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## High representation of archaea across all depths in oxic and low-pH sediment layers underlying an acidic stream

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2 **High representation of archaea across all depths in oxic and low-pH sediment layers**  
3 **underlying an acidic stream**

4  
5  
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21  
22  
23 **Abstract**

24 Parys Mountain or Mynydd Parys (Isle of Anglesey, UK) is a mine-impacted environment, which  
25 accommodates a variety of acidophilic organisms. Our previous research of water and sediments  
26 from one of the surface acidic streams showed a high proportion of archaea in the total microbial  
27 community. To understand the spatial distribution of archaea, we sampled cores (0-20 cm) of  
28 sediment and conducted chemical analyses and taxonomic profiling of microbiomes using 16S  
29 rRNA gene amplicon sequencing in different core layers. The taxonomic affiliation of sequencing  
30 reads indicated that archaea represented between 6.2% and 54% of the microbial community at all  
31 sediment depths. Majority of archaea were associated with the order Thermoplasmatales, with the  
32 most abundant group of sequences being clustered closely with the phylotype B\_DKE, followed  
33 by ‘E-plasma’, ‘A-plasma’, other yet uncultured Thermoplasmatales with *Ferroplasma* and  
34 *Cuniculiplasma* spp. represented in minor proportions. Thermoplasmatales were found at all  
35 depths and in the whole range of chemical conditions with their abundance correlating with  
36 sediment Fe, As, Cr and Mn contents. The bacterial microbiome component was largely composed  
37 in all layers of sediment by members of the phyla Proteobacteria, Actinobacteria, Nitrospirae,  
38 Firmicutes, uncultured Chloroflexi (AD3 group), and Acidobacteria. This study has revealed a

39 high abundance of Thermoplasmatales in acid mine drainage-affected sediment layers and pointed  
40 at these organisms being the main contributors to carbon, and probably to iron and sulfur cycles  
41 in this ecosystem.

42

43 **Keywords:** Acidophilic archaea and bacteria, Thermoplasmatales, '*Candidatus* Micrarchaeota',  
44 unclassified Euryarchaeota/Terrestrial Miscellaneous Euryarchaeotal Group (TMEG), acid mine  
45 drainage (AMD) systems, mine-impacted environments, sediment microbiome

46

## 47 **Introduction**

48 Parys Mountain (Parys Mt) or Mynydd Parys (Isle of Anglesey, UK) is an abandoned copper mine  
49 which contains abundant sulfidic deposits in the form of pyrite, chalcopyrite, sphalerite and galena  
50 minerals. As with many other low pH environments associated with metal mining activity, the site  
51 is characterised by the presence of acidic streams or acid mine drainage (AMD) waters, which  
52 result from the oxidative dissolution of sulfidic minerals (Johnson, 2012). Like other AMD  
53 systems, Parys Mt streams contain large concentrations of dissolved metals and metalloids which  
54 constantly flow into the Irish Sea resulting in marine pollution (Johnson, 2012). This site attracts  
55 continuous scientific interest, as reflected in the large number of studies and the identification of  
56 many new species of acidophilic bacteria and archaea (Johnson et al., 2014; Jones and Johnson,  
57 2015; Golyshina et al., 2016a).

58 Our earlier study on microbial assemblages in AMD water and sediments taken from the surface  
59 of one of acidic streams of Parys Mt revealed that archaea dominated the microbial community  
60 (Korzhenkov et al., 2019). Archaea affiliated with Euryarchaeota constituted the major group  
61 (67%) of the total shotgun reads in the community. One particular group of sequences associated  
62 with still uncultured archaea of the order Thermoplasmatales (similar to 'E-plasma' metagenomic  
63 variant) was shown to represent 58% of all metagenomic reads. In the upper sediment layer,  
64 bacterial representatives (33%) were mostly related with Proteobacteria. Other bacterial reads  
65 present in low amounts (2-6%) were largely affiliated with Actinobacteria, Nitrospirae,  
66 Bacteroidetes, Acidobacteria and Firmicutes (Korzhenkov et al., 2019).

67 However, in the lotic community, Proteobacteria, Nitrospirae, Acidobacteria and Actinobacteria  
68 did collectively outnumber archaea (Korzhenkov et al., 2019).

69 The populations of microorganisms inhabiting sediments in AMD-affected areas have been the  
70 subject of numerous studies (Kock and Schippers, 2008; Sanchez-Andrea et al., 2011; 2012; Sun  
71 et al., 2015; Zhang et al., 2019 and others). These works established that bacteria were highly  
72 abundant in AMD sediments and thus assumed they were mainly responsible for biogeochemical  
73 cycling in these ecosystems. For example, only low numbers of archaea were reported in sediments

74 of mine tailing dumps in Botswana, Germany, and Sweden and only in oxidized zones (Kock and  
75 Schippers, 2008). Although archaea of the order Thermoplasmatales are well-known inhabitants of  
76 AMD environments, including sediments, these organisms were found to be present in very low  
77 abundance and thus assumed to be unimportant (Kock and Schippers, 2008; Sanchez-Andrea et  
78 al., 2011; 2012; Sun et al., 2015; Zhang et al., 2019). Frequently however, the detailed information  
79 about the archaeal component is missing, or archaea were excluded from the analysis, leading to a  
80 potential underestimation of the ecological role of archaea in AMD ecosystems (Wakelin et al.,  
81 2012; Lukhele et al., 2019). To understand the patterns of archaeal distribution in sediments of an  
82 acidic stream at Parys Mt and to assess their potential role in elemental cycling, we collected  
83 shallow sediment cores (0-20 cm depth) from the AMD stream. We used a combination of  
84 chemical analysis and SSU rRNA gene amplicon sequencing to resolve, layer-by-layer, microbial  
85 composition changes with depth and across the chemical gradient in order to understand whether  
86 particular geochemical factors were associated with archaeal abundance and to assess their  
87 functional role *in situ*.

88

## 89 **Materials and Methods**

90 Sampling was conducted in the acidic stream located at Parys Mt (GPS location 53.38708° -  
91 4.34968°) as described previously (Fig. S1; Golyshina et al., 2016a, b; Korzhenkov et al., 2019).  
92 Intact sediment cores were taken in September 2018 at three random locations each near another  
93 (within 15 cm distance) using polycarbonate tubes (50 cm-long with inner diameter of 4 cm). The  
94 tubes were gently pressed by hand into the sediment, then plugged with a butyl rubber stopper at  
95 the top. The intact cores were then carefully removed and the base of the tubes plugged with  
96 another butyl stopper and subsequently transported back to the laboratory for analysis. Upon  
97 arrival (ca. 40 min after sampling), the cores were sliced into 2-3 cm-thick disks and transferred  
98 into sterile polypropylene 50 ml Falcon tubes for consequent chemical and microbiological  
99 analyses. pH and Eh potential in the sediment surface layers were measured in the field using a  
100 SevenGo<sup>®</sup> multimeter (Mettler-Toledo, Leicester, UK) and then again in the cores on return to the  
101 laboratory.

