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# 1 **Electrotrophy as potential primary metabolism for colonization of** 2 **conductive surfaces in deep-sea hydrothermal chimneys.**

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## 12 **Key-words**

13 Electrotrophy, hyperthermophiles, microbial electrochemical system, deep-sea hydrothermal  
14 vent, electrosynthesis

## 15 **Summary**

16 Deep-sea hydrothermal vents are extreme and complex ecosystems based on a trophic chain. We  
17 are still unsure of the first colonizers of these environments and their metabolism, but they are  
18 thought to be (hyper)thermophilic autotrophs. Here we investigate whether the electric potential  
19 observed across hydrothermal chimneys could serve as an energy source for these first colonizers.  
20 Experiments were performed in a two-chamber microbial electrochemical system inoculated with  
21 deep-sea hydrothermal chimney samples, with a cathode as sole electron donor, CO<sub>2</sub> as sole  
22 carbon source, and three different electron acceptors (nitrate, sulfate, and oxygen). After a few  
23 days of culture, all three experiments showed growth of an electrotrophic biofilm consuming  
24 directly or indirectly the electrons and producing organic compounds including acetate, glycerol,

25 and pyruvate. The only autotrophs retrieved were members of *Archaeoglobales*, in all three  
26 experiments. Various heterotrophic phyla also grew through trophic interactions, with  
27 *Thermococcales* in all three experiments and other bacterial groups specific to each electron  
28 acceptor. This electrotrophic metabolism as energy source to drive the first microbial colonization  
29 of conductive hydrothermal chimneys was discussed.

## 30 **Introduction**

31 Deep-Sea hydrothermal vents, are geochemical structures housing an extreme ecosystem rich in  
32 micro- and macro-organisms. Since their discovery in 1977 (Corliss and Ballard, 1977), they  
33 attracted the interest of researcher and, more recently, industries by their singularities. Isolated  
34 in the deep ocean, far from the sunlight and subsequent organic substrate, the primal energy  
35 sources for the development of this luxuriant biosphere remain elusive in these extreme  
36 environments rich in minerals. Since their discovery, many new metabolisms have been identified  
37 based on organic or inorganic molecules. However, the driving force sustaining all biodiversity in  
38 these environments is thought to be based on chemolithoautotrophy (Alain *et al.*, 2004). Indeed,  
39 unlike most ecosystems, deep-sea ecosystems are totally dark and microorganisms have adapted  
40 to base their metabolism on lithoautotrophy using inorganic compounds as the energy source to  
41 fix inorganic carbon sources. Primary colonizers of deep-sea hydrothermal vents are assumed to  
42 be (hyper)thermophilic microbes developing near the hydrothermal fluid, as retrieved in young  
43 hydrothermal chimneys. These first colonizers are affiliated to *Archaea*, such as *Archaeoglobales*,  
44 *Thermococcales*, *Methanococcales* or *Desulfurococcales*, and to *Bacteria* from  $\epsilon$ -*proteobacteria*  
45 and *Aquificales*. (Huber *et al.*, 2002, 2003; Nercessian *et al.*, 2003; Takai *et al.*, 2004). Recent  
46 studies have also shown that hyperthermophilic *Archaea*, which count among the supposed first  
47 colonizers, are able to quickly scan and fix onto surfaces to find the best conditions for growth  
48 (Wirth *et al.*, 2018). These hyperthermophilic microorganisms would fix inorganic carbon through

49 chemolithoautotrophic types of metabolism, using H<sub>2</sub>, H<sub>2</sub>S or CH<sub>4</sub> as energy sources and oxidized  
50 molecules such as oxygen, sulfur compounds, iron oxide or even nitrate as electron acceptors.  
51 However, the discovery of the presence of an abiotic electric current across the chimney walls  
52 (Yamamoto *et al.*, 2017) prompted the hypothesis of a new type of microorganisms called  
53 eletrotrophs having the capacity to use electrons from the abiotic electric current as an energy  
54 source coupled with carbon fixation from CO<sub>2</sub>. This metabolism was identified a few years ago on  
55 a mesophilic chemolithoautotrophic Fe(II)-oxidizing bacterium, *Acidithiobacillus ferrooxidans*  
56 (Ishii *et al.*, 2015). This strain was able to switch its source of energy from diffusible Fe<sup>2+</sup> ions to  
57 direct electron uptake from a polarized electrode. However, this feature has not yet been  
58 demonstrated in deep-sea hydrothermal vent environments. Recent studies have shown the  
59 exoelectrogenic ability of some hyperthermophilic microorganisms isolated from deep-sea  
60 hydrothermal vents, belonging to *Archaeoglobales* and *Thermococcales* (Pillot *et al.*, 2018, 2019;  
61 Yilmazel *et al.*, 2018), but no studies have been done on environmental samples potentially  
62 harboring electrotrophic communities growing naturally with an electric current as sole energy  
63 source.

64 Here, we investigate the potential presence of electrotrophic communities in deep-sea  
65 hydrothermal vents capable of using electrons directly or indirectly from the abiotic current. In  
66 this purpose, we mimic the conductive surface of the hydrothermal chimney in a cathodic  
67 chamber of Microbial Electrochemical Systems (MES) with a polarized cathode to enrich the  
68 potential electrotrophic communities inhabiting these extreme environments. The polarized  
69 cathode served as the sole energy source, while CO<sub>2</sub> bubbling served as sole carbon source. Three  
70 electron acceptors were tested separately, i.e. nitrate, oxygen, and sulfate, to show the influence  
71 of an electron acceptor on community taxonomic composition.

## 72 **Results**

### 73 **Current consumption from electrotroph enrichments**

74 Hydrothermal vents chimney samples were inoculated in MES filled with sterile mineral medium  
75 and incubated at 80°C to enrich electrotrophic communities. In the latter, the electrode served as  
76 the sole energy donor (cathode) and sparged CO<sub>2</sub> as carbon source with three different electron  
77 acceptors that were tested separately: (i) nitrate, (ii) sulfate and (iii) oxygen. The microbial  
78 electrotrophic enrichment was monitored at lowest possible potentials. These potentials were  
79 poised at -300mV/SHE in presence of oxygen and -590mV/SHE for both nitrate and sulfate,  
80 respectively. For comparison, microbial growth was also monitored without any poised potential  
81 during a month in the same conditions of incubation. Interestingly, in the latter condition, no  
82 microbial growth occurred, supported by microscope and spectrophotometric observations (data  
83 not shown). Moreover, no organic compounds were produced supported by the HPLC and NMR  
84 measurements (data not shown).

85 When potential was poised, abiotic controls containing no inoculum displayed constant currents  
86 of  $\approx 0,016 \text{ A.m}^{-2}$  at -590 mV and  $\approx 0,01 \text{ A.m}^{-2}$  at -300 mV/SHE. In both conditions, the potential  
87 hydrogen production on the cathode by water electrolysis was quantified and was under the  
88 detection threshold of the  $\mu\text{GC}$  ( $>0.001\%$  of total gas), indicating a theoretical production lower  
89 than  $34 \mu\text{M day}^{-1}$  (data not shown), similar as previously reported at 25°C (Marshall *et al.*, 2013).  
90 In comparison, experiments with the chimney sample showed current consumptions increasing  
91 shortly after inoculation (Fig. 1). Indeed, when subtracting abiotic current, the current  
92 consumptions reached a stabilized maximum of  $0.36 \text{ A.m}^{-2}$  on oxygen,  $0.72 \text{ A.m}^{-2}$  on nitrate, and  
93 up to  $1.83 \text{ A.m}^{-2}$  on sulfate corresponding therefore to an increase of 36, 45 and 114-fold  
94 compared to abiotic current, respectively. MES were autoclaved afterwards displaying decreased  
95 currents that were similar to the values of abiotic controls with a stabilized current around  $\approx 0,021$   
96  $\text{A.m}^{-2}$ .

97 At the end of monitoring of current consumption, CycloVoltamograms (CV) were performed to  
98 study reactions of oxidation and reduction that could occur in MES (Supporting Information Fig.

99 S1A). A first peak of reduction is observed at -0.295, -0.293 and -0.217 V vs SHE in presence of  
100 nitrate, sulfate and oxygen as electron acceptor, respectively (Fig. S1B). A second peak is observed  
101 at -0.639 and -0.502 V vs SHE on nitrate and sulfate, respectively. No redox peaks are detected in  
102 the abiotic controls and freshly inoculated MES, hence indicating a lack of electron shuttles  
103 brought with the inoculum (Fig. S1A).

#### 104 **Organic compounds production in liquid media**

105 During enrichment of the electrotrophic communities, the production of organic compounds was  
106 monitored in liquid media (Fig. 1). Interestingly, the glycerol, the pyruvate, and the acetate were  
107 the dominant products released in all experiment runs. Glycerol increased slowly throughout the  
108 experiments to reach a maximum of 0.47 mM on sulfate (day 11), 1.32 mM on oxygen (day 12)  
109 and 2.32 mM on nitrate (day 19). Acetate accumulated in the medium to reach 0.33 mM on  
110 oxygen (day 7), 0.75 mM on sulfate (day 13) and 1.40 mM on nitrate (day 19). Pyruvate was  
111 produced after a few days of culture with an exponential curve, reaching a maximum of 1.32 mM  
112 on oxygen (day 12), 2.39 mM on nitrate (day 9), and 3.94 mM on sulfate (day 11). Pyruvate varied,  
113 afterwards, due probably to microbial consumption or thermal degradation... Coulombic  
114 efficiency calculated on the last day of the experiment (Fig. 2) showed up to 71% (on nitrate),  
115 89% (on oxygen) and 90% (on sulfate) of electrons consumed were converted to organic  
116 compounds and released into the liquid media .The rest represents the share of electrons  
117 retained in not accumulated compounds (Table S1) and in the organic matter constituting the  
118 cells of the electrotrophic communities (estimated by qPCR to total between  $10^8$  to  $10^{10}$  16S rRNA  
119 gene copies per MES; Supporting Information Fig. S2).

#### 120 **Biodiversity of electrotrophic communities on different electron acceptors**

121 Once current consumption reached a stabilized maximum, DNAs from the biofilm and from  
122 planktonic cells in culture media were extracted and sequenced on the V4 region of the 16S rRNA

123 to study relative abundance of the biodiversity. Fig. 3 reports the taxonomic affiliation of the  
124 OTUs obtained. The chimney fragment inoculum showed a rich biodiversity (Shannon index at  
125 5.29 and Pielou's index at 0.69), with 208 OTUs mainly affiliated to *Bacteria* (99.49% vs 0.51% of  
126 *Archaea*) and more particularly to *Proteobacteria* from *Vibrionales* (34.8%), miscellaneous rare  
127 *Proteobacteria* (>33%), *Campylobacterales* (8.3%), *Thermales* (7.1%), *Aquificales* (5.62%), and  
128 *Rhodobacterales* (5.1%).

129 Enrichments in MES showed less biodiversity on cathodes and liquid media, suggesting the  
130 selective development of functional communities. The Shannon index values were 3.1 and 1.9 on  
131 nitrate, 1.7 and 1.9 on sulfate, and 4.1 and 4.2 on oxygen, with fewer OTUs associated to 72 and  
132 68 OTUs on nitrate, 39 and 53 on sulfate, and 94 and 102 on oxygen, on electrodes and liquid  
133 media, respectively. The taxonomic composition of these communities showed a larger  
134 proportion of *Archaea*, with 51% and 41.6% on nitrate, 96.8% and 96.3% on sulfate, and 7.6% and  
135 3.4% on oxygen on the electrodes and liquid media, respectively. In presence of each electron  
136 acceptor, the archaeal population on the electrode was mainly composed of *Archaeoglobales* and  
137 *Thermococcales* at different relative abundances. The latter were present at 6.7% and 28.2% on  
138 nitrate to 65.8% and 28.6% on sulfate and 3.8% and 3.6% on oxygen, respectively. Equivalent  
139 proportions of *Archaeoglobales* and *Thermococcales* were retrieved in liquid media, at 1.0% and  
140 2.5% on nitrate, 65.8% and 29.6% on sulfate and 0.3% and 2.7% on oxygen, respectively (Fig. 3).

