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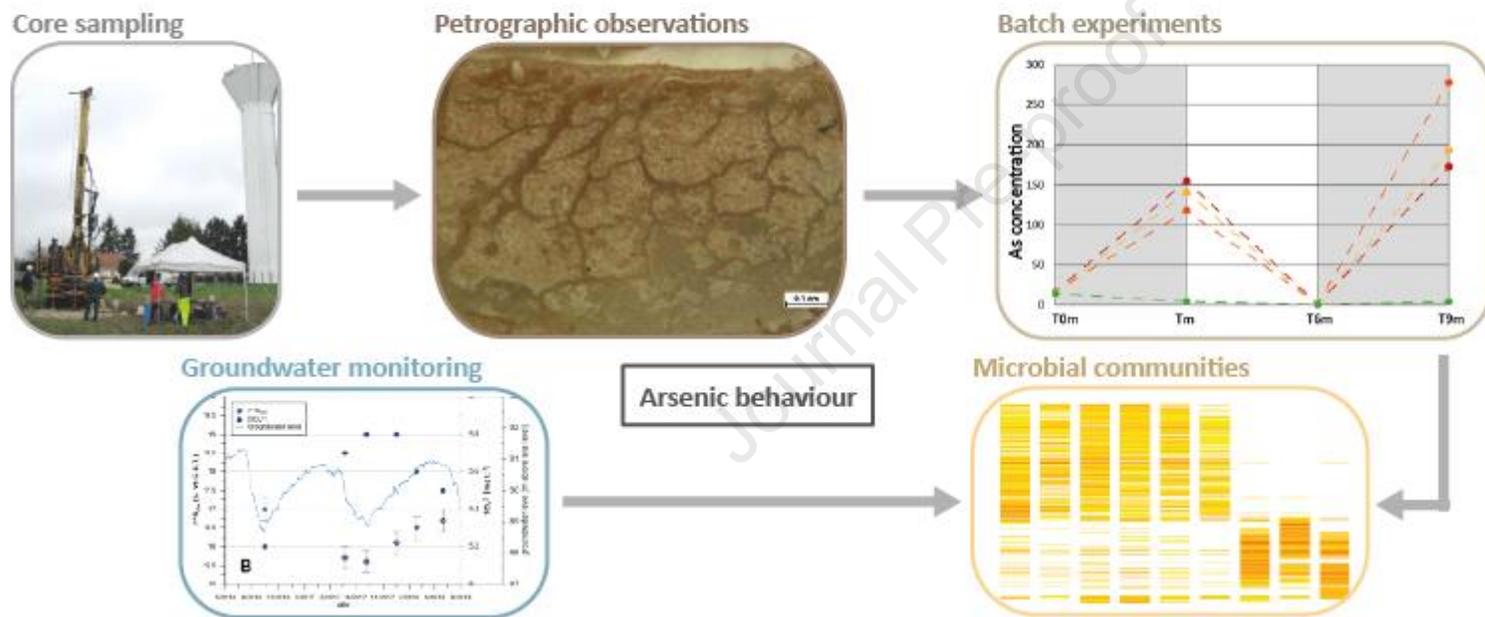
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1 **Biogeochemical behaviour of geogenic As in a confined aquifer of the Sologne**
2 **region, France**

3

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13 **ABSTRACT**

14 Arsenic (As) is one of the main toxic elements of geogenic origin that impact groundwater
15 quality and human health worldwide. In some groundwater wells of the Sologne region (Val de
16 Loire, France), drilled in a confined aquifer, As concentrations exceed the European drinking
17 water standard (10 $\mu\text{g L}^{-1}$). The monitoring of one of these drinking water wells showed As
18 concentrations in the range 20 to 25 $\mu\text{g L}^{-1}$. The presence of dissolved iron (Fe), low oxygen
19 concentration and traces of ammonium indicated reducing conditions. The $\delta^{34}\text{S}_{\text{SO}_4}$ was
20 anticorrelated with sulphate concentration. Drilling allowed to collect detrital material
21 corresponding to a Miocene floodplain and crevasse splay with preserved plant debris. The
22 level that contained the highest total As concentration was a silty-sandy clay containing 26.9
23 mg kg^{-1} As. The influence of alternating redox conditions on the behaviour of As was studied
24 by incubating this material with site groundwater, in biotic or inhibited bacterial activities

25 conditions, without synthetic organic nutrient supply, in presence of H₂ during the reducing
26 periods. The development of both AsV-reducing and AsIII-oxidising microorganisms in biotic
27 conditions was evidenced. At the end of the reducing periods, total As concentration strongly
28 increased in biotic conditions. The microflora influenced As speciation, released Fe and
29 consumed nitrate and sulphate in the water phase. Microbial communities observed in
30 groundwater samples strongly differed from those obtained at the end of the incubation
31 experiment, this result being potentially related to influence of the sediment compartment and
32 to different physico-chemical conditions. However, both included major Operating Taxonomic
33 Units (OTU) potentially involved in Fe and S biogeochemical cycles. Methanogens emerged in the
34 incubated sediment presenting the highest solubilised As and Fe. Results support the hypothesis
35 of in-situ As mobilisation and speciation mediated by active biogeochemical processes.

36 **Key words:** groundwater, arsenic, speciation, microbial communities, sediment incubation,
37 changing redox

38

39

40 **1. Introduction**

41 The presence of arsenic (As) in groundwater is a widespread environmental issue which
42 consequences affect millions of people's health (Ravenscroft et al., 2009; Chakraborti et al.,
43 2016). Chronic exposure to As was reported to increase the risk of a range of illnesses including
44 cancer (Schmidt, 2014; Tsuji et al., 2019). High As concentrations exceeding the European
45 drinking water standards (10 µg L⁻¹, EU Council Directive 98/83/EC) are generally linked to
46 geological context in many regions of the world (Podgorski and Berg, 2020). This toxic element
47 presents several oxidation states and compounds potentially changed by microbial activities
48 according to redox conditions. Arsenic release in several sedimentary and alluvial aquifers is

49 linked with low redox conditions (Ravencroft et al. 2009; Erickson et al., 2019) and reductive
50 reactions catalysed by microbial processes (Deng et al., 2018). These bio-reactions are linked
51 to the availability of electron donors, generally organic matter. Several studies reported the
52 mobilisation of As from underground sediments fuelled by organic pollutants plumes (Erickson
53 et al., 2016; Ziegler et al., 2017). However, the natural organic matter present in sediments,
54 found both in active and ancient floodplains, also plays the role of electron donor in reactions
55 that solubilise As (Sahu and Saha, 2015; Johannesson et al., 2019). The reductive dissolution
56 of As-bearing FeIII oxides minerals was evidenced as one of the main bio-processes able to
57 release As from sediments to groundwater (Welch et al., 2000; Nath et al., 2009; Duan et al.,
58 2019). However, sulphur biogeochemistry may also influence As behavior: dissolved sulphide can
59 contribute to FeIII reduction (Wang et al., 2014) and As may be adsorbed on FeS precipitates.
60 Moreover, the formation of soluble thio-As complexes may increase As mobility through
61 decreasing the adsorption of As on solid oxides (Pi et al., 2018). Several studies described the
62 strong link between As, Fe and S biogeochemical cycles and As mobility and proposed
63 geochemical models (Wang et al., 2014; Pi et al., 2018). Influence of the bio-reduction of iron
64 oxides and sulphate on As concentrations, related with fluctuations of groundwater level and
65 redox conditions, was observed (Schaefer et al., 2016; Zheng et al., 2020). Functional diversity
66 of As-rich groundwater supported these hypotheses by the presence of microorganisms able to
67 reduce AsV, either through AsV respiration or resistance mechanisms, AsIII-oxidising bacteria,
68 FeIII-reducing and SO₄-reducing bacteria (Paul et al., 2015; Cavalca et al., 2019; Zheng et al.,
69 2019; Wang et al., 2021). In addition to field studies, a range of laboratory experiments were
70 focused on the elucidation of biogeochemical processes in sediments from floodplains and
71 deltas. Mechanisms of As release were studied through long-term (Radloff et al., 2007; Gillipsie
72 et al., 2016; Duan et al., 2019; Gao et al., 2021) or short-term (Schaefer et al., 2016) incubations
73 of underground sediments. Electron donors supply generally enhanced As release in biotic and

74 anoxic conditions (Gillipsie et al., 2016; Wang et al., 2017; Deng et al., 2018), however results
75 strongly depended on the sediment geochemistry (Deng et al., 2018). Arsenic mobility was
76 hampered in presence of oxygen (Radloff et al., 2007) and mobilised during anoxic periods
77 when redox conditions were alternated (Duan et al., 2019). Microbial communities of incubated
78 sediments were rarely described, although studies performed with core materials from the
79 alluvial Jiangnan plain in the middle reaches of the Yangtze River (China) indicated the
80 presence of both FeIII- and SO₄-reducing bacteria in anaerobic microcosms showing As release
81 (Duan et al., 2019; Gao et al., 2021). However, complementary information on the influence of
82 microbial community structure and activity on the amplitude of the bio-reactions mobilising As
83 would help to improve models that predict changes in groundwater quality. In this context, the
84 present research aimed to clarify the links between microbial processes and As release in
85 groundwater, through the example of the Sologne region (Val de Loire, France), where As
86 concentrations exceeding 10 µg L⁻¹ were detected in 43 wells out of 135 that have been
87 monitored (Supplementary Figure 1, <https://ades.eaufrance.fr/>), distributed in an area of
88 approximately 5 000 km², in a geological context of Miocene sedimentary formation (Cary et
89 al., 2018). A drinking water well was monitored for two years, in order to study the evolution
90 of water parameters. In parallel, core sediment material was characterised for mineralogy and
91 geochemistry, then incubated in alternating redox conditions. Determination of microbial
92 indicators and comparison of the evolution of physico-chemical parameters in biotic and abiotic
93 conditions were performed to enlighten the biogeochemical processes influencing the mobility
94 and speciation of arsenic.