102

## 103 **DNA extraction and 16S rRNA gene amplicon sequencing**

104 DNA was extracted from 0.25 g of soil sample from each layer of three cores using the DNeasy  
105 PowerLyzer PowerSoil kit (QIAGEN) according to manufacturer's instructions. Two independent  
106 DNA extractions were carried out for each sample. Quality and concentration of extracted DNA

107 were assessed by gel electrophoresis and by Qubit™ 4.0 Fluorometer dsDNA BR Assay Kit (Life  
108 Technologies, USA).

109 Libraries of 16S rRNA gene amplicons were prepared by single PCR with double-indexed fusion  
110 primers as described previously (Fadrosh et al., 2014). Hypervariable V4 16S rRNA gene fragment  
111 was amplified using modified forward primer F515 (5'-GTGBCAGCMGCCGCGGTAA-3') and  
112 reverse R806 prokaryotic primer (5'-GGACTACHVGGGTWTCTAAT-3'), which amplify an  
113 approximately 290 bp region. Primers were designed to contain: the Illumina adapters and  
114 sequencing primers, a 12 bp barcode sequence, a heterogeneity spacer to mitigate the low sequence  
115 diversity amplicon issue, and 16S rRNA gene universal primers (Fadrosh et al., 2014). PCRs were  
116 performed using MyTaq™ Red DNA Polymerase (Bioline). All reactions were run with no-  
117 template negative controls. Thermocycling conditions were: initial denaturation at 95 °C for 2 min,  
118 followed by 30 cycles at 95 °C for 45 s, 50 °C for 60 s and 72 °C for 30 s with a final elongation  
119 at 72 °C for 5 min. Amplicons were visualised in a 1.5% tris-acetate agarose gels using a GelDoc™  
120 System (Bio-Rad, CA, USA). DNA bands of approximately 440 bp were gel-purified using  
121 QIAEX II Gel Extraction Kit (QIAGEN).

122 The purified amplicons were then quantified using Qubit 4.0 Fluorometer (Life Technologies,  
123 Carlsbad, CA, USA), pooled in equimolar amounts and the final pool was run on Illumina MiSeq  
124 platform (Illumina, San Diego, CA, USA) using 500-cycle v2 chemistry (2 × 250 bp paired-end  
125 reads) at the Centre for Environmental Biotechnology, Bangor, UK.

126

## 127 Bioinformatic analysis

128 Raw sequencing reads were processed according to previously described protocols (Fadrosh et al.,  
129 2014; Korzhenkov et al., 2019). Briefly, the data was pre-processed in order to extract the barcodes  
130 from sequences, and then cleaned of primer sequences using tagcleaner. The barcodes and the  
131 sequences were re-matched again using in-house Python scripts. The resulting filtered reads were  
132 analysed using QIIME v1.3.1. First, the libraries were demultiplexed based on the different  
133 barcodes. Then, the sequences were classified on Operational Taxonomic Units (OTUs)  
134 combining *de novo* and reference-based methods (open-reference OTU generation algorithm)  
135 using the SILVA version 132 reference database.

136 In the case of OTUs assigned to order Thermoplasmatales, a further taxonomic assignation  
137 analysis was performed using a local Blast (Camacho et al., 2008) database based on a selection  
138 of 42 Thermoplasmatales reference sequences, running a final individual blast against *nr* database  
139 for those OTU sequences with <97% of identity in their best hit against the local database.

140

## 141 Statistical analysis

142 All statistical analysis and figures were generated using the R programming language (R  
143 development core team, 2008). Principal Components Analysis (PCA) was undertaken using the  
144 *prcomp* function from package *stats*, included on basic R core. In the case of the Nonmetric  
145 Multidimensional Scaling (NMDS) analysis, we used the *vegan* package (Oksanen et al., 2019).  
146 For Canonical Correlation Analysis (CCorA) internal R scripts were developed, using basic R  
147 functions.

148

## 149 Phylogenetic analysis of Archaea

150 For phylogenetic tree construction, we selected those OTU sequences assigned to Archaea with  
151 more than 100 reads along the three cores and also 34 reference sequences belonging to different  
152 groups. Multiple alignment of sequences was developed using *Mafft* (Katoh & Standley, 2013).  
153 *UGENE* (v 1.9.8) was used for the trimming of the extremes and trimAL (Capella-Gutierrez et al.,  
154 2009) for internal trimming of the alignment, removing columns with gaps on more than the 20%  
155 of the sequences or with similarity scores lower than 0.001, producing a final multiple alignment  
156 of 293 positions. Phylogenetic tree was calculated by maximum likelihood with bootstrapping of  
157 1,000 replicates.

158

## 159 Chemical analysis

### 160 Background chemical analysis

161 Cores were divided by layers and subsamples removed for physicochemical analysis. Moisture  
162 content was determined for the < 2 mm fraction by drying at 105 °C for 24 h. The organic matter  
163 content of the sediment was measured using the loss-on-ignition method, in a muffle furnace  
164 (450 °C, 16 h; Ball, 1964). Sediment C and N content was determined after oven-drying (105 °C,  
165 24 h) using a TruSpec<sup>®</sup> CN analyzer (Leco Corp., St Joseph, MI, USA). Bulk elemental analysis  
166 on the dried, sieved fraction (40 °C, < 125 µm) was undertaken by Total Reflection X-ray  
167 Fluorescence (TXRF) using a Bruker S2 Picofox TXRF spectrometer (Bruker Inc., MA, USA).  
168 Ion chromatography (IC) was used to determine anion concentrations (F<sup>-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>) in 1:10  
169 (w/v) sediment : E-pure water (18 MΩ resistance) extracts using a 930 Compact IC Flex (Metrohm,  
170 Herisau, Switzerland).

