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## Iron Isotope Fractionation during Bio- and Photodegradation of Organoferric Colloids in Boreal Humic Waters

Olga Oleinikova, Franck Poitrasson, Olga Yu. Drozdova, Liudmila Shirokova,  
Sergey Lapitskiy, Oleg Pokrovsky

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1 Iron isotope fractionation during bio- and photo-degradation  
2 of organo-ferric colloids in boreal humic waters  
3

4 Olga V. OLENIKOVA<sup>1</sup>, Franck POITRASSON<sup>1</sup>, Olga Yu. DROZDOVA<sup>2</sup>, Liudmila S. SHIROKOVA<sup>1,3</sup>,  
5 Sergey A. LAPITSKIY<sup>2</sup>, and Oleg S. POKROVSKY<sup>1,3,4\*</sup>  
6

7 <sup>1</sup> GET (Geosciences and Environment Toulouse) UMR 5563 CNRS, 14 Avenue Edouard Belin,  
8 31400 Toulouse, France

9 <sup>2</sup> Geological Faculty of Moscow State University, 1 Leninskie Gory, 119234 Moscow, Russia

10 <sup>3</sup> N. Laverov Federal Center for Integrated Arctic Research, Russian Academy of Science, 23  
11 Naberezhnaya Sev Dviny, Arkhangelsk, Russia

12 <sup>4</sup> BIO-GEO-CLIM Laboratory, Tomsk State University, 36 Lenina av., 634050 Tomsk, Russia  
13

14 \* Corresponding author. *Email address*: oleg.pokrovsky@get.omp.eu (Oleg S. Pokrovsky).  
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19  
20  
21

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25 Key messages:

26 Adsorption of heavy Fe isotopes on heterotrophic bacteria cell surface

27 Assimilation of light Fe isotopes by live *P. aureofaciens* cells

28 Removal of heavy Fe isotopes from solution during sunlight oxidation of organo-ferric colloids

29 Generation of isotopically light Fe(II) in LMW<sub>< 1 kDa</sub> during photolysis

30 Bio- and photodegradation of organo-ferric colloids can produce very large, from -2.5 to +3.2‰

31  $\delta^{57}\text{Fe}$  isotopic variations in boreal waters  
32  
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## 41 ABSTRACT

42 Bio-degradation and photolysis of dissolved organic matter (DOM) in boreal high-latitude waters  
43 are the two main factors controlling aquatic fluxes and residence time of carbon but also metal  
44 nutrients associated with DOM such as Fe. The DOM is usually present in the form of organic and  
45 organo-mineral colloids that also account for the majority of dissolved Fe. Here we use the stable Fe  
46 isotope approach to unravel the processes controlling Fe behavior during bio- and photo-degradation  
47 of colloids in boreal Fe- and DOM-rich humic waters (a stream and a fen). The adsorption of Fe  
48 colloids onto heterotrophic bacteria *P. aureofaciens* produced enrichment in +0.4‰ ( $\delta^{57}\text{Fe}$ ) in the  
49 heavier isotopes of the cell surface relative to the remaining solution. In contrast, long-term  
50 assimilation of Fe by live cells yielded preferential incorporation of lighter isotopes into the cells (-  
51 0.7‰ relative to aqueous solution). The sunlight-induced oxidation of Fe(II) in fen water led to  
52 removal of heavier Fe isotopes (+1.5 to +2.5‰) from solution, consistent with Fe(III) hydroxide  
53 precipitation from Fe(II)-bearing solution. Altogether, bio- and photodegradation of organo-ferric  
54 colloids, occurring within a few days of exposure time, can produce several per mil isotopic  
55 excursions in shallow lentic and lotic inland waters of high latitude boreal regions. Considerable  
56 daily scale variations of Fe isotopic composition should therefore be taken into account during  
57 interpretation of riverine flux of Fe isotope to the ocean or tracing weathering processes using Fe  
58 isotopes in surface waters at high latitudes.

59

## 60 1. Introduction

61 Despite broad use of Fe isotopes for tracing various large-scale, long-term processes at the  
62 Earth's surface<sup>1,2</sup>, notably in river<sup>3-8</sup> or lake<sup>9,10</sup> waters, the fractionation of Fe isotopes during short-  
63 term processes such as bio- and photo-degradation of organic matter that binds Fe in freshwater  
64 remain poorly understood. This is especially true for organic carbon and Fe-rich boreal waters, that,  
65 from the one hand, play a primary role in the C cycle, CO<sub>2</sub> emissions and storage<sup>11</sup> and from the

66 other hand, strongly contribute to Fe and other micro-nutrient export by rivers to the coastal  
67 productive zones<sup>13,14</sup>.

68 The majority of dissolved ( $< 0.22 \mu\text{m}$ ) Fe and other metals in boreal waters are present in the  
69 form of organic and organo-mineral colloids (1 nm - 0.22  $\mu\text{m}$ ) whose transport and bioavailability  
70 differ from inorganic ions or simple organic complexes<sup>15</sup>. Two main processes responsible for fluxes  
71 and residence time of organic C and metal colloids in boreal high-latitude aquatic environments are  
72 heterotrophic bacterial respiration (degradation of DOM) and photolysis<sup>16</sup>. Colloidal transformation  
73 often occurs along the hydrological continuum of interconnected soil waters, mires, streams and  
74 large oligotrophic lakes<sup>17</sup>. Under the action of aquatic microorganisms and sunlight irradiation, the  
75 organic and organo-ferric colloids are subjected to change of dominant source and molecular weight  
76 from allochthonous large-size humic and fulvic molecules to small size autochthonous organic  
77 ligands<sup>17</sup>. Further, the DOC can be degraded into CO<sub>2</sub> or taken up by heterotrophic bacterioplankton.  
78 This may liberate Fe(III) ions, leading to Fe oxy(hydr)oxide precipitation<sup>18,19</sup>. Finally,  
79 photooxidation of DOM may lead to enrichment in low molecular weight organic ligands and their  
80 complexes with metals<sup>20</sup>.

81 In contrast to numerous works devoted to microbiological<sup>21</sup> and photochemical<sup>22</sup>  
82 mineralization of dissolved organic carbon (DOC), nitrogen and phosphorus, the bacterial and  
83 sunlight-induced transformations of Fe-rich colloids in inland waters have been little studied<sup>23,24</sup>. The  
84 transformation of colloidal Fe in boreal waters, including bogs, lakes and rivers<sup>25</sup> has been studied  
85 under the metabolic action of heterotrophic soil and aquatic bacteria<sup>18,26-27</sup> and sunlight<sup>28</sup>. In these  
86 works we demonstrated that the biodegradation of organo-ferric colloids by heterotrophic bacteria  
87 consists of *i*) element uptake inside the cells; *ii*) element adsorption at the cell surface, and *iii*) Fe  
88 hydroxide precipitation leading to scavenging of associated trace metals. During both biodegradation  
89 and photolysis of natural DOM, Fe plays a pivotal role in controlling the fate of trace elements (TE).  
90 This control occurs via *i*) formation of insoluble Fe(III) hydroxides that coprecipitate other trace

91 metals<sup>28</sup>, and *ii*) generation of low molecular weight (LMW) organic ligands that bind Fe, thus  
92 competing with strong organic complexes of other metals<sup>19,20,28</sup>.

93 The Fe isotope approach is an efficient tool for deciphering elementary processes involving  
94 microbes and aqueous solutions<sup>29-33</sup>, DOM - Fe<sup>34</sup> and mineral - Fe<sup>35,36</sup> interaction including  
95 colloids<sup>3,6, 37</sup>. The latter study demonstrated an unusual enrichment of heavier Fe isotopes in the  
96 LMW (< 1-10 kDa) fraction relative to remaining colloidal fraction (by up to +3 to +4 ‰ in  $\delta^{57}\text{Fe}$ )  
97 and suggested that bio- and/or photo-transformation of colloids may be responsible for heavy Fe  
98 isotope signatures in LMW fraction of boreal waters. However, the photo-oxidation of organo-ferric  
99 colloids removes Fe in the form of Fe(III) hydroxides<sup>19,23,38</sup> which can enrich the remaining solution  
100 in lighter Fe isotopes, consistent with numerous laboratory experiments on Fe hydroxide  
101 precipitation from Fe(II)-bearing aqueous solution<sup>39</sup>. Whereas for  $\text{Fe}^{3+}(\text{aq}) \rightarrow \text{Fe(III)}_{\text{solid}}$  (hematite)  
102 reaction at equilibrium, a negligible fractionation of  $-0.15 \pm 0.30$  ‰ ( $\Delta^{57}\text{Fe}$ ) was reported<sup>40</sup>, the  
103  $\text{Fe}^{2+}(\text{aq}) \rightarrow \text{Fe(III)}_{\text{solid}}$  (ferrihydrite) reaction has  $\Delta^{57}\text{Fe}$  of  $+4.8 \pm 0.15$  ‰<sup>39</sup>. Other experiments  
104 producing goethite reported a fractionation factor of  $+1.7 \pm 0.14$ ‰, corresponding to the  $\text{Fe(III)}_{\text{solid}}$   
105 being enriched in heavier isotopes<sup>41</sup>. As for the bacterially-induced colloid transformation, two main  
106 processes controlling the isotope fractionation during metal-live cell interaction are 1) fast adsorption  
107 of heavier Fe isotope on the cell surfaces<sup>30,31</sup>, and 2) long-term assimilation of heavier Fe isotopes as  
108 recently shown for phytoplankton<sup>42</sup> and magnetotactic bacteria<sup>33</sup>. Note however that, in contrast to  
109 the fairly good knowledge of isotope fractionation of ionic metals and their organic complexes  
110 interaction with microbial cells, virtually nothing is known on colloidal metal – cell interaction  
111 reactions. The present study is aimed at the quantification of Fe isotope signatures during photo- and  
112 bio-degradation of DOM-Fe complexes in high latitude boreal waters. We hypothesized preferential  
113 removal of heavier Fe isotopes by live cell adsorption and assimilation and Fe hydroxide  
114 precipitation in the course of bio- and photodegradation of organo-ferric colloids. We further tested  
115 the possibility of formation of isotopically heavy Fe in  $\text{LMW}_{< 1 \text{ kDa}}$  fraction of river waters, as it was  
116 put forward in a previous study<sup>37</sup>. To test these hypotheses, we selected a stream and a fen water, two

117 dominant types of Fe-rich freshwaters of the subarctic. By choosing two main processes controlling  
118 the fate of organo-ferric colloids (bio- and photo-degradation) and two representative examples of  
119 boreal Fe- and DOM-rich surface waters, we were capable to reveal the main factors that govern Fe  
120 isotope behavior on a short-term (daily) scale in large territory of subarctic landscapes.

