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1 **Mass collection of magnetotactic bacteria from the permanently stratified ferruginous**
2 **Lake Pavin, France**

3

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25 **Abstract**

26 Obtaining high biomass yields of specific microorganisms for culture-independent approaches
27 is a challenge faced by scientists studying organisms recalcitrant to laboratory conditions and
28 culture. This difficulty is highly decreased when studying magnetotactic bacteria (MTB) since
29 their unique behavior allows their enrichment and purification from other microorganisms
30 present in aquatic environments. Here, we use Lake Pavin, a permanently stratified lake in the
31 French Massif Central, as a natural laboratory to optimize collection and concentration of MTB
32 that thrive in the water column and sediments. A method is presented to separate MTB from
33 highly abundant abiotic magnetic particles in the sediment of this crater lake. For the water
34 column, different sampling approaches are compared such as *in situ* collection using a Niskin
35 bottle and online pumping. By monitoring several physicochemical parameters of the water
36 column, we identify the ecological niche where MTB live. Then, by focusing our sampling at
37 the peak of MTB abundance, we show that the online pumping system is the most efficient for
38 fast recovering of large volumes of water at a high spatial resolution, which is necessary
39 considering the sharp physicochemical gradients observed in the water column. Taking
40 advantage of aerotactic and magnetic MTB properties, we present an efficient method for MTB
41 concentration from large volumes of water. Our methodology represents a first step for further
42 multidisciplinary investigations of the diversity, metagenomic and ecology of MTB populations
43 in Lake Pavin and elsewhere, as well as chemical and isotopic analyses of their magnetosomes.

44

45 **Introduction**

46 The recent technological advances are increasingly allowing the study of microorganisms using
47 culture-independent approaches in many fields of research. These advances include the
48 development of tools to analyze genomes at the single cell scale, as well as the ultrastructure
49 and/or chemistry of microorganisms (Blainey, 2013). However, some analytical approaches
50 still require high biomass and thus often rely on cultured microorganisms or natural enrichment
51 (*e.g.* bloom). Magnetotactic bacteria (MTB) represent one example of microorganisms at the
52 origin of numerous interdisciplinary studies using culture-independent approaches over the last
53 decades mainly because most strains are recalcitrant to growth (Abreu *et al.*, 2007; Lefèvre and
54 Bazylinski, 2013; Lin *et al.*, 2017). For instance, significant progress has been achieved on
55 MTB diversity, chemistry and magnetosome biomineralization based on culture-independent
56 approaches such as single-cell analysis (Jogler *et al.*, 2010; Kolinko *et al.*, 2016), coupled FISH-
57 SEM/TEM (Li *et al.*, 2017, 2019; Kozaeva *et al.*, 2020; Liu *et al.*, 2020; Qian *et al.*, 2020),
58 comprehensive TEM (Li *et al.*, 2015; Zhang *et al.*, 2017; Li *et al.*, 2020a; Li *et al.*, 2020b) and
59 the combination of TEM and synchrotron-based STXM (Li *et al.*, 2020c). MTB can be
60 concentrated and purified magnetically from natural aquatic environments thanks to their
61 magnetosome chain at the origin of their magnetotactic behavior (Bazylinski and Frankel,
62 2004). Magnetosomes are membrane-enveloped ferrimagnetic nanocrystals, which serve as
63 magnetic field sensors (Blakemore, 1975). Their alignment in linear chains imposes a magnetic
64 moment to the bacteria, which ensures navigation in the weak geomagnetic field along vertical
65 redox gradients (Bazylinski and Frankel, 2004). This behavior, named magnetotaxis, is based
66 on MTB chemo-aerotactic capability and active swimming motility. It likely represents a
67 selective fitness advantage in their natural habitat, which is the oxic-anoxic transition zone
68 (OATZ) of aquatic ecosystems. Most cultured MTB are micro-aerophilic (Lefèvre *et al.*, 2014).
69 Consequently, sampling for MTB is generally based on the collection of sediment or water
70 samples that comprise the OATZ. MTB present in sediments can be sampled from the shore

71 (Liu *et al.*, 2018), by free diving (Lefèvre *et al.*, 2009), or using a bottom sampler (Kolinko *et*
72 *al.*, 2013). In contrast, MTB present in the water column of stratified ecosystems have
73 previously been collected using Geopump peristaltic pump (Simmons *et al.*, 2004; Moskowitz
74 *et al.*, 2008), Niskin bottle (Rivas-Lamelo *et al.*, 2017), free-flow bottle or automatic flow
75 injection sampler (Schulz-Vogt *et al.*, 2019).

76 Their detection in environmental water and sediment samples is relatively easy due to their
77 magnetotactic behavior. Indeed, MTB can be enriched magnetically by placing a bar magnet
78 adjacent to the outer wall of a bottle filled with environmental sample. If MTB are abundant in
79 the sample, a brownish or grayish-to-white spot containing mainly MTB will form next to the
80 inside of the glass wall closest to the bar magnet. Cells can be easily harvested from the bottle
81 and examined using light microscopy. Few improvements of the magnetic concentration of
82 MTB have been proposed such as (i) a glass apparatus containing a capillary end allowing the
83 concentration of MTB from a large volume of sediment when the apparatus is introduced in a
84 coiled tube that generates a homogenous magnetic field (Lins *et al.*, 2003), (ii) an apparatus
85 containing a separating chamber for an initial enrichment of MTB and a sampling chamber
86 where MTB are further concentrated and harvested (Xiao *et al.*, 2007), (iii) a “MTB trap” where
87 south- and north-seeking MTB are magnetically directed toward the tips of collection tubes,
88 from which they can be conveniently collected for further analyses (Jogler, Lin, *et al.*, 2009)
89 and (iv) a MTB column separation, the MTB-CoSe method, that allows collection of
90 magnetotactic cells directly from sediment samples (Koziaeva *et al.*, 2020). The modification
91 of the techniques commonly used for sampling and observation of environmental magnetotactic
92 microorganisms recently allowed to extend our knowledge of the diversity and ecology of
93 magnetotaxis in bacteria (Lin *et al.*, 2018; Monteil and Lefevre, 2019; Monteil *et al.*, 2019,
94 2021; Koziaeva *et al.*, 2020). Even if all these techniques allow to get biological material in
95 sufficient concentrations for biodiversity studies, the abundance of bacterial populations
96 recovered is not high enough to properly investigate some important magnetic, chemical and

97 isotopic features of the magnetic minerals they form. These aspects are yet essential to
98 understand the molecular and geochemical biomineralization processes. Indeed, the separation
99 of magnetite from MTB in the laboratory for subsequent Fe isotope analyses requires a
100 minimum of 0.1 mg of magnetite (Amor *et al.*, 2016, 2018). This represents a total amount of
101 MTB of $\sim 10^{10}$ cells, assuming typically 30 magnetosomes per cell and a magnetosome length
102 of 50 nm. To improve our ability to harvest large amount of MTB from natural environment
103 for multidisciplinary studies, including biological, mineralogical and geochemical analyses, we
104 tested and optimized several approaches to collect and concentrate MTB.

105 Lake Pavin (Massif Central, France) was selected as a natural laboratory for the following
106 reasons: (i) it is a ferruginous meromictic lake, *i.e.* permanently stratified with an OATZ located
107 in the water column instead of the sediment (Michard *et al.*, 1994; Aeschbach-Hertig *et al.*,
108 1999, 2002); (ii) the physicochemical conditions of the lake and associated sediments have been
109 studied extensively, and geochemical cycles are well constrained (Michard *et al.*, 1994; Viollier
110 *et al.*, 1995; Albéric *et al.*, 2000; Schettler *et al.*, 2007; Assayag *et al.*, 2008; Busigny *et al.*,
111 2014; Cosmidis *et al.*, 2014) and (iii) recent exploratory field trips at Lake Pavin demonstrated
112 that MTB are exceptionally abundant in concentration and biodiversity in the water column and
113 sediments and may contribute to the geochemical cycles of P, Ca and C (Miot *et al.*, 2016;
114 Rivas-Lamelo *et al.*, 2017; Monteil *et al.*, 2021).

115 Lake Pavin is the youngest crater lake of the Massif Central volcanic area (about 7000 years
116 cal BP) (Chapron *et al.*, 2010). It is redox-stratified, with anoxic and ferruginous deep waters
117 extending between 50-55 and 92 m in depth (monimolimnion), and topped by oxic waters
118 (mixolimnion) (Cosmidis *et al.*, 2014; Rivas-Lamelo *et al.*, 2017; Berg *et al.*, 2019). The
119 position of the OATZ varies slightly with time in a depth range comprised between 50 and 60
120 m (Michard *et al.*, 1994; Cosmidis *et al.*, 2014; Rivas-Lamelo *et al.*, 2017; Berg *et al.*, 2019).
121 In the deep waters, ferrous iron ($\text{Fe(II)}_{\text{aq}}$) along with NH_4^+ , are the main dissolved cations, with
122 concentrations up to 1 mM (Michard *et al.*, 1994; Viollier *et al.*, 1995; Busigny *et al.*, 2014,

123 2016). Iron is efficiently confined below the oxic-anoxic boundary due to the formation of
124 insoluble ferric iron species, Fe(III)_s, by oxidation with O₂ and other oxidants in the
125 mixolimnion (*e.g.*, NO₃⁻, Mn⁴⁺) (Lopes *et al.*, 2011). The Fe(III)_s particles settle down and are
126 reduced to soluble Fe(II)_{aq} in the anoxic waters and at the lake bottom by reaction with organic
127 matter. Dissolved Fe(II)_{aq} then diffuses upward in the water column and is finally re-oxidized
128 to Fe(III) at the redox boundary. This process, known as the “iron wheel” has been described
129 in details from available data for dissolved and particulate matter in the water column, settling
130 particles collected by sediment traps and sediment cores (Cosmidis *et al.*, 2014; Busigny *et al.*,
131 2016). Additionally, the lake’s water column has remarkably low sulfate concentrations (< 20
132 μM), which also keep low level of sulfides (Bura-Nakic *et al.*, 2009). The redox boundary fed
133 by a downward flux of dissolved O₂ and other oxidants, and an upward flux of dissolved Fe(II)
134 represents ideal conditions for magnetosome formation by MTB, as observed in similar
135 stratified aquatic environments (Simmons *et al.*, 2004; Schulz-Vogt *et al.*, 2019).

