



HAL
open science

Iron-oxidizer hotspots formed by intermittent oxic–anoxic fluid mixing in fractured rocks

Olivier Bochet, Lorine Bethencourt, Alexis Dufresne, Julien Farasin, Mathieu Pédrot, Thierry Labasque, Eliot Chatton, Nicolas Lavenant, Christophe Petton, Benjamin W Abbott, et al.

► To cite this version:

Olivier Bochet, Lorine Bethencourt, Alexis Dufresne, Julien Farasin, Mathieu Pédrot, et al.. Iron-oxidizer hotspots formed by intermittent oxic–anoxic fluid mixing in fractured rocks. *Nature Geoscience*, 2020, 13 (2), pp.149-155. 10.1038/s41561-019-0509-1 . insu-02395691

HAL Id: insu-02395691

<https://insu.hal.science/insu-02395691v1>

Submitted on 10 Dec 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Deep iron-oxidizing bacteria sustained by intermittent** 2 **oxygen delivery through fractures**

3 Olivier Bochet¹, Lorine Bethencourt², Alexis Dufresne², Julien Farasin¹, Mathieu Pedrot¹, Thierry
4 Labasque¹, Eliot Chatton¹, Nicolas Lavenant¹, Christophe Petton¹, Benjamin W. Abbott^{3,2}, Luc
5 Aquilina¹ & Tanguy Le Borgne^{1*}

6 ¹*Geosciences Rennes laboratory, UMR 6118 CNRS University of Rennes 1, France*

7 ²*Ecobio laboratory, UMR 6553 CNRS University of Rennes 1, France*

8 ³*Brigham Young University, Department of Plant and Wildlife Sciences, Provo, USA*

9 **Subsurface environments host most of the freshwater on Earth as well as diverse microor-**
10 **ganisms that may constitute a significant part of the biosphere. However, the dynamics and**
11 **spatial distribution of subsurface microorganisms and their response to hydrological pro-**
12 **cesses are poorly understood. Here we used chemical and metagenomic analysis of ground-**
13 **water in a fractured rock aquifer in western France to determine the role of fractures in the**
14 **formation of deep microbial hot spots in the subsurface. The majority of fractures, sampled**
15 **in a 130-meter-deep borehole, were anoxic, but a fracture carrying oxic groundwater was**
16 **detected at depths from 37 to 59 meters, associated with a five-fold increase in the abundance**
17 **of iron-oxidizing bacteria. We developed a mechanistic model of fluid flow and mixing in**
18 **fractures and found that such microbial hot spots are sustained by the mixing of fluids with**
19 **contrasting redox chemistries at intersections of fractures. The model predicted that meter-**
20 **scale changes in near-surface water table levels cause intermittent oxygen delivery through**

21 **deep fractures, which can extend the depth of the habitable zone for iron-oxidizing bacte-**
22 **ria hundreds of meters into the subsurface. Given that fractures are ubiquitous at multiple**
23 **scales in the subsurface, such deep microbial hot spots may substantially influence microbial**
24 **communities and their effect on Earth's biogeochemical cycles.**

25 Water flow transports nutrients and energy for microorganisms, sustaining enhanced reac-
26 tion rates by delivering missing reactants for fluid-fluid or fluid-mineral reactions¹⁻³. Highly re-
27 active hot spots often form at the intersection of hydrological flow paths, where the confluence of
28 complementary reactants promotes biogeochemical activity that can disproportionately influence
29 larger-scale production rates⁴⁻⁷. These hot spots or ecosystem control points⁸ depend on strong
30 redox and concentration gradients⁹⁻¹¹, and though they change in space and time, they often occur
31 in areas where groundwater mixes with surface water bodies^{5,12,13}. Reactive hot spots occurring
32 near the land or sediment surface can be readily identified, but deep hot spots are difficult to access
33 and generally neglected because most biogeochemical models do not predict their occurrence^{2,14}.
34 Drilling campaigns in the continental crust and in the ocean floor have revealed that deep envi-
35 ronments host diverse microbial communities¹⁵⁻²⁰, which support high rates of biogeochemical
36 transformation²¹. However, the spatial patterns of the deep biosphere remain largely unknown and
37 there is currently no mechanistic framework to predict the location and duration of deep microbial
38 hot spots^{22,23}.