## 121 **2. Materials and methods**

122 The surface waters were collected in the Northern Karelia region (NW Russia). The climate  
123 of the region is mild-cold, transitional between oceanic and continental. Average temperature in  
124 January is -13 °C, and +15 °C in July; average annual precipitation ranges between 450 and 550  
125 mm y<sup>-1</sup>. Our study area is in the most elevated part of Karelia, within a landscape of tectonic  
126 denudation hills, plateaus and ridges with an average altitude of 300-400 m, with separate insulated  
127 massifs. Predominant soils are illuvial-humic and illuvial-ferruginous-humic podzols. Coniferous  
128 forest (pine and spruce) with some deciduous trees (birch, aspen and alder) dominates the  
129 vegetation of the region. Further landscape setting is described in previous works of our group<sup>17,37</sup>.  
130 Within the hydrological continuum created by glacial processes around 8-10 thousand years ago,  
131 the water and soluble compounds travel from the ombrotrophic peat mire zone downstream the  
132 river towards a large oligotrophic lake. At the lake coast, there are minerotrophic fens located in  
133 depressions that receive their water via shallow groundwater and soil flux<sup>43</sup>.

134 A small stream and a coastal fen were collected in July 2015, during summer baseflow  
135 period. The Palojoki stream (watershed area = 32 km<sup>2</sup>, bedrock of granites, gneisses, syenites, and  
136 syenite-diorites of the early-Proterozoic and late-Archean, covered by glacial Quaternary deposits  
137 under podzol soils<sup>43</sup>) was sampled in the middle course of the flow (sample KAR-1<sup>17</sup>). The fen  
138 adjacent to the western coast of the Tsipringa Lake had an area of 1.19 km<sup>2</sup> and is underlain by  
139 Early-Archean biotite granito-gneisses (sample ZPBL<sup>17</sup>).

140 The experiments included on-site photodegradation of sterile-filtered (< 0.22 µm) stream and  
141 fen water in quartz reactors and laboratory microcosm experiments in the presence of 1 g<sub>wet</sub>/L of  
142 *Pseudomonas aureofaciens*, highly abundant culturable bacteria isolated from podzol soils<sup>44</sup>. The

143 experimental scheme described in previous works from our group<sup>27,28</sup> is shown in **Fig. 1**. The  
144 biodegradation experiments with stream and fen waters were performed after 6 months storage of  
145 sterile-filtered samples in the refrigerator. Freshly-collected bacteria at the stationary growth stage  
146 were rinsed in 0.85% NaCl and allowed to starve in nutrient-free 0.85% NaCl solution during 5 days.  
147 The cells were rinsed again 3 times before the experiment, concentrated in a mother suspension and  
148 added to sterile, acid-washed polypropylene flasks with stream or fen water (typically 2 mL of  
149 bacterial suspension to 200 mL of water) to provide the wet biomass concentration of 1 g<sub>wet</sub> L<sup>-1</sup>. Note  
150 that the amount of Fe originated from bacteria addition does not exceed 1 µg L<sup>-1</sup> for this  
151 concentration of biomass<sup>27</sup>. The flasks were shaken at 25±0.5°C and aerated via Biosilico®  
152 ventilation porous caps during 4 days. Aliquots of homogeneous suspension were sampled after 0, 1,  
153 8, 22, 30, 50 and 100 h after the addition of bacteria and filtered through 0.22 µm membrane. All the  
154 biodegradation experiments were run in duplicates. The control experiment was sterile filtered (<  
155 0.22 µm) biomass-free stream and fen water and it was processed exactly as bacterial samples.

156 For photo-degradation experiments, the stream and fen waters were collected in pre-cleaned  
157 polypropylene jars and stored in dark cold place (6±2°C) prior the experiments. The waters were  
158 processed on-site, within 2 h after sampling, in the field-constructed clean laboratory<sup>37</sup>. Sterile  
159 filtration was performed using single-used Sartorius polystyrene vacuum filtration units (0.22 µm,  
160 250 mL volume). Filtered fluids were transferred into 270-mL sterilized quartz flasks, filled with  
161 10% air headspace and covered by porous sterile stoppers. The dark control of quartz flasks was  
162 identical to the light samples except that the reactors were wrapped in Al foil. Both dark and light  
163 reactors were run in duplicates and they were placed on flat surface at the border of the lake  
164 (66°17'04"N, 30°52'05"E). The flasks were exposed to direct unshaded sunlight from July 9 to July  
165 20 during essentially anticyclonic weather. The daytime duration in this period was between 22 and  
166 20.5 hours. The temperature of the experimental reactors followed the diurnal cycle and was equal to  
167 18 ± 5°C over 250 h of exposure, which was within the range of actual water temperature in stream  
168 and fen during the month of July<sup>17,37</sup>. The quartz reactors were sampled after 0, 100 and 200 h of

169 exposure of the fen water (ZPBL) and after 0, 110 and 250 h of exposure of the stream (KAR-1). For  
170 each sampling, the whole reactor was sacrificed. The samples were immediately filtered through 0.22  
171  $\mu\text{m}$  Sartorius single-used filter into pre-cleaned polypropylene vials. These waters were then  
172 acidified using bi-distilled  $\text{HNO}_3$  for trace metals analysis or directly used, without acidification, for  
173 DOC,  $\text{UV}_{254\text{ nm}}$ , DIC and anions determinations. The 0.22  $\mu\text{m}$  filtrate from quartz reactors was  
174 additionally ultrafiltered through 1 and 10 kDa regenerated cellulose single-use filters with an  
175 Amicon 8400 frontal Ultrafiltration unit (continuously stirred 400-ml polycarbonate cell maintained  
176 under 1.5–2 atm pressure). Details of ultrafiltration procedure in humic boreal waters of North  
177 Karelia and discussion of possible artifacts are presented elsewhere<sup>17,37,43</sup>. In these experiments, we  
178 consider the behavior of Fe in  $<0.22\ \mu\text{m}$  and  $<10\ \text{kDa}$  fractions of the stream water and in  $<0.22\ \mu\text{m}$ ,  
179  $<10$  and  $<1\ \text{kDa}$  fractions of the fen water.

180 Analyses of DOC and Fe concentration in various filtered and ultrafiltered fractions were  
181 performed using Shimadzu TOC-VCSN and Agilent ICP MS, following procedures described  
182 previously<sup>27,28</sup>. The DOC was measured with an uncertainty of 3% and a detection limit of  $0.1\ \text{mg L}^{-1}$ .  
183 The total dissolved Fe represents the sum of Fe(II) and Fe(III) in the  $< 0.22\ \mu\text{m}$  fraction. The  
184 uncertainty of Fe concentration measurements ranged from 5 to 10% (1SD). The international  
185 geostandard SLRS-5 (Riverine Water Reference Material for Trace Metals certified by the National  
186 Research Council of Canada) measured each 20 samples was used to check the validity and  
187 reproducibility of Fe analysis. Good agreements were found between the replicate measurements of  
188 Fe in SLRS-5 and the certified values (relative difference  $< 20\%$  SD on the repeated measurements).

189 Assessment of Fe(II) and Fe(III) concentrations was performed using the conventional  
190 ferrozine method<sup>45</sup>. The efficiency of this method in organic-rich tropical waters was further  
191 demonstrated in Rio Negro<sup>46</sup>. Because of possible interferences from DOM in humic boreal waters,  
192 the ferrozine method was used employing a standard addition technique. This allowed to achieve a  
193 detection limit of  $15\ \mu\text{g L}^{-1}$  and an uncertainty from 10-20% at  $15 < \text{Fe(II)} < 100\ \mu\text{g L}^{-1}$  to 5-10% at  
194  $\text{Fe(II)} > 100\ \mu\text{g L}^{-1}$ . Further, we verified the stability of Fe(II) in oxygenated humic waters ( $\text{pH} = 4.6$



195 to 6.5; DOC = 20 to 50 mg L<sup>-1</sup>) using peat and moss leachate (similar to main allochthonous DOM in  
196 studied sites<sup>47</sup>). The Fe(II) concentration remained stable within ±10% over 24-180 h of exposure.

197 The Fe(III) and Fe(II) complexation with organic ligands and solution saturation degree with  
198 respect to secondary Fe hydroxide in the course of experiment were calculated using Visual  
199 MINTEQA<sup>48</sup>, in conjunction with a NICA-Donnan humic ion bonding model, as described  
200 previously<sup>17</sup>. We considered carboxylic and phenolic complexes of fulvic acids with Fe<sup>2+</sup> and Fe<sup>3+</sup>  
201 ions, as well as Fe(II) weak (electrostatic) interaction with fulvic acids.