136 This contribution first presents a method for sampling MTB from the sediments, with particular
137 emphasis on the separation between MTB and abiotic (volcanic) magnetic particles. Then, the
138 global distribution of MTB in the water column of Lake Pavin is presented. The results of
139 several tests for harvesting MTB from the water column are described and compared, including
140 water sampling using Niskin bottles and online pumping system. Finally, a technique for the
141 concentration and isolation of MTB from a large environmental water volume based on
142 magnetotaxis and chemotaxis is presented. Our results allow the identification of optimal
143 physicochemical parameters for the presence of MTB in the water column. This work is paving
144 the way for future multidisciplinary studies of MTB in Lake Pavin and others natural sites.

145

146 **Results and discussion**

147 ***Magnetotactic bacteria are abundant and diverse in the sediments of Lake Pavin***

148 Over the different sampling campaigns performed since 2016 on Lake Pavin, a large abundance
149 (up to 5.8×10^5 cells mL⁻¹ of porewater; Monteil *et al.*, 2021) and diversity of MTB
150 morphotypes has been observed in the sediments collected from the shore (Fig. 1). MTB
151 abundance determined in sediments of other aquatic systems generally falls in a range from 10^5
152 to 10^6 cells mL⁻¹ (Spring *et al.*, 1993; Jogler, Kube, *et al.*, 2009) with some studies reporting up
153 to 10^7 cells mL⁻¹ (Flies *et al.*, 2005), magnetotactic cocci being in most aquatic habitats the
154 dominant population of MTB. Although only future studies using molecular typing methods
155 will be able to specify the phylogenetic affiliation of these MTB morphotypes, the unique
156 ultrastructure of some of these MTB allows speculations on their taxonomy. First, the recently
157 published calcium-carbonate producing MTB were easy to distinguish thanks to their refractive
158 inclusions, their slow motility and their unique magnetotactic behavior when observed under
159 the light microscope (Figs. 1A and S1A) (Monteil *et al.*, 2021). This MTB morphotype was
160 among the most abundant in sediment samples along with MTB with an ultrastructure typical
161 of the Magnetococcaceae family (*e.g.* coccoid cells, helical motion, two flagella bundles) (Fig.
162 1B-E) (Bazylinski *et al.*, 2013). Cell's shapes similar to that of *Magnetospirillum* bacteria (*e.g.*
163 thin, *i.e.* < 500 nm, and long, *i.e.* > 5 μ m, helical-shaped with one chain of cuboctahedral
164 magnetosomes; Fig. 1F) could also be recognized based on TEM observations (Lefèvre *et al.*,
165 2012). Rod-shaped MTB producing polyphosphate inclusions very similar to previously
166 described MTB affiliated to the Magnetococcaceae family were also observed (Figs. 1 G and
167 H and S1B) (Spring *et al.*, 1994; Oestreicher *et al.*, 2011; Kolinko *et al.*, 2013). Magnetotactic
168 spirilla very similar (*e.g.* large, *i.e.* between 0.5 and 1 μ m, and short, *i.e.* < 3 μ m, helical-shaped
169 cells with one chain of prismatic magnetosomes) to described species of the *Magnetospira*
170 genus were also present in sediments of Lake Pavin (Fig. 1I). Then, cells resembling previously
171 described MTB of the Nitrospirae phylum (*e.g.* large rod or ovoid cells with electron dense
172 inclusions and several chain bundles of bullet-shaped magnetosomes) such as *Candidatus*
173 *Magnetoovum* sp. (Fig. 1J) (Lefèvre *et al.*, 2011; Lin *et al.*, 2012) and *Ca. Magnetobacterium*

174 bavaricum (Fig. 1K) (Spring *et al.*, 1993; Jogler *et al.*, 2010; Li *et al.*, 2020b) could also be
175 observed (Lefèvre, 2016). Finally, various rods to curved rods producing bullet-shaped
176 magnetosomes were observed and could be tentatively affiliated to the Deltaproteobacteria
177 since their ultrastructure was similar to described magnetotactic species of the *Desulfovibrio*
178 and *Desulfamplus* genera (Fig. 1L-O) (Lefèvre *et al.*, 2016; Descamps *et al.*, 2017; Pan *et al.*,
179 2019).

180 Lake Pavin sediments appeared as a natural environment particularly suitable to obtain high
181 MTB biomass. Indeed, after magnetic enrichment of sediment samples using neodymium-iron-
182 boron magnets for 2-3 h, aggregated cells formed a thin irregular whitish pellet measuring up
183 to 5 cm in diameter, containing an estimated number of up to 10^8 magnetotactic cells located
184 against the magnet. However, abiotic magnetic particles were also very abundant in the
185 sediments of this lake and were generally trapped in the pellets during MTB concentration.
186 These abiotic particles were predominantly identified as ilmenite (*i.e.* titanium-iron oxide,
187 FeTiO_3) by scanning electron microscopy analyses (Fig. S2). Ilmenite is a slightly magnetic,
188 black and steel-gray mineral. It is a paramagnetic mineral, explaining its attraction during
189 magnetic concentration of Lake Pavin sediments. At the macroscopic scale, it can easily be
190 mistaken with magnetite.

191 We attempted at purifying a maximum proportion of MTB after their magnetic concentration.
192 Our system, named “migration track”, is similar to the racetrack method developed in previous
193 studies (Wolfe *et al.*, 1987; Li *et al.*, 2010). It improved the purification of north seeking MTB
194 and was used directly on the field (Figs. 2 and S3). The pellets containing abiotic particles,
195 MTB and other microorganisms, were collected with a 1 mL micropipette on the inner wall of
196 the bottles and placed next to the north pole of a strong bar magnet (15 mT at the edge of the
197 drop). Magnetic particles were retained at this extremity while most north-seeking MTB were
198 repulsed and swam in the opposite direction where the south pole of another magnet was placed
199 (3 mT at the edge of the drop). A folded parafilm sheet was used to create a channel filled with

200 a 0.22- μm -filtered drop of Lake Pavin water overlying the sediments in the sample. Such a
201 protocol avoids the formation of chemical gradients and thus facilitates the northern migration
202 of MTB. The migration time was adjusted in order to obtain a maximum proportion of MTB
203 purified at the extremity of the device. As the majority of MTB were fast swimming bacteria
204 such as magnetotactic cocci with a motility above $200 \mu\text{m s}^{-1}$, the drop was spread (*i.e.* 4 to 4.5
205 cm length) in order to optimize the transfer of magnetotactic cells in the filtered water. Up to
206 88.0 % (71.9 % in average) of MTB were efficiently purified and concentrated after 60 min of
207 migration in the channel (Fig. 2). A whitish/greyish spot appeared close to the south pole of the
208 magnet where MTB were harvested. A majority of magnetotactic cocci along with other rod-
209 shaped magnetotactic cells composed this pellet containing more than 10^7 cells. This approach
210 combining the standard method for magnetic concentration of sediment samples and our
211 migration track technique allowed us harvesting an important quantity of MTB free of abiotic
212 magnetic particles. These MTB were composed by different populations with proportions likely
213 not representative of their proportions in the environment, as the protocols used for magnetic
214 enrichment and purification do not have the same efficiency for all MTB (e.g. Lin *et al.*, 2008).
215 Purified MTB from dozens of migration track devices were pulled together and concentrated
216 by centrifugation, thus representing a large pellet of purified MTB available for further
217 analyses.

218

219 ***Magnetotactic bacteria are distributed below the oxygen detection limit in the water column*** 220 ***of Lake Pavin***

221 Over the different field works in Lake Pavin, the abundance and proportion of the different
222 MTB morphotypes observed in the water column greatly varied depending on the season. Eight
223 MTB morphotypes could be distinguished based on microscopy observations. Among these
224 morphotypes, the most abundant (up to 5.0×10^3 cells mL^{-1}) were cells affiliated to the
225 Magnetococcaceae family that produced polyphosphate inclusions of various sizes along with

226 various organizations of magnetosomes (Figs. 3A-C and S1C) (Rivas-Lamelo *et al.*, 2017; Liu
227 *et al.*, 2020). MTB producing calcium carbonate inclusions were observed at a lower abundance
228 but could reach a cell density of 4.0×10^2 cells mL⁻¹ (Fig. 3D) (Monteil *et al.*, 2021). Two rod-
229 shaped MTB, one producing a single chain of octahedral magnetosomes along with
230 polyphosphate (Figs. 3E and S1C) and the other one producing a single chain of bullet-shaped
231 magnetosomes (Fig. 3F) were also always present in our samples but at a low cell density (< 8
232 cells mL⁻¹, the lower limit of the technique used to count MTB density using the hanging drop
233 assay). A small vibrio producing one chain of bullet-shaped magnetosomes (Fig. 3G) and some
234 helical MTB resembling cells of the *Magnetospirillum* genus (Fig. 3H) were occasionally
235 observed in samples collected in the water column of Lake Pavin. Other studies on MTB
236 populations living in the water column of stratified aquatic environments also revealed the
237 presence of a majority of magnetotactic cocci affiliated to the Magnetococcaceae family
238 (Simmons *et al.*, 2007; Schulz-Vogt *et al.*, 2019) with abundance up to 3.5×10^6 cells mL⁻¹ at
239 the top of the oxycline in Salt Pond, Massachusetts (Simmons *et al.*, 2004, 2007).