95 **2. Materials and Methods**

96 *2.1. Study site and sampling*

97 The sampling site is located on the territory of Marcilly-en-Gault village, in Sologne
98 (Supplementary Figure 1), a wetland-rich region in the center of France, South of Orleans city,

99 where sands and clays of continental detrital origin (Burdigalian, Miocene) cover the calcareous
100 aquifer of the Orléanais (Aquitanian, Miocene, Deprez and Martin, 1970). Water samplings
101 were performed in the drinking water well (reference BSS 04611X0001/FAEP, BSS001FRCU,
102 <http://ficheinfoterre.brgm.fr/InfoterreFiche/ficheBss.action?id=04611X0001/FAEP>), drilled in
103 1965, at 90.2 m total depth, the water level being at 21 m depth (without pumping) and the
104 pumping performed at 70 m depth (from the ground surface). Total historical regulatory
105 monitoring since 1986 indicates median As concentration of 23.0 $\mu\text{g L}^{-1}$ with a standard
106 deviation of 4.7 $\mu\text{g L}^{-1}$. Well water was sampled after pumping sufficiently to reach stable
107 values of pH, redox potential, dissolved oxygen concentration and conductivity. Five sampling
108 campaigns were performed at the following dates: June 2017 23th; September 2017 4th;
109 December 2017 18th; February 2018 27th, May 2018 29th. Water samples were filtrated at 0.45
110 μm (except for total organic carbon) and conditioned for further chemical analyses
111 (Supplementary table 1).

112 For molecular microbiological analyses, 10 L of groundwater were sampled at each sampling
113 date in 2 L sterile polyethylene bottles, kept on ice until filtration at the laboratory within 12
114 hours after sampling. Water samples were filtrated on sterile 0.22 μm nitrocellulose filters, that
115 were immediately stored at -20°C until microbial DNA extraction.

116 Core sampling was performed about 100 m North from the location of the well, with an auger
117 drilling machine (diameter 152 mm) (Supplementary Figure 1B). Core was performed down to
118 48.5 m depth, in March 2017 (23-24th) without adding drilling fluid. Samples of each different
119 geological facies were subsampled on-site with an alcohol-washed spatula, and immediately
120 stored in sterile glass jars, flushed with N_2 , and kept on ice until storage at 4°C in the laboratory.
121 Jars for microbial analysis and experiments were always opened in sterile conditions. Samples
122 for chemical and mineralogical analyses were kept frozen (-20°C) until analyses.

123 *2.2. Mineralogical and petrographic analysis*

124 Core samples were lyophilised and milled below 200 μm . Powder samples (infra 50 μm
125 fraction) were analysed by X-ray diffraction (XRD) using a Phillips Panalytical X'Pert Pro
126 apparatus in the same experimental conditions as described by Bassil et al. (2016). Phase
127 identification was made with the X'pert HighScore software using JCPDS Pdf2 mineralogical
128 database (Supplementary Figure 5). After deconvolution and integration of the main peak area
129 of each phase using Fytk software, the semi-quantifications (Supplementary Table 5) were
130 realised by applying modified RIR method (Chung, 1975). Thin sections (26 mm \times 46 mm)
131 were consolidated after impregnation at room temperature and under atmospheric pressure with
132 a two-component epoxy resin (Araldite 2020) and 20% acetone. The thin sections were
133 observed using an optical polarised light microscope (Nikon Eclipse E600 POL).

134 2.3. Batch experiments

135 Incubations of core solids sampled at 31.5 m depth, named M8, were prepared in 1 L-Shott
136 bottles equipped with rubber stoppers and sterilised by autoclaving. Each batch was composed
137 of 60 g solids and 600 mL of groundwater from the drinking water well (sampled on May 2018
138 29th). One batch was prepared with M8 core material submitted to γ -irradiation (Dagneux,
139 France) in order to observe the evolution of the system when biological activities are inhibited
140 (IN condition). The water was used as it was pumped for biotic experiments (BIO condition),
141 and filtrated at 0.22 μm to remove bacteria for the IN condition. Three BIO flasks were prepared
142 by distributing a single slurry of core sediment mixed with site water (magnetic stirring) in 3
143 bottles with a 25 mL sterile pipette. Alternate redox conditions were applied as follows: 3
144 months in anaerobic conditions (T0m to T3m), followed by 3 months in aerobic conditions
145 (T3m to T6m) then again 3 months in anaerobic conditions (T6m to T9m). At the beginning of
146 each anaerobic periods, the gas phase composition was imposed at 75% N_2 , 25% H_2 , and 1.25%
147 CO_2 , at a total pressure of 1 bar over the atmospheric pressure. For establishing the intermediary
148 aerobic conditions, the flasks were opened in order to replace the $\text{N}_2/\text{H}_2/\text{CO}_2$ mixture by sterile

149 air. Flasks were incubated at 20°C under reciprocal agitation. Sampling at the end of each
150 anaerobic period was performed in a glovebox (N₂ atmosphere) under agitation, in order to
151 avoid change of the solids/water ratio. Just after sampling, pH, redox potential and conductivity
152 were immediately measured. Samples (10 mL) were filtrated at 0.22 µm in a sterile Hungate
153 tube filled with N₂ and kept at 4°C until As speciation. Samples (15 mL) filtrated at 0.45 µm
154 were acidified with HNO₃ for analyses of total As and major cations. Samples (15 mL) filtrated
155 at 0.45 µm but not acidified were kept for quantification of major anions (nitrate, sulphate).
156 Slurry samples (1 mL) were stored in sterile tubes for Most Probable Number (MPN) of As-
157 transforming microbes analyses. At the end of experiment, slurries were centrifuged in sterile
158 falcon tubes, and the pellets containing both bacteria attached on sediments and suspended in
159 water were stored at -20°C until microbial DNA extraction.

160 2.4. Chemical analyses

161 Methods for analysing major chemical species and trace elements in water from the site and
162 incubations are detailed in Supplementary Table 1. Separation of As species in groundwater
163 samples was performed on site (Kim et al., 2001). This method gives precise As^{III} and As^V
164 concentrations when only these two species are present, thus results are given as As^{III}-like and
165 As^V-like species, because other As forms might be present in groundwater. Isotopy of sulphur
166 ($\delta^{34}\text{S}$) was measured by continuous flow isotope ratio mass spectrometry (CF-IRMS) after
167 precipitation as BaSO₄ and reduction/pyrolysis (Kloppmann et al., 2018). The total elemental
168 composition of the core samples (Table 2) was determined, after digestion (HNO₃/HF), by ICP-
169 OES and ICP-MS in an accredited laboratory (CRPG-CNRS, Nancy). Organic carbon
170 concentrations were determined by CHNS analysis (Flash 2000 Thermo, IC2MP, France). Total
171 As and Se were determined by ICP-MS equipped with an octopole collision/reaction cell
172 (C/RC) (Agilent 7500ce) after HNO₃/HCl digestion. The C/RC was pressurised with helium
173 and hydrogen delivered at 1 and 4 mL min⁻¹. Analyses of major cations and anions in water

174 samples from incubation experiments were performed by ion chromatography equipped with
175 conductivity detectors (Professional IC Vario, Metrohm). Anions were separated with a
176 Metrosep A Supp 16 ionic resin column (150 mm × 4 mm) and cations with a Metrosep C6
177 (150 mm × 4 mm). Speciation of As in these samples was performed by HPLC (Agilent 1100
178 series HPLC pump) coupled to ICP-MS (Agilent 7500ce) with the same C/RC operating
179 conditions as described for total analysis. Chromatographic separation was carried on using an
180 anion exchange stationary phase (Agilent G3154-65001 column and G3154-65002 guard
181 column) with an ammonium nitrate mobile phase (20 mmol L⁻¹, 2.5% (v/v) methanol and pH
182 8.5 adjusted with ammonia), delivered at 1 mL min⁻¹ flow rate. The sample injection volume
183 was 100 µL. These analytical conditions allow the separation of AsIII, AsV and mono- and di-
184 methylated AsV species (MMA^V and DMA^V) (see example chromatogram in Supplementary
185 Figure 2).