141 The MiSeq Illumina results served to study only 290 bp of 16S rRNA and thus to affiliate  
142 microorganisms confirmed at family level, but they can also provide some information on the  
143 enriched genera. In an effort to obtain more information on the probable *Archaeoglobales* and  
144 *Thermococcales* genus, we attempted a species-level identification through phylogenetic analysis.  
145 The results are presented in Fig. 4 as a Maximum Likelihood phylogenetic tree. The dominant  
146 OTUs on sulfate and oxygen were closest to *Ferroglobus placidus* and *Archeoglobus fulgidus*  
147 97.61% whereas the dominant OTU on nitrate was affiliated to *Geoglobus ahangari* with an

148 identity of 98.63% of identity. The remaining part of the biodiversity was specific to each electron  
149 acceptor used. Enrichment on nitrate showed 13.8% and 28% of *Desulfurococcales* and 46.2% and  
150 56.5% of *Thermales* on the electrode and in liquid media, respectively. Among *Thermales* that  
151 developed on the electrode, 30% were represented by a new taxon (OTU 14 in Fig. 4 and S3)  
152 whose closest cultured species was *Vulcanithermus mediatlanticus* (90% similarity). On sulfate,  
153 the remaining biodiversity represented less than 4% of the population but was mainly  
154 represented by two particular OTUs. The first OTU, accounting for up to 2.4% and 0.8% of the  
155 total population on the electrode and liquid media, respectively, was affiliated to a new  
156 *Euryarchaeota* (OTU 10 in Fig. 4 and S3) whose closest cultured match was *Methanothermus*  
157 *fervidus* strain DSM 2088, at 86% similarity. The second OTU accounted for 2.0% and 1.9% of the  
158 biodiversity on the electrode and liquid media, respectively, and was affiliated to the new  
159 *Deinococcales* (OTU 14 in Fig. 4 and S3) species, found mostly on the electrode in nitrate  
160 enrichment. In the enrichment on oxygen, the communities are dominated by 36.6% and 30.2% of  
161 *Pseudomonadales* (*Pseudomonas* sp.), 14% and 42.6% of *Bacillales* (*Bacillus* and *Geobacillus* sp.),  
162 21.3% and 7.41% of *Vibrionales* (*Photobacterium* sp.), and 9.8% and 5.1% of *Actinomycetales*  
163 (spread across 9 species) on the electrode and in liquid media, respectively, with the rest spread  
164 across *Proteobacteria* orders.

165 The clustering of the dominant OTUs (at a threshold of 0.05% of total sequences) obtained  
166 previously on the chimney sample and enrichments in MES showed a clear differentiation of  
167 communities retrieved in each sample (Supporting Information Fig. S3). The Pearson method on  
168 OTU distribution produced four clusters, one corresponding to the inoculum and the three others  
169 to each electron acceptor. Indeed, only two OTUs (OTU 4 and 36) were clearly shared between  
170 two different communities, one affiliated to *Thermococcus* spp. on nitrate and sulfate and one to  
171 *Ralstonia* sp. on the chimney sample and on nitrate. It is surprising to observe a recurrence of this  
172 last OTU which could be a contaminant specific to the extraction kit used (Salter *et al.*, 2014). The



173 other 50 dominant OTUs were specific to one community, with 21 OTUs on oxygen, 4 on sulfate, 8  
174 on nitrate, and 17 on the chimney sample. The electrotrophic communities, colonizing the  
175 cathode, were therefore different depending on electron acceptor used and their concentration  
176 was too low to be detected in the chimney sample.

## 177 **Discussion**

### 178 **Archaeoglobales as systematic (electro)lithoautotrophs of the community**

179 Herein, we evidenced the development of a microbial electrotrophic communities and metabolic  
180 activity supported by current consumption (Fig.1), product production (Fig. 2), and qPCRs (Fig. S2)  
181 suggesting that growth did occur from energy supplied by the cathode. The mechanism of energy  
182 uptake from electrode is discussed since the discovery of biofilms growing on cathode, and little is  
183 known, unlike electron transfer mechanism on anode. The two main hypotheses are the use of  
184 similar direct electron transfer pathway as on the anode, or the use of molecular H<sub>2</sub>, produced by  
185 water electrolysis, as electron mediator to the cell. In both cases, our study is the first to show the  
186 possibility of growth of biofilm from environments harboring natural electric current in absence of  
187 organic substrates. To discuss further on the putative mechanism, it is necessary to have a look on  
188 the conditions for water electrolysis. The potential for water reduction into hydrogen at 80°C, pH7,  
189 1 atm was calculated at -0.490 V vs SHE in pure water. The real operational reduction potentials is  
190 expected to be much more lower than the theoretical value due to internal resistances (from  
191 electrical connections, electrolyte, ionic membrane etc.) (Lim *et al.*, 2017). Moreover,  
192 overpotentials are expected with carbon electrodes. The decrease of this potential explains the  
193 absence of hydrogen measured in our conditions. A screening of potential in abiotic conditions  
194 confirmed the increase of current consumption and H<sub>2</sub> production only at potential lower of -0.6V  
195 vs SHE (Fig S1) Moreover several pieces of evidence indicate that direct electron transfer may have  
196 mainly participated in the development of biofilms: the growth of the similar dependent sulfate  
197 biodiversity with the cathode poised at -300 mV vs SHE (Supporting Information Fig. S4) without H<sub>2</sub>

198 production, the expression of catalytic waves observed by CV with midpoint potentials between -  
199 0.217 V to -0.639 V and the lack of similar peaks with abiotic or fresh inoculated media (Supporting  
200 Information Fig. S1), biofilm formation on the electrode (as on nitrate, Supporting Information Fig.  
201 S5), delayed production of glycerol, pyruvate and acetate (Fig. 1) fixing between 267 to 1596  
202 Coulombs.day<sup>-1</sup> (organic consumption deduced), and the recovery of electrons in all three products  
203 (Fig. 2), that largely exceeds the maximum theoretical abiotic generation of hydrogen (~3 C.day<sup>-1</sup>)  
204 by 90 to 530-fold. Thus, we can assume that the biofilm growth was largely ensured by a significant  
205 part of a direct transfer of electrons from the cathode demonstrating the presence of  
206 electrolithoautotroph microorganisms.

207 Taxonomic analysis of the enriched microbial communities at the end of the experiments showed  
208 the systematic presence on cathodes of *Archaeoglobales* (Fig. 3 and S3), whatever the electron  
209 acceptors used. The species belonging to *Archaeoglobales* order were the only enriched species in  
210 all conditions and the only known to have an autotrophic metabolism (except for *Archaeoglobus*  
211 *profundus* and *A. infectus* which are obligate heterotrophs). The *Archaeoglobales* order is  
212 composed of three genera: *Archaeoglobus*, *Geoglobus*, and *Ferroglobus*. All are hyperthermophilic  
213 obligate anaerobes with diverse metabolisms, including heterotrophy or chemolithoautotrophy.  
214 Terminal electron acceptors used by this order include sulfate, nitrate, poorly crystalline Fe (III)  
215 oxide, or sulfur oxyanions (Brileya and Reysenbach, 2014). Autotrophic growth in the  
216 *Archaeoglobales* order is ensured mainly through H<sub>2</sub> as energy source and requires both branches  
217 of the reductive acetyl-CoA/Wood-Ljungdahl pathway for CO<sub>2</sub> fixation (Vorholt *et al.*, 1997).  
218 Moreover, *Archaeoglobus fulgidus* has been recently shown to grow on iron by directly snatching  
219 electrons under carbon starvation during corrosion process (Jia *et al.*, 2018). Furthermore,  
220 *Ferroglobus* and *Geoglobus* species were shown to be exoelectrogens in pure culture in a microbial  
221 electrosynthesis cell (Yilmazel *et al.*, 2018) and were enriched during a study within a microbial  
222 electrolysis cell (Pillot *et al.*, 2018, 2019). Interestingly, some studies have shown that *Geobacter*

223 species are capable of bidirectional electron transfer using the same mechanism (Pous *et al.*, 2016).  
224 Hence, *Archaeoglobales* that have been shown already as exoelectrogens (Yilmazel *et al.*, 2018)  
225 could also be electrotrophy. It is not known how Archaea carry out exogenous electron transfer. As  
226 previously discussed, the absence of H<sub>2</sub> production and the increasing current consumptions over  
227 time suggest direct electron uptake from members of the communities developing on the  
228 electrode, as for *Acidithiobacillus ferroxidans* (Ishii *et al.*, 2015). Moreover, the qPCR (Supporting  
229 Information Fig. S2) and MiSeq data (Fig. 3) highlighted a strong correlation between current  
230 consumption and density of *Archaeoglobales* on the electrode ( $R^2=0.962$ ). In the condition with  
231 sulfate as electron acceptor, the proportion of *Archaeoglobales* represented 65.8% of total  
232 biodiversity providing 1.83 A.m<sup>-2</sup> of current consumption, compared to only 6.7% in the nitrate  
233 condition and 3.8% in the oxygen condition for 0.72 and 0.36 A.m<sup>-2</sup> of current consumption,  
234 respectively. Moreover, the majority of OTUs were affiliated to three *Archaeoglobaceae* genera,  
235 mainly *Archaeoglobus* spp. and *Ferroglobus* spp. on sulfate and oxygen and *Geoglobus* sp. on  
236 nitrate. Some *Archaeoglobus* are known to show anaerobic sulfate-reducing metabolism while  
237 *Ferroglobus* spp. are not. *Geoglobus* sp. has never been described to perform nitrate reduction so  
238 far, but it does harbor genes of nitrate- and nitrite-reductase-like proteins (Manzella *et al.*, 2015).  
239 The OTUs were related to some *Archaeoglobales* strain with 95-98% identities. Thus, we assume  
240 that in our conditions, new specific electrophyic metabolisms or new electrolythoautotrophic  
241 *Archaeoglobaceae* species were enriched on the cathode. Moreover, a member of a new  
242 phylogenetic group of Archaea was enriched up to 2.4% of total biodiversity on sulfate (OTU10 in  
243 Fig. 4). While its metabolism is still unknown, we suggest that isolation of this electrophyic  
244 archaea in MES could enable the identification of a new archaeal phylogenetic group based on  
245 electrophy.