171

### 172 Analysis of black layers (oily deposits) within the sediment

173 Two samples of sediment layers with an oily appearance and hydrocarbon-like odour were selected  
174 for further analysis. Samples were weighed out in aliquots of around 100 mg for extraction. The

175 method of extraction was modified from the EPA 3550C method for extraction of non-volatile and  
176 semi-volatile organic compounds from solids such as soils, sludges, and wastes by ultrasonic  
177 extraction (USEPA, 2007). Briefly, an equal amount of anhydrous sodium sulfate was mixed with  
178 the sample to form a free-flowing powder. The sample was then spiked with an internal standard  
179 (10 mg pristine) and extracted using 0.5 ml of a 1:1 (v/v) acetone:chloroform solution. The  
180 extraction was assisted by the use of an ultrasonic bath. The sample tube was suspended in the  
181 bath at room temperature for 1 min. After extraction, the sample was separated by centrifugation,  
182 the supernatant retained, and the pellet extracted a second time as described above. The combined  
183 organic fractions were merged and evaporated to dryness at room temperature with a gentle stream  
184 of nitrogen. Once dry, the sample was resuspended in 200  $\mu$ l of ethyl acetate and filtered (0.22  
185  $\mu$ m) prior to analysis.

186 Analysis was undertaken using a Perkin Elmer Clarus 500/580 GC-MS with a HP-5ms column  
187 (30 m, 250  $\mu$ m ID and 25  $\mu$ m film thickness). The carrier gas was helium, the split ratio set at 10:1,  
188 while the temperatures for the inlet, transfer line, and ionisation source were 250  $^{\circ}$ C, 180  $^{\circ}$ C, and  
189 200  $^{\circ}$ C, respectively. The detector was set to scan between 80-500 mu with a 3 min solvent delay.  
190 The initial oven temperature was 60  $^{\circ}$ C (10 min) followed by an 8  $^{\circ}$ C/min ramp to 300  $^{\circ}$ C followed  
191 by a 10 min hold. Approximate quantification of the analytes was achieved by comparing peak  
192 area to that of pristane and a response factor of 1 assumed. For pristine, a 6-point calibration curve  
193 was made between 0.5 and 50  $\mu$ g/ml. Retention times of the unbranched alkanes were determined  
194 using a standard mixture of C<sub>10</sub>-C<sub>19</sub>.

195

## 196 **Results and Discussion**

### 197 **Physicochemical data**

198 Cores 1, 2 and 3 showed slightly different values in pH and redox potential. Cores 1 and 2 showed  
199 a similar tendency in increasing pH with depth from 1.65-1.7 (surface) to 2.4 at a depth of 8 cm in  
200 Core 1 and to 2.68 at a depth of 15 cm for Core 2. Redox was found to be positive in all layers  
201 with insignificant variations between depths and with values always >400 mV (range 413-470  
202 mV). The three cores had visual differences in structure and exhibited mostly 'oxidized colours',  
203 from mixtures of yellow/brown, to red/brown with some ochre and in some places a completely  
204 black appearance. Core 3 was distinct in comparison to other cores, being more homogeneous and  
205 with a stable pH (2.4-2.5) across the whole depth gradient (Table S1).

206 Comparison of physical-chemical parameters between cores suggested certain variations in the  
207 content of metals and metalloids, anions, nitrogen and organic matter (Table S1). Core 1 possessed  
208 more Fe and Pb in the three upper layers (1.1, 1.2, 1.3.1) and a consistently high presence of As in

209 all layers. Core 2 demonstrated more Rb and Ti in all layers. Both Cores 1 and 2 showed an  
210 increase in Al with depth. In contrast, Core 3 exhibited high concentrations of Cu in two layers  
211 (3.4 and 3.6, depth 9-11 and 19-21 cm), Zn (layers 3.5 and 3.6, depth 13-16 and 19-21 cm) and Rb  
212 (layers 3.3 and 3.4, depth 6-9 and 9-11 cm).

213 The highest amounts of organic matter were measured for Core 1 (layers 1.2 and 1.3.1) and Core  
214 3 (layers 3.1, 3.5 and 3.6). Core 2 was found to have a low organic matter content in the sediment.  
215 The total amount of N was found to be higher in Core 2 (layers 2.2, 2.3 and 2.4) and in Core 3  
216 (3.3, 3.4, 3.5 and 3.6). The C:N ratio was significantly higher in upper layers of Core 1 (values of  
217 25.4 and 12.5 for layers 1.1 and 1.2, respectively) and Core 2 (26.7). In Core 3, an opposite pattern  
218 was apparent with C:N ratios of 12.4 and 17.6 seen in the deeper layers (3.6 and 3.7).

219 Interestingly, few fluctuations were observed in the content of fluoride, chloride, nitrate, phosphate  
220 and sulfate. Core 1 (layer 1.3.2) possessed the highest concentrations (in mg/kg) of F<sup>-</sup> (65.3), Cl<sup>-</sup>  
221 (6.5), NO<sub>3</sub><sup>-</sup> (653) and SO<sub>4</sub><sup>2-</sup> (90528). Core 3 exhibited an increased content of F<sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and SO<sub>4</sub><sup>2-</sup>  
222 in some layers (Table S1). These observations suggest a high degree of heterogeneity in chemical  
223 composition between the cores and individual subsamples.

224 We analysed 31 different chemical properties in the sediments which we divided into three  
225 categories, namely: “Carbon-Nitrogen”, “Anions” and “Other elements”. A preliminary Principal  
226 Components Analysis (PCA) showed a very complex distribution of the influence of chemical  
227 variables over the different core layers. Also, some of the chemical variables overlapped and were  
228 not used in order to reduce redundancy. Measures of total C and N (mg/kg) were removed from  
229 the analysis, while sulfate (g/kg) was included. Therefore, 28 of 31 chemical properties were  
230 included in the analysis. The analysis was divided into three different parts according to each type  
231 of chemical property. Each of these analyses is composed of a PCA where the contribution  
232 percentage of each variable has been calculated and included using a colour key, specific for each  
233 core (Fig. 1, Fig. S2-S4).

234 The PCA for “Carbon-Nitrogen” showed that Core 1 and 2 are quite distinct from each other while  
235 Core 3 remained in an intermediate position (Fig. S2).

236 PCA demonstrated that variance for F<sup>-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> are much higher on the sample 1.3.2  
237 than for the rest of the layers, being so different those four measures overlapped on the  
238 representation. The concentration of PO<sub>4</sub><sup>3-</sup> was the variable that contributed the most to the  
239 distribution of the samples in the PCA. Concentration of PO<sub>4</sub><sup>3-</sup> was below the limit of detection  
240 (0.1 mg/kg) in every layer of Core 1, was detected in 2 layers out of 6 of the Core 2 in concentration  
241 < 1 mg/kg (dry sediment), whereas 4 out of 7 layers of the Core 3 displayed values from 1.7 to 4.1  
242 mg/kg, which therefore grouped together and distinctively from the rest (Fig. S3).