186

187 2.5. Microbiological analyses

188 2.5.1. MPN of AsIII-oxidising and AsV-reducing bacteria

189 Most Probable Numbers (MPN) of active AsIII-oxidising and AsV-reducing microbes were
190 determined in slurries by the methods described in Thouin et al. (2016) and Thouin et al. (2018),
191 with the following modification: the pH of the culture media was adjusted to 7 in order to fit
192 with the groundwater pH.

193 2.5.2. Microbial DNA extraction

194 Microbial DNA was extracted from (i) the filters obtained from groundwater filtration and
195 stored at -20°C, cut into strips with a sterile scalpel just prior extraction, and (ii) the slurries
196 pellets from end of batch experiments. The FastDNA™ Spin Kit for Soil procedure (MP
197 Biomedicals) was followed, using a FastPrep-24™ instrument at a speed of 5 m s⁻¹ for 30 s.

198 DNA extract concentration was measured using the Quantifluor dsDNA sample kit and the
199 Quantus fluorimeter, according to the manufacturer's recommendations (Promega).

200 2.5.3. 16S rRNA, *aioA* and *arsB* genes copy number monitoring

201 Abundance of bacterial 16S rRNA genes and of *aioA* and *arsB* functional genes, encoding
202 respectively for the catalytic subunit of arsenite oxidase and the AsIII-efflux pump of Ars
203 resistance system, were performed by quantitative PCR (qPCR). The main characteristics of
204 qPCR reactive mixtures and programs are described in Supplementary Information 1,
205 Supplementary Table 2, and in Michel et al. (2021), Fernandez-Rojo et al. (2017) and Poirel et
206 al. (2013).

207 2.5.4. 16S rRNA gene metabarcoding

208 For next generation sequencing, amplicon libraries and sequences were generated by INRAE
209 Transfert (Narbonne, France). Briefly, the V4-V5 region of the gene coding 16S rRNA (bacteria
210 and archaea) was amplified using the barcoded, universal primer set 515WF/918WR (Wang et
211 al., 2009). PCR reactions were performed using AccuStart II PCR ToughMix kit and cleaned
212 (HighPrep PCR beads, Mokascience). Pools were submitted for sequencing on Illumina MiSeq
213 instrument at GeT-PlaGe (Auzeville, France). Sequences were processed using the FROGS
214 (v.3.2) bioinformatics pipeline (Escudié et al., 2018), implemented into the Genotoul platform
215 of the Galaxy server of Toulouse (details in Supplementary information 1). The raw datasets
216 are available on the European Nucleotide Archive system under project accession number
217 PRJEB53046.

218

219 2.5.5. Statistical analyses

220 Statistical tests were carried out using R 4.0.3 (www.r-project.org). Redundance analysis
221 (RDA) with the vegan R package, was performed to identify the effects of environmental
222 variables on bacterial composition structure, based on the relative abundance of the detected

223 OTUs in water sample from the temporal evolution. This analysis was calculated based on
224 Hellinger - transformed phylum abundance basis of 16S rRNA gene data (Borcard et al., 2011).
225 Environmental explanatory tested variables were standardised and the function ordistep in
226 vegan package was used to identify the significant explanatory forward environmental variable.
227 The significance of the RDA model was tested by ANOVA based on Monte Carlo test with 999
228 permutations. This analysis helps to determine the most influential factors and the extent that
229 various environmental parameters affected bacterial phylum, most abundant OTU composition.
230 Principal component analysis (PCA) was performed on biogeochemical parameters from the
231 batch experiment.

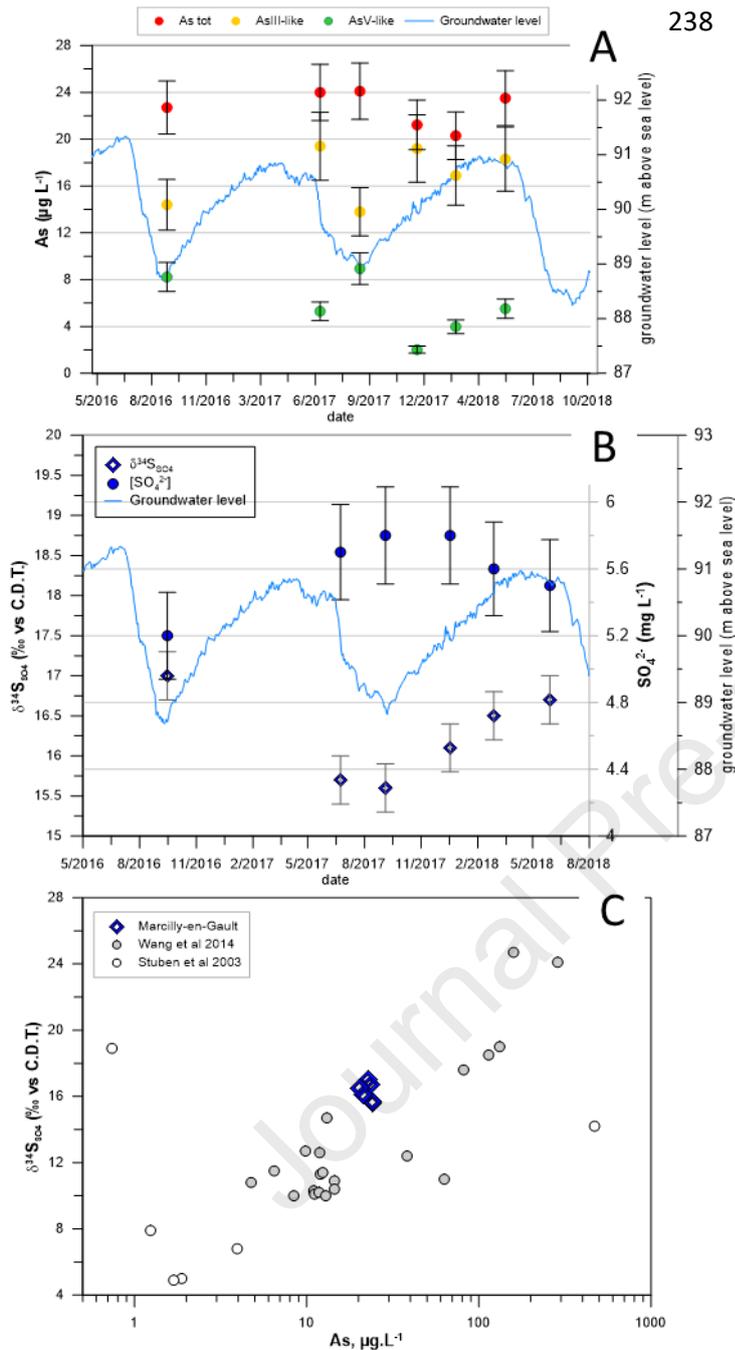
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233 **3. Results**

234 *3.1. Temporal monitoring of groundwater*

235 *3.1.1. Groundwater level and geochemistry*

236 The groundwater level variations (Fig. 1A and B) present well-marked annual cycles with high
237 level observed in late spring and the lowest level in late summer. The total amplitude of these



238 variations is in the range of 2 to 3 m.

239

240 **Fig. 1.** Evolution of groundwater geochemical parameters: (A) Total As (red) and As speciation
 241 i.e. on site separation of AsIII- (yellow) and AsV- (green) like species (error bars indicate the
 242 measurement uncertainty, and groundwater level (IGN French national altitude reference,
 243 <https://ades.eaufrance.fr/>) corresponding to the control well referenced 04612X0024/F
 244 (BSS001FRES) located 1.4 km from the drinking well; (B) $\delta^{34}\text{S}$ (open symbol) in SO_4 and SO_4
 245 concentration (closed circles), error bars indicate the analytical standard deviation; and

246 groundwater level and (C) evolution of $\delta^{34}\text{S}$ with total As concentration including data from
247 previous studies.

248
249 The total As concentration (Fig. 1A) remained between 20 and 25 $\mu\text{g L}^{-1}$ along the monitoring
250 period, with a mean of 23.6 $\mu\text{g L}^{-1}$ and a standard deviation of 1.6 $\mu\text{g L}^{-1}$, lower than the
251 measurement uncertainty (2.0-2.5 $\mu\text{g L}^{-1}$). The speciation of As seemed to be more variable
252 than total As concentration. Dissolved Fe concentration varied between 0.15 and 0.3 mg L^{-1} (\pm
253 35%), and SO_4 concentration between 5.2 and 5.8 mg L^{-1} (\pm 5%). Ammonium was always
254 detected, with concentrations generally in the range 0.07-0.1 mg L^{-1} (\pm 40%, Supplementary
255 Tables 1 and 3). For these three parameters, the temporal variations remained in the range of
256 the analytical measurement uncertainty. Results of isotopic analyses of $\text{SO}_4\text{-S}$ showed
257 variations of $\delta^{34}\text{S}$ higher than the range of analytical standard deviation (Fig. 1B).