39 A common justification for disregarding deep mixing hot spots is that groundwater flow lines
40 are not expected to intersect in homogeneous subsurface domains (Fig. 1a). In this conventional

41 view, mixing hot spots only form in groundwater discharge areas where flow lines converge⁵. As
42 water flows along subsurface flow paths, chemical species are generally consumed in the order
43 of their redox potential²¹, leading to vertical stratification of oxidized species depending on water
44 residence time (Fig. 1a). This stable redox gradient could slow down biogeochemical activity
45 and prevent the development of mixing hot spots in the deep subsurface. Here, we tested this
46 longstanding view using quantitative hydrological and microbiological methods. We hypothesized
47 that reactive microbial hot spots can occur over a large range of depths because of the presence
48 of connected fractures at multiple scales in the subsurface^{24,25}. These structural discontinuities
49 produce preferential flow paths, which can mix water with very different residence times and redox
50 properties²⁶. This short circuiting of hydrological flow paths can produce reactive hot spots at much
51 greater depths than predicted for homogeneous environments (Fig. 1b).

52 **Observation of subsurface microbial mats in fractured rock**

53 To test the hypothesis of fracture-induced microbial hot spots in the deep biosphere, we analyzed
54 the biological and chemical properties of fracture fluid in a borehole where a massive microbial
55 mat was formed by iron-oxidizing bacteria (FeOB) dozens of meters below the surface (see Sup-
56 plementary Movie) in the fractured rock observatory of Ploemeur, France, part of the H+ net-
57 work of hydrogeological observatories (<http://hplus.ore.fr/en/ploemeur>). FeOB are emblematic
58 of mixing zones, where they take advantage of redox gradients from groundwater inflows rich
59 in reduced iron encountering oxygenated environments²⁷. At circumneutral pH, soluble Fe(II) is
60 readily oxidized by O_2 , forming iron oxyhydroxides which immediately precipitate from solution.

61 Neutrophilic FeOB are only able to outcompete this abiotic oxidation in specific microaerobic
62 conditions ($0.1 < O_2 < 1.5 \text{ mg.L}^{-1}$)²⁸, where they can form thick microbial mats, for instance, in
63 stream beds^{29,30}, intertidal zones³¹, groundwater seeps³², and deep-sea vents³³. As Fe cycling is
64 coupled with the biogeochemical cycles of carbon, nitrogen, and sulfur through both biotic and abi-
65 otic reactions²⁷, FeOB may exert an important control on the biogeochemistry of both continental³⁴
66 and oceanic^{23,35} subsurface environments. In continental systems, neutrophilic, oxygen-dependent
67 iron-oxidizing bacteria are mostly Betaproteobacteria represented by the *Leptothrix* genus³⁰ and
68 members of the Gallionellaceae family: *Gallionella*, *Sideroxydans*, and *Ferriphaselus*^{36,37}. Gal-
69 lionellaceae are thought to be autotrophs that use dissolved CO₂ as a carbon source^{36,37}. In under-
70 ground ecosystems, this primary production could be an important organic carbon source fueling
71 heterotrophic microorganisms. Gallionellaceae appear to be dominant in fractured rocks³⁴, where
72 fluids are rich in dissolved iron, serving as electron donor for FeOB. However, the pathways and
73 dynamics of electron acceptor delivery (e.g. dissolved oxygen or nitrate) in these environments are
74 poorly understood.

75 The studied fractured rock aquifer is partitioned into two chemically distinct zones: recharge
76 areas, located in high topographic positions, where water is oxygen-rich, and discharge areas, lo-
77 cated in low topographic positions, which are poor in oxygen and rich in dissolved iron (see Meth-
78 ods and Extended Data Fig. 1). In recharge areas, downward oxygen fluxes are driven by a positive
79 difference between near surface and deep hydraulic heads. In discharge areas, upward dissolved
80 iron fluxes are driven by negative difference between these hydraulic heads (see Extended Data
81 Fig. 1). Flow primarily occurs in a network of fractures that extends through granite and micas-

82 chist bedrock (see Methods)^{38,39}. The PZ26 borehole is a 130 m deep artesian borehole located in
83 a discharge area, where a borehole camera survey revealed the presence of a massive rust-colored
84 mat (Fig. 2a, Extended Data Fig. 4, and Supplementary Movie). Borehole optical logs and video
85 images showed that the borehole walls were entirely covered from the surface to approximately a
86 60 m depth by the rust-colored mat. Growth was particularly profuse where the main permeable
87 fractures intersected the borehole, in places completely clogging the borehole. Below 60 m, only
88 traces of the mat were visible, though there was still evidence of microbial growth at the main frac-
89 tures. All fractures are under pressure and constantly produce flow towards the top of the borehole
90 (see Methods and Extended Data Fig. 5). Therefore, dissolved oxygen cannot diffuse from the sur-
91 face through the borehole and any oxic fluid contributing to the observed microbial iron oxidation
92 must flow through the fracture network from the surface down to 60 meters. Along this fracture
93 pathway, the transported oxic fluid is likely to have multiple opportunities to mix with iron rich
94 fluid at intersections with anoxic fractures (Fig. 1b). We combined hydrogeological, geochemical,
95 and metagenomic analysis to investigate the resulting development of deep microbial hot spots of
96 iron oxidizing bacteria within the fracture network. Because mixing of different fracture fluids in
97 the borehole could influence chemical and biological properties, we collected fracture fluid directly
98 from fractures using packers^{40,41} (hydraulic obturators, see Methods and Extended Data Fig. 5),
99 which allow isolating the fractures from the borehole and sampling undisturbed fracture fluid, i.e.
100 not influenced by the borehole.