240 From April 2016 to October 2019, ten vertical profiles of MTB distribution along with several
241 physicochemical parameters were obtained across the OATZ of the water column using a
242 Niskin bottle for water collection (Figs. 4 and S4). These profiles could bring important
243 information concerning the MTB distribution in the water column of Lake Pavin. For instance,
244 although there are important variations of the oxygen and redox gradients over the seasons and
245 years, MTB were always distributed below the oxygen detection limit (Figs. 4 and S4). The
246 maximum abundance of MTB in the water column was systematically detected near the
247 maximum decrease of the redox potential, *i.e.* between 51.0 and 57.2 m depth depending on
248 seasons/years (Fig. 4). The peak of turbidity, corresponding mainly to the precipitation of iron
249 phosphate and (oxy)hydroxide (Cosmidis *et al.*, 2014; Busigny *et al.*, 2016), was either at the
250 same depth level as the peak of MTB abundance or just below. In most cases, their depths
251 evolved similarly depending on the season (Figs. 4 and S4). However, it should be noted that

252 the intensities of the turbidity and MTB peaks did not correlate with each other. For instance,
253 in October 2017, a high MTB concentration of 7.4×10^3 cells mL⁻¹ was observed at 51.0 m
254 depth while the peak of turbidity was very weak. Reciprocally, in April 2016, a strong peak of
255 turbidity was observed at 54.6 m depth while the number of MTB reached only 1.4×10^3 cells
256 mL⁻¹. Accordingly, the contribution of MTB populations to the turbidity of the water column
257 is insignificant and the turbidity value cannot be used unequivocally as a proxy for MTB. The
258 peak of MTB was always observed at the level of or below the break of conductivity at value
259 of 78.9 ± 13.0 $\mu\text{S cm}^{-1}$ (n = 10 sampling campaigns using Niskin bottles) which corresponds to
260 the region where iron concentration starts to increase (Cosmidis *et al.*, 2014; Busigny *et al.*,
261 2016).

262 In spring and summer, the maximum number of MTB counted in the water column was located
263 between 54.3 and 57.2 m depth, while in autumn, the maximum number of MTB was generally
264 located at a lower depth in the water column, between 51.0 and 53.0 m (Figs. 4B and S5). This
265 zonation can be explained by the dynamics of the upper water column. In winter, shallow waters
266 (0-10 m) cool down and eventually become denser than deeper waters (10 to *ca.* 50-55 m) thus
267 creating an instability and a mixing of the upper water column (*i.e.* mixolimnion). This brings
268 dissolved O₂ to the deep part and slightly deepens the OATZ, turbidity peak and the maximum
269 MTB abundance zone. In contrast, during summer and autumn, shallow waters are warmer and
270 the water column is strongly stratified by the thermocline (*ca.* 8-12 m depth) that limits O₂
271 renewal in the hypolimnion, inducing an upward shift of chemocline, turbidity and MTB peaks
272 to shallower depth (due to upward Fe(II) diffusion from deep anoxic waters).

273 Sampling with a Niskin bottle is thus an efficient method to decipher the global distribution of
274 MTB in the water column. Nevertheless, the use of Niskin bottles is associated with several
275 drawbacks. First, the bottles are, in general, vertical and, in our case, with a height of ~50 cm
276 for the 5.7-L bottle and ~100 cm for the 20-L bottle (Fig. 5). As a result, this method provides
277 a limited depth resolution for the assessment of MTB distribution in the water column, not

278 better than 50 to 100 cm. However, near the OATZ of Lake Pavin, the strong stratification at
279 relatively small scale, as measured by *in situ* probes, requires a better depth resolution for water
280 collection. Moreover, water collection takes a long time, especially at a 60 m depth: about 30
281 minutes in total are required for bottle opening, descent, closing, ascent, and emptying on the
282 platform. Then, when a water sample is collected, the only direct control over the depth is a
283 meter counter connected to the pulley of the winch, which provides a poor precision. The depth
284 is precisely known thanks to a depth gauge fixed to the Niskin bottle only when the bottle is
285 pulled out from the water. Thus, there is no direct indication that the bottle is located at the peak
286 of MTB abundance. Therefore, the method of water collection using Niskin bottle is adapted
287 for a study of global MTB distribution in the water column but (i) can hardly target the peak of
288 MTB abundance reproducibly and (ii) cannot be used to establish a precise distribution profile
289 of MTB at high depth resolution.

290

291 ***Online pumping allows a sampling at high depth resolution, showing that MTB distribution***
292 ***is correlated with conductivity and oxygen***

293 We developed an alternative method for water sampling using a vertical graduated pipe settled
294 at depth and connected at the other side of the pipe to a submersible pump (Fig. 5). The water
295 transfer from depth to surface can be rapid and efficient but depends on the pump flow capacity.
296 In the present work, the maximum pump flow reached 8 L min⁻¹ (BLDC Pump, model DC50E-
297 1250), filling a 20-L Nalgene bottle in *ca.* 150 s. This is much faster compared to the 30 minutes
298 required to collect 20 L with a Niskin bottle. A clear advantage of our online sampling system
299 is that several Nalgene bottles could be filled from the same depth on a time scale where
300 evolution of the water physico-chemistry and MTB abundance are insignificant. We compared
301 MTB abundances in several Nalgene bottles successively filled and found them statistically
302 identical (data not shown). Moreover, the abundance of cells collected at the same depth using
303 a Niskin bottle was similar or lower compared to our pipe system showing that the flow

304 generated by the pump has no impact on MTB motility and integrity. Indeed, electron
305 microscopy analyses of cells sampled with the two approaches show similar MTB morphology
306 and magnetosomes organization. For sampling another depth in the water column, the
307 graduated pipe can simply be shifted to a shallower or deeper position. A waiting time is
308 required to flush the water contained in the 60-m-long pipe, corresponding roughly to 150 s.
309 Another benefit of our online sampling system is that it allows the collection of water at
310 relatively high depth resolution of approximately 10 to 20 cm.

311

312 In Lake Pavin, although MTB-rich waters can be efficiently collected at a specific depth using
313 our online sampling system, imprecisions persist because the depth of the MTB abundance peak
314 can change over days, and even hours, due to slight variation of the redox boundary and internal
315 waves of the lake (Bonhomme, 2008; Bonhomme *et al.*, 2011). In order to overcome this
316 problem, we evaluated whether physicochemical parameters of the water could be used as a
317 proxy for MTB abundance in the water column. Over several days in July 2019, we monitored
318 MTB cell density around the peak of MTB abundance identified beforehand, as well as
319 temperature, conductivity at 25°C (*i.e.* C₂₅), dissolved oxygen concentration and pH of the
320 water samples (measured *in situ* directly at the extremity of the pipe). The results indicate that
321 MTB cell density ranged from 13 to 1.18×10^4 cells mL⁻¹, temperature from 6.3 to 7.0°C, C₂₅
322 from 63.3 to 78 µS cm⁻¹, and pH from 6.1 to 6.3. Figure 6 illustrates that MTB cell density is
323 not correlated with pH and temperature, but displays specific distribution with C₂₅ and
324 dissolved O₂ concentration. MTB cell density tends to increase with decreasing O₂ level, and
325 MTB > 4.0×10^3 cells mL⁻¹ are only found when dissolved O₂ is lower than 2 µmol L⁻¹.
326 However, low MTB cell densities are also observed in some water samples with low O₂ (*i.e.*
327 around 2 µmol L⁻¹; Fig. 6A) showing that O₂ concentration is an important parameter
328 controlling MTB distribution but is not sufficient to monitor online MTB abundance. The
329 conductivity is also a relevant parameter since the peak of MTB abundance was systematically

330 found in a restrictive range of C_{25} values, between 64.7 and 67.1 $\mu\text{S cm}^{-1}$ (Fig. 6B). Considering
331 the twelve richest samples with $\text{MTB} > 4.0 \times 10^3 \text{ cells mL}^{-1}$, the average C_{25} value is 65.8 ± 1.4
332 $\mu\text{S cm}^{-1}$ (2σ). The conductivity represents the concentration of dissolved ionic species,
333 including iron. Thus, the peak of MTB abundance in the water column of Lake Pavin occurs at
334 an ideal set of conditions including an oxygen concentration below 2 $\mu\text{mol L}^{-1}$ and a
335 conductivity around 65.8 $\mu\text{S cm}^{-1}$. The conductivity can be instantaneously measured in the
336 water collected by our online system and was further used as a proxy for MTB abundance.

337 The MTB abundance, O_2 concentrations and specific conductivity measured along three depth
338 profiles in Lake Pavin water column are reported in Figure 7. While C_{25} showed a progressive
339 increase with depth for the different sampling days, MTB cell density and O_2 concentration
340 showed stronger variabilities and scattering. Oxygen concentration typically decreased with
341 depth but displayed unexpected bursts at depths of 51.5 m (July 14th 2019) and 52.0 m (July
342 15th 2019). This O_2 increase was not observed in July 17th 2019 although there might be subtle
343 variations at low level. The presence of O_2 anomalies (*i.e.* concentration burst in anoxic waters)
344 may reflect an inflow of a sub-lacustrine intermittent spring, as described previously in Lake
345 Pavin at a depth ranging between 50 and 55 m (Bonhomme *et al.*, 2011). Interestingly, the MTB
346 concentration is anticorrelated with these O_2 burst, with no MTB in elevated O_2 regions (Fig.
347 7).

348 Using the online pumping system, we noticed that the MTB abundance exhibited several peaks
349 at different depths in the water column. Two peaks were observed on July 14th and 15th, and
350 three peaks on July 17th (Fig. 7). This was not the case on July 9th and during the other sampling
351 campaigns while performing the MTB abundance profiles with the Niskin bottle. Although
352 MTB are distributed along several meters in the water column, a high depth resolution is thus
353 required to study precisely MTB distribution in the water column of Lake Pavin. The origin of
354 multiple MTB peaks is still poorly constrained. Future studies will have to examine the potential
355 link between physico-chemical conditions and associated MTB populations by monitoring in

356 detail physico-chemical properties of the water, MTB morphotypes and molecular typing
357 methods.

358

359 Finally, two important questions must be discussed concerning our online pumping system.

360 First, when the pipe is left at depth for relatively long time (*e.g.* several minutes or more), where

361 is the water collected at the pipe mouth coming from? It is unknown if the water collected for

362 a long time is derived from a roughly constant depth level, all around, below or above the pipe

363 mouth. Because of strong vertical density gradient in the vicinity of the oxic-anoxic transition

364 zone in Lake Pavin, we can reasonably assume that the zone of water collection around the pipe

365 mouth is similar at each sampling depth (regardless of the water flow direction). This is

366 supported for instance by the progressive increase in conductivity with depth on the different

367 profiles of July 2019 (Fig. 7, in particular the smooth shape of the profiles in July 14th and 15th).