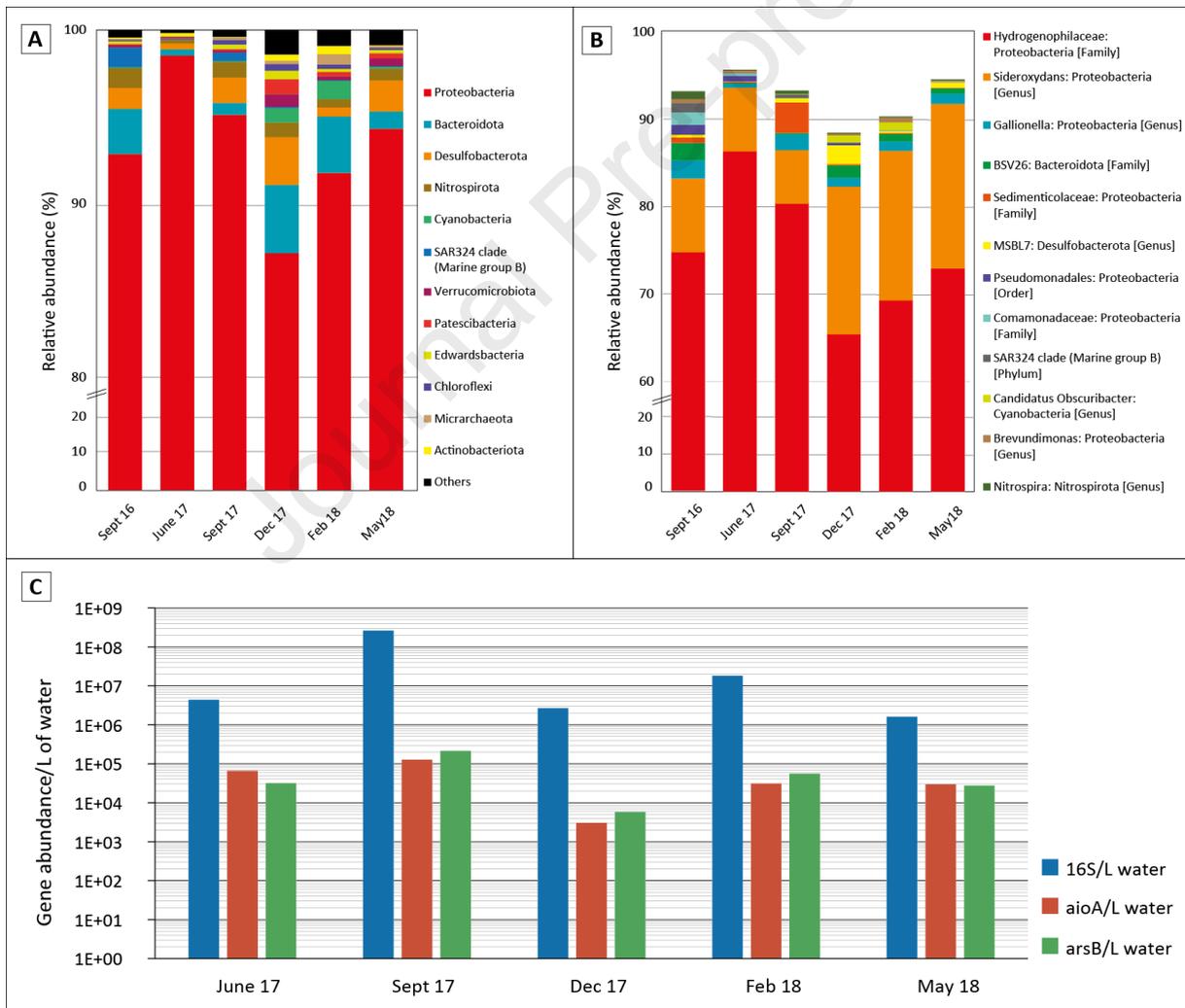
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259 3.1.2. Microbial parameters

260 Rarefaction curves (Supplementary Figure 3) together with richness and diversity indexes
261 (Supplementary Table 4) indicated a relatively low bacterial diversity. Bacterial communities
262 of the groundwater were composed of a majority of Proteobacteria (Fig. 2A). The most
263 abundant OTU found in groundwater was affiliated to Hydrogenophilaceae family, with an
264 unknown genus representing 69 to 86% of the total sequences (Fig. 2B). The second main OTU
265 belonged to the Gallionellaceae family, gathering autotrophic FeII-oxidisers (Hallbeck and
266 Pedersen, 2014). Autotrophic bacteria using FeII as energy source, identified at the genus level,
267 (*Gallionella*, *Ferriphaselus* and *Sideroxydans* genera), were always present in groundwater
268 samples (from 0.5 to 2.2 % of the sequences). Sulphate-reducing bacteria (SRB), including
269 *Desulfovibrio* and *Desulfatirhabdium* genera were always present but in small abundance (total
270 from 0.1 to 0.5 % of the sequences), and this abundance did not vary with seasons. Bacteria

271 involved in denitrification and able to use sulphur compounds as energy source, identified at
 272 the genus level (*Sulfuritalea*, *Denitratisoma*, *Sulfuricella*) were always present as minor OTUs
 273 (total from 0.01 to 0.1% of the sequences) in groundwater. The global abundance of the
 274 universal bacterial 16S rRNA gene did not follow a clear seasonal tendency (Fig. 2C). The two
 275 functional genes, i.e. *aioA* and *arsB* genes, involved in As biotransformations were always
 276 present, and their abundance ranged between 1.6×10^6 and 2.6×10^8 gene copies L^{-1} for *aioA*,
 277 and between 5.8×10^3 and 2.2×10^5 gene copies L^{-1} for *arsB*.

278

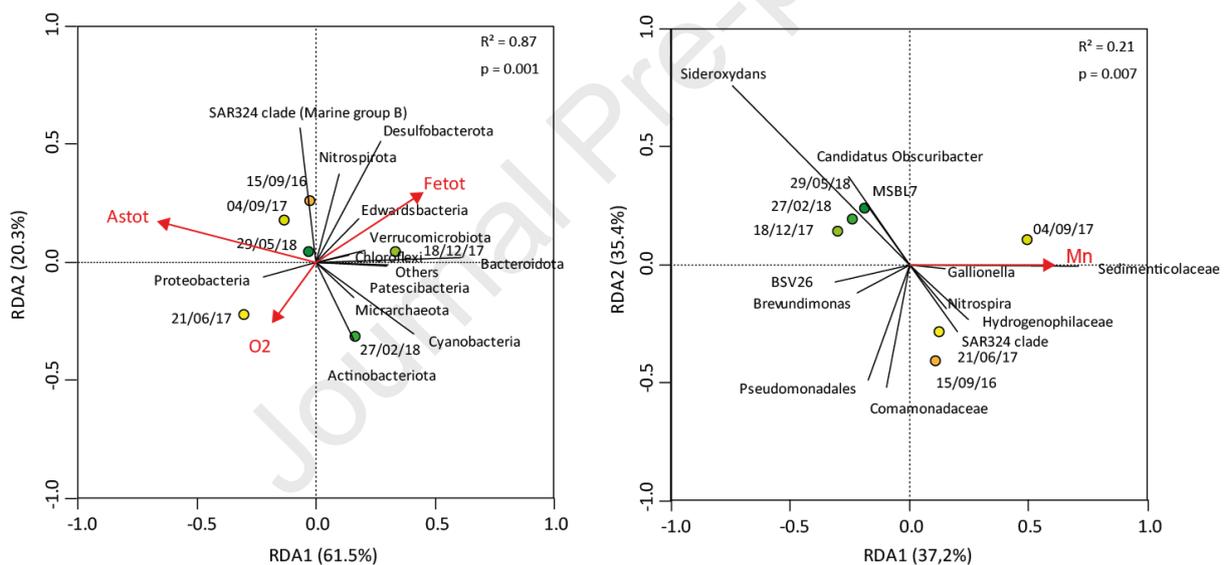


279

280 **Fig. 2.** Drinking water well of Marcilly-en-Gault, evolution of (A) main bacterial phyla; (B)
 281 most abundant OTUs; (C) total biomass (16S rRNA gene copies) and functional genes *aioA*
 282 and *arsB* copies respectively involved in the biological oxidation and reduction of arsenic.

283

284 A RDA was performed to identify the influence on microbial community structure of physico-
 285 chemical characteristics of groundwater (Fig. 3). The results indicated that total As, total Fe
 286 and dissolved oxygen concentration were explanatory variables that significantly influenced the
 287 community structure at the phylum level (Fig. 3A), whereas only Mn concentration
 288 significantly influenced the community structure at the genus level (Fig. 3B).



289

290 **Fig. 3.** RDA of sequence abundance assigned at phylum level (A) and genus level (B) of
 291 microbial communities in groundwater samples in relation to environmental variables. Red
 292 lines represent the significant environmental variables, black lines represent the phylum or
 293 genus and circles represent the samples, from orange to dark green following the chronology.