101 **Fracture distribution and chemical composition**

102 The depth and hydraulic properties of the main permeable fracture were quantified from borehole
103 flowmeter logging and optical borehole logging (see Fig. 2b, Methods and Extended Data Table
104 1). More than half of the flow in borehole PZ26 was produced by a highly permeable zone at the
105 bottom of the borehole (below 94 m). The rest of the flux came from various shallower fractures,
106 each constituting less than 10 % of the total flow. Estimation of fracture dips and density from
107 optical and acoustic logs (Fig. 2c) indicated that the density of fractures was on average 1.5
108 fractures per meter in the first 60 meters and close to 1 fracture per meter in the lower part of the
109 borehole. The average fracture dip was about 60 degrees above 60 meters, and about 40 degrees
110 below 60 meters. The zone with the maximum average fracture dip (60 to 80 degrees) was located
111 between 45 and 65 meters (Fig. 2c).

112 To characterize the properties of fracture fluid and investigate the existence of natural micro-
113 bial hot spots within the fracture network, we performed vertically-discrete sampling of pristine
114 fracture fluid with an inflatable packer capable of blocking vertical flow (see Methods). We sam-
115 pled water from each of the main fractures along the entire length of the borehole and analyzed ma-
116 jor ions, dissolved gases and microbial diversity (Fig. 2d, Fig. 3 and Methods). The depths of the
117 sampled fractures were known with a good precision from the optical and flow logs (Extended Data
118 Table 1). Based on the volume pumped before sampling ($V = 100L$) and the estimated fracture
119 aperture, the sampling radius ranged from 4 to 8 meters from the borehole (Extended Data Table
120 1). Hence, combining optical and flow logs with packer sampling allowed precise characterization

121 of the spatial distribution of chemical properties and microbial communities in the subsurface. Iron
122 concentration generally increased with depth, while oxygen concentration decreased, but there was
123 a significant anomaly of high oxygen concentration (4 mg.L^{-1}) in the F54 fracture intersecting the
124 borehole at 54 m, suggesting a high degree of connectivity of the associated fracture network with
125 the surface (Fig. 2d). This was consistent with the high chlorofluorocarbon (CFC) 113 concen-
126 tration measured at the same depth, indicating short residence-time⁴², and with the increase of the
127 average fracture dip between 45 and 65 meters, indicating the presence of numerous sub-vertical
128 fractures, which may facilitate connectivity with the surface (Fig. 2c). Ground Penetrating Radar
129 imaging of saline tracer transport in fractures obtained in a nearby borehole indicated the existence
130 of numerous intersections between permeable fractures³⁹. These intersections create high vertical
131 connectivity of the fracture network and act as hydrological mixing zones and biogeochemical hot
132 spots when intersecting fractures carry chemically distinct fluids. The 3D representation of the
133 permeable fractures intersecting the borehole provided a view of the fracture network organiza-
134 tion, suggesting that connections between the oxic fracture and anoxic fractures occurred outside
135 of the borehole (Fig. 2e). Comparison of borehole oxygen profiles measured during a recharge
136 period (April 2017) and a dry period (July 2017) showed that oxygen delivery at depth through
137 fractures was intermittent in time (Fig. 2f and Extended Data Fig. 3). During the recharge period,
138 most fractures above 60 meters delivered oxygen at depth, while no oxygen was detected in the
139 borehole during the dry period.

140 **Deep hot spot of iron oxidizing bacteria**

141 Scanning electron microscope observations performed on samples from batch experiments and
142 from the borehole showed typical structures of known iron-oxidizing bacteria (see Methods). The
143 most abundant structures were sheaths (Fig. 3a), which can be formed by *Leptothrix ochracea*,
144 and twisted stalks (Fig. 3b), typical of *Gallionella ferruginea*⁴³ and *Ferriphaselus amnicola*⁴⁴.
145 Batch incubations were performed to characterize the mechanisms of biofilm formation and its
146 effect on water chemistry (see Methods). For these experiments, water was sampled from the top
147 of the borehole, which represents a mixture of water produced from the different fractures in this
148 artesian borehole (all fractures are under pressure and produce water that is constantly flowing out
149 of the borehole). Batch results confirmed that the main reaction occurring when mixing fluid from
150 different fractures was iron oxidation by FeOB. Carbon needed for bacterial growth was provided
151 by dissolved inorganic carbon. Such reactions are expected to occur naturally at intersections of
152 oxic and anoxic fractures (Fig. 1b).

