In the Vilhelmsborg S ϕ sediment cores with added acetylene, the surface
285 $^{15}\text{N-N}_2$ production was also negligible indicating the almost complete inhibition of nitrification
286 activity by the acetylene.

287 For the $^{15}\text{NO}_3^-$ nitrification experiment in Yarra River sediments, no significant difference was seen in
288 D_{14} (the rate of denitrification of ambient $^{14}\text{NO}_3^-$) with the presence of cable bacteria (Fig 4b, $p = 0.95$
289 and 0.1 after log-transformation). The solid lines in Fig 4b show regressions for the “no-cables”
290 treatment, and the dashed lines show the expected value of the “active-cables” treatment if an
291 anoxic rate of $5 \mu\text{mol m}^{-2} \text{h}^{-1}$ anoxic nitrification were occurring (representing 0.1% of the calculated
292 anodic electron transfer), with overlap of the denitrification and anoxic nitrification zone modelled
293 as a square root function with no overlap at zero and complete reduction of the produced $^{14}\text{NO}_3^-$
294 reduced to $^{29}\text{N}_2$ at $5000 \mu\text{mol L}^{-1}$. Note that the data presented in Figure 4b come from two separate
295 experiments covering the ranges of $0 - 400 \mu\text{mol L}^{-1}$ and $500 - 5000 \mu\text{mol L}^{-1}$ nitrate (see Table 1),
296 resulting in the discontinuity at $400 \mu\text{mol L}^{-1}$.

297

298 **Discussion**

299

300 **1. Nitrate reduction performed by or in the presence of cable bacteria**

301 The enhancement of DNRA in the active-cables treatment (Fig 2a) indicates that cable bacteria can
302 influence nitrate reduction, but does not differentiate between two possible mechanisms: direct
303 cathodic reduction by the cable bacteria, or an indirect influence on the N cycle due to their
304 biogeochemical fingerprint (i.e. Fe-DNRA stimulation). The second nitrate reduction experiment
305 tested these hypotheses by adding a third treatment (inactivated-cables, Fig 2b). In this experiment,
306 there is no difference between denitrification, DNRA or %DNRA between the active-cables and
307 inactivated-cables treatments. Therefore, despite the known ability of cable bacteria to use nitrate
308 (or nitrite) as the cathodic electron acceptor (Marzocchi et al. 2014), it appears that the cable
309 bacteria themselves do not contribute significantly to DNRA in the presence of oxygen. Higher DNRA
310 rates and %DNRA in the inactivated-cables compared to the no-cables treatment indicates that
311 sediments with a history of cable bacteria exhibit increased DNRA, even though direct DNRA by the
312 cable bacteria was precluded. Therefore, we conclude that some by-product of cable bacteria's
313 biogeochemical fingerprint leads to increased DNRA rates.

314 Recent studies have established a relationship between DNRA and Fe²⁺ in Yarra River sediments
315 (Kessler et al. 2018; Roberts et al. 2014; Robertson et al. 2016), and so we propose that the
316 increased pore water Fe²⁺ concentration as induced by the activity of cable bacteria (Sulu-Gambari et
317 al. 2016a; Sulu-Gambari et al. 2016b) may be responsible for the enhanced DNRA observed in the
318 active-cables treatment. In the second nitrate reduction experiment, Fe²⁺ was significantly enhanced
319 in the presence of cable bacteria (Fig 3a), presumably because of solubilisation of FeS by the acidity
320 generated by the cable bacteria's anodic reaction and/or equilibrium dissolution due to depletion of
321 pore water sulphide (Rao et al. 2016; Risgaard-Petersen et al. 2012; Sulu-Gambari et al. 2016a; Sulu-
322 Gambari et al. 2016b) (see Fig 1c). This result is also consistent with the recent findings of Otte et al.
323 (2018), who found significant correlations of both Fe²⁺-oxidising and Fe³⁺-reducing bacteria with
324 cable bacteria in both marine and freshwater systems. Specifically, the Fe²⁺-oxidising genera
325 *Pedomicrobium*, *Hoeflea*, *Chlorobium* and *Rhodopseudomonas* were identified as being correlated
326 with cable bacteria. Notably, a member *Hoeflea* has been associated with nitrate-dependent iron
327 oxidation (Sorokina et al. 2012), though there are many other possible candidates that may be
328 present in our sediments. The contribution of DNRA to nitrate reduction was weakly correlated with
329 Fe²⁺, with higher Fe²⁺ increasing %DNRA. While the weakness of this correlation reflects the
330 complexity of the relationship between Fe²⁺ and nitrate reduction pathways, this general response is
331 consistent with our previous observations (Kessler et al. 2018; Roberts et al. 2014; Robertson et al.
332 2016) showing a link between Fe²⁺ availability and DNRA.