294 3.2. Solids description

295 3.2.1. Sedimentological description

296 The sedimentological description (Supplementary Figure 4) begins at 13.5 m depth; this section
297 shows at the base the dominant clays with roots traces and plant debris (48.0 m to 35.5 m).
298 These clays are followed by poorly sorted medium and coarse sands (35.5 m to 31.5 m). Finally,
299 the youngest levels (31.5 m to 13.6 m) correspond to an alternation of well-sorted medium
300 sands and clays with plant debris. The profile represents a part of “Sables et Argiles de
301 Sologne” formation (Desprez and Martin, 1970) that covers the aquifer limestone formations
302 “Marne de l’Orléanais” and “Calcaire de Pithiviers ». The sedimentary filling is characterised
303 by clays and heterogeneous sands (well to poorly sorted fine to coarse sands). These clays with
304 root traces, organic matter and plant debris correspond to floodplain deposits. The thick sandy
305 part (poorly-sorted) probably corresponds to meandering channel (river) and the isolated sandy
306 levels (well-sorted) in the floodplain clays corresponds to crevasse splay deposits.

307 3.2.2. Mineralogy

308 The mineralogy of the sediments was determined by XRD on 13 different samples
309 corresponding to 13 visually different geological layers, with one of the layer (M9, 37.5 m deep)
310 divided in two sub-samples (XRD spectra given in supplementary Figure 5). Total As
311 concentration was also determined on these samples (Supplementary Table 5). The upper 8
312 layers (M1 – M8) represent a floodplain clays with crevasse splay sands. Two samples, M7 and
313 M8 (floodplain and crevasse splay facies), present the two highest As concentrations of the
314 profile, (> 20 ppm), and relatively high clay content. These two layers are visually characterised
315 by different colours; blue for M7, and red-brown for M8. The M9 layer presents heterogeneous
316 facies, marly in the upper part (M9a). This level is close to the interface between two different
317 geological formations, the sands and clays of Sologne above, and the underlying calcareous
318 formation. The deeper samples (M10-M13) are mostly calcareous facies (M11 and M13) or
319 marl-calcareous (M10 and M12).

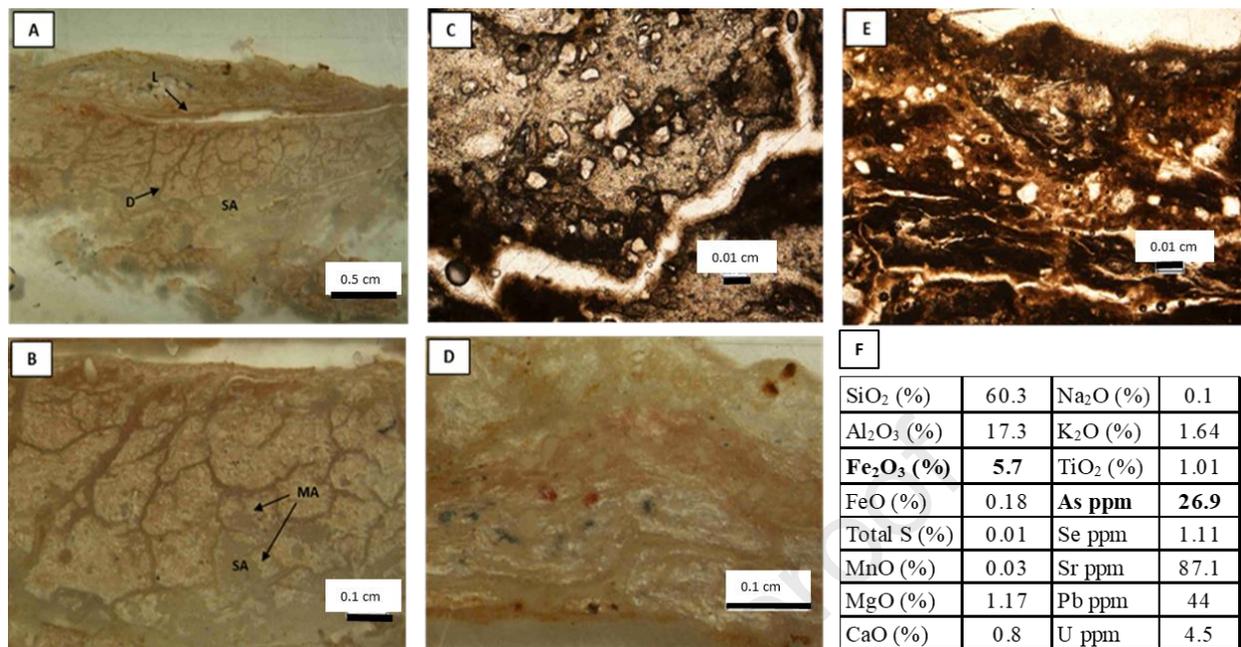
320 3.2.3. Chemical composition

321 Three samples, corresponding to the 2 clayey levels with high As concentrations (M7 and M8),
322 and one of the underlying calcareous levels (M12) were characterised in terms of major and
323 trace elements contents (Supplementary Table 6 and Fig. 4 for M8). These analysis confirmed
324 the siliceous feature of M7 and M8 compared with the characteristics of M12 that defined this
325 level as calcareous. Iron, sulphur and organic carbon contents were similar in the 3 samples,
326 with significant Fe contents but very low S and C concentrations. Arsenic and metals (Pb and
327 U) were enriched in M7 and M8 compared with M12.

328 *2.3.4. Petrographic description*

329 Petrographic observations of resin-impregnated M8 sample (Fig. 4) showed that the material
330 was mainly composed of beige siliceous clay showing numerous desiccation traces (arrow D in
331 Fig. 4A), and a laminar structure (arrow L in Fig. 4A). Desiccation cracks form sub-polygonal
332 structures where the siliceous matrix can be partially or entirely transformed into clay (Fig. 4B,
333 arrows MA), as indicated by the presence of remaining white matrix in clayey phases (Fig. 4C,
334 D, E). The laminations are composed of successive layers of clear siliceous material and clayey
335 beige material. Small black, dark brown and red pellets are observed inserted into the layers
336 (Fig. 4D).

337



338

339 **Fig. 4.** Microscopic petrographic observations of thin sections (A to D) and chemical
 340 composition (F) of M8 sample. Letters inside the pictures correspond to: SA:
 341 Siliceous/Argillaceous mud; L: Laminar structures; MA: Sub-polygonal structures (partly
 342 argillaceous siliceous matrix); D: Desiccation traces.

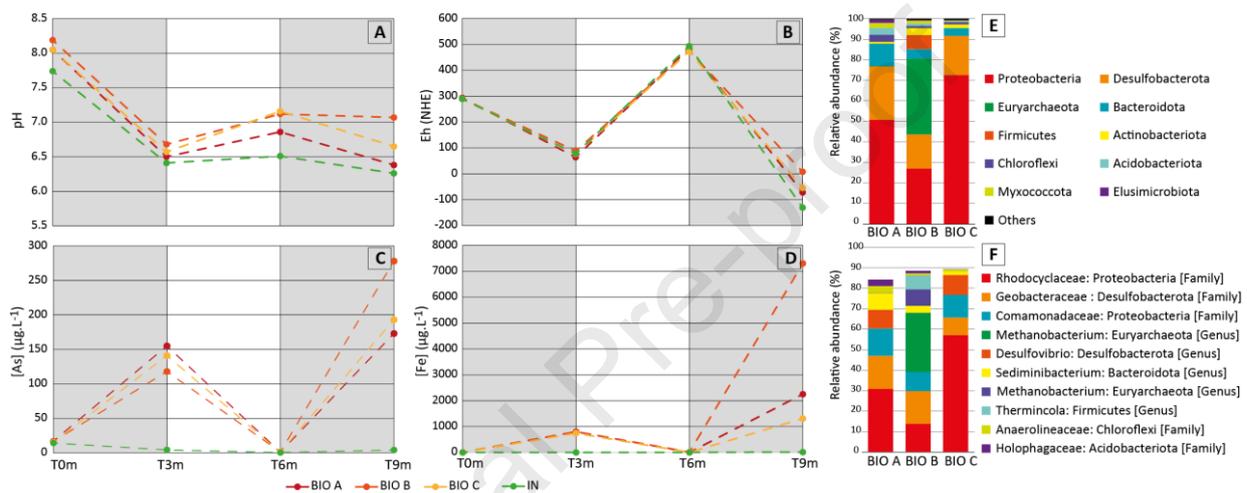
343 3.3. Batch experiments with M8

344 3.3.1. Water geochemistry

345 Total As concentrations increased during the anaerobic periods and decreased during the
 346 aerobic step in the BIO slurries (Fig. 5). These variations in As concentrations were not
 347 observed in the IN condition. At the end of the first anaerobic period (T3m), total As
 348 concentrations were in the range 100-150 $\mu\text{g L}^{-1}$ in BIO slurries, and the three parallel
 349 microcosms behaved in the same way. Conversely, at the end of the second anaerobic period
 350 (T9m), the BIO B slurry presented higher As concentration (275 $\mu\text{g L}^{-1}$) than A and C (between
 351 150 and 200 $\mu\text{g L}^{-1}$). Total dissolved Fe followed the same evolution as total As, with
 352 concentrations close to 900 $\mu\text{g L}^{-1}$ at the end of the first anaerobic period, and up to 7000 $\mu\text{g L}^{-1}$

353 ¹ in B slurry at the end of the second anaerobic period. The redox potential was lower at the end
 354 of the second anaerobic period in all flasks, independently from the presence of active bacteria,
 355 and pH was higher in BIO than in IN conditions all along the experiment. During the anaerobic
 356 periods, pH tended to decrease, probably because of CO₂ present in the gas phase. Acidification
 357 was less important in B slurry than in A and C, at the end of experiment.