153 To assess the diversity and the relative abundance of FeOB from water samples collected
154 in fractures, we performed a metagenomic characterization of batch incubation and groundwater-
155 associated communities. Taxonomic classification of metagenomic reads from fluids sampled in
156 fractures showed highly diverse communities with more than 155 phyla and candidate divisions
157 of Bacteria and Archaea (see Supplementary Information). Metagenomes from water enriched
158 in FeOB during batch incubations were further used to reconstruct genomes of the main FeOB
159 from assembly and binning of metagenomic sequences (see Supplementary Information). This

160 yielded nine draft genomes (i.e. clusters of large genomic fragments or contigs) affiliated with the
161 Gallionellaceae family (see Supplementary Table S1). Phylogenomic analyses based on universal
162 protein-coding genes showed that seven genomes were related to the three Gallionellaceae genera
163 already described. Two additional genomes appeared more distantly related, suggesting that they
164 could belong to a new lineage of undescribed Gallionellaceae (see Supplementary Fig. S4 and Fig.
165 S5). Abundant microtubular sheaths, until now attributed to *Leptothrix ochracea*³⁰, were observed
166 in batch and borehole samples, but the *Leptothrix* genome was not detected in our samples.

167 The proportion of reads aligning with the Gallionellaceae genomes was variable among frac-
168 tures ranging from 0.25 % in the fractures located above 30 meters and below 60 meters depth to
169 1.5 and 1.3 % respectively in fractures F37 and F54/59 (Fig. 3c). This large increase in relative
170 abundance with respect to the background (about 600 % in F37 and 500 % in F54/59) placed FeOB
171 in second position in the ranking of bacteria family in F37 and in first position in F54/F59 (see Fig.
172 S3 in the Supplementary Information). In this context of highly diverse subsurface bacterial com-
173 munities, the relative abundances of the dominant bacteria in fractures did not exceed one to two
174 percent, as found in other studies using a similar genome-resolved approach⁴⁵. Hence, the two
175 criteria that we use to define the FeOB hot spot are: i) a 500 to 600 % increase of the FeOB rela-
176 tive abundance in fractures F37 and F54/59 relative to the background, ii) a respective second and
177 first position of FeOB in the relative abundance ranking of bacteria families in these two fractures,
178 while they were ranked much lower in other fractures.

179 The detected microbial hot spot coincided with the location of the oxic fracture zone that

180 delivered oxygen from the surface to depth through a network of mostly anoxic fractures, rich
181 in dissolved iron (Fig. 2). Therefore the origin of this hot spot was likely the intersection oxic
182 and anoxic fractures that created locally favorable conditions for microbial iron oxidation in the
183 fracture network (Fig. 1b and 2f). Genes associated with denitrification pathways were detected
184 in some of the assembled Galionellaceae genomes (data not shown). However, measured nitrate
185 concentration was always below the detection limit, suggesting that oxygen was the main electron
186 acceptor for iron oxidation. Note that the continuous presence of a dense microbial mat in the
187 borehole from 60 meters to the top of the borehole was likely due to the upflow of fracture fluid
188 from fractures F37 and F54/F59 to the surface because of the artesian nature of the borehole. This
189 allowed for the colonization of the upper part borehole by FeOB, which are known to create large
190 amounts of mat. Because iron oxidation does not yield much energy, FeOB need to oxidize a large
191 quantity of reduced iron to sustain their metabolism^{27,29}.

192 **Fracture fluid mixing and intermittent oxygen delivery**

193 We combined flow, fracture, and oxygen logs with chemical composition of fracture fluids in the
194 borehole to develop a mechanistic model explaining and predicting quantitatively the formation
195 of deep microbial hot spots in the subsurface. During recharge periods, sub-vertical fractures act
196 as preferential flow paths to transport oxygen-rich water from the surface to the deep subsurface.
197 Because such transport occurs through a multiscale fracture network composed of fractures of
198 broadly different lengths and orientations⁴⁶, there are multiple opportunities for mixing with old
199 groundwater that is rich in dissolved mineral species at intersections of oxic and anoxic fractures.