333 There are two possible explanations for the influence of Fe²⁺ on nitrate reduction pathway. First,
334 several studies have suggested that Fe²⁺ can be a direct electron donor for DNRA bacteria (Coby et
335 al. 2011; Kessler et al. 2018; Roberts et al. 2014; Robertson et al. 2016; Robertson and Thamdrup
336 2017; Weber et al. 2006), as depicted in Fig 1b. Mostly, it is suggested that Fe²⁺ reacts with nitrite
337 (NO₂⁻) rather than nitrate, and that the earlier step of nitrate reduction to nitrite is performed by
338 other members of the denitrifying community (Robertson et al. 2016). This first step is usually slow,
339 and nitrite does not usually accumulate in these sediments, instead being rapidly reduced to N₂ by
340 denitrification and/or NH₄⁺ by DNRA, depending on which community dominates (Roberts et al.
341 2014). The first nitrate reduction experiment supports this hypothesis, though the effect is smaller in
342 the second experiment (Fig 2b). Secondly, it is known that Fe²⁺ can inhibit denitrification by
343 disrupting intracellular electron transport (Carlson et al. 2012), which would lead to a similar

344 increase in %DNRA. Both the active-cables and inhibited-cables treatments appear to have slightly
345 reduced denitrification in both experiments (Fig 2a and b), although because neither denitrification
346 nor DNRA rates are generally correlated with Fe^{2+} , this study cannot conclusively differentiate these
347 two effects. It is likely that both effects are relevant, depending on other conditions, and that other
348 factors influence both denitrification and DNRA. For example, microbes utilising the well-established
349 sulphide-driven DNRA pathway (An and Gardner 2002; Brunet and Garcia-Gil 1996) may scavenge
350 sulphide released by FeS dissolution in the anoxic zone. As the microbial communities responsible
351 for Fe^{2+} - and sulphide-driven DNRA are not well established, it is difficult to separate these effects.
352 Similarly, sulphide has known toxic effects on denitrification (Sørensen et al. 1980). Despite these
353 additional influences, which may account for the weak correlation in Fig 3b, it would appear that the
354 role of cable bacteria in the nitrogen cycle is to enhance the relative importance of DNRA by other
355 members of the sediment microbial community through increasing Fe^{2+} availability.

356

357 **2. Anoxic nitrification at the cable bacteria anode**

358 No evidence for anoxic nitrification was observed at either site (Fig 4). The measured nitrification
359 rates in the anoxic bottom layer (where the $^{15}\text{NH}_4^+$ was added) are consistently slightly higher in the
360 active-cables treatments, but this effect is never statistically significant. The $^{15}\text{N-N}_2$ measured at the
361 surface sediment is presumably due to a small leak of $^{15}\text{NH}_4^+$ solution to the surface through pores
362 and fractures in the sediment during the injection. Indeed the high variation in the mean rates in the
363 surface layers (s.e. = 20 % - 150 %) are consistent with random fractures in the sediment. It is
364 possible that if DNRA dominates (see earlier discussion), then $^{15}\text{NO}_3^-$ produced by anoxic nitrification
365 would be reduced back to $^{15}\text{NH}_4^+$, which we would not detect using this method. As the %DNRA was
366 never above 50 % in either nitrate reduction experiment, and was usually approximately 10 % (Fig 2),
367 we would still expect denitrification to be measureable in this case. With the sediment from
368 Vilhelmsborg Sø, the finding that anoxic nitrification is negligible is consistent with the experiment
369 from the Yarra River.

370 For the $^{15}\text{NO}_3^-$ experiment in Yarra River sediments, varying concentrations of $^{15}\text{NO}_3^-$ were added to
371 the oxic water overlying the sediment. This should have resulted in increasingly deeper penetration
372 of NO_3^- , and therefore an increasingly deep zone of denitrification. If anoxic nitrification occurred, it
373 would be expected that there is an additional source of $^{14}\text{NO}_3^-$ in the zone of denitrification,
374 increasing as the depth of the denitrification zone increases. Therefore, there D_{14} should be
375 enhanced in the presence of cable bacteria if anoxic nitrification is occurring. There is no evidence of
376 deviation of the “active-cables” treatment toward the dashed line in Fig 4b. Thus, this experiment
377 provides additional evidence that anoxic nitrification does not occur either as part of cable bacteria
378 activity, or by nitrifiers in the presence of (anodic) cable bacteria. It is noteworthy that the higher
379 concentration treatment shows an increase in D_{14} with $^{15}\text{NO}_3^-$ concentration. This indicates that one
380 or more of the assumptions of the isotope pairing technique are not met in this experiment, most
381 likely that the system has not reached a steady state (Nielsen 1992; Risgaard - Petersen et al. 2003).
382 As the present experiment is in any case exploiting a weakness in the isotope pairing technique, this
383 does not invalidate the above finding, but does mean that the rates of D_{14} found cannot be treated
384 as representative.