368 The second important question to address is whether the physico-chemical conditions of water

369 are preserved or not during transfer through the pipe from depth to surface. Again, the smooth

370 shape of the conductivity profile suggests that the water was well preserved during ascension.

371 In addition, we noted a clear oxic-anoxic transition of the water samples (even though the oxic

372 zone was not monitored and reported here due to the absence of MTB). The anoxic conditions

373 (*i.e.* below probe detection limit) measured at depth > 52.5 m (Fig. 7) indicate that O₂ diffusion

374 through the pipe did not occur or was negligible during water ascension. The only parameter

375 significantly modified was temperature due to high exchange surface with ambient water

376 around the pipe. The water temperature measured on the platform was between 6.3 and 7°C

377 (Fig. 6), while *in situ* measurement provided values near 4°C. Overall our online pumping

378 system allows to achieve a high spatial resolution together with consistent water conditions and

379 associated MTB abundance.

380

381

382 *Chemotaxis and magnetotaxis allow an efficient MTB concentration from large water*
383 *samples*

384 Most previous studies of environmental MTB focused on water-saturated sediment samples
385 (Jogler *et al.*, 2010; Lefèvre *et al.*, 2011; Lin *et al.*, 2013, 2018) and a few explored the stratified
386 water column of freshwater or saline lakes (Simmons *et al.*, 2004; Rivas-Lamelo *et al.*, 2017;
387 Schulz-Vogt *et al.*, 2019). In all of these studies, MTB were isolated using magnetic enrichment
388 (Jogler and Schüller, 2009; Lefèvre and Bazylinski, 2013; Lin *et al.*, 2017). This magnetotaxis-
389 based procedure is well adapted for samples of small volume (*i.e.* below 1 L) but is not efficient
390 for large volumes such as 10 or 20 L, which are needed for studies that require high MTB
391 biomass, for instance, to combine mineralogical, biological and geochemical analyses of
392 magnetosomes.

393 In order to concentrate MTB cells from a 20-L volume to 1 L and subsequently perform
394 magnetic enrichment, we explored the possibility to concentrate them using their aerotaxis.
395 Indeed, MTB are known to display strong microaerophilic, aerophilic or -phobic response
396 (Frankel *et al.*, 1997; Lefèvre *et al.*, 2014; Popp *et al.*, 2014). Using our online pumping system,
397 we collected water samples from Lake Pavin (near the peak of MTB abundance) and filled six
398 Nalgene bottles of 20 L each. After sampling, the bottles were left open to air so that O₂ could
399 diffuse into the water (Fig. 8). MTB were expected to swim downwards to the bottom of the
400 bottle. Because O₂ diffusion in water was extremely slow, the experiment was speeded up by
401 gently removing water from the upper part of the bottle by pumping 4 L every 30 or 60 minutes,
402 so that O₂ could diffuse deeper in the bottle while aerotactic cells were swimming downward.
403 Among the six bottles, three of them were pumped every 30 minutes and the three others every
404 60 minutes. This provided a triplicate of each condition (30 or 60 minutes idle time between
405 pumping). For every volume interval, water was collected and MTB concentration was
406 quantified in triplicate under a light microscope. The distribution of MTB concentration was
407 very consistent and reproducible between triplicates (see error bars on Fig. 8). The results of

408 the experiment with 30 minutes idle time between pumping showed that MTB swam and
409 concentrated in the bottom water. However, only $42.3 \pm 4.1\%$ of the total MTB cells were found
410 in the bottom water (1 L), while $\sim 60\%$ were lost in the upper oxygenated waters. In contrast,
411 the experiment with 60 minutes idle time gave a higher concentration yield with $80.7 (\pm 0.8)\%$
412 of the total MTB cells present in the bottom water. Overall, this shows that downwards MTB
413 migration imposed by O_2 diffusion in the bottle is an efficient pre-concentration procedure.
414 These data allowed to estimate the motion speed of the slowest MTB from Lake Pavin water
415 column. The vertically travelled distance in the 20 L Nalgene bottle was 10 cm in 60 minutes.
416 This corresponds to MTB motion speed *ca.* $28 \mu\text{m s}^{-1}$, which is in the speed range to that
417 previously described for MTB (Lefèvre *et al.*, 2014) and in agreement with light microscope
418 observation where we could distinguish highly motile magnetotactic cocci and some slow
419 motile magnetotactic large rods. A further magnetic enrichment of the chemotactically enriched
420 samples allowed the formation of a large pellet against the magnet that could be easily harvested
421 and used for further analyses of magnetotactic cells or their magnetosomes. For a 20 L bottle
422 sampled at the peak of MTB abundance in Lake Pavin water column, more than 10^8 cells could
423 be collected. Similarly to the magnetic concentration carried out in the sediments, coupling
424 aerotactic and magnetic concentration allows to obtain large pellets of cells composed by
425 several populations of MTB in proportions likely different from the natural ones as the
426 sensitivity to oxygen probably differs between the different MTB present in our samples.

427

428 **Conclusion**

429 The present work on Lake Pavin provides several analytical methods for optimizing MTB
430 collection in sediments and the water column. In sediments, MTB were efficiently separated
431 from magnetic abiotic particles (*i.e.* volcanic ilmenite) using a “migration track” system, where
432 MTB are left swimming towards a magnet. In the water column, a comparison of sampling
433 protocols based on Niskin bottle and online pumping system demonstrates a stronger potential

434 of online technique, with fast collection of large volumes (20 L in 150 s) and at high depth
435 resolution (about 10-20 cm). Another strong advantage of the online pumping technique is the
436 capacity to evaluate the zone of maximum MTB abundance using empirical calibration
437 coupling dissolved oxygen and conductivity measurements. While only one peak of MTB was
438 identified on depth profile performed using Niskin bottles, high resolution profile using online
439 pumping system indicates that two to three peaks of MTB are present. Likewise, we anticipate
440 a stratification of MTB morphotypes and a potential link to geochemical parameters in the water
441 column. This aspect will need to be explored in future studies and may help to decipher the
442 ecological niche specific of the different MTB populations present in the water column.

443 After collection of large volumes of water at depth, MTB can now be efficiently recovered by
444 coupling their aerotactic and magnetic behaviors. This opens a new avenue for chemical and
445 isotopic studies that require large amount of MTB and are usually limited to laboratory cultures.
446 In the case of Lake Pavin, MTB present at the OATZ of the water column show a maximum
447 concentration slightly higher than 10^4 cells mL^{-1} . Therefore, the mass requirement for Fe
448 isotope analysis (~ 0.1 mg of magnetite, *i.e.* 10^{10} cells) implies that about 1000 L of water will
449 have to be collected and treated. Although this represent a large volume of water, this can
450 reasonably be achieved by the present protocols. The application of these methods to others
451 chemically stratified aquatic environments such as Salt Pond, Massachusetts (Simmons *et al.*,
452 2004) or the Black Sea (Schulz-Vogt *et al.*, 2019) could be of high interest to carry out a
453 comparative study of MTB ecology, diversity and isotope characteristics from different
454 ecosystems.

455

456 **Experimental procedures**

457 *Sample collection and in situ physicochemical measurements*

458 Sampling in the water column was carried out at ten different dates between April 2016 and
459 October 2019. Samples were collected from a platform located near the center of Lake Pavin

460 (45.495792°N, 2.888117°E) using a 5.7 L or 20 L Niskin bottle (General Oceanics, USA)
461 connected to a winch or by pumping directly the water using a speed-adjustable 12 V brushless
462 pump (see details in the Results section). For initial MTB counting, water collected at the
463 targeted depths was transferred in one-liter glass bottles filled to their capacity and tightly
464 closed. *In situ* environmental parameters of the water column were acquired with different
465 profiler probes: YSI 6600 (CTD-O₂-pH-ORP), EXO2 (CTD-O₂-pH-ORP-turbidity-
466 chlorophyll-phycoyanine-fDOM), SDOT 300 nke (O₂-T), AQUAlogger 210TYPT Aquatec
467 (turbidity-T-depth), STBD 300 nke (turbidity-T-depth), where CTD stands for conductivity-
468 temperature-depth, ORP for oxidation reduction potential and fDOM for fluorescent dissolved
469 organic matter. Detection limit for dissolved O₂ is *ca.* 0.1% or about $0.3 \pm 0.2 \mu\text{M}$.

470 Sediments were sampled along the edge of the dock, near the main access road to Lake Pavin
471 (45.499162°N, 2.886399°E). They were recovered onshore with a scooper by fully filling one-
472 liter glass bottles with 300-400 mL of sediments and overlying 600-700 mL water. During
473 sampling, air bubbles were avoided. Once in the laboratory, bottles were stored with their cap
474 closed, under dim light and at room temperature (~25°C).

475

476 ***Magnetic enrichment and light microscope observation***

477 North-seeking magnetic cells were concentrated by placing the south pole of a magnetic stirring
478 bar or neodymium-iron-boron magnet (disc magnet with a diameter of 5 mm and a height of 5
479 mm) for 1-3 hours next to the bottle wall, above the sediment-water interface or at mid-height
480 of the water bottle. Examination of magnetically concentrated cells was carried out using the
481 hanging drop technique (Schüler, 2002) under a Zeiss Primo Star light microscope equipped
482 with phase-contrast, differential interference contrast optics and a camera AxioCam 105.
483 Magnetotaxis was evidenced by rotating a stirring bar magnet at 180° on the microscope stage
484 to reverse the local magnetic field.

485

486 ***Cell count profiles***

487 For counting MTB cells in the water column, two 1-L bottles of water were collected at the
488 different investigated depths. For each bottle, three 40- μ L drops were immediately observed
489 using the hanging drop technique. Magnetotactic cells accumulated at the edge of the drops
490 when a magnetic field was applied at one side of the drop and were subsequently counted under
491 the light microscope. The counting occurred two minutes after the cells in the hanging drop
492 were exposed to the magnetic field. The number of cells counted in each drop was multiplied
493 by a factor of 25 to obtain the abundance of cells per milliliter. Cell counts were reported as the
494 means of two triplicates counts for each depth. Using this counting method, the lower limit of
495 MTB abundance is 8 cells mL⁻¹ (*i.e.* minimum of 1 cell /3 replicates \times 25).