200 This effectively creates a short-circuiting of the stratified redox sequence expected for homoge-
201 neous media (Fig. 1a). Iron-oxidizing microbial communities can then develop at intersections
202 between oxygenated and anoxic fractures at much greater depths than expected in the conventional
203 view (Fig. 1b). Seasonal fluctuations in recharge (Extended Data Fig. 3a) generate intermittent
204 oxygen delivery, likely inducing temporally dynamic microbial growth (Fig. 2f). The proposed
205 mechanism of fracture-induced mixing may be particularly significant in the fractured rocks that
206 constitute continental aquifers^{21,34} as well as in the oceanic crust where other taxa of iron-oxidizing
207 bacteria are present¹⁸. Our model predicts the range of depths where microaerobic conditions nec-
208 essary for FeOB are met⁴⁷ by quantifying change in oxygen due to biogeochemical reactions along
209 the fracture flow path and mixing at fracture intersections.

210 We considered an intersection between two fractures that connected oxidized and reduced
211 flow paths (Fig. 1c), characterized respectively by the hydraulic transmissivities T_{ox} and T_{red} and
212 the oxygen concentrations $O_2^{ox} \gg O_2^{red}$. We assumed that water flow in the oxidized fracture Q_{ox}
213 was driven by the local hydraulic head difference between the shallow and deep subsurface dh (Fig.
214 1b), and that flow in the reduced fracture Q_{red} was driven by a regional hydraulic head gradient ∇h_r
215 (Fig. 1c). Furthermore, we assumed that oxygen consumption followed a first order kinetic when
216 transported by fracture flow. The oxygen concentration at fracture intersections was then calculated
217 assuming complete mixing at fracture intersections from a flux balance for different depths and
218 hydrological parameters (see Methods and Supplementary Information). Baseline values for all
219 parameters were estimated from observed conditions at the field site (Table 1).

220 As hydraulic transmissivity values can span orders of magnitude in fractured media^{24,38}, we
221 tested the sensitivity to this parameter of the location of the microbial oxidation zone (defined as
222 the range of depths where $0.1 < O_2 < 1.5 \text{ mg.L}^{-1}$)²⁸ (Fig. 4a), holding all other factors constant.
223 For low transmissivity ratios $T_{ox}/T_{red} < 10^{-3}$, the microbial oxidation zone was constrained to
224 shallow depths, but for intermediate ratios $10^{-2} < T_{ox}/T_{red} < 10^{-1}$, the reactive zone occurred
225 over a large range of depths (Fig. 4a). At larger ratios, chemical oxidation became dominant.
226 When model parameters were set to match observed conditions at the field site (Table 1), the
227 transmissivity ratio was 10^{-2} , which corresponds to the transition between shallow and deep mi-
228 crobial oxidation regimes. In this situation, the depths favorable for FeOB development ranged
229 from approximately 450 to 40 meters, which includes the depth of the observed microbial mat
230 formation (60 meters). While current representations dissociate surface, oxygen-rich ecosystems
231 from reduced, deep ecosystem, this result reveals the existence of a thick reactive zone where
232 mixed conditions can occur at fracture intersections, potentially contributing to recycling chemical
233 species originating from rock weathering²¹.

234 Because the flux of water and associated dissolved oxygen is proportional to the hydraulic
235 head difference dh between the near-surface and deep compartments, the range of depths favorable
236 for FeOB development is expected to vary seasonally (Fig. 4b), following the intermittent oxygen
237 delivery by fractures. At the field site, the near-surface hydraulic head varies between the recharge
238 period and the dry season, and the deep hydraulic head is relatively stable (Extended Data Fig. 3a).
239 According to model predictions (Fig. 4b), the resulting meter-scale fluctuations of the hydraulic
240 head difference between the shallow and deep compartments dh can generate hundred-meter-scale

241 fluctuations of the maximum depth of the FeOB habitable zone. This is consistent with observed
242 large changes in oxygen fluxes through permeable fractures observed between the recharge and
243 dry seasons at our field site (Fig. 2f and Extended Data Fig. 3b). This result demonstrates that the
244 depth of the microbial oxidation zone is highly sensitive to fluctuations in recharge at the surface,
245 which challenges the general view that deep subsurface environments respond slowly and weakly
246 to surface changes.

247 The presented model provides a mechanistic explanation for the observed deep microbial
248 hot spots and allows exploration of the sensitivity of this phenomenon to hydrological flow path
249 structure and temporal dynamics. This quantitative framework could be used to assess the like-
250 lihood of occurrence of different types of biogeochemical reactions besides iron-oxidation and
251 their consequences for the transport and transformation of chemical elements, nutrients, and con-
252 taminants in hydrological systems^{21,22,27}, particularly in the context of critical zone studies that
253 investigate coupled processes in the aquifer-catchment-atmosphere continuum². While the deep
254 subsurface is usually considered as biogeochemically buffered³⁴, because of its supposed isolation
255 from surface hydrological fluctuations, our results suggest that subterranean biogeochemistry may
256 be spatially heterogeneous and temporally dynamic. Favorable conditions for reactive hot spot
257 development are likely seasonal, creating hot moments of microbial activity at different depths
258 and different times of the year. Because the subsurface represents considerable volumes of water
259 and fluxes of dissolved elements⁴⁸, the identification of fracture-induced hot spots could influence
260 landscape-scale biogeochemical cycles²¹. More generally, the presence of large and dynamic sub-
261 surface microbial communities has fundamental implications for our understanding of the location