243 The specific PCA was conducted based on concentrations of “Other elements”, primarily metals  
244 and metalloids (Fig. S4). Iron (Fe), Arsenic (As) and Manganese (Mn) were the elements which  
245 had greatest influence on the PCA; concentrations of Fe and As were much higher in Core 1, while  
246 Mn was greatest in Cores 1 and 2, but lower in Core 3. On the other side, Zinc (Zn), Copper (Cu)  
247 and Yttrium (Y) were specifically higher in some sublayers of Core 3; however, these are the  
248 variables showing less contribution percentage to the patterns shown in the PCA.

249

## 250 Principal components analysis using all chemical properties

251 All chemical parameters were then analysed and included in the same PCA (Fig. 1). Again, the  
252 sublayer 1.3.2 dropped far away from the rest of ‘the cloud’ due to its drastic shift in values on  
253 anions concentrations, except  $\text{PO}_4^{3-}$ . For this reason, the group of the Core 1 showed a very high  
254 variance (represented by a big ellipse). However, it is also evident how the remaining variables  
255 influenced the separation of the rest of layers groups, with Fe and As concentration pushing for  
256 Core 1 group as long as Pb and Br (which was not so clear in the specific PCA for elements) (Fig.  
257 1).

258

259 Comparison of chemical composition of sediment from the surface and overlying waters  
260 established previously (Korzhenkov et al., 2019) and in this study showed that Al was represented  
261 in significantly higher quantities across the gradient, exceeding its concentrations on the surface  
262 up to 5-9 times. Concentrations of K and Ti determined in sediment core layers at various depths  
263 were >2-fold higher than at the surface, whereas Cr and Mn were present at lower concentrations.  
264 Ni, Zn, Ca, As, and Sr had about the same concentrations across samples with few exceptions (e.g.  
265 more abundant in some layers). Pb was generally detected in lower quantities than on the surface,  
266 however, there were few exceptions. Fe was found in high various quantities in different layers of  
267 sediment, comparable with those at the surface (66.7 g/kg) (Table S1).

268 Total carbon and nitrogen were less abundant in deeper layers in comparison to those at the surface  
269 (2.8% and 0.3%, respectively) (Table S1). C:N ratio was highly variable (0.8-26.7) across the  
270 different layers and was not dependent on depth (Table S1).

271 GC-MS analysis of black layers (oily deposits) from Parys Mt acidic stream sediment identified  
272 hydrocarbons, specifically unbranched alkanes with  $\text{C}_{17}$  being the most abundant type.

273

## 274 **Microbial content**

275 Taxonomic composition of microbial communities in sediment layers

276 Archaea

277 Archaeal sequences were found in all three cores (Fig. 2), which is in accordance with previous  
278 studies investigating surface sediments (0-3 cm) at this site (Korzhenkov et al., 2019). Across  
279 different sediment depths, archaea represented a dominant group, as judged from the total number  
280 of reads and numbers of OTUs, particularly in cores 1 and 2. In Core 3, a very large number (ca  
281 30%) of archaeal reads were observed in the upper sediment layer; all deeper layers displayed a  
282 consistent decrease of archaeal reads (down to 6%) and increase in various bacterial groups.

283 Archaeal diversity has been mostly restricted to Euryarchaeota (or Thermoplasmata, according  
284 to the GDTB taxonomy <https://gtdb.ecogenomic.org>), and among those, mainly to the members of  
285 the order Thermoplasmatales. In this study, Thermoplasmatales reads were detected in high  
286 abundance as follows: (i) in Core 1 it ranged from 49% of the total reads at the surface to 39.5%  
287 at a depth of 6-8 cm; (ii) in Core 2 they represented 54.1% of total reads at the surface to 51.5% at  
288 a depth of 10-15 cm; (iii) in Core 3 they represented 39.9% at the surface to 6.2% at a depth of 20  
289 cm.

290 Among Thermoplasmatales, the most abundant group of sequences across the depth gradient was  
291 affiliated to B\_DKE metagenomic assembly. These sequences represented 5-45% of the total with  
292 the greatest abundance seen in Core 2. This group has also previously been reported in pyrite mine  
293 biofilm (Harz Mountains, Germany) by Krause et al. (2017). These archaea were followed by the  
294 'E-plasma' variant which was present in all three cores with varying numbers (0.5-15%) depending  
295 upon depth. In addition, within Cores 1 and 2, sequences similar to the phylotype with accession  
296 number FR683002 and to other unclassified Thermoplasmatales were detected (<0.5-10%). Reads  
297 related with 'A-plasma' metagenomic assembly, *Ferroplasma acidiphilum*- (both in quantities  
298 <0.5-5%) and *Cuniculiplasma divulgatum*-related (with a relative abundance of <0.5%) organisms  
299 were also identified. These phylotypes clustered with known taxonomic clades of archaea or  
300 reference organisms, as demonstrated in Fig. 3 and Table S2. No correlation of relative numbers  
301 of these taxonomic groups with sediment depth was seen. However, in the case of 'A-plasma'-  
302 and *Ferroplasma acidiphilum*-related organisms, their abundance gradually increased with depth  
303 down to the black-colored layer (Fig. 2). Maximal numbers of 'A-plasma' were observed at 8-10  
304 cm (Core 2), and for *Ferroplasma*-like sequences at 6-8 cm (Core 1), 4-15 cm (Core 2) and 9-11  
305 cm (Core 3). Interestingly, *Ferroplasma* reads were not detected in upper layers of all three cores,  
306 and their presence has not previously been reported in any other parts of the Parys Mt ecosystem  
307 (Korzhenkov et al., 2019). *Cuniculiplasma* spp. was the lowest-abundance group among other  
308 Thermoplasmatales with a relative abundance <0.5% across all layers and depths. These archaea  
309 were also earlier shown to only comprise a minor group in the upper sediment/water stream  
310 community (Korzhenkov et al., 2019).