385

386 **3. Implications for cable bacteria-rich environments**

387 Since the discovery of cable bacteria and their complex metabolism, many questions have arisen
388 about their ability to affect other biogeochemical processes. There is strong evidence that cable
389 bacteria can reduce nitrate (or nitrite) at their cathode (see Fig 1) (Marzocchi et al. 2014). That work
390 was performed under laboratory conditions in high-nitrate ($> 250 \mu\text{M}$), anoxic water. As yet, *in situ*
391 observations of cable bacteria have not been reported in such an environment, but this remains a
392 viable ecological niche for such activity. The present work shows that when the overlying water is
393 oxygenated, cable bacteria do not contribute significantly to DNRA, as might be expected from
394 traditional thermodynamic redox cascades (Froelich et al. 1979), or at least that cable bacteria DNRA
395 occurs at low rates relative to total nitrate reduction.

396

397 Cable bacteria appear to play a role in the N cycle through the dissolution of FeS by the acid-
398 generating anodic half-reactions. This increased Fe^{2+} pool then serves as a driver for DNRA following
399 recent observations Fe^{2+} directly and indirectly enhancing DNRA, including in the Yarra River (Kessler
400 et al. 2018; Roberts et al. 2014; Robertson et al. 2016; Robertson and Thamdrup 2017). As the
401 relative rates of DNRA and denitrification are of global interest as global N loads increase (Conley et
402 al. 2009; Gruber and Galloway 2008; Steffen et al. 2015), understanding the conditions under which
403 DNRA may be enhanced (or denitrification suppressed) is critical. If cable bacteria are significantly
404 enhancing DNRA, then the stable, seasonally hypoxic systems most closely associated with cable
405 bacteria (Burdorf et al. 2017; Burdorf et al. 2016; Malkin et al. 2014; Nielsen 2016; Seitaj et al. 2015)
406 may become more N-recycling during the seasonal cable bacteria dominance. This is particularly
407 interesting as the Fe-cycling associated with cable bacteria has been shown to buffer against euxinia
408 (Seitaj et al. 2015). The proposed mechanism is that the Fe^{2+} solubilised at the anodic end of cable
409 bacteria diffuses upwards, creating an iron oxide layer at the surface. This iron oxide layer provides a
410 firewall against free sulphide diffusing out of the sediment once sulphide supply exceeds cable
411 bacteria demand. This work suggests that the net value of cable bacteria as mediators of water
412 quality may be limited, as the same Fe^{2+} release may inhibit N removal from estuarine and coastal
413 waters by directly inhibiting denitrification and/or favouring its recycling through DNRA.

414

415 **Acknowledgements**

416 We thank V Eate, D Brehm and S Koh for assistance in the field and laboratory. This work was
417 supported by Australian Research Council Discovery Project grant DP150101281 to Cook, Glud &
418 Meysman, by a Monash University ResearchFirst Award to Wawryk and Kessler, and from the
419 European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-
420 Curie grant agreement No 656385 to Marzocchi. Risgaard-Petersen was financially supported by the
421 Danish National Research Foundation DNRF136.