262 and timing of biogeochemical activity in the Earth's ecosystems¹⁴. The existence of fractures at
263 multiple scales in the Earth's subsurface²⁴ suggests that the mechanisms described here may be
264 pervasive and potentially involve a large range of biogeochemical processes, driven by mixing of
265 fluids with different residence times and chemical compositions¹⁰.

266 **References**

- 267 1. Maher, K. & Chamberlain, C. Hydrologic regulation of chemical weathering and the geologic
269 carbon cycle. *Science* **343**, 1502–1504 (2014).
- 270 2. Li, L. *et al.* Expanding the role of reactive transport models in critical zone processes. *Earth-*
271 *Science Reviews* **165**, 280–301 (2017).
- 272 3. Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M. & Packmann, A. I. The ecology
273 and biogeochemistry of stream biofilms. *Nature Reviews Microbiology* **14**, 251–263 (2016).
- 274 4. McMahon, P. Aquifer/aquitard interfaces: Mixing zones that enhance biogeochemical reac-
275 tions. *Hydrogeology Journal* **9**, 34–43 (2001).
- 276 5. McClain, M. *et al.* Biogeochemical hot spots and hot moments at the interface of terrestrial
277 and aquatic ecosystems. *Ecosystems* **6**, 301–312 (2003).
- 278 6. Frei, S., Knorr, K. H., Peiffer, S. & Fleckenstein, J. H. Surface micro-topography causes hot
279 spots of biogeochemical activity in wetland systems: A virtual modeling experiment. *J. of*
280 *Geophys. Res. : Biogeosciences* **117**, G00N12 (2012).

- 281 7. Cardenas, M. B. Hyporheic zone hydrologic science: A historical account of its emergence
282 and a prospectus. *Water Resources Research* **51**, 3601–3616 (2015).
- 283 8. Bernhardt, E. S. *et al.* Control points in ecosystems: Moving beyond the hot spot hot moment
284 concept. *Ecosystems* 1–18 (2017).
- 285 9. Kallmeyer, J. & Wagner, D. *Microbial Life of the Deep Biosphere* (De Gruyter, 2014).
- 286 10. Chapelle, F. *Ground-Water Microbiology and Geochemistry* (John Wiley and Sons, 2001).
- 287 11. Brune, A., Frenzel, P. & Cypionka, H. Life at the oxic anoxic interface: microbial activities
288 and adaptations. *FEMS Microbiology Reviews* **24**, 691–710 (2000).
- 289 12. Boano, F. *et al.* Hyporheic flow and transport processes: Mechanisms, models, and biogeo-
290 chemical implications. *Rev. Geophys.* **52**, 603679 (2014).
- 291 13. Stegen, J. C. *et al.* Groundwater-surface water mixing shifts ecological assembly processes
292 and stimulates organic carbon turnover. *Nature Communications* **7**, 11237 (2015).
- 293 14. Long, P., Williams, K., Hubbard, S. & Banfield, J. Microbial metagenomics reveals climate-
294 relevant subsurface biogeochemical processes. *Trends in Microbiology* **24**, 600–610 (2016).
- 295 15. Pedersen, K. Microbial life in deep granitic rock. *FEMS Microbiology Reviews* **20**, 399–414
296 (1997).
- 297 16. Breuker, A., Köweker, G., Blazejak, A. & Schippers, A. The deep biosphere in terrestrial
298 sediments in the Chesapeake Bay area, Virginia, USA. *Frontiers in Microbiology* **2**, 1–13
299 (2011).