202 For Fe isotope analysis, filtered and acidified water samples were evaporated in the clean  
203 laboratory. After acid digestion of the residue using a mixture of HCl and HNO<sub>3</sub>, Fe was purified via  
204 anion exchange chromatography with HCl using Bio Rad AG1 X4 resin, 200–400 mesh to remove  
205 all matrix elements<sup>37</sup>. For this, we used thermoretractable Teflon columns with an internal diameter  
206 of 4 mm<sup>8</sup>. The resins were conditioned using 6 M of HCl prior to the sample loading in 0.5 ml of 6  
207 M HCl. The matrix species were eluted in 3 ml of the same acid and, subsequently, Fe was  
208 quantitatively eluted with 2 ml of 0.05 M HCl. The purified Fe solutions were evaporated at 120°C.  
209 After evaporation, purified Fe samples were redissolved in a 0.05 M HCl solution that was used for  
210 the MC-ICP-MS analysis. Iron and the internal standard, Ni, were set to concentration producing  
211 signals of ca. 40 V of <sup>56</sup>Fe and 20V for <sup>60</sup>Ni. Iron isotope measurements were performed at the  
212 CNRS-INSU National MC-ICP-MS facility in Lyon using a Thermo Electron Neptune Plus and at  
213 GET-CNRS in Toulouse using a Thermo Electron Neptune MC-ICP-MS (Bremen, Germany)<sup>49</sup>. All  
214 analyses are reported in the delta notation relative to the IRMM-014 standard, expressed as δ<sup>57</sup>Fe,  
215 which represents the deviation in per mil relative to the reference material:

$$216 \quad \delta^{57}\text{Fe} (\text{‰}) = \left( \frac{(^{57}\text{Fe}/^{54}\text{Fe})_{\text{sample}}}{(^{57}\text{Fe}/^{54}\text{Fe})_{\text{IRMM14}}} - 1 \right) * 1000$$

217 We also obtained δ<sup>56</sup>Fe values but, since the relationships between δ<sup>56</sup>Fe and δ<sup>57</sup>Fe of the  
218 samples plot on a single mass fractionation line (δ<sup>57</sup>Fe = 1.466 × δ<sup>56</sup>Fe + 0.005, R<sup>2</sup> = 0.9995, p <  
219 10<sup>-4</sup>), only δ<sup>57</sup>Fe values are discussed in this paper. Data quality was checked by the analysis of our

220 in-house hematite standard every 5 samples in the analytical sequence. Our mean value of  
221  $0.771\pm 0.047\%$  (2SD) for this standard obtained between Lyon and Toulouse from pooling 51  
222 individual analyses by groups of 6 was consistent with the values of  $0.766\pm 0.072\%$  reported  
223 previously<sup>8</sup>.

224

### 225 3. Results and Discussion

#### 226 3.1. Iron in the $< 0.22 \mu\text{m}$ fraction during biodegradation experiments: Heavier isotopes 227 adsorption onto and lighter isotopes uptake by live cells of *P. aureofaciens*

228 The studied surface waters were oxygenated, organic- and Fe-rich (pH = 7.1, DOC = 12 mg  
229  $\text{L}^{-1}$ , Fe = 208  $\mu\text{g L}^{-1}$  in KAR-1; pH = 5.4, DOC = 38.7 mg  $\text{L}^{-1}$ , and Fe = 4310  $\mu\text{g L}^{-1}$  in ZPBL). The  
230 stream water contained ~24% of Fe(II) and the fen contained ~20% of Fe(II); the  $\text{LMW}_{< 1 \text{ kDa}}$   
231 fraction of DOC was sizably higher in the stream compared to the fen (77 and 29%, respectively).  
232 The experimental results are reported in **Table 1** whereas those of the control experiments are listed  
233 in **Table S1**. The pH value increased by 0.6 and 1.2 unit over 4 days of *P. aureofaciens* reaction  
234 with stream water and fen water, respectively (**Table 1**). There was no sizeable DOC decrease over  
235 the first 1 h of reaction. The long-term removal (1 – 100 h) of DOC by *P. aureofaciens* was not  
236 pronounced in the stream water but was high in the fen water ( $-33\pm 5\%$ ).

237 Iron exhibited initial adsorption after 1 h of reaction as it is seen from the difference between  
238 the stream and the fen water control and bacterial suspension at the beginning of experiment (**Fig. 2**  
239 **A and B and Table 1**). The proportions of fast adsorption (0-1 h) and intracellular  
240 assimilation/Fe(III) hydroxide (1-93 h) relative to total Fe removal in the experiments were  
241 respectively equal to 35% and 37% in the stream water and 30% and 25% in the fen water. In the  
242 latter, the concentration of divalent Fe decreased almost 3-fold over the first day of reaction relative  
243 to control (**Fig. 2 C**). No sizeable change of Fe(II) in stream water could be assessed because the  
244 Fe(II) concentration in KAR-1 (not shown) was  $30\pm 20 \mu\text{g/L}$  (~16% of total dissolved Fe), with a  
245 quantification limit of Fe(II) by this method of  $15 \mu\text{g/L}$ <sup>45</sup>.

246 All initial solutions were undersaturated with respect to Fe-bearing minerals. Calculated  
247 change of Fe(II) and Fe(III) speciation in the course of experiments demonstrated the dominance of  
248 Fe(III)-fulvic acid (FA) complexes. The Fe(II) bound to FA fully disappeared in the stream and  
249 decreased by a factor of 3 in the fen waters (**Fig. S1**). Specifically, the Fe(III) complex with  
250 carboxylic groups of FA degraded 4-fold in both fen and stream water, whereas phenolic complex of  
251 Fe(III) remained stable (**Table S2**). The proportion of Fe(II) complex with carboxylates of FA  
252 increased whereas Fe(II)-weak electrostatic complex completely disappeared. This is consistent with  
253 highly reactive behavior of dissolved Fe(II) in biodegradation experiments.

254 The second  $< 0.22 \mu\text{m}$  filtration step in the laboratory of the control water samples showed  
255 that the 6 months storage in the fridge did not affect stream water chemical composition but it  
256 induced a decrease of Fe concentration in the fen water from  $4310 \mu\text{g/L}$  to  $2470 \mu\text{g/L}$ , accompanied  
257 by a change in  $\delta^{57}\text{Fe}$  from  $0.25 \pm 0.06\text{‰}$  to  $1.06 \pm 0.04\text{‰}$ . A strong  $\delta^{57}\text{Fe}$  increase from  $1.59 \pm 0.05\text{‰}$  to  
258  $2.87 \pm 0.07\text{‰}$  also resulted from the storage for the stream water (Table 1 and S1). This change could  
259 be linked to some  $\text{Fe}(\text{OH})_3$  precipitation due to either aggregation of Fe(III)-rich organic colloids or  
260 Fe(II) oxidation. To preserve at least the Fe isotopic composition of DOC-rich waters over several  
261 months, immediate freezing after filtration in the field on the sampling site is preferable<sup>50</sup>, but this is  
262 not always possible for logistical reasons.

263 Although the fractionation of Fe between bacterial cells and organo-ferric colloids has not been  
264 studied previously, the adsorption of metal cations such as  $\text{Zn}^{2+}$  at the microorganism cell surface is  
265 known to favor the heavier isotopes<sup>51</sup> and both  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ions follow this rule<sup>30,31</sup>. The heavier Fe  
266 isotopes are preferentially adsorbed onto solid surfaces during equilibrium isotope fractionation  
267 processes<sup>36</sup>. Experiments that lasted 11 days with anaerobic phototrophic Fe-oxidizing, aerobic  
268 neutrophilic Fe-oxidizing, and heterotrophic Fe-reducing bacteria demonstrated that metabolically-  
269 produced hydrous ferric oxide (HFO) exhibited heavier isotopic composition than the initial Fe(II),  
270 with a fractionation factor ( $\Delta^{57}\text{Fe}$ ) of  $+2.2 \pm 0.3\text{‰}$ <sup>52</sup>. Further, depending on the phytoplankton species  
271 and the composition of the culture medium, the cell surface was found to be enriched in heavier

272 isotopes by  $+2.4\pm 0.6\text{‰}$  to  $+2.9\pm 0.1\text{‰}$  for  $\text{Fe}^{2+}$  containing solution and by  $+0.4\pm 0.2$  to  $+1.0\pm 0.2\text{‰}$   
273 for  $\text{Fe}^{3+}$  solution<sup>30</sup>. More recent measurements of Fe isotope fractionation during Fe adsorption onto  
274 phytoplankton cells quantitatively confirmed these data<sup>31</sup>. The value  $\Delta^{57}\text{Fe}_{\text{cell-solution}}$  of  $+0.5\pm 0.1\text{‰}$  for  
275 the river water inferred from the present study suggests essentially  $\text{Fe}^{3+}$  ion adsorption onto *P.*  
276 *aurefaciens* cell surfaces, presumably forming Fe(III)-phosphoryl complexes<sup>44</sup>. Note however that  
277 the competition between surface organic moieties and aqueous organic complexes for heavy Fe  
278 isotopes can decrease the magnitude of isotopic offset in humic waters compared to experimental  
279 solutions with low DOC. At present, the isotopic offset for adsorption of organo-ferric colloids onto  
280 organic surfaces is not known and this should be the subject of future research.

281 The interaction of stream water with heterotrophic bacterium produced first, a drop in  $\delta^{57}\text{Fe}$   
282 of ca 0.4‰ in solution due to adsorption of heavier isotopes on the cell surface, and then a gradual  
283 increase in aqueous  $\delta^{57}\text{Fe}$  from  $+2.5\pm 0.1\text{‰}$  to  $+3.2\pm 0.1\text{‰}$  between 1 and 24 h (**Fig. 3 A**) yielding  
284  $\Delta^{57}\text{Fe}_{\text{cell-solution}}$  of  $-0.7\text{‰}$ . This implies removal of lighter Fe isotopes due to their preferential  
285 intracellular uptake. After an initial drop in the stream water  $\delta^{57}\text{Fe}$ , the long-term removal of lighter  
286 Fe isotopes by live bacteria produced ca 0.7‰ increase in  $\delta^{57}\text{Fe}$  over the first 20 h of reaction that  
287 remained constant to 50 h (**Fig. 3A**). This strongly supports biological mechanism of lighter Fe  
288 isotopes uptake inside cells rather than metabolically-induced  $\text{Fe}(\text{OH})_3$  precipitation. In the latter  
289 case, the solid phase would be enriched in heavy isotope ( $\Delta^{57}\text{Fe}_{\text{FeOOH-Fe(aq)}} = +1.5$  to  $+4\text{-}5\text{‰}$ )<sup>32,53,54</sup> as  
290 also supported by natural observations<sup>9,53</sup>. The present result is at variance with previous Fe  
291 assimilation by magnetotactic bacteria<sup>33</sup> or phytoplankton<sup>42</sup> that inferred a heavier Fe isotope uptake  
292 inside the cells due to a combined Fe oxidation. However, the Fe(III)-DOM complexes in solution  
293 retain heavy isotope as it is known for  $\text{Fe}^{3+}(\text{H}_2\text{O})_6$  –Fe(III) chelate (DFO-B): the organic complexes  
294 are enriched in heavy isotopes with an isotopic offset of  $+0.90\pm 0.23\text{‰}$ <sup>55</sup>, and overall, there is a  
295 strong positive correlation between Fe fractionation factors and the Fe-binding affinity of the  
296 ligands<sup>56</sup>. According to vMinteq calculation, the majority of Fe in our experimental solutions was  
297 bound to phenolic complexes with fulvic acids (Fig. S1, Table S2). Therefore, we suggest that strong

298 complexation of isotopically heavy Fe(III) with DOM prevents the intracellular uptake of heavier Fe  
299 isotopes.