422 **References**

423

- 424 An, S., and W. S. Gardner. 2002. Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen
425 link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas).
426 *Marine Ecology Progress Series* **237**: 41-50.
- 427 Berg, P., L. Klemetsson, and T. Rosswall. 1982. Inhibitory effect of low partial pressures of acetylene
428 on nitrification. *Soil Biology and Biochemistry* **14**: 301-303.
- 429 Brunet, R., and L. Garcia-Gil. 1996. Sulfide-induced dissimilatory nitrate reduction to ammonia in
430 anaerobic freshwater sediments. *FEMS Microbiology Ecology* **21**: 131-138.
- 431 Burdorf, L. D. and others 2017. Long-distance electron transport occurs globally in marine sediments.
432 *Biogeosciences* **14**: 683.
- 433 Burdorf, L. D. W., S. Hidalgo-Martinez, P. Cook, and F. Meysman. 2016. Long-distance electron
434 transport by cable bacteria in mangrove sediments. *Marine Ecology Progress Series* **545**: 1-8.
- 435 Carlson, H. K., I. C. Clark, R. A. Melnyk, and J. D. Coates. 2012. Toward a mechanistic understanding
436 of anaerobic nitrate-dependent iron oxidation: balancing electron uptake and detoxification.
437 *Frontiers in microbiology* **3**.
- 438 Coby, A. J., F. Picardal, E. Shelobolina, H. Xu, and E. E. Roden. 2011. Repeated anaerobic microbial
439 redox cycling of iron. *Applied and environmental microbiology* **77**: 6036-6042.
- 440 Conley, D. J. and others 2009. Controlling eutrophication: nitrogen and phosphorus. *Science* **323**:
441 1014-1015.
- 442 Crawley, M. J. 2012. *The R book*. John Wiley & Sons.
- 443 Damgaard, L. R., N. Risgaard-Petersen, and L. P. Nielsen. 2014. Electric potential microelectrode for
444 studies of electro-biogeophysics. *Journal of Geophysical Research: Biogeosciences*:
445 2014JG002665.
- 446 Dunn, R. J., D. Robertson, P. R. Teasdale, N. J. Waltham, and D. T. Welsh. 2013. Benthic metabolism
447 and nitrogen dynamics in an urbanised tidal creek: Domination of DNRA over denitrification
448 as a nitrate reduction pathway. *Estuarine, Coastal and Shelf Science* **131**: 271-281.
- 449 Froelich, P. N. and others 1979. Early oxidation of organic matter in pelagic sediments of the eastern
450 equatorial Atlantic: suboxic diagenesis. *Geochimica et cosmochimica acta* **43**: 1075-1090.
- 451 Gardner, W. S., M. J. McCarthy, S. An, D. Sobolev, K. S. Sell, and D. Brock. 2006. Nitrogen fixation and
452 dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas
453 estuaries. *Limnology and Oceanography* **51**: 558-568.
- 454 Giblin, A. E., C. R. Tobias, B. Song, N. Weston, G. T. Banta, and V. H. Rivera-Monroy. 2013. The
455 importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of
456 coastal ecosystems. *Oceanography* **26**: 124-131.
- 457 Ginestet, P., J. M. Audic, V. Urbain, and J. C. Block. 1998. Estimation of nitrifying bacterial activities
458 by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and
459 azide. *Applied and Environmental Microbiology* **64**: 2266-2268.
- 460 Gruber, N., and J. N. Galloway. 2008. An Earth-system perspective of the global nitrogen cycle.
461 *Nature* **451**: 293.
- 462 He, Z., J. J. Kan, Y. B. Wang, Y. L. Huang, F. Mansfeld, and K. H. Neelson. 2009. Electricity Production
463 Coupled to Ammonium in a Microbial Fuel Cell. *Environmental Science & Technology* **43**:
464 3391-3397.
- 465 Hulth, S., R. C. Aller, and F. Gilbert. 1999. Coupled anoxic nitrification/manganese reduction in
466 marine sediments. *Geochimica et Cosmochimica Acta* **63**: 49-66.
- 467 Kessler, A. J., K. L. Roberts, A. Bissett, and P. L. M. Cook. 2018. Biogeochemical controls on the
468 relative importance of denitrification and dissimilatory nitrate reduction to ammonium in
469 estuaries. *Global Biogeochemical Cycles* **32**: 1045–1057.

470 Lowy, D. A., L. M. Tender, J. G. Zeikus, D. H. Park, and D. R. Lovley. 2006. Harvesting energy from the
471 marine sediment–water interface II: Kinetic activity of anode materials. *Biosensors and*
472 *Bioelectronics* **21**: 2058-2063.

473 Malkin, S. Y. and others 2014. Natural occurrence of microbial sulphur oxidation by long-range
474 electron transport in the seafloor. *ISME J* **8**: 1843-1854.

475 Marzocchi, U. and others 2014. Electric coupling between distant nitrate reduction and sulfide
476 oxidation in marine sediment. *The ISME journal* **8**: 1682-1690.

477 Meysman, F. J. 2017. Cable Bacteria Take a New Breath Using Long-Distance Electricity. *Trends in*
478 *microbiology*.

479 Mortimer, R. J. and others 2004. Anoxic nitrification in marine sediments. *Marine Ecology Progress*
480 *Series* **276**: 37-51.

481 Muller, H. and others 2016. Long-distance electron transfer by cable bacteria in aquifer sediments.
482 *ISME J* **10**: 2010-2019.

483 Nielsen, L. P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS*
484 *Microbiology Letters* **86**: 357-362.

485 Nielsen, Lars p. 2016. Ecology: Electrical Cable Bacteria Save Marine Life. *Current Biology* **26**: R32-
486 R33.

487 Nielsen, L. P., and N. Risgaard-Petersen. 2015. Rethinking sediment biogeochemistry after the
488 discovery of electric currents. *Annual review of marine science* **7**: 425-442.