358



359

360 **Fig. 5.** Evolution of pH (A), redox potential (Eh, B), total As (C) and Fe (D) concentrations in
 361 the incubated slurries; anaerobic periods are presented in grey background. IN: inhibited
 362 biological activities; BIO: microcosms with active biological activities. T0m: starting up; T3m:
 363 3 months; T6m: 6 months; T9m: 9 months. Major phyla (E) and 10 major OTUs (F) found in
 364 the BIO slurries at the end of the second anaerobic period (T9m).

365

366 The incubation conditions influenced the speciation of As in the water phase (Table 1). In the
 367 IN condition, As^{III} was the major initial species, then As^V became the main form of As until
 368 the end of experiment. In the BIO condition, at the end of the anaerobic periods, As-containing
 369 species were detected, with retention times that did not match any available standard. They
 370 could not be precisely quantified because of the lack of standards, however as estimated based

371 on AsV standard additions slope, they represented more than 50% of total As in A, B and C at
 372 the end of the first anaerobic period and in A and C at the end of the second anaerobic period.
 373 The unidentified As species were not detected at the end of the aerobic incubation period,
 374 however at the end of this experimental step, dimethyl-arsenate (DMA^V) was present in BIO
 375 condition, representing nearly 50% of total As. From the end of the first incubation period,
 376 dissolved As concentration remained very low but mainly in the form of AsV in the IN
 377 microcosm. This result might be linked to the adsorption of AsIII followed by abiotic oxidation
 378 at the surface of clay material (Lin and Puls, 2000).

379 **Table 1.** Total As, As speciation in the water phases of incubated slurries, and MPN of AsIII-
 380 oxidising and AsV-reducing microbes.

Time	Condition	Total As (µg L ⁻¹)	AsIII (%)	AsV (%)	DMA ^V (%)	Un. species 1* (%)	Un. species 2* (%)	Un. species 3* (%)	MPN AsIII-ox g ⁻¹ solids	MPN AsV-red g ⁻¹ solids
Beginning (T0m)	BIO A	17.0	40.0	60.0	ND	ND	ND	ND	< LQ	3.16×10 ³
	BIO B	16.7	37.2	62.8	ND	ND	ND	ND	< LQ	3.76×10 ³
	BIO C	15.3	36.9	63.1	ND	ND	ND	ND	8.00×10 ¹	9.20×10 ²
	IN	14.3	95.5	4.5	ND	ND	ND	ND	< LQ	< LQ
End of first anaerobic period (T3m)	BIO A	155.0	4.8	0.8	ND	6.8	8.6	79.0	1.84×10 ³	2.52×10 ³
	BIO B	118.0	4.5	0.7	ND	9.8	7.9	77.0	9.60×10 ³	5.20×10 ³
	BIO C	141.0	6.7	1.3	ND	7.7	9.0	75.3	3.60×10 ³	9.60×10 ⁴
	IN	4.4	3.5	96.5	ND	ND	ND	ND	< LQ	< LQ
End of aerobic period (T6m)	BIO A	1.2	1.8	44.6	53.6	ND	ND	ND	5.20×10 ⁵	9.60×10 ⁵
	BIO B	1.4	1.5	46.2	52.3	ND	ND	ND	1.32×10 ⁵	1.96×10 ⁵
	BIO C	1.1	1.7	50	48.3	ND	ND	ND	1.32×10 ⁵	5.20×10 ⁶
	IN	0.5	4.0	95.0	1.0	ND	ND	ND	< LQ	< LQ
End of second anaerobic period (T9m)	BIO A	173.0	14.1	0.9	ND	10.3	5.9	68.8	1.96×10 ⁵	3.68×10 ⁷
	BIO B	278.0	88.7	2.3	ND	8.5	0.5	ND	9.60×10 ⁵	6.80×10 ⁶
	BIO C	193.0	25.7	1.6	ND	5.8	6.3	60.6	8.80×10 ⁵	5.20×10 ⁶
	IN	4.2	10.6	89.4	ND	ND	ND	ND	< LQ	< LQ

381 ND: not detected, (*) unidentified As-containing species, estimated indicative values calculated with
382 AsV standard addition slope; LQ = $4.00 \times 10^1 \text{ g}^{-1}$.
383

384 Concentrations of Cl^- , Na^+ , Ca^{2+} and Mg^{2+} remained stable and identical in BIO and IN
385 conditions all along experiment (Supplementary Figure 6), whereas NO_3^- and SO_4^{2-} tended to
386 be more impacted by redox conditions in BIO than in IN condition, particularly between T0m
387 and T6m.

388

389 3.3.2. Microbial parameters

390 The quantification of As-transforming microbes by MPN showed the growth of active AsIII-
391 oxidising and AsV-reducing bacteria throughout the incubation (Table 1), independently of
392 aerobic or anaerobic periods, whereas in IN condition the quantity of As-transforming microbes
393 remained lower than the quantification limit all along the experiment. AsV-reducing bacteria
394 were always more abundant than AsIII-oxidising micro-organisms. At the end of the second
395 anaerobic period, the order of magnitude of MPN in sediments from parallel microcosms A, B
396 and C was 10^6 g^{-1} for AsIII-oxidising bacteria and 10^7 g^{-1} for AsV-reducing bacteria.

397 The PCA built with all physico-chemical and biological parameters measured during the
398 incubation (Supplementary Figure 7) illustrates the contrast between the evolution of IN and
399 BIO slurries. Arsenic concentration was correlated with Fe concentration, and negatively
400 correlated with sulphate concentration. Moreover, this analysis suggests an early divergence,
401 detectable at the end of the first incubation period, between the microcosm B and the two
402 microcosms A and C.

403 The bacterial diversity in the slurries at the end of the second anaerobic period (supplementary
404 Table 4) was higher than that of the groundwater bacterial communities, in spite of a lower
405 richness. The corresponding 16S rRNA gene sequences (Fig. 5 E and F) showed close diversity

406 profiles in batches A and C, that differed from batch B that contained Euryarchaeota (Archaea
407 domain) in higher abundance, with a significant proportion of sequences affiliated to
408 methanogenic micro-organisms. All slurries contained, as major OTUs, sequences that were
409 only affiliated at the family level, first to Rhodocyclaceae, then to Geobacteraceae. Sum of
410 sulphate-reducers sequences (Supplementary Table 7) represented nearly 10% in A and C, but
411 only 0.3% in B. Conversely, sum of methanogens sequences represented 37% of the sequences
412 in B, but less than 0.2% in A and were not detected in C.

413

414 **4. Discussion**

415 *4.1. Geological context and origin of As in the confined aquifer*

416 Macroscopic and microscopic observations of the core material suggest that the sands and clays
417 covering the calcareous confined aquifer were deposited in a meandering river system
418 environment composed of channels, crevasse splay and floodplain facies. These last two facies
419 are enriched in As and other trace elements. The As enrichment in sedimentary facies linked
420 with high groundwater As concentrations was already reported (Chatterjee et al., 2003; Sahu
421 and Saha, 2015). In these formations, As-rich levels are generally described as containing
422 elevated organic matter concentrations. Here, the clayey levels presenting the highest As
423 contents displayed low organic carbon concentrations (0.01 to 0.02%). However, macroscopic
424 observation of plant debris in the core suggested that organic matter is present but distributed
425 in nuggets.

426

427 *4.2. As release mechanisms implying Fe and S cycles*

428 The geochemical composition of the confined groundwater, with absence of nitrate (< 0.5 mg
429 L^{-1}) and presence of ammonium, dissolved Mn and Fe, suggests anaerobic conditions. The
430 dissolved O_2 concentrations (0.9 to 2.3 mg L^{-1}) might be related to the diffusion of this gas into
431 the well which creates local suboxic conditions. Low concentrations of SO_4^{2-} associated with
432 high $\delta^{34}S$ of sulphate (mean of 16.3 ‰) would be consistent with in-situ sulphate reduction
433 (Stueben et al., 2003; Wang et al., 2014). The evolution of the $\delta^{34}S_{SO_4}$ of the water sampled in
434 the drinking water well of Marcilly-en-Gault according to total sulphate concentration suggests
435 variations of the in-situ activity of SRB, that might be influenced by evolution of redox
436 conditions or organic matter supply. These $\delta^{34}S$ values are also associated to notable
437 concentrations of As (mean 22.6 $\mu g L^{-1}$) as already described by various authors e. g., (Fig.
438 1C; Pi et al., 2018, Stueben et al., 2003, Wang et al., 2014). In a complementary way, the
439 development of active As-transforming microbes during batch incubations confirmed the
440 presence of living micro-organisms in the confined aquifer. These results support the hypothesis
441 of active biogeochemical reactions influencing the release and speciation of As in groundwater.