300 In the fen water, we observed a similar  $\delta^{57}\text{Fe}$  evolution during biodegradation experiments,  
301 though with smaller variations. The isotopic composition of dissolved Fe did not appreciably change  
302 during *P. aureofaciens* short-term interaction with fen water: we observed only weak adsorption of  
303 heavy isotope ( $<0.1\text{‰}$ ) and a weak assimilation of light isotopes  $\Delta^{57}\text{Fe}_{\text{cell-solution}} \leq 0.1\text{‰}$  over first 40  
304 h (**Fig. 3 B**). Relative to the sterile control, a decrease in  $\delta^{57}\text{Fe}$  at the beginning of experiment  
305 signified preferential adsorption of heavy isotopes with  $\Delta^{57}\text{Fe}_{\text{cell-solution}} = +0.2 \pm 0.1\text{‰}$ . Over 100 h of  
306 reaction, light Fe isotopes were taken up by the cells as the  $\delta^{57}\text{Fe}$  of solution increased from  
307  $+0.86 \pm 0.1\text{‰}$  to  $+1.05 \pm 0.05\text{‰}$ .

308 Overall, the effect of bio-transformation of Fe isotopic signature were much stronger in the  
309 stream water compared to the fen water, which may be due to different concentration of Fe in these  
310 two samples (200 and 4300  $\mu\text{g/L}$ ) at otherwise similar bacterial concentration ( $1\text{g}_{\text{wet}}/\text{L}$ ). As a result,  
311 there was an order of magnitude higher ligand : metal ratio ((cell surface sites) : Fe fraction) in the  
312 stream compared to the fen water.

313

314 *3.2. Iron concentrations and isotopic signatures evolution during photodegradation of organo-ferric*  
315 *colloids: Removal of heavier isotopes due to Fe(III) hydroxide formation and appearance of*  
316 *isotopically light Fe(II) in the LMW fraction*

317 There was no pH variation in the course of photodegradation experiments: within the uncertainty  
318 of duplicates ( $< 0.1\text{-}0.2$  pH unit), the pH in the dark control and the light experiment was identical  
319 and equal to  $7.0 \pm 0.2$  and  $5.3 \pm 0.1$  in the stream and fen water, respectively (Table 1 and S1). The  
320 DOC concentration in  $<0.22 \mu\text{m}$  fraction of stream and fen water decreased by 30 and 50%,  
321 respectively, over 200 to 250 h of photodegradation experiment (Table 1). There was a good  
322 correlation between ultraviolet absorbency ( $\text{UV}_{254 \text{ nm}}$ ) and Fe concentration in the fen water ( $R^2 =$   
323  $0.928$ ) which was absent in the stream water in the course of experiment ( $R^2 = 0.19$ ).

324 In the stream water, Fe concentration remained generally constant within the uncertainties  
325 over 250 h of exposure to sunlight, relative to the dark control, and no formation of flocculent  
326 material was noted (**Fig. 4 A**). There was 50 to 80% removal of Fe(II), between 110 and 250 h of  
327 exposure, which occurred for both  $<0.22\ \mu\text{m}$  and  $<10\ \text{kDa}$  fractions (from 50 to 15  $\mu\text{g/L}$  and from 20  
328  $\mu\text{g/L}$  to  $<$  limit of quantification, respectively, **Fig. 4 B**).

329 The removal of Fe during fen water exposure to sunlight was strong in the  $<0.22\ \mu\text{m}$  fraction  
330 ( $\sim -75\%$  of initial concentration) and did not occur in the  $<10\ \text{kDa}$  fraction ( $\leq 7\%$  of the initial  
331 concentration, **Fig. 4 D and Table 1**). A notable increase in total Fe concentration (ca. 210%) in the  
332  $\text{LMW}_{<1\ \text{kDa}}$  fraction was observed over 200 h of exposure (**Fig. 4 D**). The divalent Fe concentration  
333 decreased by 50% in the  $<0.22\ \mu\text{m}$  fraction but strongly increased ( $\sim 500\%$ ) in the  $<1\ \text{kDa}$  fraction  
334 (**Fig. 4 E and Table 1**). Over 196 h of irradiation, the Fe(II) proportion increased from 20 to 50% in  
335 the  $<0.22\ \mu\text{m}$  fraction and from 35 to 55% in the  $<1\ \text{kDa}$  fraction. The  $<10\ \text{kDa}$  fraction did not  
336 demonstrate any difference in Fe(II) concentration between the sunlight-irradiated samples and the  
337 dark control.

338 During sunlight exposure, the  $<0.22\ \mu\text{m}$  fraction of the fen water became depleted in Fe as  
339 the molar ratio of  $C_{\text{org}}:\text{Fe}$  increased from 50 to 100, as can be calculated from evolution of  
340 concentrations listed in Table 1. This trend mainly stems from more efficient Fe removal compared  
341 to DOC. The change of this ratio in the stream water was within the experimental uncertainty. The  
342  $C_{\text{org}}:\text{Fe}$  ratio in the fen water colloids ( $1\ \text{kDa} - 0.22\ \mu\text{m}$ ) did not change in the course of photo-  
343 degradation, from 31 at the beginning of experiment to 32 after 193 h of sunlight exposure. In the  
344  $\text{LMW}_{<1\ \text{kDa}}$  fraction of fen water, the  $C_{\text{org}}:\text{Fe}$  ratio decreased from 247 to 126.

345 All size fractions of the initial stream solution and the  $\text{LMW}_{<1\ \text{kDa}}$  fraction of the fen water  
346 were undersaturated with respect to Fe-bearing minerals. The initial  $<0.22\ \mu\text{m}$  fen water was  
347 supersaturated with respect to goethite, lepidocrocite and magnetite (Saturation Indexes = 1.3, 0.62,  
348 and 3.6, respectively). The speciation calculation using vMinteq also demonstrated that Fe(III) is  
349 fully complexed with DOM (**Fig. S2**). Therefore, the removal of Fe in the form of particulate Fe

350 hydroxide could be only due to liberation of part of Fe from organic complexes after photolytic  
351 degradation of colloidal DOM that stabilized Fe (III) polymers in solutions. Calculated Fe(II) and  
352 Fe(III) speciation in the fen water before and after photodegradation experiment demonstrated an  
353 appearance of ~2% of inorganic Fe(II) and approx. 2-fold increase of Fe(II) bound to DOM, in both  
354 < 0.22  $\mu\text{m}$  and < 1 kDa size fraction (**Fig. S2**). In the stream water, there was a 3-fold decrease of  
355 Fe(II) bound to DOM. Specifically, the Fe(III) complex with carboxylic groups of FA decreased and  
356 increased 3-fold in fen and stream waters, respectively (**Table S3**). Phenolic complexes of Fe(III)  
357 which accounted for >93...99% of all Fe, remained constant in the course of experiment. In the fen  
358 water, the proportion of Fe(II)-weak electrostatic complex strongly decreased (by a factor of 4.5 and  
359 1.5 in < 0.22  $\mu\text{m}$  and < 1 kDa fractions, respectively) whereas Fe(II) complex with FA carboxylates  
360 increased in < 0.22  $\mu\text{m}$  fraction and stayed constant in < 1 kDa fraction (**Table S3**). According to  
361 vMinteq predictions,  $4.5 \times 10^{-5}$  M of ferrihydrite should could have precipitated from the fen water  
362 over 200 h of solar irradiation. This is equivalent to ~2250  $\mu\text{g/L}$  of dissolved Fe concentration  
363 decrease, which is comparable to the range encountered in the experiment (ca. 3400  $\mu\text{g/L}$ , see **Fig. 4**  
364 **D** and **Table 1**).

365 The isotopic signature of stream water samples remained constant in the < 0.22  $\mu\text{m}$  fraction  
366 of dark control and sun-light irradiated quartz reactors (**Fig. 5A**). The  $\delta^{57}\text{Fe}$  value of the < 10 kDa  
367 fraction decreased from  $+2.93 \pm 0.05\text{‰}$  to  $+1.92 \pm 0.1\text{‰}$  after first 110 h of exposure and then  
368 remained constant. Note that the initial < 10 kDa fraction of stream water was ca. 1.5 ‰ isotopically  
369 heavier than the < 0.22  $\mu\text{m}$  fraction, in agreement with previous measurement of Fe isotopic  
370 composition in colloids of Northern Karelian streams<sup>37</sup>.

371 The isotopic ratio in the dark control of fen water remained stable at  $\delta^{57}\text{Fe} = 0.2 \pm 0.3\text{‰}$ ,  
372 except in the  $\text{LMW}_{<1\text{ kDa}}$  fraction, where a  $+0.6\text{‰}$  increase was observed, related to the Fe and DOC  
373 loss (Table S1). Similar effect was reported in the organic-rich Negro River where it was attributed  
374 to the loss of isotopically light aggregates of Fe(III) with OM<sup>50</sup>. In contrast to dark controls, sunlight  
375 irradiation of the fen water strongly impacted  $\delta^{57}\text{Fe}$  in all 3 fractions, <0.22  $\mu\text{m}$ , <10 kDa and <1

376 kDa (**Fig. 5 B**). Over first 100 h of reaction, there was sizeable, from 1 to 2‰, decrease of  $\delta^{57}\text{Fe}$  in  
377 the  $<0.22\ \mu\text{m}$  and the  $<1\text{-}10\ \text{kDa}$  fraction, respectively. Over the next 100 h, the  $\delta^{57}\text{Fe}$  further  
378 decreased by 1.5 ‰ in the  $<0.22\ \mu\text{m}$  fraction and stabilized at  $\delta^{57}\text{Fe} = -2.2\pm 0.2\%$  in the  $<1$  and  $<10$   
379 kDa fractions.