246 The growth of *Archaeoglobales* species in presence of oxygen is a surprising finding.  
247 *Archaeoglobales* have a strictly anaerobic metabolism, and the reductive acetyl-CoA pathway is

248 very sensitive to the presence of oxygen (Fuchs, 2011). This can be firstly explained by the low  
249 solubility of oxygen at 80°C combined with the electrochemically oxygen reduction on electrode  
250 in controls (data not shown). It hence results in an low oxygen or oxygen-free environment within  
251 the carbon cloth mesh for anaerobic development of microorganisms into a protective biofilm  
252 (Hamilton, 1987). This observation was also supported by the near absence of *Archaeoglobales* in  
253 the liquid media (Fig. 3). In absence of other electron acceptors, some *Archaeoglobales* perform  
254 carboxydrotrophic metabolism to grow from CO, as demonstrated for *Archaeoglobus fulgidus*  
255 (Sokolova and Lebedinsky, 2013; Hocking *et al.*, 2015). This fermentative CO metabolism leads to  
256 the production of acetate and transient accumulation of formate via the Wood-Ljungdahl  
257 pathway, but no net ATP is really produced (Henstra *et al.*, 2007). The energy conservation  
258 through this metabolism in *Archaeoglobus fulgidus* is still poorly understood (Hocking *et al.*,  
259 2015). A second hypothesis concerns direct interspecies electron transfer (DIET) (Kato *et al.*,  
260 2012; Lovley, 2017), with *Archaeoglobales* transferring electrons to another microorganism as an  
261 electron acceptor. Research into DIET is in its early stages, and further investigations are required  
262 to better understand the diversity of microorganisms and the mechanism of carbon and electron  
263 flows in anaerobic environments (Lovley, 2017) such as hydrothermal ecosystems.

#### 264 **Electrosynthesis of organic compounds**

265 The pyruvate, the glycerol and the acetate accumulated, while another set of compounds that  
266 appear transiently were essentially detectable in the first few days of biofilm growth (Supporting  
267 Information Table S1). They included amino acids (threonine, alanine) and volatile fatty acids  
268 (formate, succinate, lactate, acetoacetate, 3-hydroxyisovalerate) whose concentrations did not  
269 exceed one micromole. Despite their thermostability, this transient production suggests they  
270 were used by microbial communities developing on the electrode in interaction with the primary  
271 producers during enrichment.

272 On the other hand, in presence of nitrate, sulfate and oxygen as electron acceptors, the liquid  
273 media accumulated three main organic products acetate, glycerol, and pyruvate (Fig. 1).  
274 Coulombic efficiency calculations (Fig. 2) showed that redox levels of the carbon-products  
275 represented 71%–90% of electrons consumed, and only about 10%–30% of net electrons  
276 consumed by electrotrophy during growth was used directly for biomass or transferred to an  
277 electron acceptor. This concurs with the energy yield from the Wood-Ljungdahl pathway of  
278 *Archaeoglobales*, with only 5% of carbon flux directed to the production of biomass and the other  
279 95% diverted to the production of small organic end-products excreted from the cell (Fast and  
280 Papoutsakis, 2012).

281 However, the production of pyruvate and glycerol warrants further analysis. Pyruvate is normally  
282 a central intermediate of CO<sub>2</sub> uptake by the reducing route of the acetyl-CoA/WL pathway (Berg  
283 *et al.*, 2010). It can be used to drive the anabolic reactions needed for biosynthesis of cellular  
284 constituents. Theoretically, the only explanation for improved production and accumulation of  
285 pyruvate (up to 5 mM in the liquid media of sulfate experiment) would be that pyruvate-using  
286 enzymes were inhibited or that pyruvate influx exceeded its conversion rate. Here we could  
287 suggest that in-cell electron over-feeding at the cathode leads to significant production of  
288 pyruvate. Indeed, in a physiological context, the production of pyruvate from acetyl-CoA via  
289 pyruvate synthase requires the oxidation of reduced ferredoxins for CO<sub>2</sub> fixation (Furdui and  
290 Ragsdale, 2000). The continuous electron uptake from the cathode would lead to a significant  
291 reduction in electron carriers (including ferredoxins, flavins, cytochromes, and/or nicotinamides),  
292 thus forcing the electrotrophic microbial community to produce pyruvate as a redox sink.

293 In the same context of pyruvate production, glycerol is produced by reduction of  
294 dihydroxyacetone phosphate a glycolytic intermediate, to glycerol 3-phosphate (G3P) followed by  
295 dephosphorylation of G3P to glycerol. In some yeasts, glycerol production is essential for  
296 osmoadaptation but equally for regulating the NADH surplus during anaerobic growth (Björkqvist

297 *et al.*, 1997). A similar mechanism may operate in our conditions for the probable excess of NADH  
298 pool due to the electrode poised at -590 mV vs SHE, which would explain the accumulation of  
299 glycerol found in our experiments.

300 In an ecophysiological context, a similar pyruvate and glycerol production could occur on  
301 hydrothermal chimney walls into which electric current propagates (Yamamoto *et al.*, 2017). The  
302 electrotoph biofilms would continually receive electrons, leading to the excess of intracellular  
303 reducing power that would be counterbalanced by the overproduction of glycerol and pyruvate.  
304 Moreover, glycerol is an essential compound in the synthesis of membrane lipids in *Archaea* and  
305 probably also in biofilm formation and osmoadaptability (Desai *et al.*, 2013; Shemesh and Chai,  
306 2013). Pyruvate unites several key metabolic processes, such as its conversion into carbohydrates,  
307 fatty acids or some amino acids. Furthermore, these products can serve as carbon and energy  
308 sources for heterotrophic microorganisms or for fermentation. In our experiments, pyruvate and  
309 glycerol concentrations varied over time, suggesting they were being consumed by heterotrophic  
310 microorganisms. Acetate production would thus result from the fermentation of pyruvate or  
311 other compounds produced by electrotophic *Archaeoglobales*.

### 312 **Enrichment of rich heterotrophic biodiversity from electrotophic Archaeoglobales** 313 **community**

314 During our enrichment experiments, the development of effective and specific biodiversity was  
315 dependent on the electron acceptors used (Fig. 3). Heatmap analyses (Supporting Information Fig.  
316 3) showed four distinct communities for the three electron acceptors and the initial inoculum. Thus,  
317 at the lower taxonomic level of the biodiversity analysis, most OTUs are not shared between each  
318 enrichment, except for one OTU of *Thermococcales* that was shared between the nitrate and sulfate  
319 experiments. This suggests a real specificity of the communities and a specific evolution or  
320 adaptation of the members of the shared phyla to the different electron acceptors available in the  
321 environment. However, the various enrichments also showed the presence of *Thermococcales*

322 regardless of the electron acceptors used, thus demonstrating a strong interaction between  
323 *Thermococcales*, heterotrophs, and *Archaeoglobales*, the only autotrophs. In a previous study,  
324 enrichments on the anode of a microbial electrolysis cell showed a similar tendency, with  
325 *Archaeoglobales* strongly correlated to *Thermococcales* (Pillot *et al.*, 2018, 2019). Moreover,  
326 members of these two groups have frequently been found together in various hydrothermal sites  
327 on the surface of the Earth (Corre *et al.*, 2001; Nercessian *et al.*, 2003; Takai *et al.*, 2004; Jaeschke  
328 *et al.*, 2012), where they are considered as potential primary colonizers of their environments (33–  
329 36). This could point to a co-evolution and metabolic adaptation of these microorganisms to their  
330 unstable environmental conditions in hydrothermal settings. After *Thermococcales*, the rest of the  
331 heterotrophic biodiversity was specific to each electron acceptor.

332 On nitrate, two additional phylogenetic groups were retrieved: *Desulfurococcales* and *Thermales*.  
333 OTUs of *Desulfurococcales* are mainly affiliated to *Thermoterrivibrio* or *Aeropyrum* species, which are  
334 hyperthermophilic and heterotrophic *Crenarchaeota* growing by fermentation of complex organic  
335 compounds or sulfur/oxygen reduction (Huber and Stetter, 2015).

336 Concerning *Thermales*, a new taxon was enriched on cathode and only affiliated with 90 %  
337 similarity to *Vulcanithermus mediatlanticus*. On sulfate, beside the large majority of  
338 *Archaeoglobales* and *Thermococcales* (up to 94%–96%), this new taxon of *Thermales* (OTU 14, Fig.  
339 S3) has also been enriched on the cathode, representing 2% of total biodiversity. *Thermales* are  
340 thermophilic (30°C–80°C) and heterotrophic bacteria whose only four genera (*Marinithermus*,  
341 *Oceanithermus*, *Rhabdothermus*, and *Vulcanithermus*) are all retrieved in marine hydrothermal  
342 systems. They are known to be aerobic or microaerophilic. Some strains grow anaerobically with  
343 several inorganic electron acceptors such as nitrate, nitrite, Fe (III) and elemental sulfur  
344 (Albuquerque and Costa, 2014). All of the species *Thermales* can utilize the pyruvate as carbon  
345 and energy source. The produced pyruvate would be a substrate of choice for this new taxon  
346 which would use the sulfate and nitrate as electron acceptors.

347 *Pseudomonadales* and *Bacillales* were found in the oxygen experiment. Most *Pseudomonas* are  
348 known to be aerobic and mesophilic bacteria, with a few thermophilic species, including the  
349 autotrophic *Pseudomonas thermocarboxydovorans* that grows at up to 65°C (Lyons *et al.*, 1984;  
350 Palleroni, 2015). There have already been reports of mesophilic *Pseudomonas* species growing in  
351 thermophilic conditions in composting environments (Droffner *et al.*, 1995). Moreover, some  
352 *Pseudomonas* sp. are known to be electroactive in microbial fuel cells, through long-distance  
353 extracellular electron transport (Shen *et al.*, 2014; Maruthupandy *et al.*, 2015; Lai *et al.*, 2016),  
354 and were dominant on the cathodes of a benthic microbial fuel cell on a deep-ocean cold seep  
355 (Reimers *et al.*, 2006). In *Bacillales*, the *Geobacillus* spp. and some *Bacillus* sp. are known to be  
356 mainly (hyper)thermophilic aerobic and heterotrophic *Firmicutes* (Vos, 2015).

### 357 **Hydrothermal electric current: a new energy source for the development of primary** 358 **producers**

359 The presence of so many heterotrophs in an initially autotrophic condition points to the  
360 hypothesis of a trophic relationship inside the electrotrophic community (Fig. 5). This suggests  
361 that the only autotrophs retrieved in all communities, the *Archaeoglobales*, might be the first  
362 colonizer of the electrode, using CO<sub>2</sub> as carbon source and cathode as energy source. Studies have  
363 shown how modeling and field observations can be usefully combined to describe the relationship  
364 between chemical energy conditions and metabolic interactions within microbial communities  
365 (Lin *et al.*, 2016; Dahle *et al.*, 2018). However, the models predicted low abundances of  
366 *Archaeoglobales* (<0.04%) whereas on-field detection found abundances of more than 40% in the  
367 inner section of the studied hydrothermal chimney (Dahle *et al.*, 2018). Indeed, in these models,  
368 the predicted H<sub>2</sub> concentration, based on observations, would be too low to support the growth  
369 of hydrogenotrophic or methanogenic species (Lin *et al.*, 2016). The authors concluded on a  
370 probable H<sub>2</sub> syntrophy, with hydrogen being produced by heterotrophic microorganisms such as  
371 fermentative *Thermococcales* species. Our study is the first evidence of the development of



372 hyperthermophilic electrotrophic/heterotrophic communities directly enriched from the natural  
373 environment known to harbor natural electric current as a potential energy source. We can thus  
374 conclude that this kind of electrolithoautotrophic metabolism is highly likely in deep-sea  
375 hydrothermal ecosystems, which raises the question of the importance of this metabolism in the  
376 primary colonization of hydrothermal vents. The hydrothermal electric current could make up for  
377 the lack of H<sub>2</sub> normally needed to sustain the growth of hydrogenotrophic microorganisms.  
378 Indeed, the constant electron supply on the surface of a conductive chimney allows a new energy  
379 source and long-range transfer between the electron donor (represented here by reduced  
380 molecules such as H<sub>2</sub>S electrochemically oxidized on the inner surface of the chimney wall) and  
381 the electron acceptor (O<sub>2</sub>, sulfur compounds, nitrate, metals) present all over the external surface  
382 of the chimney. This electrical current would thus allow primary colonizers to grow not just on all  
383 the surface but also in the chimney structure. These primary colonizers would release organic  
384 compounds used by the heterotrophic community for growth, as observed with the successive  
385 production and consumption of organic compounds in our experiments. Moreover, migrating out  
386 to larger potential growth surface would help to meet a wider range of physiological conditions  
387 through pH, temperature and oxidoreduction gradients. This allows a wider diversity of growth  
388 patterns than through chemolithoautotrophy, which is restricted to unstable and limited contact  
389 zones between reduced compounds (H<sub>2</sub>, H<sub>2</sub>S) in the hydrothermal fluid and electron acceptors  
390 around the hydrothermal chimneys, often precipitating together.