489 Nielsen, L. P., N. Risgaard-Petersen, H. Fossing, P. B. Christensen, and M. Sayama. 2010. Electric
490 currents couple spatially separated biogeochemical processes in marine sediment. *Nature*
491 **463**: 1071-1074.

492 Otte, J. M. and others 2018. The distribution of active iron - cycling bacteria in marine and
493 freshwater sediments is decoupled from geochemical gradients. *Environmental*
494 *microbiology*.

495 Pfeffer, C. and others 2012. Filamentous bacteria transport electrons over centimetre distances.
496 *Nature* **491**: 218-221.

497 Qu, B., B. Fan, S. K. Zhu, and Y. L. Zheng. 2014. Anaerobic ammonium oxidation with an anode as the
498 electron acceptor. *Env Microbiol Rep* **6**: 100-105.

499 Rao, A. M., S. Y. Malkin, S. Hidalgo-Martinez, and F. J. Meysman. 2016. The impact of electrogenic
500 sulfide oxidation on elemental cycling and solute fluxes in coastal sediment. *Geochimica et*
501 *Cosmochimica Acta* **172**: 265-286.

502 Risgaard-Petersen, N., L. R. Damgaard, A. Revil, and L. P. Nielsen. 2014. Mapping electron sources
503 and sinks in a marine biogeochemical battery. *Journal of Geophysical Research: Biogeosciences*:
504 2014JG002673.

505 Risgaard-Petersen, N. and others 2015. Cable Bacteria in Freshwater Sediments. *Applied and*
506 *Environmental Microbiology* **81**: 6003-6011.

507 Risgaard-Petersen, N., A. Revil, P. Meister, and L. P. Nielsen. 2012. Sulfur, iron-, and calcium cycling
508 associated with natural electric currents running through marine sediment. *Geochimica et*
509 *Cosmochimica Acta* **92**: 1-13.

510 Risgaard-Petersen, N., N. P. Revsbech, and S. Rysgaard. 1995. Combined microdiffusion-hypobromite
511 oxidation method for determining nitrogen-15 isotope in ammonium. *Soil Science Society of*
512 *America Journal* **59**: 1077-1080.

513 Risgaard - Petersen, N., L. R. Damgaard, A. Revil, and L. P. Nielsen. 2014. Mapping electron sources
514 and sinks in a marine biogeochemical battery. *Journal of Geophysical Research: Biogeosciences* **119**:
515 1475-1486.

516 Risgaard - Petersen, N., L. P. Nielsen, S. Rysgaard, T. Dalsgaard, and R. L. Meyer. 2003. Application of
517 the isotope pairing technique in sediments where anammox and denitrification coexist.
518 *Limnology and Oceanography: Methods* **1**: 63-73.

519 Roberts, K. L., V. M. Eate, B. D. Eyre, D. P. Holland, and P. L. Cook. 2012. Hypoxic events stimulate
520 nitrogen recycling in a shallow salt - wedge estuary: The Yarra River estuary, Australia.
521 *Limnology and Oceanography* **57**: 1427-1442.

522 Roberts, K. L., A. J. Kessler, M. R. Grace, and P. L. M. Cook. 2014. Increased rates of dissimilatory
523 nitrate reduction to ammonium (DNRA) under oxic conditions in a periodically hypoxic
524 estuary. *Geochimica et Cosmochimica Acta* **133**: 313-324.

525 Robertson, E. K., K. L. Roberts, L. D. Burdorf, P. Cook, and B. Thamdrup. 2016. Dissimilatory nitrate
526 reduction to ammonium coupled to Fe (II) oxidation in sediments of a periodically hypoxic
527 estuary. *Limnology and Oceanography* **61**: 365-381.

528 Robertson, E. K., and B. Thamdrup. 2017. The fate of nitrogen is linked to iron(II) availability in a
529 freshwater lake sediment. *Geochimica et Cosmochimica Acta* **205**: 84-99.

530 Seitaj, D. and others 2015. Cable bacteria generate a firewall against euxinia in seasonally hypoxic
531 basins. *Proceedings of the National Academy of Sciences* **112**: 13278-13283.

532 Sørensen, J., J. Tiedje, and R. Firestone. 1980. Inhibition by sulfide of nitric and nitrous oxide
533 reduction by denitrifying *Pseudomonas fluorescens*. *Applied and Environmental*
534 *Microbiology* **39**: 105-108.

535 Sorokina, A. Y., E. Y. Chernousova, and G. A. Dubinina. 2012. *Hoeflea siderophila* sp. nov., a new
536 neutrophilic iron-oxidizing bacterium. *Microbiology* **81**: 59-66.