380 Sunlight exposure of stream water did not produce any sizable change of Fe concentration and  
381 isotopic signature in the  $< 0.22\ \mu\text{m}$  fraction (**Fig. 4A and 5A**), in general agreement with stability of  
382 boreal high latitude metal concentration in riverwaters with respect to sunlight irradiation<sup>28,57</sup>. After  
383 100 h of sunlight irradiation, the  $< 10\ \text{kDa}$  fraction of stream water became 1.5‰ lighter compared  
384 to the initial value or the dark control (**Fig. 5A**), although the Fe concentration in this fraction  
385 decreased only between 100 and 250 h of exposure (**Fig. 4 A**). A plausible explanation for this  
386 isotopic pattern invokes the presence of strong low molecular weight ( $< 10\ \text{kDa}$ ) Fe(III)-organic  
387 ligand (chelate) complexes which are enriched in heavy isotopes<sup>55</sup> and which are capable to stabilize  
388 dissolved Fe. For example, aquatic prokaryotes produce the LMW (0.3 – 1 kDa) Fe(III)  
389 siderophores<sup>58</sup>. These light sensitive, presumably aromatic Fe complexes represent a small iron  
390 fraction of overall  $\text{Fe}_{<10\ \text{kDa}}$  pool, but our results indicate that it exhibits a very high  $\delta^{57}\text{Fe}$  value. The  
391 isotopic signature of  $\text{LMW}_{<1\text{-}10\ \text{kDa}}$  Fe poor, C-rich fraction of Karelian waters reaches  $+4.2\%$ <sup>37</sup>. We  
392 hypothesize that these isotopically heavy Fe-organic complexes have low residence time in the river  
393 channel and are produced due to periphyton or plankton metabolic activity.

394 The Fe in the fen water was strongly sensitive to sunlight irradiation as  $> 70\%$  Fe was removed  
395 from the  $<0.22\ \mu\text{m}$  fraction and all filtrates and ultrafiltrates became strongly impoverished in heavy  
396 isotopes (**Fig. 4 D, 5 B**). The removal of Fe followed that of  $\text{SUVA}_{254}$  decrease<sup>28</sup>, suggesting that the  
397 majority of Fe that is removed from the fen water was bound to aromatic (colored) organic carbon.  
398 This is also confirmed by the dominance of Fe(III)-phenolic groups of FA in Fe speciation (**Table**  
399 **S3**). It is possible that the photolysis of Fe-DOM complexes liberates ionic Fe which could be  
400 removed from solution in the form of isotopically heavy Fe hydroxides. The removal of heavy  
401 isotopes by sunlight oxidation of organo-ferric colloids observed in this study ( $+2.3\pm 0.1\%$ ) is



402 generally consistent with known fractionation of Fe between Fe(III) hydroxide and Fe(II) solution:  
403 ( $\Delta^{57}\text{Fe}_{\text{FeOOH-Fe(aq)}} = +2.3 \pm 0.3\text{‰}$ , ref. 52-54).

404 In contrast to what was hypothesized in our earlier work on these waters<sup>37</sup>, no enrichment in  
405 heavy isotopes of LMW fraction was observed during photolysis of humic waters. Here we suggest  
406 that Fe(II) generated in the < 1 kDa fraction is enriched in light isotopes compared with initial Fe(III)  
407 in colloids, thus producing overall negative  $\delta^{57}\text{Fe}$  value in  $\text{LMW}_{< 1 \text{ kDa}}$  after irradiation. Assuming an  
408 equilibrium fractionation factor between Fe(II) and Fe(III) of  $-4.3\text{‰}$ <sup>59</sup> and considering the starting  
409 water  $\delta^{57}\text{Fe}$  close to  $0\text{‰}$  (**Fig. 5B**), the 55% of Fe(II) in < 1 kDa fraction (**Fig. 4 F**) provides  $0.55 \times (-$   
410  $4.3\text{‰}) = -2.4\text{‰}$  of  $\delta^{57}\text{Fe}$ , in full agreement with values shown in **Fig. 5 B**. This  $< 0.22 \mu\text{m}$  fraction  
411 comprises two Fe pools: (1) Fe remaining after  $\text{Fe}^{2+}(\text{aq}) \rightarrow \text{Fe}(\text{OH})_3$  and after  $\text{Fe}^{3+}(\text{colloidal}) \rightarrow$   
412  $\text{Fe}(\text{OH})_3$  precipitation, and (2) isotopically light  $\text{Fe}^{2+}$  produced in the LMW fraction. It is important  
413 to note that the photolysis of organo-ferric colloids which represents 80% of Fe in  $< 0.22 \mu\text{m}$  fraction,  
414 removes Fe(III) in the form of  $\text{Fe}(\text{OH})_3$  hydroxides corresponding to  $\Delta^{57}\text{Fe}_{\text{Fe(III)hydroxide-Fe(aq)}}$  of  
415  $2.4 \pm 0.1\text{‰}$ , **Fig 5B**. The observed enrichment of solid phase in heavier isotopes is therefore much  
416 lower than  $\text{Fe}^{2+}(\text{aq}) \rightarrow \text{Fe}(\text{OH})_3$  reaction ( $\Delta^{57}\text{Fe}_{\text{Ferrihydrite-Fe(II)}} = 4.7 \text{‰}$ <sup>39</sup>) and opposite in sign to  
417  $\text{Fe}^{3+}(\text{aq}) \rightarrow \text{Fe}(\text{OH})_3$  reaction ( $\Delta^{57}\text{Fe}_{\text{Fe(III)hydroxide-Fe(aq)}} = -0.9 \text{‰}$ <sup>60</sup>). Lower value of isotopic  
418 enrichment compared to the equilibrium fractionation factor between  $\text{Fe(II)aq}$  and pure ferrihydrite<sup>39</sup>  
419 may be due to Fe(II) sorption onto hydrous Fe(III)-oxides and subsequent atom (isotope) exchange  
420 between Fe(II) and hydrous Fe(III)-oxides. These processes are known to be responsible for  
421 substantial Fe isotope fractionation in a number of organic-free systems<sup>36, 61-64</sup>. However, in humic  
422 waters, both remaining  $\text{Fe(II)}_{\text{aq}}$  ions and  $>\text{FeOOH}$  surface sites of newly precipitated Fe(III)  
423 hydroxides are likely to be bound to carboxylic and phenolic groups of fulvic acids. Organic matter  
424 associated with Fe(III) hydroxide and surface organic - Fe complexes may modify the magnitude of  
425 Fe(II)-Fe(III) hydroxide isotope exchange<sup>34</sup>. Thus, Chanda et al<sup>65</sup> argued that the presence of organic  
426 C causes distortion of the Fe octahedral structure and decreases by ca.  $1.2 \text{‰}$  ( $^{57}\text{Fe}/^{54}\text{Fe}$ ) the  
427 fractionation factor between Fe(II) and NOM-ferrihydrite. Similar mechanisms may operate in case

428 of isotopic exchange between Fe(II) and organo-ferric aggregates produced by photolysis of Fe(III)-  
429 rich colloids.

430 We observed notable differences in the degree of Fe concentrations and Fe isotopic  
431 composition during photolysis of the fen and the stream water. These differences may stem from the  
432 combination of two main factors controlling the degree of DOM and Fe photo-reactivity in natural  
433 waters: the fen water is a 1.5 unit of pH lower than the stream and has a factor of 30 higher in Fe  
434 concentration. The water acidity is known to exert a strong positive effect on the photolysis of  
435 DOM<sup>65,66</sup> and the DOM photobleaching is enhanced by elevated Fe concentration via the photo-  
436 Fenton effect below pH 6<sup>67</sup>. Overall, in natural settings, one may expect large variation of Fe and  
437 DOM photo-liability depending on the environmental context. Thus, slightly alkaline (pH = 8)  
438 surface water from a temperate peatland in China exhibited quite higher photo-stability of Fe<sup>68</sup>,  
439 whereas acidic DOC-rich waters from a subtropical swamp under UV irradiation demonstrated 10  
440 times more efficient removal of Fe relative to DOC<sup>69</sup>. As such, depending on the lithological context  
441 of peatlands (i.e., acidic (felsic) or carbonate (sedimentary) rocks), the degree of Fe chemical and  
442 isotopic composition change under sunlight may be dramatically different.

443

444 *3.3. Complexity of Fe isotope fractionation in boreal humic waters and implication for inland*  
445 *waters Fe isotope budget*

446 We hypothesize several processes responsible for chemical removal and isotopic redistribution of  
447 Fe among different colloidal pools in DOM- and Fe-rich stream and fen water, shown schematically  
448 in **Fig. 6**. High molecular size (10 kDa – 0.45  $\mu\text{m}$ ) organo-ferric colloids representing the majority of  
449 dissolved (<0.22  $\mu\text{m}$ ) Fe<sup>17,37</sup> are stabilized by organic ligands originating from topsoil and bog peat  
450 leaching. In addition, a small fraction of Fe(III) could be linked to highly specific, LMW<sub><1-10 kDa</sub>  
451 ligands having a short residence time in the river channel; these complexes could be produced via in-  
452 stream plankton and periphyton activity. In the fen water, a sizeable fraction of Fe is in the form of  
453 Fe(II) inorganic and organic complexes<sup>70</sup>. In the river water, the HMW colloids are subjected to

454 biologically-controlled transformations via *i*) adsorption onto surfaces of aquatic bacteria, favoring  
455 heavier isotopes to the cells, with an overall  $\Delta^{57}\text{Fe}_{\text{cell ads-solution}} = +0.4 \pm 0.1\text{‰}$ , and *ii*) assimilation by  
456 live bacteria, favoring lighter Fe isotopes to the cells with  $\Delta^{57}\text{Fe}_{\text{cell incorp-solution}} = -0.7 \pm 0.1\text{‰}$ . On a  
457 short-term scale (hours), the humic waters, once placed in contact with bacteria, are therefore  
458 becoming enriched in lighter Fe isotopes, followed, on a long-term scale (days), by enrichment in  
459 heavier Fe isotopes. Considering available data on preferential heavy isotope adsorption onto  
460 phytoplanktonic and periphytic cyanobacteria inhabiting natural waters (from +0.4 to +2.9‰, ref.  
461 30), and neglecting Rayleigh distillation processes given that we are dealing with open systems  
462 (hydrological continuum, ref. 17), the overall magnitude of diurnal variation of dissolved ( $< 0.45$   
463  $\mu\text{m}$ )  $\delta^{57}\text{Fe}$  in small streams and stagnant surface waters in the presence of common soil bacteria may  
464 range from +2.7‰ to +0.7‰. This greatly exceeds the range of Fe isotopic excursions in various  
465 sediments and in all possible bedrocks<sup>71</sup>.