496 Magnetotactic cells in sediment samples were counted using a Malassez counting chamber.
497 Measurements were systematically carried out on three replicates. Cells were not immobilized
498 during counting. Although some MTB are fast swimmers, we were able to count the number of
499 cells in the chamber reproducively. Counts were performed as fast as possible, a task facilitated
500 by the fact that most MTB cells contain refractile inclusions (e.g. sulphur or calcium carbonate)
501 which makes their identification under the microscope easy.

502

503 ***Transmission and scanning electron microscopy (TEM and SEM)***

504 The morphology of MTB as well as the shape and arrangement of magnetosomes were observed
505 after fixation of the cells on a TEM carbon-coated grid. Electron micrographs were recorded
506 with a Tecnai G² BioTWIN (FEI Company) equipped with a CCD camera (Megaview III,
507 Olympus Soft imaging Solutions GmbH) using an accelerating voltage of 100 kV. Z-contrast
508 imaging in the high-angle annular dark field (STEM-HAADF) mode, and elemental mapping
509 by X-ray energy-dispersive spectrometry (XEDS) were carried out using a JEOL 2100F
510 microscope. This machine, operating at 200 kV, was equipped with a Schottky emission gun,
511 an ultrahigh resolution pole piece, and an ultrathin window JEOL XEDS detector.

512 Bulk magnetic particles extracted from the sediments were observed by scanning electron
513 microscopy (SEM) in back-scattered electron and secondary electron imaging modes to
514 characterize both the shape and chemical composition of particles in the samples. Analyses
515 were carried out at Institut de Physique du Globe de Paris (IPGP) using a Carl Zeiss EVO MA10
516 SEM. Standard operating conditions for SEM imaging and EDS (energy dispersive
517 spectroscopy) analyses were 15 kV accelerating voltage, working distance of 12 mm, and
518 electron beam current of 2-3 nA. Samples were coated with a few nanometers of Au prior to
519 analysis.

520

521 *Statistical analyses*

522 Statistical analyses were performed in R, version 4.0.0 (R Core Team, 2018). For each variable,
523 groups' averages were compared either by a paired Student's t-test or by a paired non-
524 parametric Mann–Whitney U test if variables were not normally distributed. Differences were
525 considered as significant when P-values were below 0.05.

526

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539

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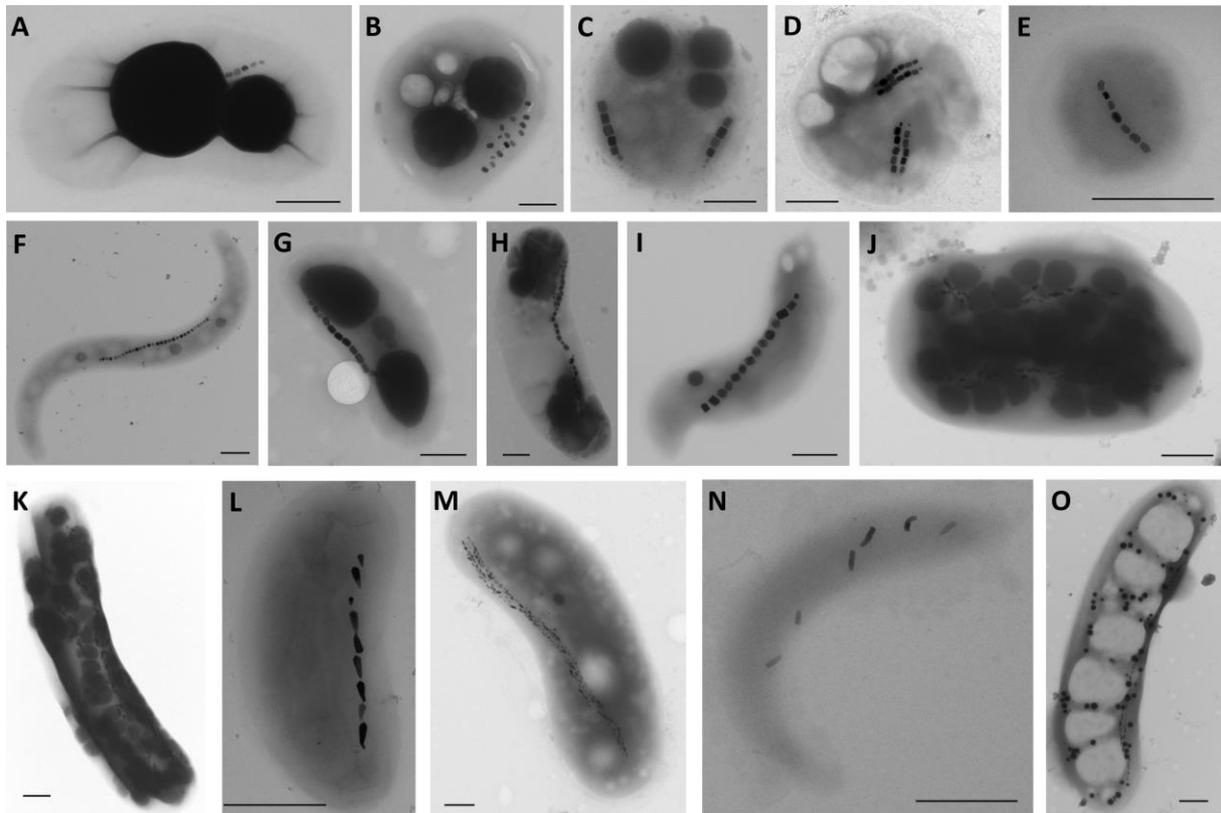
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770 **Figure and Figure legends**

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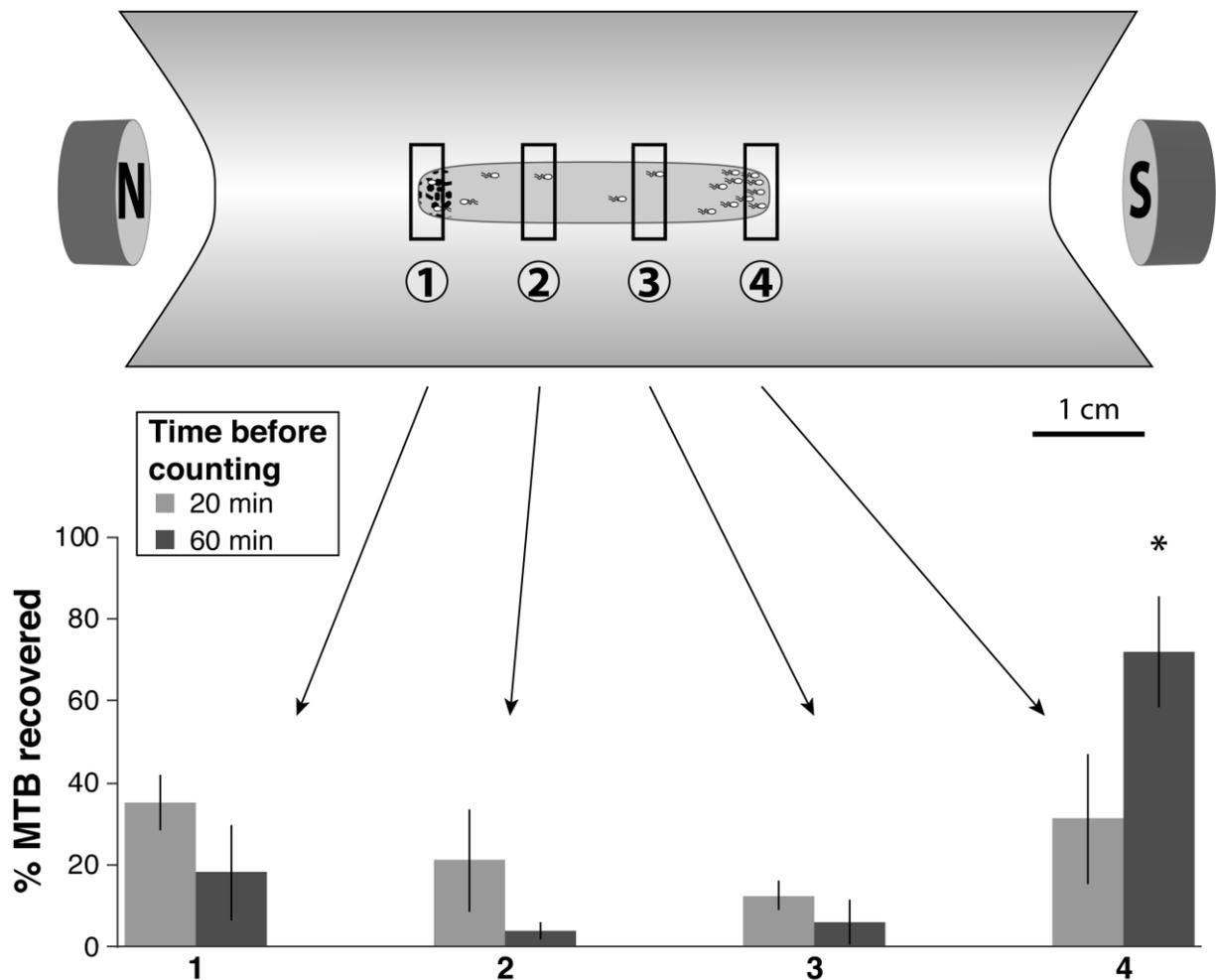
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773 **Fig. 1.** Transmission electron microscope images of different magnetotactic bacteria
774 morphotypes observed in Lake Pavin sediments. See main text for morphotypes description.