## 391 **Conclusion**

392 Taken together, the results found in this study converge into evidence of the ability of indigenous  
393 microorganisms from deep hydrothermal vents to grow using electric current and CO<sub>2</sub>. This ability  
394 seems to be spread across diverse phylogenetic groups and to be coupled with diverse electron  
395 acceptors. Through their electro-litho-auto-trophic metabolism, *Archaeoglobaceae* strains  
396 produce and release organic compounds into their close environment, allowing the growth of

397 heterotrophic microorganisms, and ultimately enabling more and more diversity to develop over  
398 time. This metabolism could be one of the primary energies for the colonization of deep-sea  
399 hydrothermal chimneys and the development of a complex trophic network driving sustainable  
400 biodiversity. A similar mechanism could have occurred during the Hadean, allowing the  
401 emergence of life in hydrothermal environments by constant electron influx to the first proto-  
402 cells.

## 403 **Experimental procedures**

### 404 **Sample collection and preparation**

405 A hydrothermal chimney sample was collected on the acidic and iron-rich Capelinhos site on the  
406 Lucky Strike hydrothermal field (37°17.0'N, MAR) during the MoMARSat cruise in 2014  
407 (<http://dx.doi.org/10.17600/14000300>) led by IFREMER (France) onboard R/V *Pourquoi Pas?*  
408 (Sarradin and Cannat, 2014). The sample (PL583-8) was collected by breaking off a piece of a high-  
409 temperature active black smoker using the submersible's robotic arm, and bringing it back to the  
410 surface in a decontaminated insulated box (<http://video.ifremer.fr/video?id=9415>). Onboard,  
411 chimney fragments were anaerobically crushed in an anaerobic chamber under H<sub>2</sub>:N<sub>2</sub> (2.5:97.5)  
412 atmosphere (La Calhene, France), placed in flasks under anaerobic conditions (anoxic seawater at  
413 pH 7 with 0.5 mg L<sup>-1</sup> of Na<sub>2</sub>S and N<sub>2</sub>:H<sub>2</sub>:CO<sub>2</sub> (90:5:5) gas atmosphere), and stored at 4°C.  
414 Prior to our experiments, pieces of the hydrothermal chimney were removed from the sulfidic  
415 seawater flask, crushed with a sterile mortar and pestle in an anaerobic chamber (Coy  
416 Laboratories, Grass Lake, MI), and distributed into anaerobic tubes for use in the various  
417 experiments.

### 418 **Electrotrophic enrichment on nitrate, sulfate, and oxygen**

419 MES were filled with 1.5 L of an amended sterile mineral medium as previously described (Pillot  
420 *et al.*, 2018) without yeast extract, and set at 80°C and pH 6.0 throughout on-platform monitoring.  
421 The electrode (cathode) composed of 20 cm<sup>2</sup> of carbon cloth was poised at the lowest potential

422 before initiation of abiotic current consumption (Supporting Information Fig S6) using SP-240  
423 potentiostats and EC-Lab software (BioLogic, France). We thus used a potential of -590 mV vs( in  
424 the nitrate and sulfate experiments and -300 mV vs SHE in the oxygen experiment. A similar  
425 experiment at -300 mV vs SHE has been initiated in presence of sulfate (see SI Fig. S4) to confirm  
426 the growth of electrolithoautotroph microorganisms without any H<sub>2</sub> production possible. The  
427 electrode poised as cathode served as the sole electron donor for electrotrroph growth. For the  
428 nitrate experiment, one system was supplemented with 4 mM of sodium nitrate. For the sulfate  
429 experiment, a second system was supplemented with 10 mM of sodium sulfate, and the cathodic  
430 chambers were sparged with N<sub>2</sub>:CO<sub>2</sub> (90:10, 100 mL/min). For the oxygen experiment, a third  
431 system was sparged with N<sub>2</sub>:CO<sub>2</sub>:O<sub>2</sub> (80:10:10, 100 mL/min) with initially 10% oxygen as electron  
432 acceptor. All three systems were inoculated with 8 g of the crushed chimney (~0.5% (w/v)).  
433 Current consumption was monitored via the chronoamperometry method with current density  
434 and readings were taken every 10 s. An abiotic control without inoculation showed no current  
435 consumption during the same experiment period. CycloVoltammograms (scan rate: 20 mV/s)  
436 were analyzed using QSoas software (version 2.1). Coulombic efficiencies where calculates with  
437 the following equation:

$$438 \quad CE (\%) = \frac{F \cdot n_e \cdot \Delta[P] \cdot V_{\text{catholyte}}}{\int_{t_0}^t I(t) \cdot dt} \cdot 100$$

439 I(t): current consumed between t<sub>0</sub> and t (A)

440 F: Faraday constant

441 n<sub>e</sub>: number of moles of electrons presents per mole of product (mol)

442 Δ[P]: variation of the concentration of organic product between t<sub>0</sub> and t (mol.L<sup>-1</sup>)

443 V<sub>catholyte</sub>: volume of catholyte (L)

444 **Identification and quantification of organic compound production**

445 To identify and quantify the production of organic compounds from the biofilm, samples of liquid  
446 media were collected at the beginning and at the end of the experiment and analyzed by  $^1\text{H}$  NMR  
447 spectroscopy. For this, 400  $\mu\text{L}$  of each culture medium, were added to 200  $\mu\text{L}$  of PBS solution  
448 prepared in  $\text{D}_2\text{O}$  (NaCl, 140 mM; KCl, 2.7 mM;  $\text{KH}_2\text{PO}_4$ , 1.5 mM;  $\text{Na}_2\text{HPO}_4$ , 8.1 mM, pH 7.4)  
449 supplemented with 0.5 mmol  $\text{L}^{-1}$  of trimethylsilylpropionic acid- $d_4$  (TSP) as NMR reference. All the  
450 1D  $^1\text{H}$  NMR experiments were carried out at 300 K on a Bruker Avance spectrometer (Bruker,  
451 BioSpin Corporation, France) operating at 600 MHz for the  $^1\text{H}$  frequency and equipped with a 5-  
452 mm BBFO probe.

453 Spectra were recorded using the 1D nuclear Overhauser effect spectroscopy pulse sequence (Trd-  
454  $90^\circ\text{-T}_1\text{-}90^\circ\text{-tm-}90^\circ\text{-Taq}$ ) with a relaxation delay (Trd) of 12.5 s, a mixing time (tm) of 100 ms, and a  
455  $\text{T}_1$  of 4  $\mu\text{s}$ . The sequence enables optimal suppression of the water signal that dominates the  
456 spectrum. We collected 128 free induction decays (FID) of 65,536 datapoints using a spectral  
457 width of 12 kHz and an acquisition time of 2.72 s. For all spectra, FIDs were multiplied by an  
458 exponential weighting function corresponding to a line broadening of 0.3 Hz and zero-filled before  
459 Fourier transformation. NMR spectra were manually phased using Topspin 3.5 software (Bruker  
460 Biospin Corporation, France) and automatically baseline-corrected and referenced to the TSP  
461 signal ( $\delta = -0.015$  ppm) using Chenomx NMR suite v7.5 software (Chenomx Inc., Canada). A 0.3 Hz  
462 line-broadening apodization was applied prior to spectral analysis, and  $^1\text{H}\text{-}^1\text{H}$  TOCSY (Bax and  
463 Davis, 1985) and  $^1\text{H}\text{-}^{13}\text{C}$  HSQC (Schleucher *et al.*, 1994) experiments were recorded on selected  
464 samples to identify the detected metabolites. Quantification of identified metabolites was done  
465 using Chenomx NMR suite v7.5 software (Chenomx Inc., Canada) using the TSP signal as the  
466 internal standard.

#### 467 **Biodiversity analysis**

468 Taxonomic affiliation was carried out according to (Zhang *et al.*, 2016). DNA was extracted from 1  
469 g of the crushed chimney and, at the end of each culture period, from scrapings of half of the WE

470 and from centrifuged pellets of 50 mL of spent media. The DNA extraction was carried out using  
471 the MoBio PowerSoil DNA isolation kit (Carlsbad, CA). The V4 region of the 16S rRNA gene was  
472 amplified using the universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-  
473 GGA CTA CNN GGG TAT CTA AT-3') (Bates *et al.*, 2011) with Taq&Load MasterMix (Promega). PCR  
474 reactions, qPCR, amplicon sequencing and taxonomic affiliation were carried as previously  
475 described (Pillot *et al.*, 2018). The qPCR results were expressed in copies number of 16s rRNA  
476 gene per gram of crushed chimney, per milliliter of liquid media or per cm<sup>2</sup> of surface of the  
477 electrode. To analyze alpha diversity, the OTU tables were rarefied to a sampling depth of 9410  
478 sequences per library, and three metrics were calculated: the richness component, represented  
479 by number of OTUs observed, the Shannon index, representing total biodiversity, and the  
480 evenness index (Pielou's index), which measures distribution of individuals within species  
481 independently of species richness. Rarefaction curves (Supporting Information Fig. S7) for each  
482 enrichment approached an asymptote, suggesting that the sequencing depths were sufficient to  
483 capture overall microbial diversity in the studied samples. The phylogenetic tree was obtained  
484 with MEGA software v10.0.5 with the MUSCLE clustering algorithm and the Maximum Likelihood  
485 Tree Test with a Bootstrap method (2500 replications). The heatmap was obtained using RStudio  
486 software v3. The raw sequences for all samples can be found in the European Nucleotide Archive  
487 (accession number: PRJEB35427).