466 Further, photolysis of DOM and Fe-DOM complexes in surface waters, which operates at the  
467 time scale comparable to water and solute residence time in these waters (1-10 days), is capable to  
468 dramatically enrich in lighter Fe isotopes (by 1.5 to 2.5‰) of both dissolved ( $< 0.22 \mu\text{m}$ ) and LMW<sub><</sub>  
469 <sub>1-10 kDa</sub> water fraction. We suggest that the two major processes of organo-ferric colloid  
470 transformation under sunlight in natural waters include: *(i)* degradation of the organic part of colloids  
471 (1 kDa – 0.22  $\mu\text{m}$ ) and production of low molecular weight ( $< 1$  kDa) organic ligands including  
472 carboxylic and aromatic chelates capable of strong binding of Fe ions; *(ii)* aggregation of HMW  
473 organo-ferric colloids and precipitation of Fe-rich oxy(hydroxide) after the solar radiation-induced  
474 removal of stabilizing organic matter.

475 The enigmatic enrichment of the LMW fraction ( $< 1-10$  kDa) of the stream water in heavy  
476 isotopes<sup>37</sup> is therefore most likely linked to strong, presumably aromatic, isotopically heavy Fe(III)-  
477 organic complexes. These compounds have low residence time and are produced in the river channel  
478 due to periphyton or plankton metabolic activity. In addition, sunlight irradiation of subarctic humic  
479 waters may produce 10-fold increase in aliphatic and aromatic carbonic acids<sup>72</sup> capable to bind both

480 Fe(II) and Fe(III). Altogether, bio- and photo-degradation of dissolved Fe in river, stream and bog  
481 waters can produce from -2 to +3 ‰  $\delta^{57}\text{Fe}$  variation on time scales of a few days. Because this time  
482 is generally shorter than the water residence time in surface waters, considering of Fe isotopic  
483 signature of rivers and streams as a conservative value inherited from soils and using it for tracing  
484 the sources and weathering regime on watersheds are not warranted at short time- and length-scales.  
485 Therefore, naturally-induced variations in biological activity (switching from heterogenic bacteria  
486 uptake to adsorption onto aquatic phototrophs) and sunlight illumination can modify the overall Fe  
487 isotopic signatures of surface waters by several permil. Moreover, when considering the processes  
488 responsible for Fe chemical and isotopic transformation in organic-rich streams, one has to assess  
489 both dissolved ( $<0.45$  or  $<0.22$   $\mu\text{m}$ ) and  $\text{LMW}_{< 1-10 \text{ kDa}}$  fractions of metal, since their isotopic  
490 signatures and photo-susceptibility may be dramatically (by 1 to 3‰) different.

491

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493

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498

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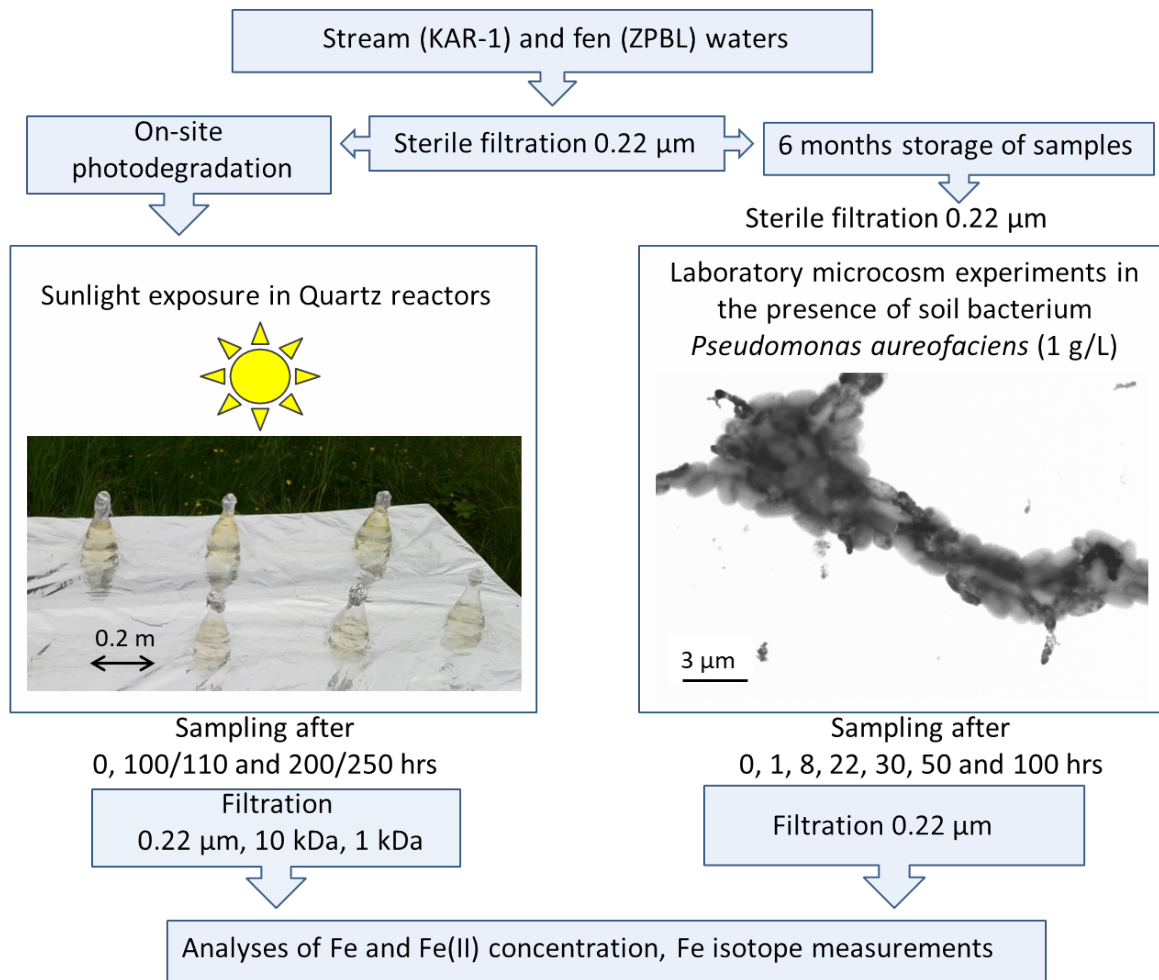
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708 **Table 1.** Measured pH, DOC, Fe concentrations and isotopic ratios, relative to IRMM-14, during  
 709 bio-and photo-degradation experiments. Note that the DOC in biodegradation experiments  
 710 of stream water slightly increased due to cell lysis and exometabolite production. The  
 711 standard error was calculated using the Student's t factor:  $SE = (t \times SD) / \sqrt{N}$ , where N is the  
 712 number of measurements.  
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<b>Bio-degradation</b> (1 g <sub>wet</sub> /L of <i>Pseudomonas aureofaciens</i> )											
hrs	Fen water ZPBL <0.22 µm					hrs	Stream water KAR-1 <0.22 µm				
	pH	DOC, mg L <sup>-1</sup>	Fe, µg L <sup>-1</sup>	Fe(II), µg L <sup>-1</sup>	δ <sup>57</sup> Fe ±2 SE, ‰		pH	DOC, mg L <sup>-1</sup>	Fe, µg L <sup>-1</sup>	Fe(II), µg L <sup>-1</sup>	δ <sup>57</sup> Fe ±2 SE, ‰
0	4.9	37.4	2470±45	645±20	1.06±0.05	0	6.6	12.4	180±10	30±20	2.87±0.07
1	5.2	37.0	1690±20	620±20	0.86±0.07	1	6.4	13.6	115±7	< LOQ*	2.54±0.06
8	5.4	35.8	1520±20	290±10	0.85±0.12	8	6.4	13.0	82±10	< LOQ*	2.68±0.16
22	5.7	34.8	1365±15	no data	0.88±0.07	22	6.7	14.0	63±3	< LOQ*	3.21±0.15
30	5.8	26.6	1290±5	145±5	0.99±0.10	50	7.0	14.1	56±2	< LOQ*	3.26±0.13
50	5.8	23.3	1197±5	120±10	1.03±0.05	100	7.2	15.2	50±2	< LOQ*	no data
100	6.1	25.2	1054±5	84±5	1.04±0.04						
<b>Sunlight exposure</b>											
hrs	Fen water ZPBL <0.22 µm					hrs	Stream water KAR-1 <0.22 µm				
0	5.4	38.7	4310±100	840±50	0.25±0.07	0	7.2	11.9	208±20	50±20	1.59±0.05
100	5.1	20.3	1840±400	770±170	-0.8±0.38	110	6.8	10.4	193±25	60±25	1.75±0.2
200	5.3	18.7	915±30	450±10	-2.63±0.03	250	6.7	8.2	175±25	15±5	2.1±0.12
hrs	Fen water ZPBL <10 kDa					hrs	Stream water KAR-1 <10 kDa				
0	5.3	26.5	1125±50	620±20	0.19±0.02	0	6.8	10.2	35±5	20±10	2.93±0.06
100	5.2	18.7	1000±50	510±20	-1.98±0.13	110	6.9	10.0	49±10	30±20	1.95±0.06
200	5.3	17.9	945±50	470±30	-2.49±0.15	250	6.8	8.1	14±2	< LOQ*	1.89±0.07
hrs	Fen water ZPBL <1 kDa					* Limit of quantification					
0	5.6	10.6	200±20	70±10	-0.18±0.03						
100	5.3	11.5	560±30	300±100	-2.34±0.11						
200	5.4	16.8	620±30	340±100	-2.43±0.12						