775 Scale bars represent 0.5 μm.

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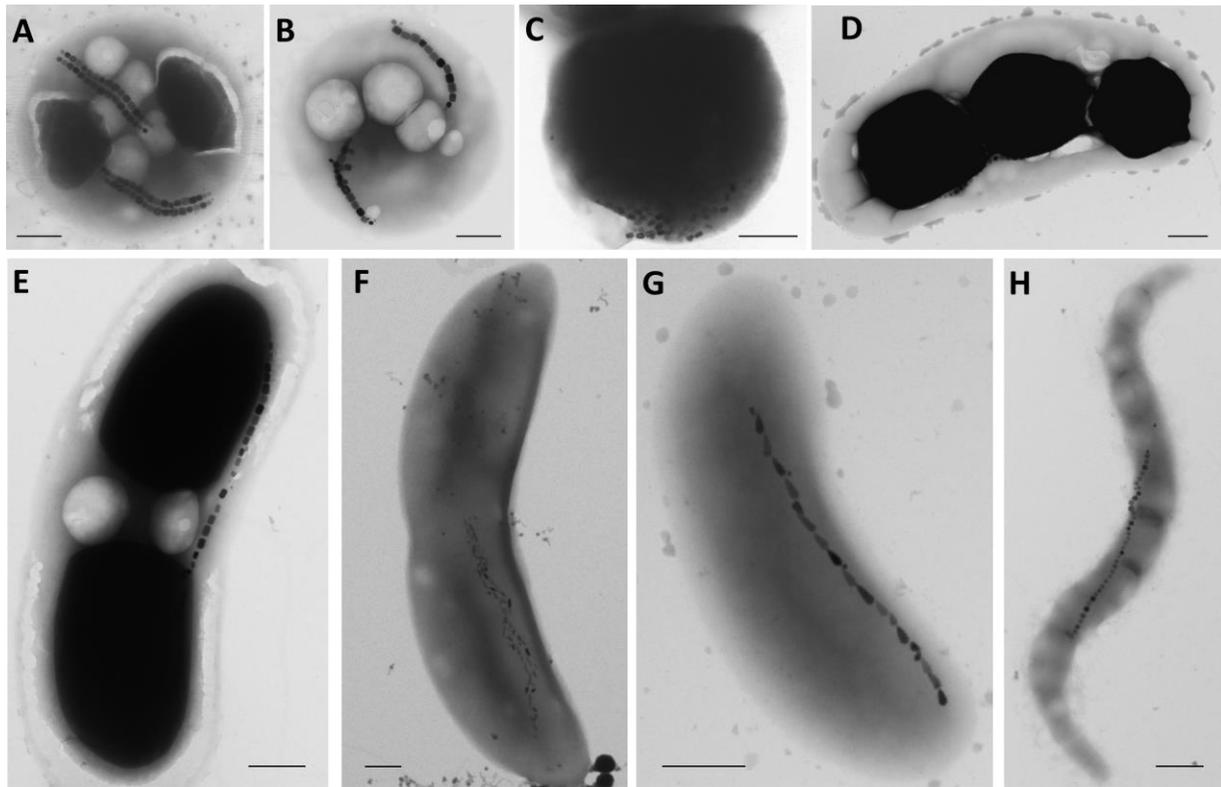
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779 **Fig. 2.** Magnetic purification of MTB from Lake Pavin sediments using the migration track
 780 device, developed in the present work. The pellet of magnetically enriched cells is deposited on
 781 the left side of the system (*i.e.* position 1) and magnetic particles (represented by the black dots)
 782 and south seeking MTB are trapped by the left magnetic bar. North seeking MTB swim toward
 783 the right magnetic bar with the majority accumulating at position 4. Histograms on the lower
 784 part of the figure represent the proportion of cells counted at each position (1, 2, 3 and 4) after
 785 20 or 60 min of magnetic purification, illustrating the efficiency of our migration track device.
 786 Cell counts are reported as the mean of triplicate measurements, with vertical lines indicating
 787 2σ uncertainties. The zone 4 after 60 min of magnetic purification is the only condition that
 788 concentrates significantly MTB relative to others. This is supported by a pairwise student t-test
 789 ($p < 0.034$).

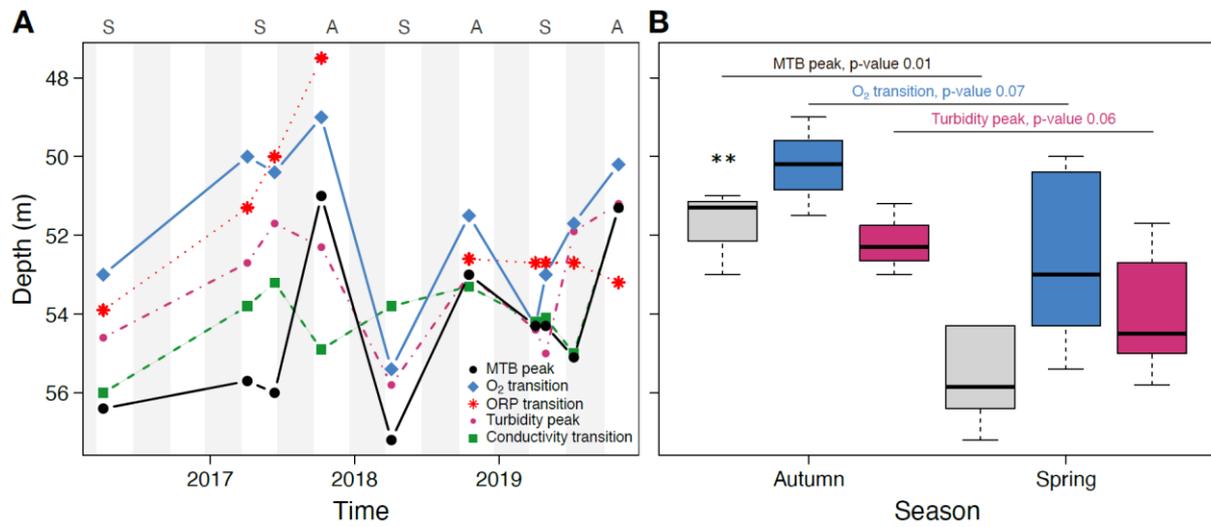
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792 **Fig. 3.** Transmission electron microscope images of different magnetotactic bacteria
793 morphotypes from Lake Pavin water column. See main text for morphotypes description. Scale
794 bars represent 0.5 μm.

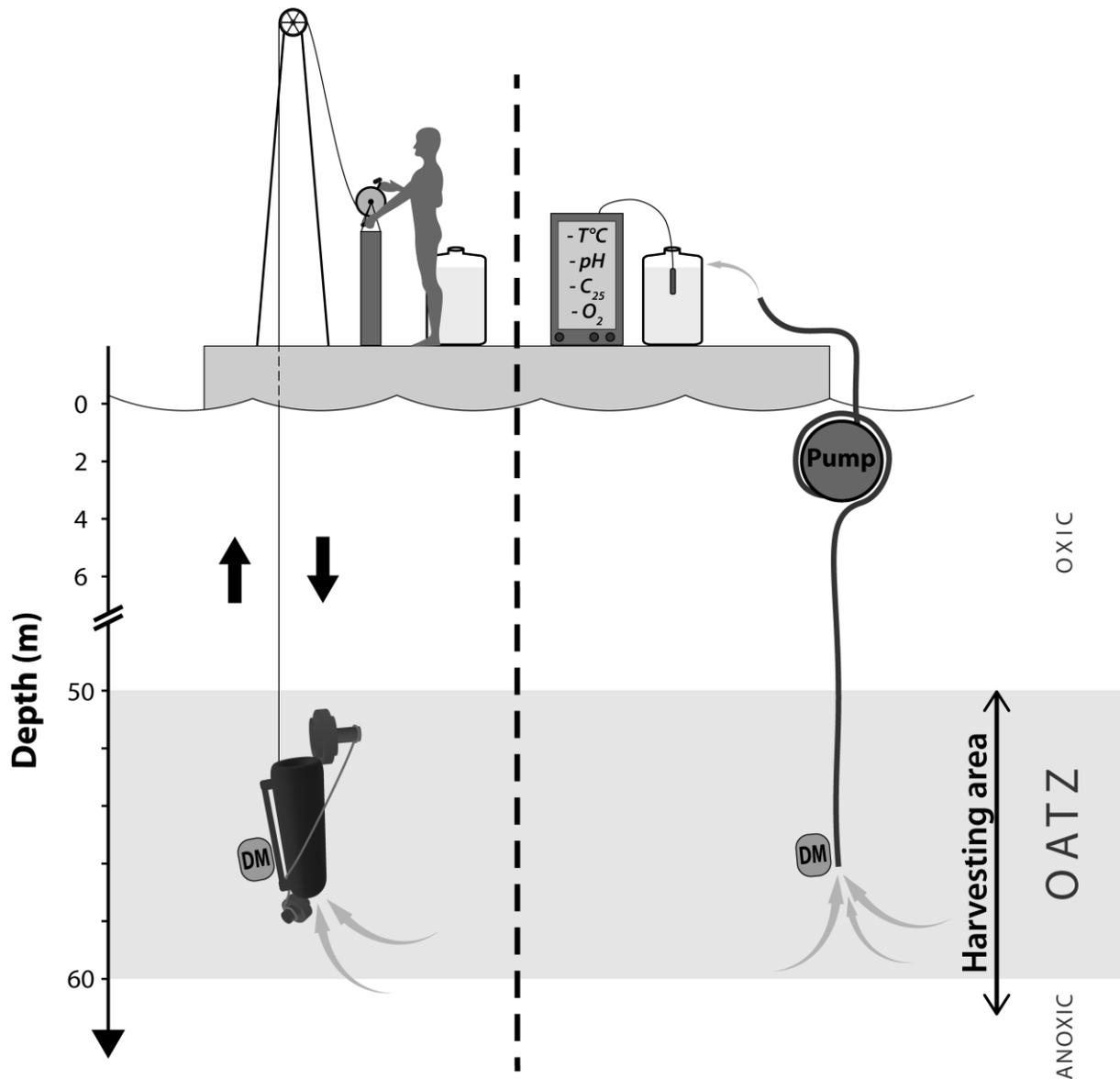
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797 **Fig. 4.** A) Depth variations of the MTB and turbidity peaks, as well as conductivity, oxygen
 798 and redox potential transitions upper boundary (*i.e.* depth where the maximum gradient starts)
 799 over time. B) Box plots showing the distribution of these depths as a function of the sampling
 800 season for the variables with the most significant mean differences according to a pairwise
 801 Student's t-test ($p < 0.1$).