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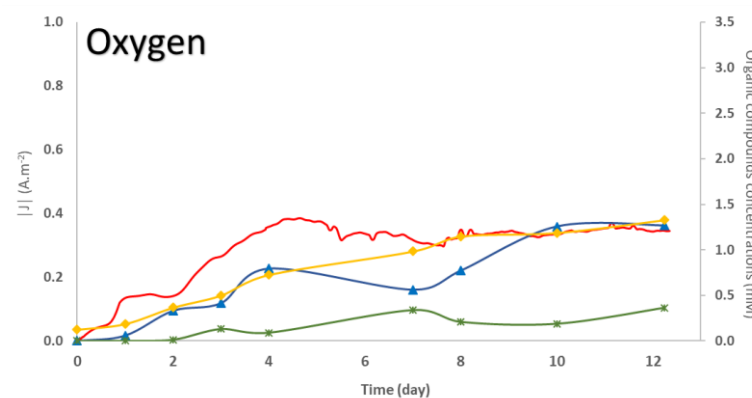
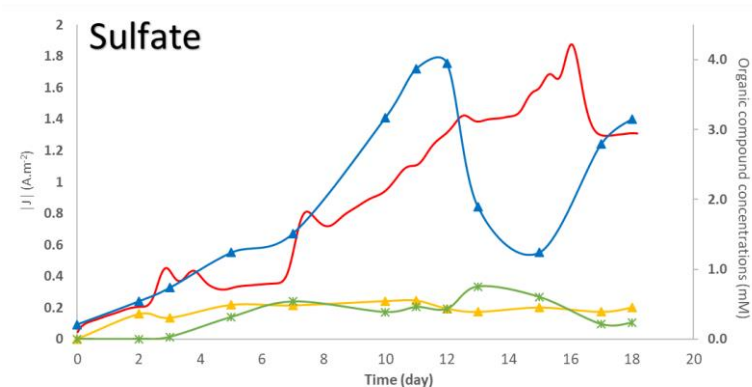
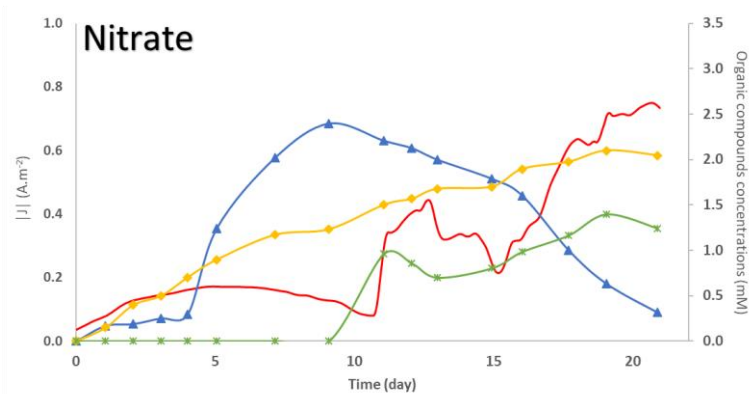
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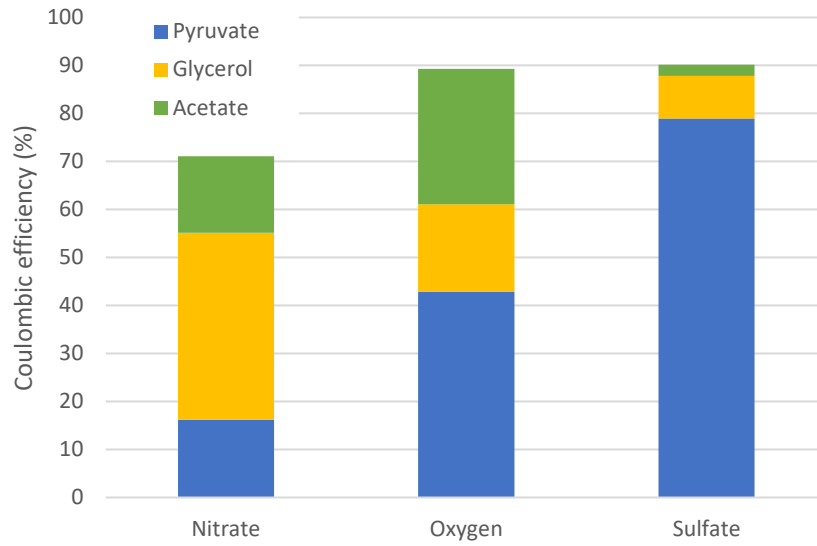
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648 **Figures**



652 **Figure 1.** Current consumption (red continuous line); pyruvate (blue triangle), glycerol (yellow  
653 square) and acetate (green cross) productions over time of culture for each electron-acceptor  
654 experiment. The current was obtained from a poised electrode at -590 mV vs SHE for nitrate and  
655 sulfate experiments and -300 mV vs SHE for oxygen.

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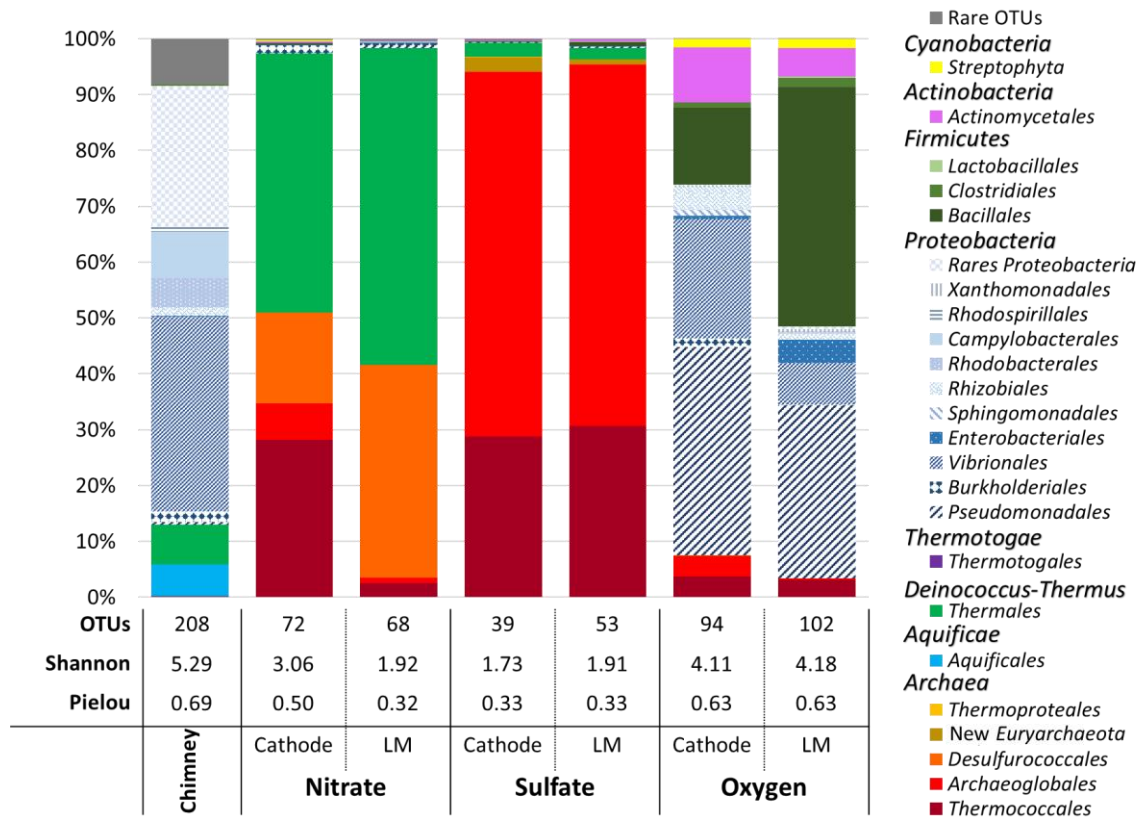


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658 **Figure 2.** Coulombic efficiency for organic products in presence of the different electron

659 acceptors.

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**Figure 3.** Dominant taxonomic affiliation at order level and biodiversity indices of microbial

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communities from a crushed chimney sample from Capelinhos vent site (Lucky Strike

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hydrothermal vent field), as plotted on the cathode and liquid media (LM) after the weeks of

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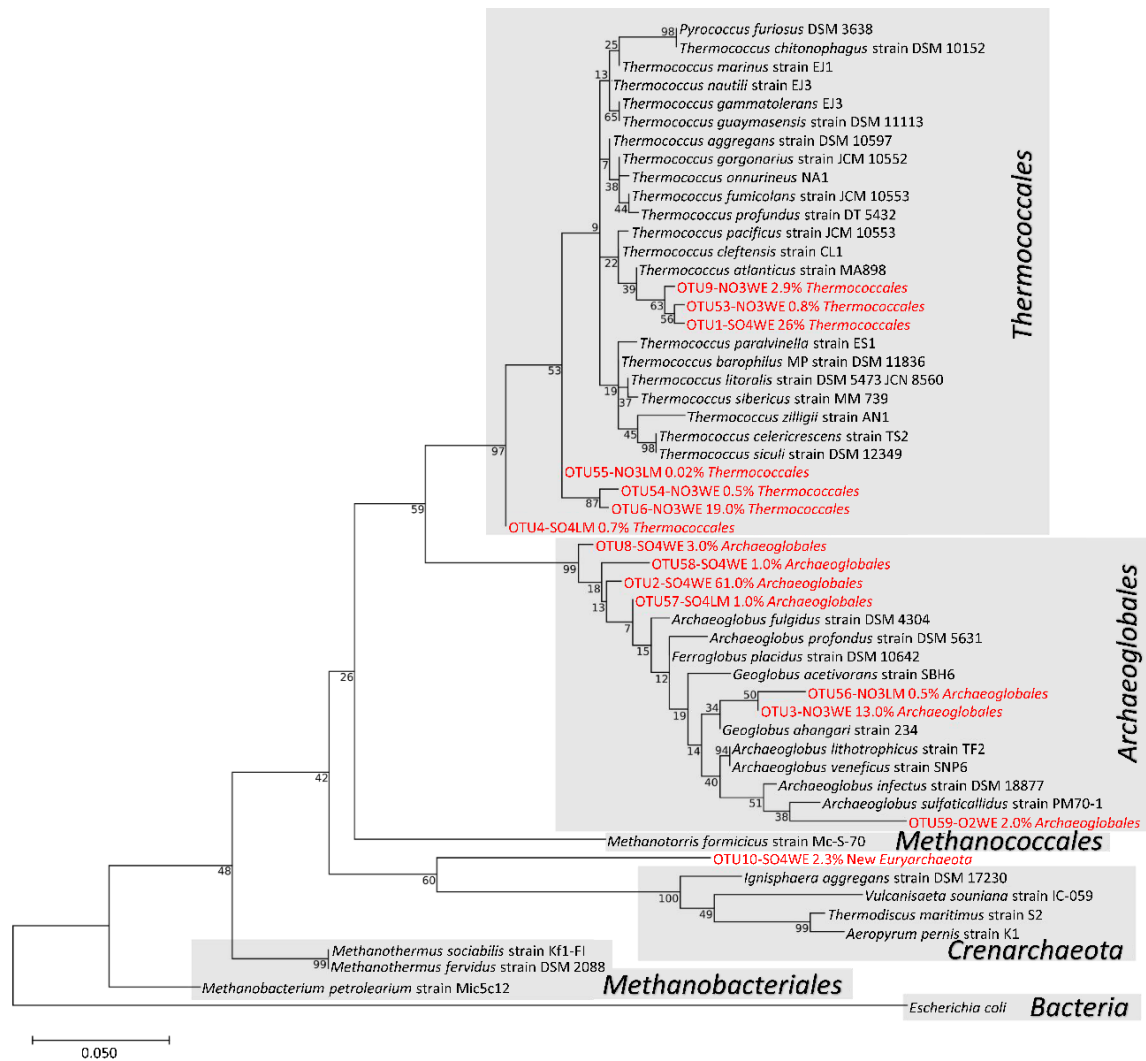
culture. OTUs representing less than 1% of total sequences of the samples are pooled as 'Rare

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OTUs'.