- 300 17. Nyysönen, M. *et al.* Taxonomically and functionally diverse microbial communities in deep
301 crystalline rocks of the Fennoscandian shield. *ISME Journal* **8**, 126–138 (2014).
- 302 18. Santelli, C. M. *et al.* Abundance and diversity of microbial life in ocean crust. *Nature* **453**,
303 653–656 (2008).
- 304 19. Ménez, B., Pasini, V. & Brunelli, D. Life in the hydrated suboceanic mantle. *Nature Geo-*
305 *science* **5**, 133–137 (2012).
- 306 20. Trias, R. *et al.* High reactivity of deep biota under anthropogenic CO₂ injection into basalt.
307 *Nature Communications* **8**, 1063 (2017).
- 308 21. Kolbe, T. *et al.* Stratification of reactivity determines nitrate removal in groundwater. *Pro-*
309 *ceedings of the National Academy of Sciences* **116**, 2494–2499 (2019).
- 310 22. Edwards, K. J., Becker, K. & Colwell, F. The deep, dark energy biosphere: intraterrestrial life
311 on earth. *Annual Review of Earth and Planetary Sciences* **40**, 551–568 (2012).
- 312 23. Alain, K. *et al.* *Microbial Life of the Deep Biosphere*, vol. 1 (Walter de Gruyter, 2014).
- 313 24. Bonnet, E. *et al.* Scaling of fracture systems in geological media. *Rev. Geophys.* **39**, 347–383
314 (2001).
- 315 25. St Clair, J. *et al.* Geophysical imaging reveals topographic stress control of bedrock weather-
316 ing. *Science* **350**, 534–538 (2015).
- 317 26. Martinez-Landa, L. *et al.* Mixing induced reactive transport in fractured crystalline rocks.
318 *Applied geochemistry* **27**, 479–489 (2012).

- 319 27. Melton, E. D., Swanner, E., Behrens, S., Schmidt, C. & Kappler, A. The interplay of micro-
320 bially mediated reactions in the biogeochemical fe cycle. *Nature Reviews Microbiology* **12**,
321 797–808 (2014).
- 322 28. Anderson, C. R. & Pedersen, K. In situ growth of Gallionella biofilms and partitioning of
323 lanthanides and actinides between biological material and ferric oxyhydroxides. *Geobiology*
324 **1**, 169–178 (2003).
- 325 29. Quaiser, A. *et al.* Unraveling the stratification of an iron-oxidizing microbial mat by metatran-
326 scriptomics. *Plos One* **9**, 1–9 (2014).
- 327 30. Fleming, E. J., Cetinić, I., Chan, C. S., King, D. W. & Emerson, D. Ecological succession
328 among iron-oxidizing bacteria. *The ISME journal* **8**, 804–815 (2014).
- 329 31. McAllister, S. M. *et al.* Dynamic hydrologic and biogeochemical processes drive microbially
330 enhanced iron and sulfur cycling within the intertidal mixing zone of a beach aquifer. *Limnol-
331 ogy and Oceanography* **60**, 329–345 (2015).
- 332 32. Emerson, D. & Revsbech, N. P. Investigation of an iron-oxidizing microbial mat community
333 located near aarhus, denmark: field studies. *Applied and Environmental Microbiology* **60**,
334 4022–4031 (1994).
- 335 33. Emerson, D. & Moyer, C. L. Neutrophilic fe-oxidizing bacteria are abundant at the loihi
336 seamount hydrothermal vents and play a major role in fe oxide deposition. *Applied and Envi-
337 ronmental Microbiology* **68**, 3085–3093 (2002).

- 338 34. Ben Maamar, S. *et al.* Groundwater isolation governs chemistry and microbial community
339 structure along hydrologic flowpaths. *Frontiers in Microbiology* **6**, 1–13 (2015).
- 340 35. Bach, W. & Edwards, K. J. Iron and sulfide oxidation within the basaltic ocean crust: implica-
341 tions for chemolithoautotrophic microbial biomass production. *Geochimica et Cosmochimica*
342 *Acta* **67**, 3871–3887 (2003).
- 343 36. Emerson, D. *et al.* Comparative genomics of freshwater Fe-oxidizing bacteria: Implications
344 for physiology, ecology, and systematics. *Frontiers in Microbiology* **4**, 1–17 (2013).
- 345 37. Kato, S. *et al.* Comparative genomic insights into ecophysiology of neutrophilic, mi-
346 croaerophilic iron oxidizing bacteria. *Frontiers in Microbiology* **6**, 1–16 (2015).
- 347 38. Le Borgne, T., Bour, O., Paillet, F. L. & Caudal, J. P. Assessment of preferential flow path con-
348 nectivity and hydraulic properties at single-borehole and cross-borehole scales in a fractured
349 aquifer. *Journal of Hydrology* **328**, 347–359 (2006).
- 350 39. Dorn, C., Linde, N., Le Borgne, T., Bour, O. & Baron, L. Single-hole gpr reflection imaging
351 of solute transport in a granitic aquifer. *Geophysical Research Letters* **38** (2011).
- 352 40. Purkamo, L. *et al.* Dissecting the deep biosphere: Retrieving authentic microbial communities
353 from packer-isolated deep crystalline bedrock fracture zones. *FEMS Microbiology Ecology* **85**,
354 324–337 (2013).
- 355 41. Sorensen, J. P. R. *et al.* Using Boreholes as Windows into Groundwater Ecosystems. *PLoS*
356 *ONE* **8** (2013).