311 In this study, minor quantities (0.1-0.5%) were affiliated with TMEG-related organisms  
312 (Terrestrial Miscellaneous Euryarchaeal group, or ambiguous taxa in the class *Thermoplasmata*,  
313 as per the SILVA database v.132). The relative abundance of this group were relatively constant  
314 with depth in Core 1, but were mostly detected in the upper sections of cores 2 and 3.  
315 Furthermore, 'Ca. Micrarchaeota' was present in very low abundance (<0.5%) across almost all  
316 sediment depths. Both groups were shown previously to inhabit the uppermost layer of sediments  
317 and can also be found in the overlying stream water (Korzhenkov et al., 2019).

318 All archaea of the order Thermoplasmatales described so far are prominent inhabitants of acidic  
319 environments and exhibit a heterotrophic lifestyle, which is reflected in their preferential growth  
320 on complex polypeptides (Golyshina, 2011; Golyshina et al., 2016a). *Thermoplasma* was also  
321 shown to possess the potential for sulfur-driven respiration with organic carbon as an electron  
322 donor (Darland et al., 1970). Furthermore, members of the family Ferroplasmaceae are able to  
323 undertake iron oxidation/reduction (Golyshina, 2011). Heterotrophy and iron redox cycling (iron  
324 is highly available under oxidative redox conditions and low pH) together with facultatively  
325 anaerobic capability are likely present among archaeal components of these sediment  
326 communities. The occurrence of Fe (III) reduction in acidic sediments at low oxygen concentration  
327 was reported previously (Küsel et al., 2002). Sulfur respiration could potentially be another trait  
328 of these archaea. Iron redox cycling and heterotrophy were confirmed experimentally for cultured  
329 mesophilic species of *Ferroplasma acidiphilum* and *Cuniculiplasma divulgatum*, respectively  
330 (Golyshina et al., 2000; 2016a). However, since the majority of archaea populating this  
331 environment are uncultured, their metabolic properties remain to be confirmed.

332 It should be noted that all these archaeal phylotypes are widely found in a range of acidic  
333 environments. Archaea designated as B\_DKE were identified in enrichment cultures established  
334 with biofilms obtained from a pyrite mine (Harz Mountains, Germany) (Krause et al., 2017). The  
335 organism was shown to grow in anaerobic enrichment culture when the medium was supplemented  
336 with polypeptides and ferric sulfate; furthermore, the authors suggested that these archaea could  
337 undertake ferric iron reduction (Krause et al., 2017). Similar features are highly likely for B\_DKE  
338 archaea although their physiological properties still need to be confirmed in pure culture. Similar  
339 organisms are present in various low-pH environments (Krause et al., 2017). For example, almost  
340 identical SSU rRNA gene sequences with accession numbers HQ730609, EU370309, HM745409  
341 and EF396244 were detected in anaerobic sediments and biofilm communities from Rio Tinto  
342 (Spain), an extremely acidic, metal-rich stream (Huelva, Spain), and in La-Zarza-Perrunal acid  
343 mine effluent (Spain) (Sanchez-Andrea et al., 2011; Rowe et al., 2007; Gonzalez-Torril et al.,  
344 2011). Moreover, similar phylotypes were recovered from a low temperature (8.5 °C) underground

345 mine at Cae Coch (GU229859, Wales, UK) (Kimura et al., 2011), and in an acidic geothermal area  
346 (35 °C) of Copahue (KP204537, Neuquen, Argentina) (Urbieto et al., 2015).

347 Other most-abundant phylotypes from Parys Mt sediments were clustered with the sequence with  
348 the accession number FR683002 from the microbial community of Pb-Zn mine, and also in acid  
349 mineral bioleaching systems of Dongxiang copper mine, Yinshan Lead-Zink mine and Yun-Fu  
350 pyrite mine (DQ464162; FN386445), all places located in China (Xiao et al., 2008; Huang et al.,  
351 2011; Tan et al., 2009). Furthermore, similar sequences were present in macroscopic filaments  
352 from Rio Tinto (Spain) (DQ303253, Garcia-Moyano et al., 2007), in cave wall biofilms from the  
353 Frasassi cave system, Italy (DQ499229; Macalady et al., 2007), in Iron Mountain AMD system,  
354 USA (AF544220; Baker and Banfield, 2003), in thermal and acidophilic biofilms, Mexico  
355 (KJ907756; unpublished) and in endolithic microbial community from Rio Tinto basin, Spain  
356 (EF441883; unpublished).

357 Other archaea identified in Parys Mt sediments and still awaiting their isolation are ‘E-plasma’  
358 and ‘A-plasma’ (Baker and Banfield, 2003). Firstly detected in Iron Mountain (USA)  
359 metagenomic datasets, these organisms were found in the Parys Mt acidic stream surface sediment,  
360 with ‘E-plasma’ as a dominant phylotype (Korzhenkov et al., 2019). Their metabolism was  
361 predicted as heterotrophic, which involves iron oxidation/reduction (Yelton et al., 2013). It is  
362 worth noting that both are also ubiquitous: they were found e.g. in macroscopic filaments  
363 (DQ303254 and EF441874, correspondingly) (Garcia-Moyano et al., 2007), and in endolithic  
364 communities in the Rio Tinto basin (EF441884 and EF441874). The ‘A-plasma’ phylotype was  
365 also detected in anaerobic sediments from Rio Tinto (HQ730610; Sanchez-Andrea et al., 2011).  
366 Furthermore, 16S rRNA gene amplicon reads of both archaea were found in forested wetland  
367 sediment samples influenced by waste coal deposits, USA (AF523940, AF523941; Brofft et al.,  
368 2002), in terrestrial subsurface cave systems, Italy (KM410353, AF523941; Hamilton et al., 2015)  
369 and in a thermal acidic biofilm, Mexico (KJ907754, KJ907758; unpublished). Additionally,  
370 sequences clustering with the ‘A-plasma’ were present in the metagenomic data from a terrestrial  
371 acidic spring field, Japan (AB600341; Kato et al., 2011).