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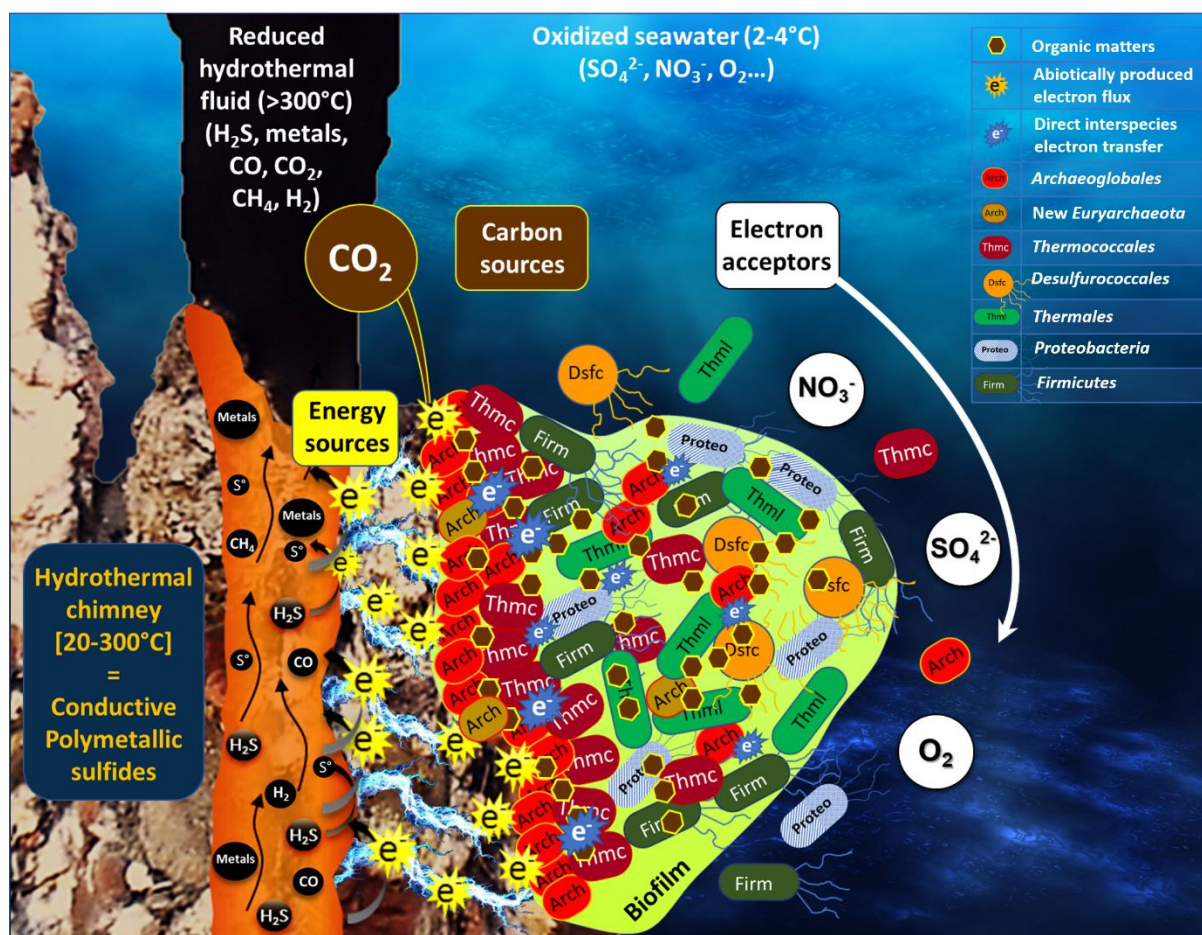


669 **Figure 4.** Maximum Likelihood phylogenetic tree of archaeal OTUs retrieved on various  
 670 enrichments on the 293pb 16S fragment obtained in the barcoding 16S method (LM: Liquid  
 671 Media; WE: Working Electrode, cathode). Numbers at nodes represent bootstrap values inferred  
 672 by MEGAX. Scale bars represent the average number of substitutions per site.

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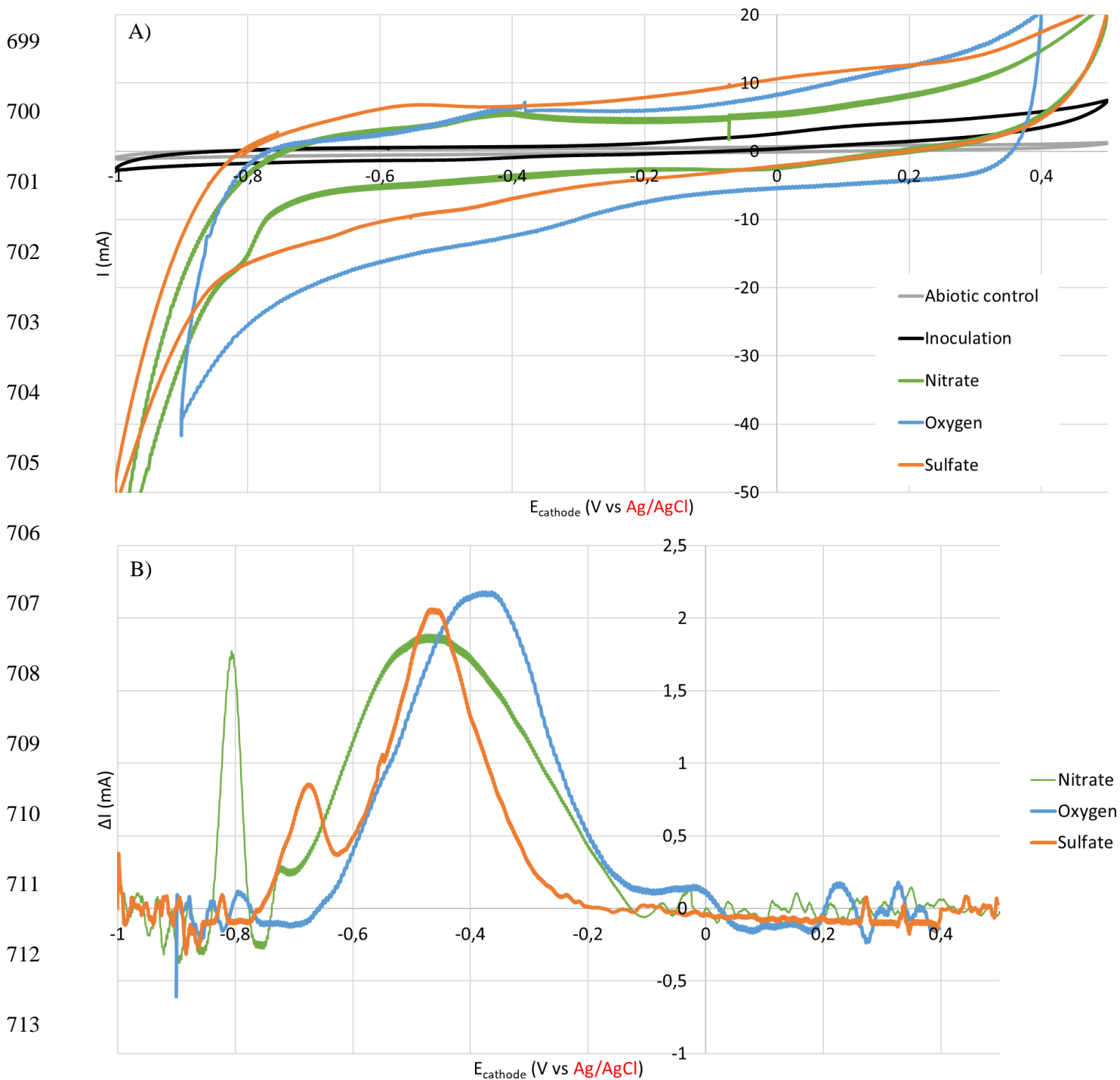
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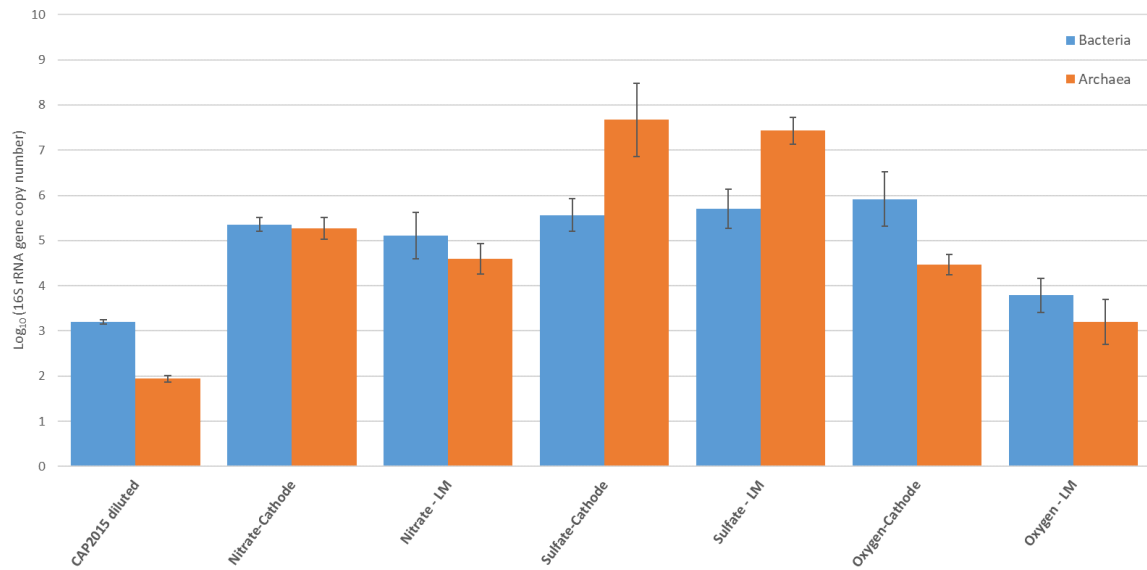
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677 Figure 5: Schematic representation of microbial colonization of iron-rich hydrothermal  
 678 chimney (Capelinhos site on the Lucky Strike hydrothermal field) by  
 679 electrolithoautotrophic microorganisms. The production of an abiotic electrical current by  
 680 potential differences between the reduced hydrothermal fluid (H<sub>2</sub>S, metals, CO, CH<sub>4</sub>, H<sub>2</sub>...) and  
 681 oxidized seawater (O<sub>2</sub>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>2-</sup>) (Yamamoto et al. 2017) leads to the formation of  
 682 electron flux moving towards the chimney surface. This electrons flux can serve directly as  
 683 an energy source to enable the growth of electrolithoautotrophic and hyperthermophilic  
 684 *Archaeoglobales* using the CO<sub>2</sub> as carbon source and nitrate and/or sulfate as electron  
 685 acceptors. In the absence of a usable electron acceptor, *Archaeoglobales* would be likely  
 686 to perform direct interspecies electron transfer to ensure their growth. The electron  
 687 acceptor fluctuations, correlated to the continual influx of electric current would favor the  
 688 production of organic matters (amino acid, formate, pyruvate, glycerol...) by the  
 689 *Archaeoglobales*. This organic matter is then used by heterotrophic microorganisms by  
 690 fermentation or respiration (anaerobic or aerobic) thus providing the primal food web  
 691 initially present into the hydrothermal ecosystems. *The electrical current also could favor*  
 692 *the electrolysis water leading to the abiotic H<sub>2</sub> production (not measurable in our abiotic*  
 693 *conditions), which would serve as chemical energy source. Arch: Achaeoglobales; Thmc:*  
 694 *Thermococcales; Dsfc: Desulfurococcales; Thml: Thermales; Prot: Proteobacteria; Firm:*  
 695 *Firmicute; NO<sub>3</sub><sup>-</sup>: nitrate; SO<sub>4</sub><sup>2-</sup>: sulfate; O<sub>2</sub>: dioxygen; CH<sub>4</sub>: Methane; CO<sub>2</sub>: Carbon Dioxide;*  
 696 *CO: Carbon monoxide; H<sub>2</sub>S : Hydrogen sulfide ; S<sup>0</sup>: sulfur; Metals: Fe, Mn, Cu, Zn...*  
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698 SUPPLEMENTARY INFORMATION



714 **Supplementary Information Figure S1:** A) Cyclic Voltammograms (scan rate = 20 mV/s) of the abiotic  
715 control, and of the experiments at inoculation time and after 30 days for each condition (Nitrate, Oxygen and  
716 Sulfate). B) Reduction peaks extracted from Cyclic Voltammograms (scan rate = 20 mV/s) where the baseline  
717 have been subtracted with the software QSoas. The  $\Delta I$  of reduction peaks are expressed in inversed values.  
Cyclic voltammograms carried out with a 3 M Ag/AgCl reference electrode ( $E = +0.165$  V vs SHE at 80°C).