537 Steffen, W. and others 2015. Planetary boundaries: Guiding human development on a changing
538 planet. *Science* **347**: 1259855.

539 Stookey, L. L. 1970. Ferrozine---a new spectrophotometric reagent for iron. *Analytical chemistry* **42**:
540 779-781.

541 Sulu-Gambari, F., D. Seitaj, T. Behrends, D. Banerjee, F. J. R. Meysman, and C. P. Slomp. 2016a.
542 Impact of cable bacteria on sedimentary iron and manganese dynamics in a seasonally-
543 hypoxic marine basin. *Geochimica et Cosmochimica Acta* **192**: 49-69.

544 Sulu-Gambari, F., D. Seitaj, F. J. R. Meysman, R. Schauer, L. Polerecky, and C. P. Slomp. 2016b. Cable
545 Bacteria Control Iron-Phosphorus Dynamics in Sediments of a Coastal Hypoxic Basin.
546 *Environmental Science & Technology* **50**: 1227-1233.

547 Tender, L. M. and others 2002. Harnessing microbially generated power on the seafloor. *Nat Biotech*
548 **20**: 821-825.

549 Van De Velde, S. and others 2016. The impact of electrogenic sulfur oxidation on the
550 biogeochemistry of coastal sediments: A field study. *Geochimica et Cosmochimica Acta* **194**:
551 211-232.

552 Vasquez-Cardenas, D. and others 2015. Microbial carbon metabolism associated with electrogenic
553 sulphur oxidation in coastal sediments. *The Isme Journal* **9**: 1966.

554 Vilajeliu-Pons, A., C. Koch, M. D. Balaguer, J. Colprim, F. Harnisch, and S. Puig. 2018. Microbial
555 electricity driven anoxic ammonium removal. *Water research* **130**: 168-175.

556 Viollier, E., P. Inglett, K. Hunter, A. Roychoudhury, and P. Van Cappellen. 2000. The ferrozine method
557 revisited: Fe (II)/Fe (III) determination in natural waters. *Applied geochemistry* **15**: 785-790.

558 Weber, K. A., M. M. Urrutia, P. F. Churchill, R. K. Kukkadapu, and E. E. Roden. 2006. Anaerobic redox
559 cycling of iron by freshwater sediment microorganisms. *Environmental Microbiology* **8**: 100-
560 113.

561 Xu, X. and others 2015. Nitrification, Denitrification, and Power Generation Enhanced by
562 Photocatalysis in Microbial Fuel Cells in the Absence of Organic Compounds. *Energy & Fuels*
563 **29**: 1227-1232.

564

565

Tables

Table 1: Details of experiments performed. "Details" provides the incubation times and/or nitrate concentrations used in that experiment. All experiments were performed with Yarra River Sediments except the third $^{15}\text{NH}_4^+$ nitrification experiment, which was performed with Vilhelmsborg S \emptyset sediment as indicated.

Sample collection	Treatments	Details	Cut dates†	Experiment date
Nitrate reduction experiments				
30/01/17	12 x active-cables 12 x no-cables	2, 4, 6, 8 h	06/02, 13/02, 15/02, 17/02, 19/02, 1 h before expt.	23/02/16
16/02/18	8 x active-cables 8 x inactivated-cables 8 x no-cables	6 h	Daily 17-20/02, Twice daily 21-25/02, 1 h before expt.	26/02/18
$^{15}\text{NH}_4^+$ nitrification experiments				
17/11/16	9 x active-cables 12 x inactivated-cables	20, 40, 60 min	1 h before expt.	06/12/16
30/01/17	8 x active-cables 8 x no-cables	30, 60 min	06/02, 13/02, 15/02, 17/02, 19/02, 1 h before expt.	20/02/17
28/02/17 (Vilhelmsborg S \emptyset)	12 x active-cables 12 x inactivated-cables	30, 75, 100, 120 min	1 h before expt.	30/03/17
$^{15}\text{NO}_3^-$ nitrification experiments				
17/11/16	12 x active-cables 12 x inactivated-cables	10, 30, 100, 400 $\mu\text{M } ^{15}\text{NO}_3^-$	1 h before expt.	07/12/16
30/01/17	12 x active-cables 12 x no-cables	500, 1000, 2500, 5000 $\mu\text{M } ^{15}\text{NO}_3^-$	06/02, 13/02, 15/02, 17/02, 19/02, 20/02, 21/02, 1 h before expt.	22/02/17

† Active-cables treatments were never cut. Inactivated-cables treatments were cut only once, on the day of the experiment. No-cables treatments were cut approximately every two days as described.