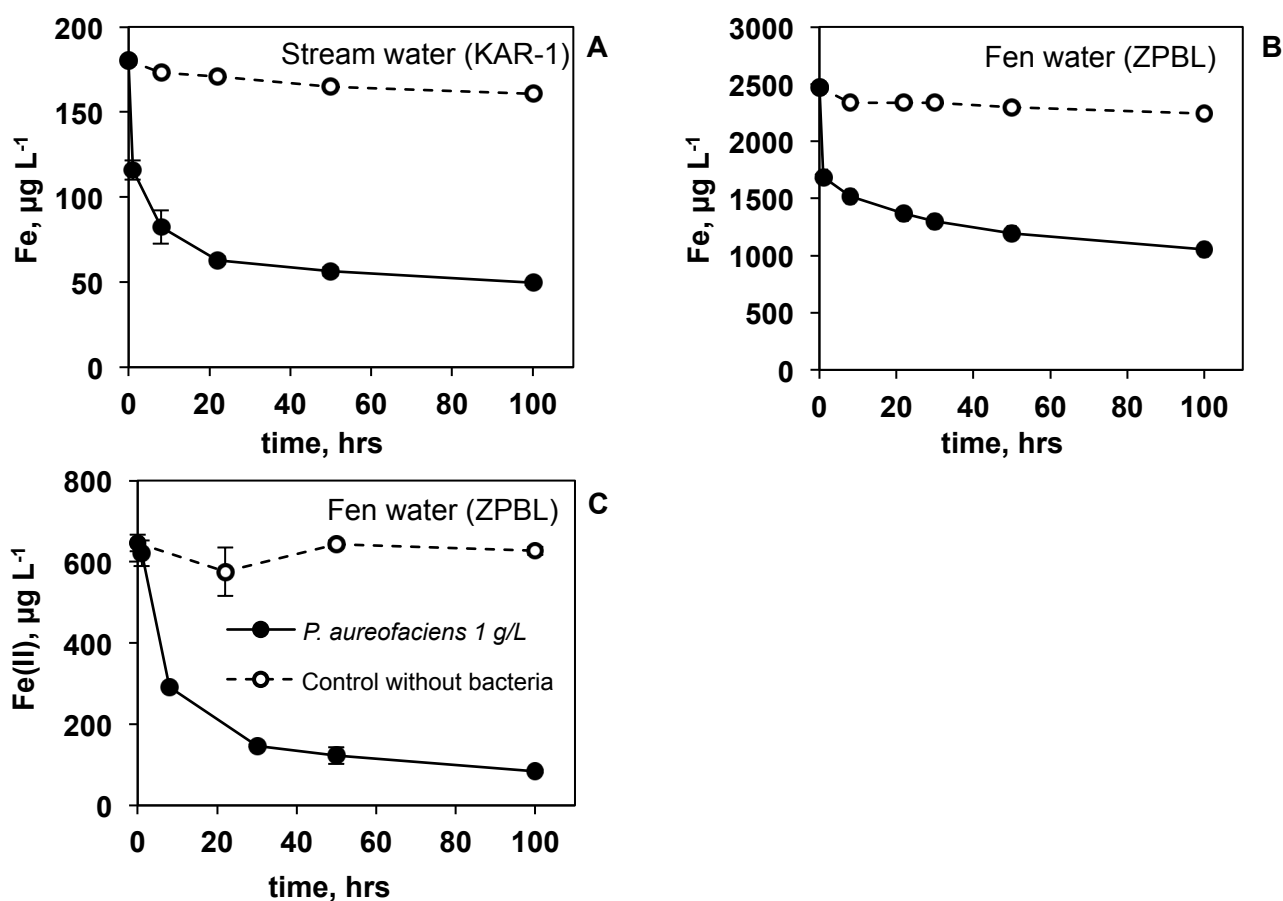
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717 **Fig. 1.** Conceptual scheme of experiments (run in duplicates): a photo of quartz reactors exposed to  
 718 sunlight and a Transmission Electron Microscopy (TEM) image of bacterial cells with  
 719 precipitated Fe hydroxides. The photodegradation experiments were performed on-site,  
 720 immediately after water sampling and sterile filtration. The biodegradation experiments  
 721 required sterile laboratory environments and were run after 6 months of water storage. The  
 722 biodegradation experiments included only  $< 0.22 \mu\text{m}$  filtration after sampling. In  
 723 photodegradation experiments, the stream water was processed for the  $< 0.22 \mu\text{m}$  and the  $<$   
 724  $10 \text{ kDa}$  filtration and the fen water was filtered through  $0.22 \mu\text{m}$ ,  $10 \text{ kDa}$  and  $1 \text{ kDa}$ .

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726 **Fig. 2.** Evolution of total dissolved Fe concentration during biodegradation experiments of stream  
 727 (A) and fen (B) water. (C): Fe(II) concentration evolution in the fen water during  
 728 biodegradation. The error bars of biotic experiments represent 1 SD of duplicates. In most  
 729 cases, they are smaller than the symbol size. Bacterial experiments are shown by solid  
 730 circles and bacteria-free control is represented by open circles for all three panels as  
 731 indicated in (C). A 10 to 12 % decrease of Fe concentration in the bacteria-free control  
 732 experiment may be due to precipitation of small amount of Fe(III) hydroxide.

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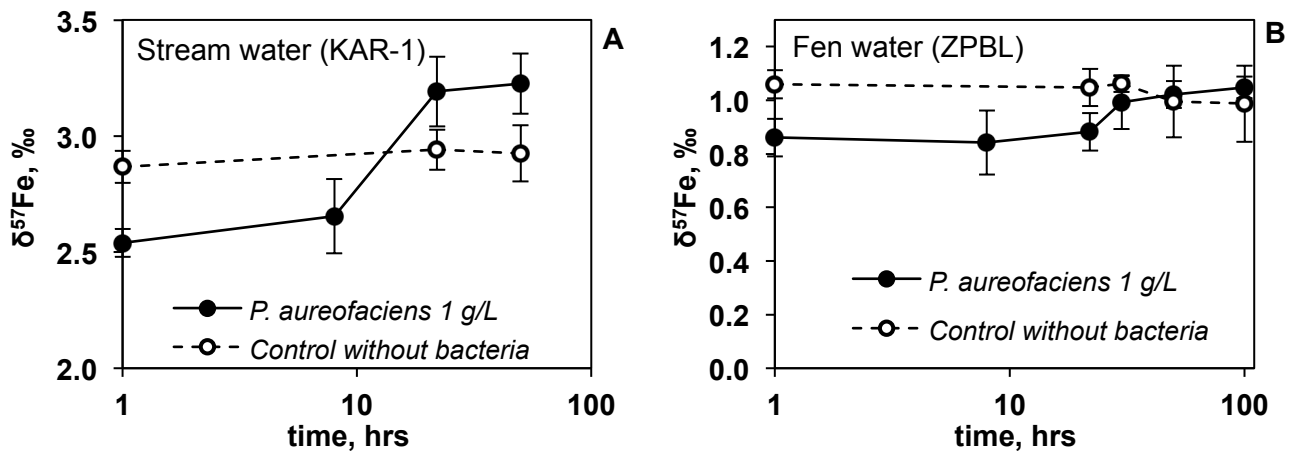
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740 **Fig. 3.** Evolution of isotopic ratios  $\delta^{57}\text{Fe}$  during biodegradation experiments of stream (A) and fen  
 741 (B) water. Note log scale for time axis..