372 In relation to our results, a few points need to be highlighted. Firstly, there is still an extremely  
373 small number of archaeal taxa cultured from AMD, in comparison to bacteria. Bacterial  
374 acidophilic diversity associated with AMD sites is assigned to more than 13 genera belonging to  
375 various phyla (Acidobacteria, Actinobacteria, Firmicutes, Nitrospirae and Proteobacteria)  
376 (Mendez-Garcia et al., 2015; Gavrillov et al., 2019). However, all cultured archaea from similar  
377 AMD environments with validly published names are affiliated with the single order,  
378 Thermoplasmatales of the phylum Euryarchaeota (genera *Ferroplasma*, *Acidiplasma* and  
379 *Cuniculiplasma*) (Golyshina et al., 2000; 2009; 2016a; Hawkes et al., 2008). Thermophilic

380 crenarchaeon *Metallosphaera prunae* isolated from a uranium mine is the only example of cultured  
381 representatives from another higher archaeal taxon (Fuchs et al., 1995). Thus, organisms of the  
382 order Thermoplasmatales are considered to be the most successful archaeal colonisers of mining  
383 sites, natural or anthropogenic environments with moderate temperatures, benefitting from low pH  
384 and oxygen levels. The second important point to consider when assessing sequencing data from  
385 similar environments is that the sequences submitted to the databases with the 16S rRNA sequence  
386 identity levels below 94% with the reference isolates, are often wrongly qualified as  
387 *Thermogymnomonas* spp. or *Thermoplasma* spp., which creates confusion and leads to incorrect  
388 interpretation. Importantly, *Thermogymnomonas* or *Thermoplasma* spp. were so far not detected  
389 in the low- or moderate-temperature AMD environments.

390 Other archaea inhabiting Parys Mt sediment belonged to ‘*Ca. Micrarchaeota*’ detected at different  
391 depths of the three cores. These sequences showed 98-99% 16S rRNA gene identity levels to  
392 organisms from volcanic environments (GQ141757; KJ907762) and from Parys Mt surface parts  
393 (Golyshina et al., 2019). 16S rRNA sequence identity of these sediment variants to ‘*Ca.*  
394 *Mancarchaeum acidiphilum*’, Mia14 was found to be 91.8%.

395

## 396 Bacteria

397 Among bacteria, members of the phylum Proteobacteria were most abundant in all cores,  
398 comprising on average  $26.0 \pm 3.5\%$  of the community across all depths. *Firmicutes* in all layers  
399 reached moderate numbers representing  $7.2 \pm 3.8\%$  of the total community (Fig. 2). Other bacterial  
400 groups consistently present in all layers were from the phyla Nitrospirae, Actinobacteria,  
401 uncultured Chloroflexi (AD3 group), Acidobacteria and others (Fig. 2).

402 No correlation of Proteobacteria distribution with sediment depth was observed. Among  
403 Proteobacteria, classes Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria  
404 signatures were the most prominent. Gammaproteobacteria were represented mostly by three  
405 groups of organisms: the unclassified Gammaproteobacteria, order Xanthomonadales (family  
406 Xanthomonadaceae) and the cluster RCP1-48.

407 Xanthomonadaceae (0.5-45%) were represented mostly by organisms closely related to  
408 *Metallibacterium scheffleri*, described as facultatively anaerobic, iron-reducing organisms  
409 (Ziegler et al., 2013). In addition, some *Stenotrophomonas* spp. and *Pseudoxanthomonas* spp. were  
410 detected. Also, *Acidithiobacillus* spp.-related OTUs, with a rather low sequence identities with  
411 type strains (<96-97%) were observed in minor amounts (<0.5-1%). Similarly, low numbers of  
412 *Acidithiobacillus* were earlier detected in the surface sediment and water, suggesting that this  
413 particular environment is not very favourable to these organisms (Korzhenkov et al., 2019). A  
414 possible reason is the extremely low pH (<2), high redox and abundance of Fe (III) in Parys Mt

415 AMD; these factors were previously considered as less advantageous for these organisms  
416 (Rawlings et al., 1999). Alphaproteobacteria were detected in quantities from 0.1% to a maximum  
417 of 7.3% at all sediment depths. Representatives of Rhodospirillales (family Acetobacteraceae)  
418 were seen mostly in OTUs with a very distant phylogenetic position from *Rhodophila* spp.,  
419 *Acidisoma* spp. and *Acidisphaera rubrifaciens*, making it challenging to speculate on their  
420 metabolism.

421 Deltaproteobacteria were associated with the order Bdellovibrionales, family Bacteriovoraceae, in  
422 which the sequences showed low homology (less than 90%) to described isolates. Patchiness was  
423 observed for the vertical distribution of these bacteria. Some increase in numbers of  
424 Bacteriovoraceae with depth was observed. Another relatively abundant bacterial phylum was  
425 Actinobacteria (<0.5-15%) with OTUs affiliated mostly with Acidimicrobiales. Among them, the  
426 sequences similar to *Aciditerrimonas* (95% identity to *Atn. ferriducens*), *Acidimicrobium* (95%  
427 identity to *Am. ferrooxidans*) and *Ferrimicrobium acidiphilum* (100%) were detected.  
428 *Aciditerrimonas* was described as facultatively anaerobic, heterotrophic and autotrophic organism,  
429 able to undertake dissimilatory reduction of ferric iron (Itoh et al., 2011). *Acidimicrobium* and  
430 *Ferrimicrobium* are known inhabitants of acidic environments, with the ability to undertake iron  
431 oxidation to undergo heterotrophic growth (Mendez-Garcia et al., 2015).

432 The consistent presence of Nitrospirae (1-20%) was demonstrated at various depths in all cores.  
433 Of note, at the depth of 18-20 cm in Core 3, the Nitrospirae OTUs reached 52.1%, with affiliation  
434 of all sequences to *Leptospirillum* spp. (Markosyan, 1972; Hippe, 2000; Coram & Rawlings,  
435 2002), represented mostly by *L. ferrooxidans*-related organisms and by new species of this genus.  
436 All validly published leptospirilli were described as aerobic and autotrophic (ferrous iron  
437 oxidising) organisms (Markosyan, 1972; Hippe, 2000; Coram & Rawlings, 2002).