442 A link between biogeochemical processes and As mobilisation from sediments to groundwater,
443 implying diverse mechanisms and especially iron and sulphur cycles, was evidenced in several
444 other sites. The mechanism most often at the origin of As release is the biological reduction of
445 Fe oxides (Stueben et al., 2003; Zheng et al., 2004; Nath et al., 2009; Erickson et al., 2016;
446 Ziegler et al., 2017; Johannesson et al., 2019). The confined groundwater of Marcilly-en-Gault
447 contains dissolved Fe, the analysis of core materials indicated the presence of FeIII in the As-
448 rich levels, and our slurry incubation experiment showed As and Fe release during anaerobic
449 periods (Figure 5), and correlation between these two parameters (Supplementary Figure 7).
450 These results support the hypothesis that Fe oxides reduction plays a major role in the release
451 of As. Sulphate reduction was also invoked in previous studies as possibly contributing to As
452 release through (1) indirect chemical reduction of Fe oxides by H_2S , and (2) the formation of

453 soluble thio-arsenate complexes (Wang et al., 2014; Gao et al., 2021). These processes might
454 have modestly contributed to As mobility in our microcosms. Pi et al. (2018) observed profiles
455 of biogeochemical Fe reduction and sulphate reduction controlling both As speciation and
456 concentration in groundwater of Datong basin, China. They hypothesised that vertical
457 variations of As concentrations and forms were associated with availability of organic matter,
458 providing energy to microbial reactions. Our incubations of core materials showed that bacterial
459 activities influenced As speciation, producing As species different from AsIII and AsV. In
460 anaerobic conditions, non-identified species might correspond to thio-arsenate complexes that
461 were already associated with bacterial sulphate reduction in core sediments microcosms (Gao
462 et al., 2021). In aerobic conditions, up to 50% of total As was present as DMA^V, a biologically
463 produced methylated species (Dombrowski et al., 2005). Yet, these species exclusively
464 produced when bacterial communities are active could be less efficiently adsorbed on Fe oxides
465 surfaces than AsV or AsIII (Lafferty and Loeppert, 2005; Couture et al., 2013). Thus, in
466 addition to the reduction of iron oxides, bio-reduction of sulphate and direct As transformation
467 by microbes could contribute to the mobility of this toxic element.

468

469 *4.3. Microbial assemblages*

470 *4.3.1. Groundwater*

471 The main major OTU found in Marcilly-en-Gault groundwater was affiliated to
472 Hydrogenophilaceae family, currently composed of 5 genera, *Thiobacillus*, *Hydrogenophilus*,
473 *Sulfuricella*, *Petrobacter*, and *Tepidiphilus* (Garrity et al., 2005; Orlygsson and Kristjansson,
474 2014). This family includes chemolithotrophic or mixotrophic bacteria able to oxidise inorganic
475 substrates, such as sulphur compounds or hydrogen, possibly using nitrate as electron acceptor
476 (*Sulfuricella*, Kojima and Fukui, 2010). The second main metabolic group found in

477 groundwater is composed of OTUs affiliated to Galliolenaceae, gathering autotrophic
478 microorganisms oxidising FeII in micro-aerophilic environments. Their abundance in the
479 sampled groundwater is consistent with the presence of dissolved Fe, and it might be assumed
480 that they could form biofilms onto surfaces of the drinking water well (Stuetz and McLaughlan,
481 2004). They may also grow in areas of the aquifer where small quantities of oxygen could
482 diffuse or be transported. Glodowska et al. (2021) already quantified non negligible proportions
483 of Galliolenaceae and other putative FeII-oxidisers in As-bearing groundwater where they are
484 assumed to favour As immobilisation with FeIII oxides formation in environments of low-
485 oxygen availability. Bacteria that could contribute directly or indirectly to iron oxides reduction,
486 such a sulphate reducers (Desulfurivibrionaceae) and FeIII reducers (*Georgfuchsia*) were
487 always detected in groundwater samples, but in relatively low abundance. Interestingly,
488 *Georgfuchsia* was described as able to degrade aromatic compounds (Weelink et al., 2009), that
489 could be an asset to use organic molecules derived from buried plant debris. *Sphingopyxis*,
490 another heterotroph found in the groundwater, is also known for its ability to degrade complex
491 molecules (Sharma et al., 2021).

492 4.3.2. Batch experiments

493 The microbial communities retrieved at the end of the incubation experiment clearly differed
494 from those observed in groundwater samples. This difference can be explained by the strong
495 reducing conditions and hydrogen supply during the last incubation period, while in the
496 drinking well, diffusion of oxygen could create microaerophilic conditions, specific to the local
497 environment of this water source. Moreover, the sediment is a source of nutrients and carries
498 some bacteria. Indeed, bacterial communities present in the water phase of aquifers may differ
499 from those attached to solid phases (Smith et al., 2018). In these incubated slurries, the two
500 most abundant OTUs were affiliated, at the family level, with Rhodocyclaceae and
501 Geobacteraceae. Rhodocyclaceae family includes many genera presenting diverse

502 metabolisms; thus it is hardly possible to infer a role of members of this OTU. However, a
503 recent report of microcosm experiment performed with groundwater and sediments supplied
504 with hydrogen also led to enrichment of Rhodocyclaceae that could be related to the genus
505 *Dechloromonas*, a hydrogenotrophic denitrifier (Duffner et al., 2021). This OTU was clearly
506 enriched during incubation, but was also present in all groundwater samples, in small
507 abundance. Geobacteraceae members share the ability to use FeIII as terminal electron acceptor
508 and can use either small organic compounds or hydrogen as electron source (Röling, 2014).
509 Their presence as a major OTU in our system can be related with the Fe oxides dissolution
510 process which could mobilise As. This OTU was very rare in the groundwater samples, only
511 sparsely detected in September 2016 and May 2018. Incubation also led to enrichment of
512 Comamonadaceae, a very metabolically diverse family. The next groups of less abundant but
513 major OTUs marked differences between microcosms: they included either SRB in A and C,
514 or methanogens in B. This strong contrast between B compared with A/C microbial
515 communities might be considered in the light of the difference in final geochemical profiles of
516 the slurries: B presented much more dissolved Fe and As than A and C. In terms of sulphate
517 concentrations, the three microcosms behaved similarly. The divergence of results in B might
518 be linked to the initial heterogeneity of the solid phase inherent in the nature of the actual
519 material that induced heterogeneity in initial microbial communities. Such phenomenon was
520 previously reported in parallel slurries of underground sediment mixed with ferrihydrite (Kwon
521 et al., 2016) which evolved differently in terms of geochemistry and microbial communities,
522 this result being related with subtle differences in the initial composition of the microbial
523 communities. High As concentrations in zones of active methanogenic activities have already
524 been reported (Wang et al., 2015; Glodowska et al., 2021) whereas the biogeochemical link
525 between methanogenesis and As release is not clearly elucidated. According to Glodowska et
526 al. (2021), organic carbon input due to hydrogeological conditions fuels fermentation producing

527 hydrogen, thus, indirectly, methanogenic metabolisms and efficient reduction of As-bearing Fe
528 minerals. Methane oxidation might also be coupled to FeIII reduction. In our systems, hydrogen
529 was provided, thus could be consumed for both FeIII reduction and methanogenesis. One
530 important question would be the possible availability of hydrogen or other electron donors in
531 the confined aquifer. Previous studies showed that intrinsic organic matter of aquifer sediments
532 could fuel anaerobic respiration processes (Duan et al., 2019; Gao et al., 2021). However,
533 organic carbon concentration was very low in M8 sample, (0.02%) compared with that reported
534 in previous studies (0.16 to 0.70% in Duan et al., 2019; 0.22% in Gao et al., 2021). The classical
535 hypothesis of hydrogen production through fermentation of organic matter could hardly be
536 considered in our system except if nuggets of organic matter, such as the plant debris
537 macroscopically observed in the sedimentary profile, could be used as organic substrates. As a
538 fact, the microbial community of incubated slurries contained the fermentative genus
539 *Propionivibrio*, in low abundance (0.1 to 0.6%) but in all microcosms. Species of this genus,
540 belonging to Rhodocyclaceae, are aerotolerant anaerobes performing fermentation of aromatic
541 compounds (Brune et al., 2002); their emergence in the incubated slurries might indicate the
542 intrinsic organic matter could be metabolised.