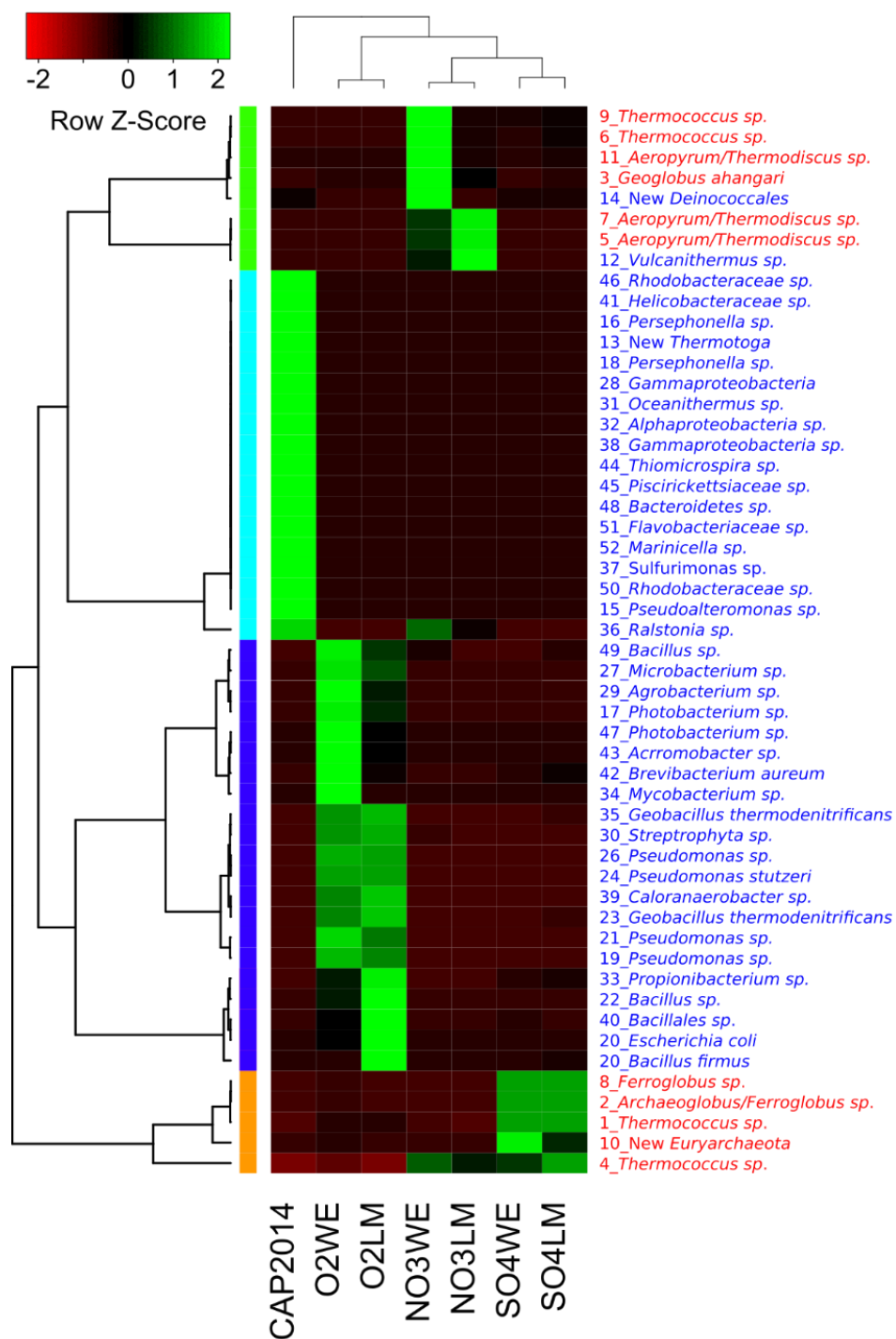
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719 **Supplementary Information Figure S2.** Quantification of 16S rRNA gene copies from Bacteria

720 (blue) or Archaea (orange) per gram of crushed chimney, per milliliter of liquid or per cm<sup>2</sup> of

721 working electrode. Error bar represent the standard deviation obtained on triplicates.





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723 **Supplementary Information Figure S3.** Heatmap representation of the distribution of dominant

724 OTUs (>0.05%) over the different electron acceptors (LM: Liquid Media; WE: Working Electrode,

725 cathode). OTUs and samples clustering were performed with centroid average method and with

726 Pearson distance measurement method. The red taxa represent the Archaea members and blue

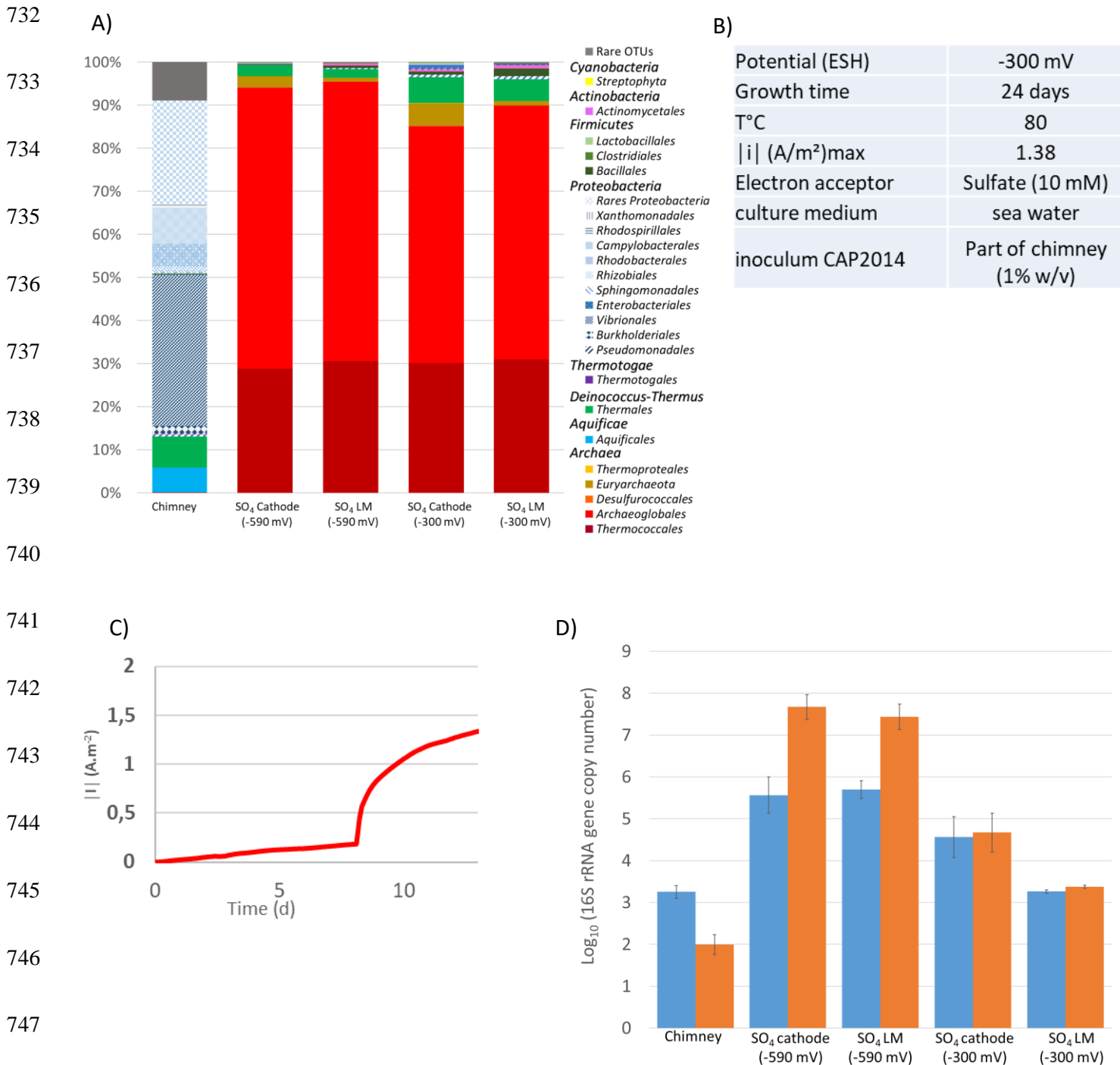
727 taxa, the Bacteria.

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**Supplementary Information Figure S4:** A) Biodiversity comparisons developing it on poised cathode at -590 mV and -300 mV vs SHE in presence of sulfate. B) Parameters of growth with the cathode poised at -300 mV vs SHE. C) current consumption correlated to the growth of biofilm. D) Quantification of 16S rRNA gene copies from Bacteria (blue) or Archaea (orange) per gram of crushed chimney, per milliliter of liquid or per cm<sup>2</sup> of cathode.

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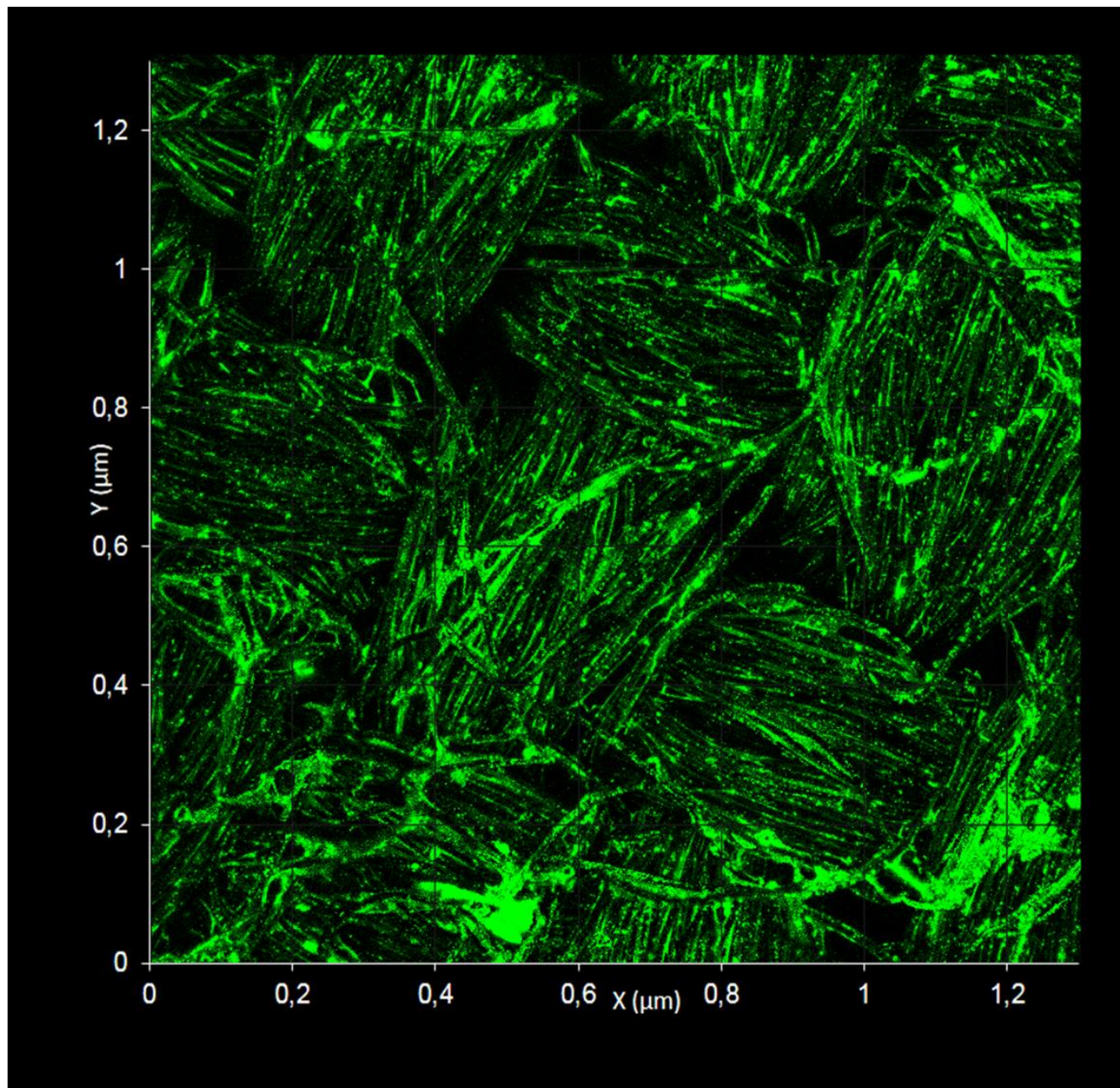
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768 **Supplementary information Figure S5:** Representative confocal microscopy of the biofilm after 30 days of  
769 experiment on nitrate as electron acceptor. The green signal, corresponding to cells stained with Syto9 dye, allow  
770 to show the biofilm covering the interwoven carbon fibers.