438 Firmicutes were found to increase their numbers with depth in Core 1 and varied in numbers in  
439 other cores, in line with the physicochemical heterogeneity of the sediments. Among them, the  
440 sequences of *Sulfobacillus*, YNPFFP6 group of Sulfobacillaceae-, *Alicyclobacillus*- and  
441 *Desulfosporosinus*-related bacteria were the most representative OTUs. *Sulfobacillus* and  
442 *Alicyclobacillus* spp. are well-known inhabitants of AMD systems with facultatively anaerobic  
443 lifestyles and capable of iron oxidation and reduction, oxidation of sulfur compounds and  
444 heterotrophic or autotrophic types of carbon assimilation (Mendez-Garcia et al., 2015). Sulfate-  
445 reducing *Desulfosporosinus* members were also previously shown to inhabit AMD sediments  
446 (Alazard et al., 2010; Sánchez-Andrea et al., 2015). Firmicutes were found to be highly represented  
447 in the black-colored layers, reaching proportionally high numbers of 30-50% of total reads. Of  
448 note, at a depth of 9-11 cm in Core 3, Firmicutes represented 75.2% of the total reads. The majority  
449 of OTUs found were either *Sulfobacillus*- and *Alicyclobacillus*-related sequences, only distantly

450 affiliated to the species with the established taxonomy. Other Firmicutes belonged to  
451 *Desulfosporosinus* and other bacteria of the family Peptococcaceae (Clostridiales). Moreover,  
452 sequences distantly related to other families of the order Clostridiales were identified in the  
453 sequencing data of ‘black layers’. During the sampling, while inserting sampling corers into the  
454 sediments and reaching the ‘black horizon’, we observed the development on the water surface of  
455 a thin hydrophobic film, highly likely, of hydrocarbons. We measured hydrocarbons in two  
456 selected samples of ‘black layers’ and identified the *n*-heptadecane as a major component (19 and  
457 43 mg/kg). This compound is known to be the most abundant product in cyanobacteria, but can  
458 also hypothetically be formed from fatty acids through reactions catalysed by reductases and  
459 decarboxylases (Kang & Nielsen, 2017). Whatever the origin, this compound can be metabolised  
460 by acidophilic bacteria, including *Sulfobacillus* spp., as demonstrated previously (Hamamura et  
461 al., 2005; Ivanova et al., 2013).

462 Across the depths, other bacteria were represented by uncultured Chloroflexi (AD3 group/ JG 37-  
463 AG-4) in numbers between 0.5-1% for Cores 2 and 3 and ca. 5% within Core 1. The metabolic  
464 features of these organisms previously detected in acidic ecosystems, remain unknown (Gavrilov  
465 et al., 2019).

466

467 In order to assess how the abundance profiles differs in the three cores, a Non-Metric  
468 MultiDimensional Scaling (NMDS) was performed, using Bray-Curtis distances (Fig. 4). The  
469 NMDS of the whole community suggests that the most abundant groups were in general not  
470 defining very well the differences over the 3 cores, hence these groups are mostly concentrated  
471 close to the center of the diagram. Additionally, NMDS results emphasised that microbial  
472 community stability decreases with depth. So, all samples from Core 1 kept a more similar  
473 taxonomic distribution profile than Cores 2 and 3 (see Fig. 4). This can also be observed in their  
474 ellipse ranges, based on layers variance. Finally, separation among samples seems to be the result  
475 of less-abundant taxa, especially in Core 3. For instance, TMEG was detected at very low  
476 quantities in all samples, however, the layer 3.1 showed the biggest relative abundance (0.336 %)   
477 which is about 12-fold higher than the average of TMEG numbers (0.027%). This was also the  
478 case with *Sulfobacillus*, which was especially abundant in layer 3.4 (>70%) (Fig. 4A).

479 If we focus on the NMDS representation for Archaea, we can see a very large difference in the  
480 distribution. Samples from the Core 1 clustered very compactly showing a very similar distribution  
481 of all archaeal groups. In contrast, samples from Core 3 showed a large amount of scatter and  
482 largest variance on their ellipse (Fig. 4B).

483

484 Correlation analysis between microbial diversity and chemical properties

485 Canonical correlation analysis (CCorA) was used to demonstrate the relationship between  
486 chemical properties and microbial community composition (in this case, treating microbial groups  
487 as variables). According to the CCorA, B\_DKE phylotype and also other Thermoplasmatales were  
488 the groups with highest correlation with chemical variables, specifically to As, Fe, Cr and Mn, in  
489 comparison with bacteria (Fig. 5). All Thermoplasmatales phylotypes and '*Ca. Mancarchaeum*  
490 *acidiphilum*' possess a high genomic potential for metal resistance, as suggested previously in  
491 acidophiles (Dopson and Holmes, 2014). Thus, metallochaperones, heavy metal reductases,  
492 mercury (II) reductases, CopP type ATPases, arsenic efflux pump-related proteins (ArsA, ArsB  
493 and ArsR) were found in the genomic data of reference organisms (Table S3). Genes encoding  
494 these proteins were shown previously to be often located on 'defence' genomic islands (Golyshina  
495 et al., 2016b; 2017).

496

#### 497 Comparison with other acidic sediments

498 In comparison to other AMD sediments, this particular system is characterised by positive redox  
499 potential and relatively low pH (1.7-2.5). The high abundance of archaea shown in this study seems  
500 different from earlier analyses due to a lower redox and higher pH values in the latter (Sanchez-  
501 Andrea et al., 2011, 2012, Sun et al., 2015). However, Parys Mt and Rio Tinto sediment archaeal  
502 phylotypes were found to be similar, supporting the prediction of the versatility of uncultured  
503 Thermoplasmatales in relation to the oxygen tolerance and pointing at their potential facultative  
504 anaerobic lifestyle. As in the present study, archaea of the order Thermoplasmatales were reported  
505 independently of sampling depth and spot at the Rio Tinto mine site (Sanchez-Andrea et al., 2011,  
506 2012).

507 Diverse archaeal sequences were earlier revealed in the arsenic-rich creek sediment of Carnoules  
508 Mine, France (Volant et al., 2012). Archaea (Thermoplasmatales/Euryarchaeota together with  
509 Thaumarchaeota) were suggested to be important contributors to carbon and nitrogen cycles in  
510 microniches within the sediment. No overlap in archaeal phylotypes from Parys Mt and Carnoules  
511 Mine sediments could be observed, while the latter hosted archaea very distantly related with all  
512 cultured Thermoplasmatales (Volant et al., 2012). However, a relatively high similarity (about 97-  
513 98% SSU rRNA gene sequence identity) was recorded for reads from Carnoules Mine and Los  
514 Ruedos biofilm communities (Mendez-Garcia et al., 2014).

515 The bacterial component in Carnoules Mine included members of genera *Gallionella*, *Thiomonas*,  
516 *Acidithiobacillus* and *Acidiphilium*, all of which are indicative to pH values higher than in Parys  
517 Mt sediment (Bruneel et al., 2011).