543 *4.3.3. Linking microbial assemblages with biogeochemical processes*

544 SRB were always detected both in groundwater samples and microcosms. However, they were
545 present in relatively low abundance in groundwater, and in B microcosm in which the highest
546 release of As was observed. In-situ evolution of SO_4 and $\delta^{34}\text{S}$ and the development of SRB in
547 A and C slurries support the hypothesis of active sulphate-reduction in the confined aquifer.

548 Both groundwater and microcosms hosted microbial assemblages containing OTUs whose
549 known members are involved in iron and sulphur cycles, but only a few methanogens except in
550 one of the microcosms, where this metabolic group was abundant. All these types of microbial
551 metabolisms were already observed in other As-bearing groundwater systems (Table 2).

552 Globally, the major abundance of bacterial OTUs involved in Fe cycling, i.e. FeII oxidation in
 553 groundwater samples, and FeIII-reduction in all incubated slurries, strongly suggests a major
 554 role of Fe biogeocycle in the confined aquifer. The Geobacteraceae-affiliated OTU massively
 555 present at the end of slurry incubations, and putatively involved in Fe oxide reduction, was only
 556 sparsely detected in groundwater samples, possibly because they were not mobile but attached
 557 to solid phases, or because the local suboxic condition of the well was not suitable for their
 558 growth. However another OTU, affiliated to *Georgfuchsia*, a known FeIII reducer (Weelink et
 559 al., 2009), was present in low abundance but regularly along all seasons in groundwater.

560

561 **Table 2.** Main characters of microbial communities in arsenic-rich groundwater,
 562 groundwater-related sediments, and related laboratory experiments

Environments	Main OTUs (16S genus level)	Main detected functions	References
Groundwater, Inner Mongolia	<i>Pseudomonas</i> , <i>Acinetobacter</i> , <i>Brevundimonas</i> , <i>Aquabacterium</i> , <i>Psychrobacter</i> , <i>Geobacter</i> , <i>Arthrobacter</i> , <i>Massilia</i> , <i>Dietzia</i> , <i>Sphingomonas</i> , <i>Planococcus</i> , <i>Methanosaeta</i> , <i>Nitrosophaera</i> , <i>Thermoprotei</i>	Not mentioned	Li et al., 2013
Holocene and Pleistocene sediments, Cambodia	<i>Geobacter</i> , <i>Propionivibrio</i> , <i>Aeromonas</i> , <i>Acidobacteria</i> , <i>Pelagibius</i> , <i>Enterobacter</i> , <i>Pseudomonas</i> , <i>Sphingomonas</i> , <i>Pseudolabrys</i> , <i>Methylobacterium</i> , <i>Microvirga</i> , <i>Delftia</i> , <i>Tumebacillus</i> , <i>Euzebya</i> , <i>Dietzia</i>	Not mentioned	Hery et al., 2015

Groundwater, West Bengal, India	<i>Pseudomonas, Rhizobium, Brevundimonas, Rhodococcus, Rheinheimera, Phyllobacterium, Staphylococcus, Herbaspirillum, Acinetobacter, Arthrobacter, Bacillus, Stenotrophomonas, Hydrogenophaga</i>	As resistance, AsV respiration, AsIII oxidation, FeIII respiration, NO ₃ and SO ₄ respiration	Paul et al., 2015
Groundwater, Cremona, Italy	<i>Sphingopyxis, Sphingomonas, Thiobacillus, Methylophilus, Sulfuricurvum, Thiotrix, Pseudomonas, Azotobacter, Methylomonas Rhodobacter, Leptothrix, Geobacter, Geothrix, Ferribacterium</i>	AsIII oxidation, As resistance, AsV respiration (few), C fixation, FeIII-reduction	Cavalca et al., 2019
Groundwater, central Yangtze River Basin, China	<i>Sideroxydans, Gallionella, Geobacter, Methylothermus, Methylosinus, Methylomonas, Methylocaldum, Sulfurospirillum, Sulfuritaela, Methanospirillum, Methanosarcina, Methanosateia, Methanomassilicoccus, Methanobacterium, Desulfovibrio, Rhodoferrax, Pseudomonas, Microbacterium, Flavobacterium, Aquabacterium, Acidovorax</i>	FeII oxidation, FeIII reduction	Zheng et al., 2019

Groundwater, Inner Mongolia		AsV respiration, As resistance	Wang et al., 2021
Groundwater, Central Yangtze River basin, China		SO ₄ reduction (<i>dsrB</i> gene of <i>Desulfobulbus</i> , <i>Desulfocapsa</i> , <i>Desulfomonile</i> , <i>Desulfobacca</i> , <i>Desulfovibrio</i> , <i>Desulfurispora</i> , <i>Desulfatiglans</i>)	Zheng et al., 2020
Groundwater, Sologne, France	<i>Gallionella</i> , <i>Brevundimonas</i> , <i>Nitrospira</i> , <i>Georgfuchsia</i> , <i>Sphingopyxis</i> , <i>Perlucidibaca</i> , <i>Desulfurivibrio</i> , <i>Delftia</i> , <i>Sphingobium</i> , <i>Sediminibacterium</i> , <i>Magnetovibrio</i> , <i>Ferriphaseus</i>	AsIII-oxidation, AsV-reduction	Present study
Incubations with lactate + acetate, Holocene and Pleistocene sediments, Cambodia	<i>Geobacter</i> , <i>Sulfurospirillum</i> , <i>Desulfovibrio</i> , <i>Pelosinus</i> , <i>Hydrogenophaga</i> , <i>Dechloromonas</i> , <i>Desulfobulbaceae</i>	AsV respiration	Hery et al., 2015
Microcosms, Jiangnan Plain, China	<i>Pseudomonas</i> , <i>Pedobacter</i> , <i>Paenibacillus</i> , <i>Cellulomonas</i> , <i>Clostridium</i> , <i>Thiobacillus</i> , <i>Geobacter</i> , <i>Dechloromonas</i> , <i>Desulfosporosinus</i> , <i>Clostridium</i>	Sulphate reduction, FeIII-reduction, As resistance	Deng et al., 2018

Microcosms	<i>Desulfovibrio</i> , <i>Desulfomicrobium</i> , <i>Geobacter</i> , <i>Methanosarcina</i> , <i>Methanobacterium</i>	Sulphate- reduction, AsV respiration	Gao et al., 2021
Microcosms, Sologne, France	<i>Methanobacterium</i> , <i>Desulfovibrio</i> , <i>Sediminibacterium</i> , <i>Thermincola</i> , <i>Sulfuritalea</i> , <i>Lacibacter</i> , <i>Propionivibrio</i> , <i>Haliangium</i> , <i>Methyloversatilis</i> , <i>Sandaracinus</i>	AsIII- oxidation, AsV-reduction	Present study

563

564 **5. Conclusion**

565 The occurrence of As in the confined aquifer of Sologne appears to find its origins in anoxic
566 bioprocesses, as already described in other sedimentary paleo-environments. In particular, the
567 Fe oxides detected in As-rich floodplain and crevasse splay clays (fluvial environment)
568 covering the calcareous aquifer (lake environment) could be biologically reduced. The major
569 role of FeIII reduction in As release was supported by high Fe concentrations in water and solid
570 phases, and proportions of microbial groups involved in Fe bio-geocycle, both in groundwater
571 samples and microcosms. Other microbial reactions could play non negligible roles in As
572 mobilisation, namely sulphate reduction, AsV reduction, AsIII oxidation and As methylation.
573 Corresponding bacterial activities drive the production of diverse As species whose proportions
574 may fluctuate and influence the distribution of As between solids and water. Even if phenomena
575 globally followed the same patterns in all microcosms, our results underline the possibility of
576 strong variations in the amplitude of the bio-reactions driving As release from solids to water,
577 linked to subtle heterogeneities that induced important divergence of microbial communities
578 compositions. This phenomenon, generally ignored or underestimated, deserves to be explored
579 further to improve the accuracy of predictive models taking into account biogeochemical

580 reactions. In the context of global change and increasing anthropic pressure on water resources,
581 managers will need reliable tools to anticipate the evolution of groundwater quality. At larger
582 scale, important spatial heterogeneities of organic matter distribution and As concentrations,
583 linked to geological context, i.e. sedimentary deposition in the meandering river system
584 (channels, crevasse splay and floodplain), may occur and induce local variations of the intensity
585 of biogeochemical processes. The mechanisms of As release in the sub-surface and their
586 potential evolution with groundwater table level would need to be better understood through
587 long-term monitoring of dissolved As species, and development of experimental systems and
588 models taking into account heterogeneities at different scales.

589

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HIGHLIGHTS

- Arsenic of sedimentary origin influences quality of a French groundwater
- Temporal variations of As- and S- cycles bio-indicators are observed on site
- Incubations of core material show biologically related As solubilisation
- Incubated core slurries and groundwater present distinct bacterial communities
- As concentration and speciation is driven by bacterial activities

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Declaration of competing interest

The authors declare that they have no known competing interest that could have influenced this research results and interpretation.

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