Figure captions

Figure 1: Schematic of cable bacteria showing typical sediment depth-profiles of O₂ (red), H₂S (green) and pH (black). Also shown in italics are the anodic and cathodic half-equations for cable bacteria metabolism and the proposed reactions involving the N cycle: denitrification and DNRA at the cathode (A), Fe-DNRA at the pH minimum (B) and anoxic nitrification (NIT) at the anode (C).

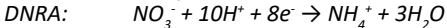
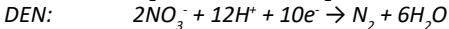
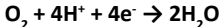
Figure 1 (print version): Schematic of cable bacteria showing typical profiles of O₂ (solid), H₂S (long dash) and pH (short dash). Also shown in italics are the anodic and cathodic half-equations for cable bacteria metabolism and the proposed reactions involving the N cycle: denitrification and DNRA at the cathode (A), Fe-DNRA at the pH minimum (B) and anoxic nitrification (NIT) at the anode (C).

Figure 2: summary of nitrate reduction experiments results. Shown are rates of denitrification and DNRA and %DNRA for the active-cables (A), inactivated-cables (I) and no-cables (N) treatments. (a) shows the first experiment (23/02/2016, N=12) and has only treatments A and N. (b) shows the second experiment (26/02/2018, N=8), with all three treatments. Note the different axes to assist visualisation. Error bars represent standard error.

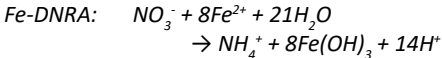
Figure 3: The link between cable bacteria, Fe²⁺ and %DNRA. (a) average Fe²⁺ concentration in the upper 20 mm of sediment is significantly lower in the no-cables treatment than the active-cables or inactivated-cables treatments ($p = 0.01$). Data shown is from the same experiment as shown in Fig 2b. Error bars represent standard error. $N = 6-8$. (b) %DNRA is weakly correlated with Fe²⁺ for the same data shown in panel a ($p = 0.2$). Marker colour denotes the data as being part of the active-cables (A), inactivated-cables (I) and no-cables (N) treatments. $N = 20$.

Figure 4: Results of anoxic nitrification experiments in Yarra River (YR) and Vilhelmsborg SØ (VS) sediments. (a) Rate of ¹⁵N-N₂ production in the ¹⁵NH₄⁺ nitrification experiment. Each experiment shows both an active-cables (A) and either an inactivated-cables (I) or no-cables (N) treatment. Surface and bottom refer to the surface and deep sediment layers. $N = 3$ for experiment 1 & 3 and $N = 4$ for experiment 2. "surface" is 0-10 mm depth for YR and 0-3 mm depth for VS. "bottom" is 10-20 mm depth for YR and 3-21 mm depth for VS. Note that rates are minimum rates, as ¹⁵N₂ lost to the overlying water column is not considered. (b) D₁₄ during the ¹⁵NO₃⁻ nitrification experiment using Yarra River sediment. The dashed line represent the deviation expected if anoxic nitrification occurred at a rate of 5 $\mu\text{mol m}^{-2} \text{h}^{-1}$ (0.1 % of total cable bacteria anodic electron transfer) and all of this nitrification resulted in ²⁹N₂ at 5000 $\mu\text{mol L}^{-1}$ nitrate. Error bars represent standard error. $N = 3$.

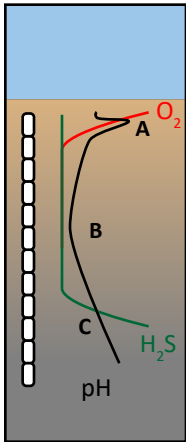
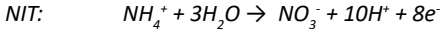
A. Cathodic reaction of cable bacteria:

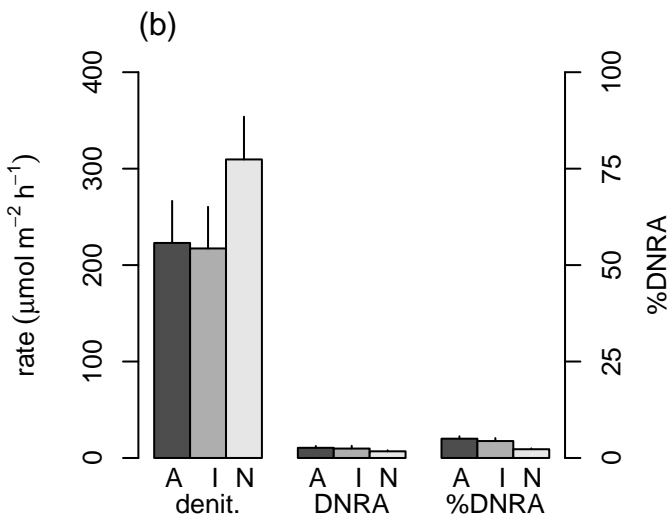
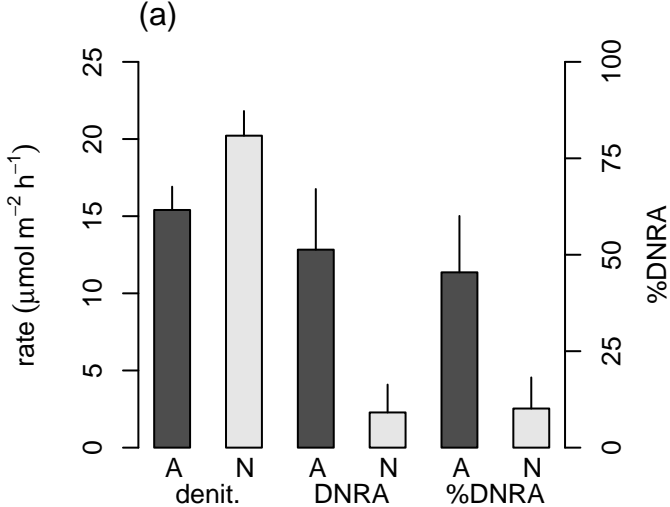


B. pH minimum

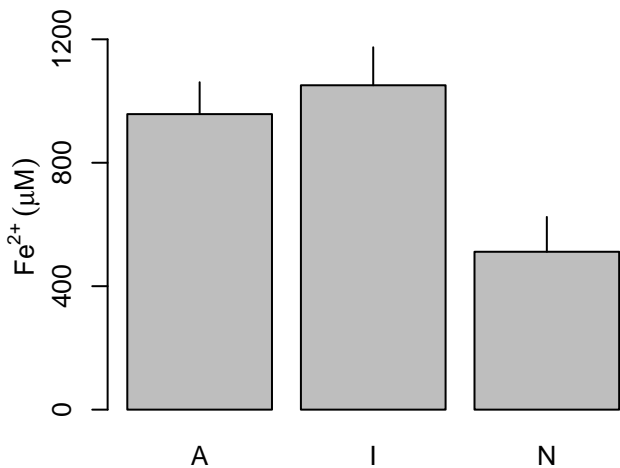


C. Anodic reaction of cable bacteria:

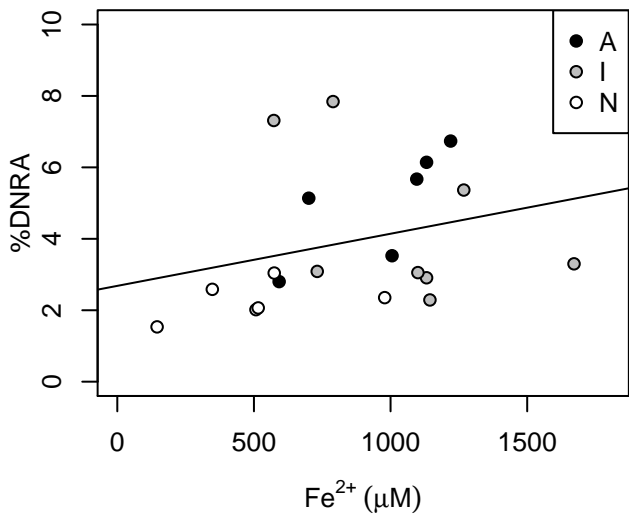


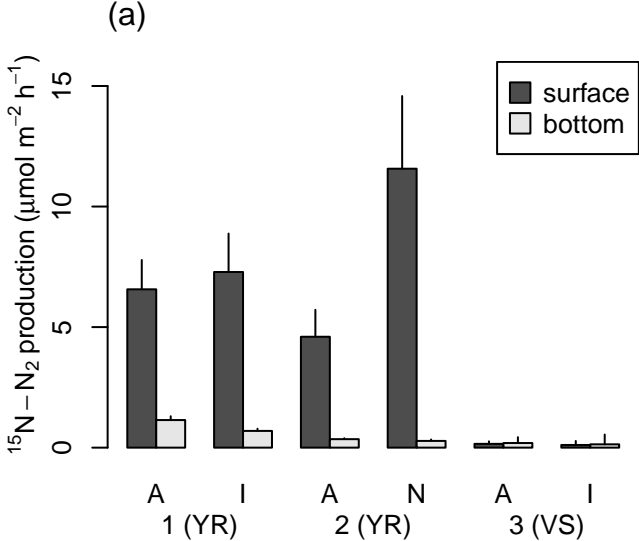


(a)



(b)





(b)