518 Low pH favours the presence of other extremely acidophilic microorganisms, e.g. *Leptospillum*  
519 spp. in the sediment samples. These organisms were shown to be completely absent in anoxic and  
520 higher pH sediments of Rio Tinto (Sanchez-Andrea et al., 2011, 2012). Other bacterial groups  
521 were found to be rather typical and characteristic for AMD sediments. Probably the lack of  
522 Bacteroidetes could be noted as a discrepancy in this context, because of the oxic conditions being  
523 inhibitory to the acidophilic members of this phylum. Other bacteria presented in large quantities  
524 in sedimental microniches, such as Gammaproteobacteria (*Acidibacter ferrireducens*,  
525 *Metallibacterium scheffleri* and RCP1-48 group) together with Actinobacteria (*Aciditerrimonas*,  
526 *Acidimicrobium* and *Ferrimicrobium*) point at the importance of iron metabolism in this  
527 ecosystem. Furthermore, their involvement in heterotrophic and autotrophic loops of the carbon  
528 cycle Parys Mt sediment is supported by presence of these very phylotypes. Apart from these  
529 microorganisms, Sulfo bacillaceae and Alicyclobacillaceae families might take part in carbon and  
530 iron transformations in Parys Mt, which was also found in AMD sediments in other locations  
531 (Sanchez-Andrea et al., 2011, 2012).

532 Interestingly, the high abundance of particular archaeal taxa of the order Thermoplasmatales in  
533 Parys Mt sediments occurred across all samples, independently of variations in pH, Eh and depth.  
534 However, once again, this group of organisms and overall the archaeal members of low-pH  
535 environments are significantly lagging behind their much better metabolically characterised  
536 bacterial counterparts. This is primarily associated with the difficulties of cultivation of archaea,  
537 for which (i.e. for the vast majority of members of Thermoplasmatales) only genome-informed  
538 predictions of metabolic traits are available. We suggest that the lifestyles and ecological roles of  
539 archaea in sediments of Parys Mt are based on the degradation of organic compounds from primary  
540 producers and e.g. scavenging protein/polypeptide-rich biomass detritus and on the inorganic iron  
541 and sulfur compounds conversions. Further research is needed to understand the contribution of  
542 particular archaeal organisms inhabiting this ecosystem.

543

## 544 **Conclusions**

545 The environmental conditions in Parys Mt sediment underlying the AMD stream determined the  
546 make-up of the microbial community with a large proportion of Thermoplasmatales archaea,  
547 which were abundant at various depths and sediment layers. Bacterial community members,  
548 generally less abundant than archaea, varied in numbers more significantly across different depths,  
549 their taxonomic affiliations pointed at their involvement in metabolism of carbon, iron and sulfur  
550 elements. The decisive factors favouring high archaeal numbers are the low pH (1.7-2.4), the  
551 positive redox potential, availability of carbon sources (polypeptides-rich detritus/dead biomass),  
552 electron donors (ferrous iron, sulfur compounds or carbon) and acceptors (ferric iron and oxygen).

553 Importantly, a positive relationship was identified between Fe, As, Cr and Mn contents and archaeal  
554 abundance, which points towards a strong tolerance of Thermoplasmatales to the high  
555 concentrations of dissolved metals and metalloids. Significant numbers of archaea in AMD  
556 sediments and the ubiquity of similar systems on our planet suggest Thermoplasmatales may have  
557 a greater impact on the global carbon, sulfur and iron cycling than currently assumed. Further  
558 efforts are required to investigate their roles in the environment through cultivation and omics-  
559 driven analyses of their physiology and metabolism.

560

#### 561 **Data Availability Statement**

562 The datasets presented in this study can be found in online repositories. The names of  
563 repository/repositories and accession number(s) can be found in the article/**Supplementary**  
564 **Material**.

565

#### 566 **Author contributions**

567 PG and OG conception of the work; PG, MD, GW, and FB undertook the field and laboratory  
568 work. FB, SW and DJ undertook the chemical analysis, MD, EL and RB acquisition of the data  
569 for the work. RB, EL, ST, MY, PG, and OG interpretation of the data for the work. OG, RB, and  
570 PG drafted the manuscript with further contribution from all authors. All authors contributed to  
571 the article and approved the submitted version.

572

#### 573 **Conflicts of interest**

574 The authors declare no conflict of interest.

575

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584

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834

### 835 **Figure Captions**

836

837 **Figure 1.** PCA including all chemical parameters Analysis by Principal Components Analysis  
838 (PCA) of the influence of all chemical properties measured on the three cores. Contribution of  
839 each variable (chemical properties) to this graphical representation is shown by a color key from  
840 medium grey (less contribution) to violet (highest contribution). Ellipses and open dots represent  
841 the variance and mean for each core, respectively. Anion concentrations are showing the highest  
842 percentages of contribution due to the higher figures on these values for measured on layer 1.3.2,  
843 which is disrupting the variance (ellipse) corresponding to Core 1.

844

845 **Figure 2.** Relative abundance of various taxonomic groups in Parys Mt sediments. OTUs found  
846 after analysis of the sequencing results were grouped by lineage on those most abundant taxons,  
847 from lowest to higher levels, with genus as the basic clustering level where possible. The final  
848 table was generated with 30 taxonomic groups. From this table, a balls diagram was produced  
849 showing the relative abundance of these taxonomic groups.

850

851 **Figure 3.** Phylogenetic tree of Archaea. The tree was developed to include the most abundant  
852 OTU (>100 reads) sequences found along the three cores. Bootstrap values are shown on main  
853 parental nodes, where open dots represent bootstrap values under 80, while closed black dots  
854 represent values equal or higher to 80. OTU sequences are represented by coloured squares  
855 corresponding to their assigned taxonomy (see bioinformatics analysis in the Materials and  
856 Methods section), while size corresponds to their relative abundance (%) relative to the amount of  
857 Archaea present. Reference sequences are represented by their accession number on Genbank or  
858 IMG/M system.

859

860 **Figure 4.** NMDS based on the taxonomic profiles in each sediment core. A: NMDS regarding the  
861 distribution of the whole community. B: NMDS regarding only distribution of *Archaea*. Grey  
862 squares show the relative abundance of each taxonomic group in all layers on the three cores. Open  
863 dots show the mean of each layer while ellipse lines are based on the variance observed among  
864 each group of layers on each core. Stress level of analysis return a value of 0.118 and 0.108, which  
865 is considered a good or very good model adjustment over the 2D plane.

866

867 **Figure 5.** Canonical correlation analysis between chemical variables and microbial community.  
868 Panel showing the CCorA among Taxonomy distribution, chemical parameters and the samples  
869 representation over the canonical variates. Top panels are separate representations of variables (A)  
870 and samples (B). Below, same both representations overlapped adding the relative abundance of  
871 each taxonomic group along the whole core (C).

872