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804 **Fig. 5.** Schematic representation of the methods used to sample the water column of Lake Pavin

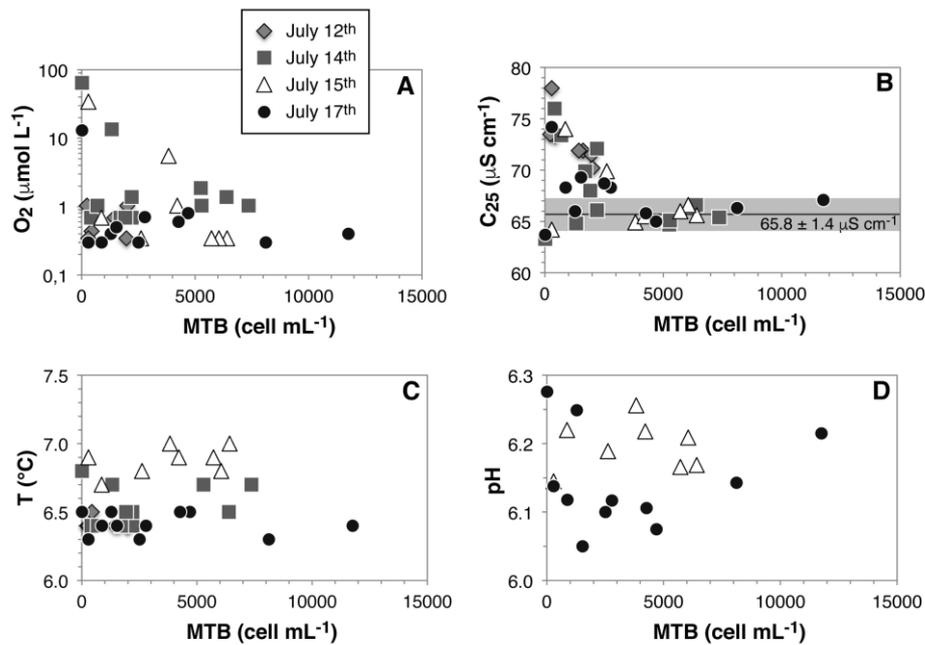
805 using a Niskin bottle (left side) and online pumping (right side). DM refers to the depthmeter

806 used to control the pipe depth. The oxic-anoxic transition zone (OATZ) represents the region

807 where oxygen concentrations are at the micromolar to sub-micromolar levels and where

808 populations of magnetotactic cells are present.

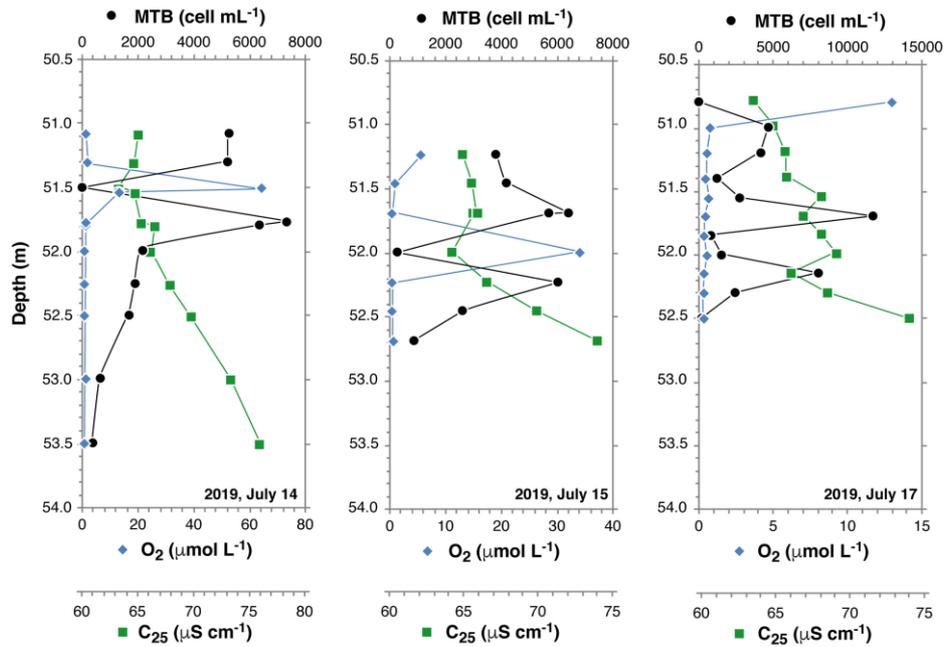
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811 **Fig. 6.** Relations between MTB cell abundance and various physicochemical parameters from
 812 Lake Pavin water column in the vicinity of the redox boundary, during the July 2019 sampling
 813 campaign. (A) Dissolved oxygen concentration ($\mu\text{mol L}^{-1}$), (B) specific conductivity at 25°C
 814 ($\mu\text{S cm}^{-1}$), (C) temperature ($^{\circ}\text{C}$), and (D) pH. All samples were collected with our online
 815 pumping system (Fig. 5 left panel). Note that the temperature measured on the platform could
 816 slightly differ from the temperature at depth (i.e. approximately 4°C (Michard *et al.*, 1994;
 817 Bonhomme *et al.*, 2011)) due to thermal exchange in the 60-m long pipe.

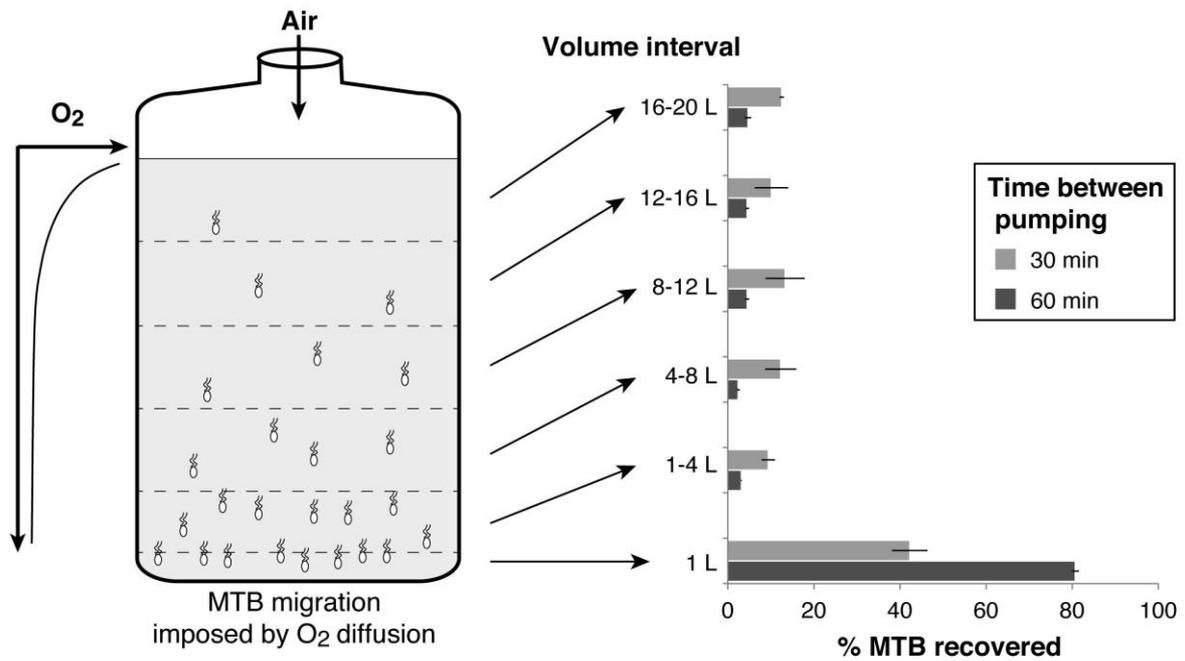
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820 **Fig. 7.** Magnetotactic cell abundance (cell mL^{-1}), dissolved oxygen concentration ($\mu\text{mol L}^{-1}$)
 821 and specific conductivity ($\mu\text{S cm}^{-1}$) profiles from Lake Pavin water column, on July 14th, 15th
 822 and 17th 2019. The high-spatial resolution of water collection was reached using online
 823 collecting system.

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826 **Fig. 8.** Schematic representation (left panel) of the aerotactic concentration of magnetotactic
 827 bacteria after collection from the water column with the online pumping system and transferred
 828 in a 20-L Nalgene bottle. Histograms (right panel) represent the percentage of MTB cells
 829 recovered in each water layer after 30 or 60 min of idle time between pumping, showing the
 830 efficiency of our device. The percentage of MTB recovered are reported as the mean of
 831 triplicate counts and line extensions represent the positive and negative standard deviations.
 832 The thickness of each water layer in the Nalgene bottle was about 10 cm.

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834 **Supporting Information**

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836 **Mass collection of magnetotactic bacteria from the permanently stratified ferruginous**