- 357 42. Ayraud, V. *et al.* Compartmentalization of physical and chemical properties in hard-rock
358 aquifers deduced from chemical and groundwater age analyses. *Applied Geochemistry* **23**,
359 2686–2707 (2008).
- 360 43. Hallbeck, L., Stahl, F. & Pedersen, K. Phylogeny and phenotypic characterization of the
361 stalk-forming and iron-oxidizing bacterium *Gallionella ferruginea*. *Journal of General Micro-*
362 *biology* **139**, 1531–1535 (1993).
- 363 44. Kato, S., Krepski, S., Chan, C., Itoh, T. & Ohkuma, M. *Ferriphaselus amnicola* gen. nov.,
364 sp. nov., a neutrophilic, stalk-forming, iron-oxidizing bacterium isolated from an iron-rich
365 groundwater seep. *International Journal of Systematic and Evolutionary Microbiology* **64**,
366 921–925 (2014).
- 367 45. Castelle, C. J. *et al.* Extraordinary phylogenetic diversity and metabolic versatility in aquifer
368 sediment. *Nature communications* **4**, 2120 (2013).
- 369 46. Jimenez-Martinez, J. *et al.* Temporal and spatial scaling of hydraulic response to recharge in
370 fractured aquifers: Insights from a frequency domain analysis. *Water Resources Research* **49**,
371 3007–3023 (2013).
- 372 47. Druschel, G. K., Emerson, D., Sutka, R., Suchecki, P. & Luther, G. W. Low-oxygen and
373 chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing mi-
374 croorganisms. *Geochimica et Cosmochimica Acta* **72**, 3358–3370 (2008).
- 375 48. Gleeson, T., Befus, K. M., Jasechko, S., Luijendijk, E. & Cardenas, M. B. The global volume
376 and distribution of modern groundwater. *Nature Geoscience* **9**, 161–167 (2016).

377 **Corresponding Author**

378 Correspondence and requests for materials should be addressed to Tanguy Le Borgne, tanguy.le-
379 borgne@univ-rennes1.fr

380 **Acknowledgements** Funding was provided by ERC project ReactiveFronts (648377), ANR projects CRI-
381 TEX (ANR-11-EQPX-0011), Subsurface mixing and reactions (ANR-14-CE04-0003) and Stock-en-Socle
382 (ANR-13-SEED-0009), ADEME and Région Bretagne. We thank the Ploemeur observatory (H+ network
383 hplus.ore.fr and OZCAR network of critical zone observatories) for providing data and field support for
384 this study. All the data used in this study and the fracture mixing code are accessible from the following
385 URL: <http://hplus.ore.fr/bochet-et-al-2019-data>. We thank L. Longuevergne and O. Bour, respectively PI
386 of the Ploemeur site and of the H+ network, for providing access to the site and to the data, and support for
387 the organization of field campaigns. We thank M. Bouhnik-Le-Coz and P. Petitjean for chemical analysis,
388 CMEBA for SEM imaging, CONDATE EAU for dissolved gas analysis, M. Chorin and A. Quaiser for light
389 and fluorescence microscopy. S. Michon-Coudouel, M. Biget for metagenome sequencing and S. Gu for the
390 chinese translation of the abstract. We finally thank Y. Duclos, S. Ben Maamar, T. Babey, G. Baby, P. Davy
391 for their help and stimulating scientific discussions.

392 **Author Contributions**

393 O.B. lead the field campaigns, batch tests, data interpretation, model development and results for-
394 matting, L.B. carried out metagenomic analysis, A.D. supervised metagenomic data interpretation,
395 J.F. managed bacteria sampling and characterization in field campaigns and batch experiments, and

Table 1: Baseline parameters for the fracture mixing model estimated from field data (see Supplementary Information).

Parameter	Estimated value	Parameter	Estimated value
z	50 m	T_{ox}	$10^{-5} \text{ m}^2.\text{s}^{-1}$
α	60°	z_c	850 m
∇h_r	3.10^{-3}	z_d	23 m
dh	8 m	O_2^0	4.5 mg.L^{-1}
T_{red}	$10^{-3} \text{ m}^2.\text{s}^{-1}$	O_2^{min}	0.1 mg.L^{-1}

396 performed hydrochemical borehole logging, M.P. carried out chemical analysis in field campaigns
397 and batch experiments, T.L. managed measurements of dissolved gases and CFC in field cam-
398 paigns, N.L. developed the packer system and managed borehole flow and pressure measurements,
399 C.P. handled logistics related to field campaigns, B.W.A. contributed to formalize biogeochemi-
400 cal implications of results, manuscript editing and proofreading, L.A. supervised geochemical and
401 metagenomic data interpretation, T.L.B. designed the research and supervised data interpretation,
402 modelling and manuscript writing.