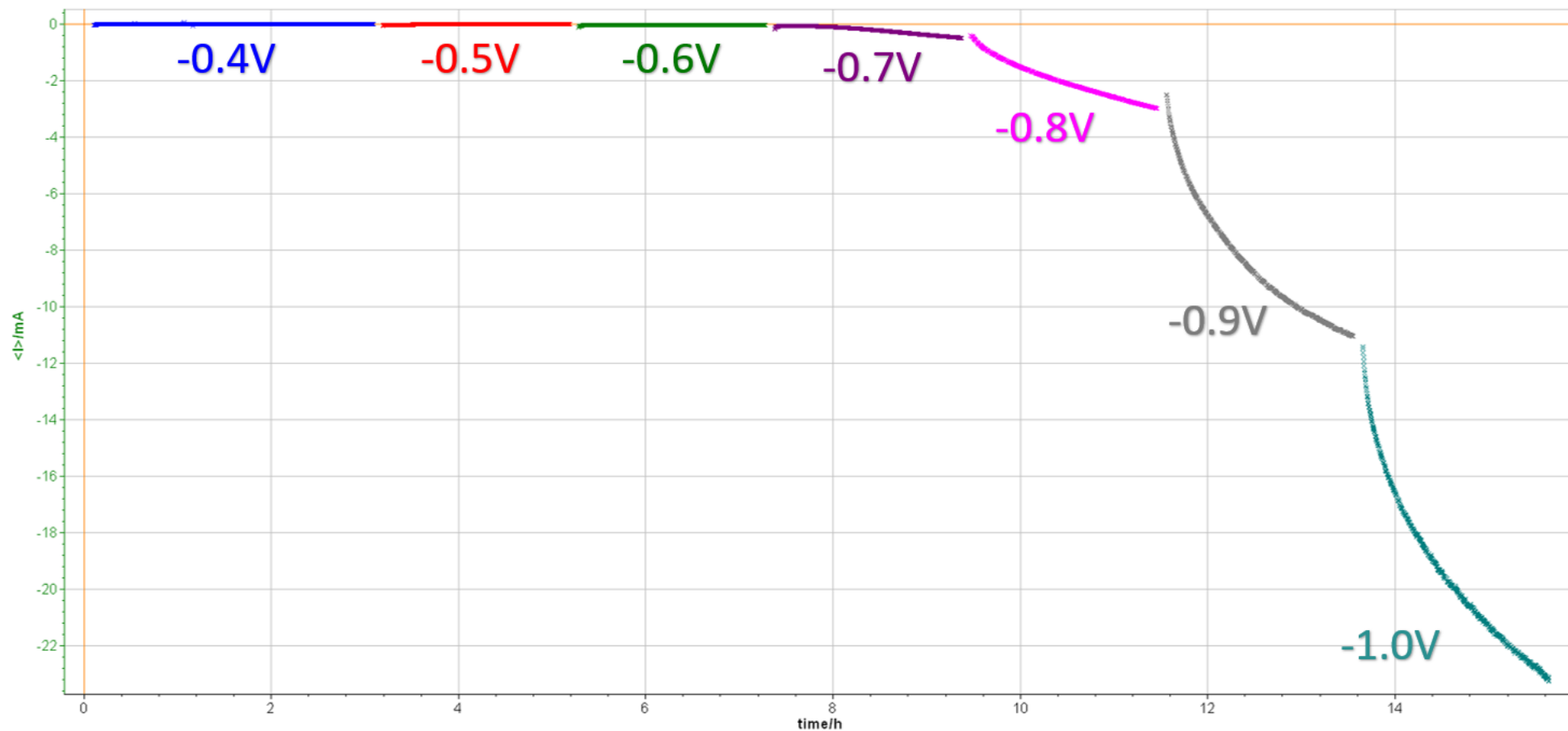
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| Time (days) | Benzoate like | Methanol | Formate | Cystine | Acetoacetate | Lactate | Threonine | Succinate | Ethanol | Alanine | Acetamide | 2-Aminoisobutyric acid | 3-hydroxyisovalerate |
|-------------|---------------|----------|---------|---------|--------------|---------|-----------|-----------|---------|---------|-----------|------------------------|----------------------|
| 0           | 0,0056        | 0,0032   | 0,0085  | 0,0123  | 0,024        | 0,0066  | 0,0019    | 0,0013    | 0,0022  | 0       | 0         | 0,0048                 | 0,0046               |
| 1           | 0             | 0        | 0,0615  | 0,0355  | 0            | 0,03    | 0,0115    | 0         | 0,177   | 0       | 0         | 0,067                  | 0,0375               |
| 2           | 0,0294        | 0        | 0,0084  | 0,0302  | 0            | 0,024   | 0,0026    | 0,0022    | 0,0406  | 0       | 0         | 0                      | 0,0076               |
| 3           | 0,0198        | 0,0108   | 0,0549  | 0,0237  | 0            | 0,015   | 0,0036    | 0         | 0,0375  | 0,0108  | 0         | 0,0051                 | 0,0282               |
| 4           | 0,0242        | 0,1606   | 0,0218  | 0,0306  | 0            | 0,0106  | 0,004     | 0         | 0,0425  | 0,012   | 0,02      | 0,0042                 | 0,0066               |
| 5           | 0,0255        | 0,0315   | 0,0705  | 0,0236  | 0            | 0,0315  | 0,003     | 0         | 0,0475  | 0,0105  | 0,0255    | 0,0065                 | 0,0345               |
| 7           | 0,0544        | 0,009    | 0,0234  | 0,0177  | 0            | 0,0092  | 0,0042    | 0         | 0,0298  | 0,0138  | 0,0486    | 0,0152                 | 0,0094               |
| 9           | 0,055         | 0,036    | 0,063   | 0,0138  | 0,044        | 0,037   | 0         | 0         | 0,086   | 0       | 0,054     | 0,028                  | 0,081                |
| 11          | 0,0766        | 0,0092   | 0,0258  | 0,0096  | 0,0046       | 0,011   | 0,0022    | 0         | 0,0168  | 0,0202  | 0,0576    | 0,0196                 | 0,0138               |
| 12          | 0,0762        | 0        | 0,017   | 0,0078  | 0,0154       | 0,0122  | 0,002     | 0         | 0       | 0       | 0,0692    | 0,0216                 | 0,008                |
| 13          | 0,0948        | 0        | 0,0326  | 0,0012  | 0            | 0,0172  | 0         | 0         | 0,0336  | 0,0542  | 0,0394    | 0,012                  | 0,0144               |
| 15          | 0,1034        | 0,0072   | 0,0236  | 0,0026  | 0            | 0,016   | 0,002     | 0         | 0       | 0,0936  | 0,0358    | 0,0132                 | 0,0112               |
| 16          | 0,1161        | 0        | 0       | 0       | 0            | 0,0276  | 0,0087    | 0         | 0,0579  | 0,1785  | 0,0189    | 0,0081                 | 0,0234               |
| 18          | 0,099         | 0,0046   | 0,0134  | 0       | 0            | 0,011   | 0,0012    | 0         | 0       | 0,1798  | 0,0106    | 0                      | 0,0098               |
| 21          | 0,1284        | 0,0054   | 0,022   | 0,0194  | 0            | 0,013   | 0,004     | 0,0012    | 0,0064  | 0,2366  | 0,0064    | 0                      | 0,0146               |

773 **Supplementary information Table 1:** Evolution of transient compounds (in mM) over the enrichment on Nitrate measured by NMR

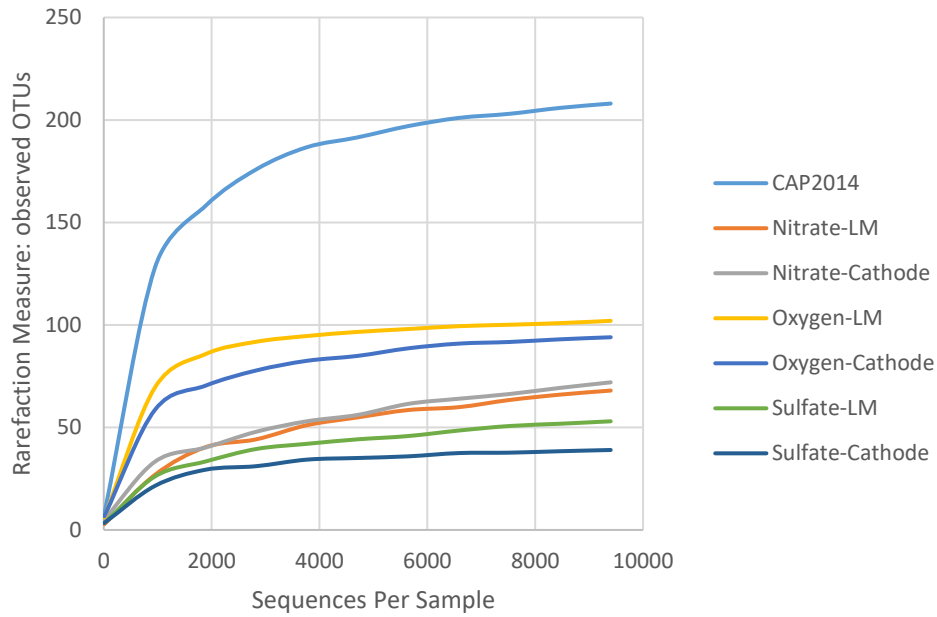
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776 **Supplementary information Figure S6:** Screening of potentials in abiotic and anaerobic conditions to select the lowest potential before water electrolysis.

777 Potential are expressed vs. SHE.



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779 **Supplementary Information Figure S7.** Rarefaction curves of 16S rDNA sequences for bacterial

780 and archaeal diversities in the different samples. Curves were calculated on OTUs at 97%

781 similarity.

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