837 **Lake Pavin, France**

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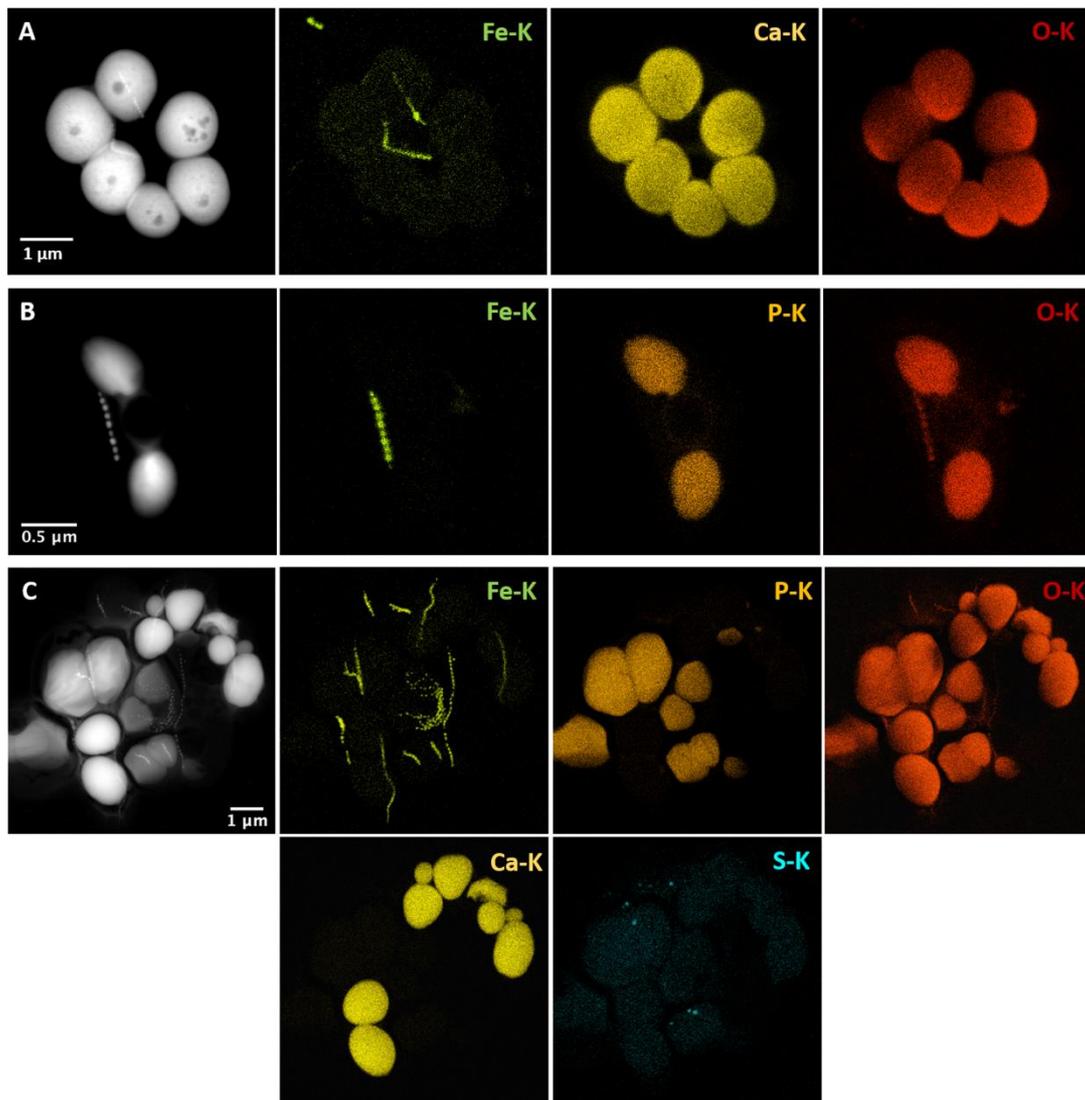
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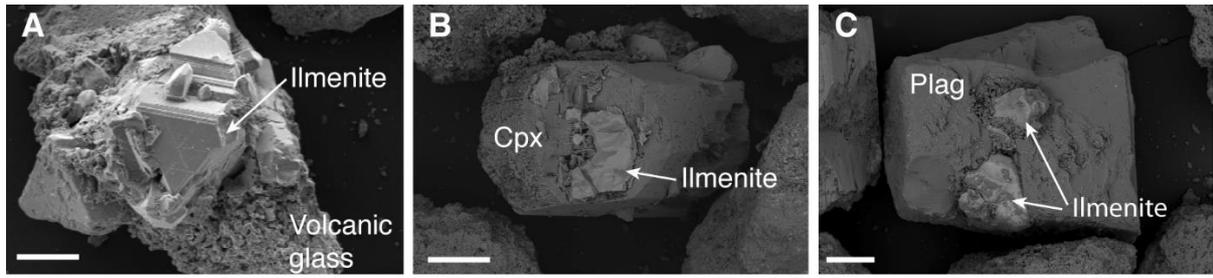
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859

860 **Fig. S1.** Z-contrast imaging in the high-angle annular dark field (STEM-HAADF) mode, and
 861 elemental mapping by X-ray energy-dispersive spectrometry (XEDS) showing the chemical
 862 composition of inclusions formed by magnetotactic bacteria present in the sediments (A and
 863 B) and in the water column (C) of Lake Pavin.



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865 **Fig. S2.** Scanning electron microscope images of ilmenite inclusions in volcanic glass (A), and

866 clinopyroxene (Cpx) (B) and plagioclase (Plag) (C) crystals. This illustrates the volcanic origin

867 of ilmenite from Lake Pavin sediments. Scale bars represent 100 μm .

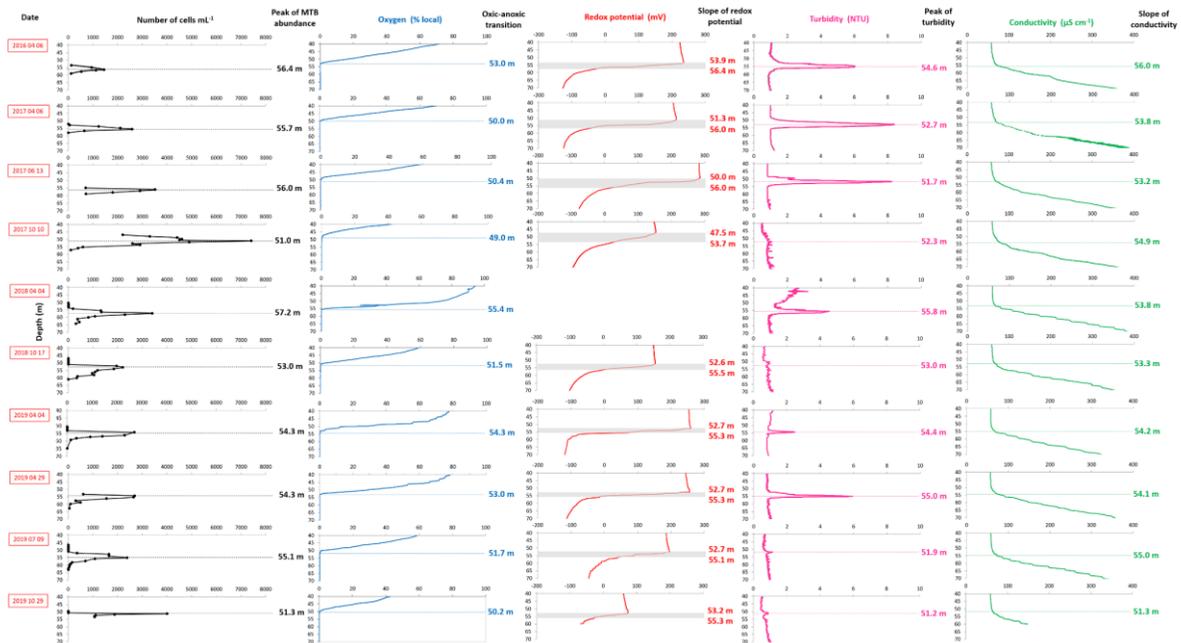
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870 **Fig. S3.** Picture of the migration track system. Edges of the drop exposed to a magnetic field

871 generated by the two disc magnets are shown by arrows. The scale is shown by a ruler.



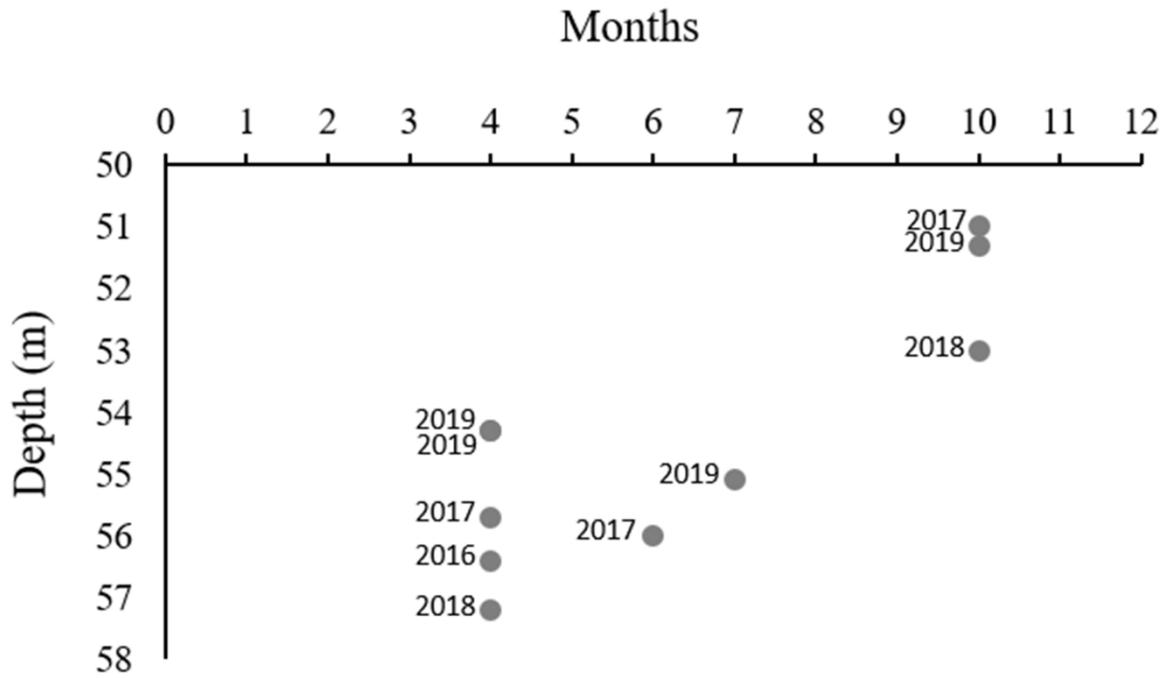
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874 **Fig. S4.** Vertical profiles of magnetotactic cells abundance (A), dissolved oxygen concentration (B), redox potential (C), turbidity (D) and
875 conductivity (C₂₅) (E) through the water column. Sampling campaigns were carried out at ten different dates between April 2016 and October 2019.
876 Note that measurements extend through the oxic-anoxic interface and the deeper regions of the anaerobic zone of the water column. Samples used
877 for magnetotactic cells counting were collected using a Niskin bottle. All other parameters correspond to continuous profiles measured using *in situ*
878 probes.

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882 **Fig. S5.** Depth of the maximum abundance of MTB counted in the water column of Lake Pavin
 883 as a function of the month. It illustrates that MTB are generally located shallower in the water
 884 column in autumn compared to spring and summer.

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