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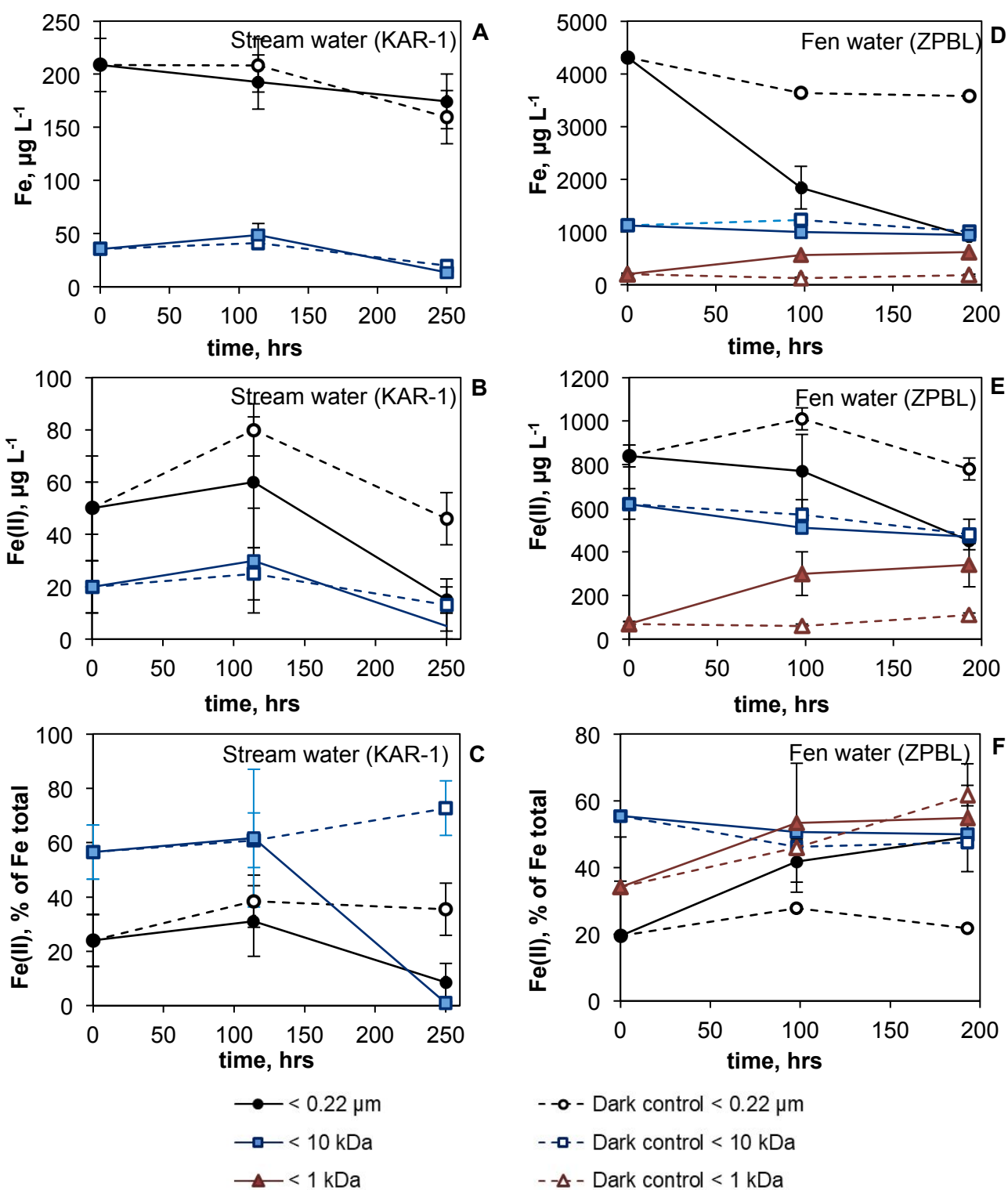
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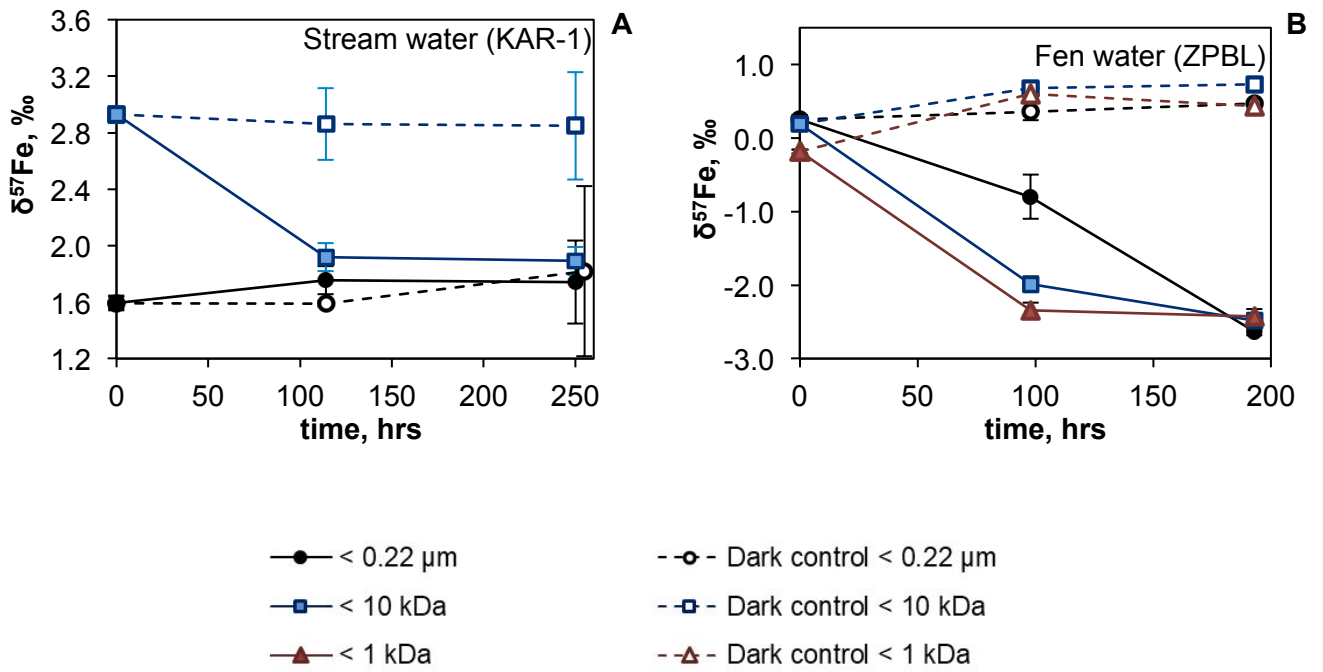
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752 **Fig. 4.** Evolution of  $Fe_{tot}$  and  $Fe(II)$  concentration during photodegradation experiments of  
 753 stream (A, B, C) and fen (D, E and F) water in quartz reactors. Note a drop in  $Fe_{tot}$  and  $Fe(II)$   
 754 concentration of the < 0.22 µm dark control at 200 and 250 h not observed in previously stored  
 755 samples in biodegradation experiments (Fig. 2), which may be due to partial precipitation fo  $Fe(III)$   
 756 hydroxide in freshly sampled natural water upon storage.

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760 **Fig 5.** Evolution of isotopic ratios  $\delta^{57}\text{Fe}$  during photodegradation experiments of stream (A) and fen  
 761 (B) water. There is a clear decrease in  $\delta^{57}\text{Fe}$  value in all fractions of photodegraded samples  
 762 of fen water, but this decrease is only pronounced for the < 10 kDa fraction of stream water.  
 763 Note that a weak increase in  $\delta^{57}\text{Fe}$  for the <0.22 μm dark control in KAR-1 is within the  
 764 uncertainty of replicates. An increase in  $\delta^{57}\text{Fe}$  for the < 1 kDa and < 10 kDa dark control of  
 765 fen waters is due to Fe loss of these unstable, freshly sampled waters.

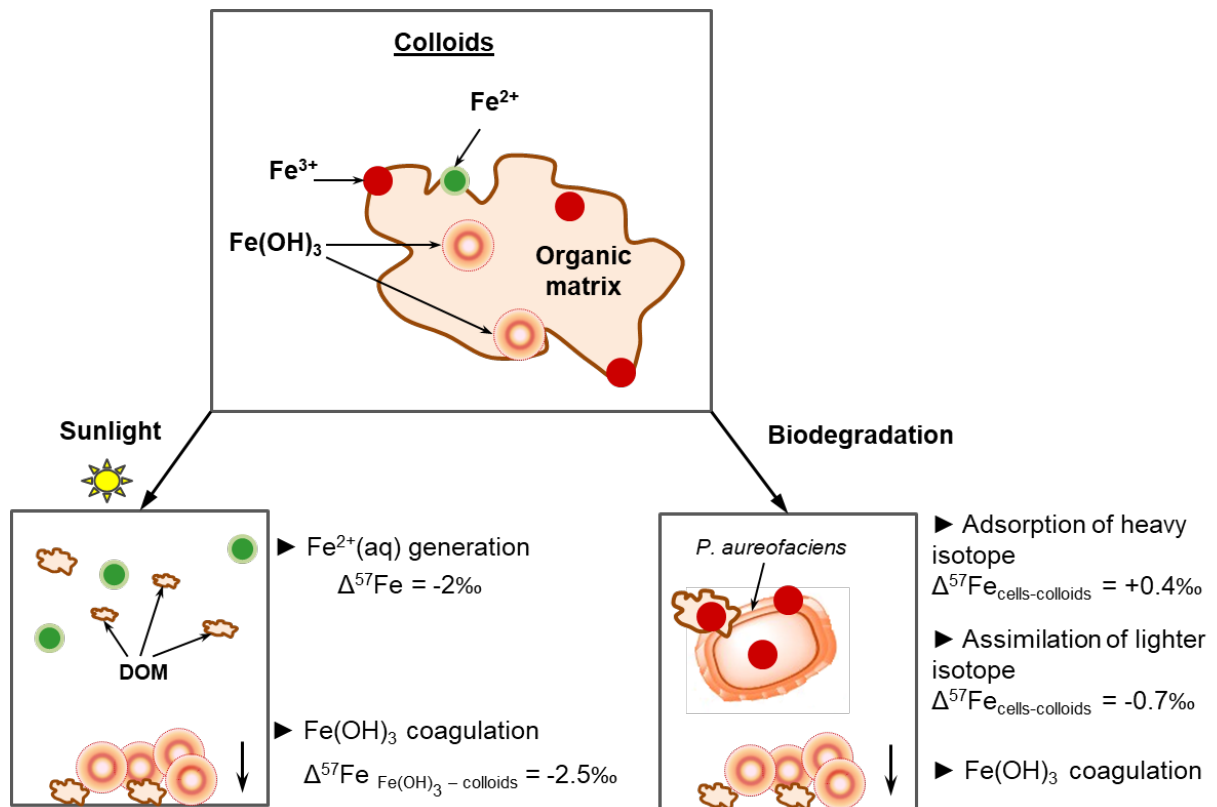
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772 **Fig. 6.** Cartoon of Fe isotope fractionation under sunlight-induced transformation (left) and  
 773 biodegradation (right) of organic and organo-ferric colloids (1 kDa - 0.22 μm). Live heterotrophic *P.*  
 774 *aureofaciens* bacteria adsorb heavier and assimilate lighter Fe isotopes. Sunlight irradiation  
 775 generates isotopically light Fe(II) in low molecular weight (< 1 kDa) fraction and produces heavy  
 776 isotope enrichment in particulate fraction relative to total dissolved (< 0.22 μm) form. After bio- or  
 777 photo-degradation of organic matter which constitutes organo-ferric colloids, the liberation of Fe(III)  
 778 ions and precipitation of Fe(III) hydroxide occur. This removes heavier isotopes from solution into  
 779 the solid phase. Altogether, bio- and photodegradation of organo-ferric colloids can produce very Fe)  
 780 large, from -2 to +3‰ isotopic variations ( $\delta^{57}\text{Fe}$ ) in boreal humic waters.

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**SUPPORTING INFORMATION:**

**Measured pH, DOC, Fe concentrations and isotopic ratios in control reactors used in bio-and photo-degradation experiments; results of vMinteq calculation of Fe(II) and Fe(III) speciation in experimental solutions**



## SUPPORTING INFORMATION:

### Measured pH, DOC, Fe concentrations and isotopic ratios in control reactors used in bio-and photo-degradation experiments; results of vMinteq calculation of Fe(II) and Fe(III) speciation in experimental solutions

**Table S1.** Measured pH, DOC, Fe concentrations and isotopic ratios, relative to IRMM-14, in control reactors used in bio-and photo-degradation experiments. Note that abiotic < 0.22  $\mu\text{m}$  controls of biodegradation experiments were more stable in terms of both [Fe] and  $\delta^{57}\text{Fe}$  than the dark controls of photodegradation experiments. Freshly sampled natural water used for photodegradation experiments could be subjected to certain transformation, re-equilibration and even coagulation of Fe and DOC. In contrast, aged filtered water was used in biodegradation experiments and it produced more stable pattern of concentration and isotope signature in control reactors.

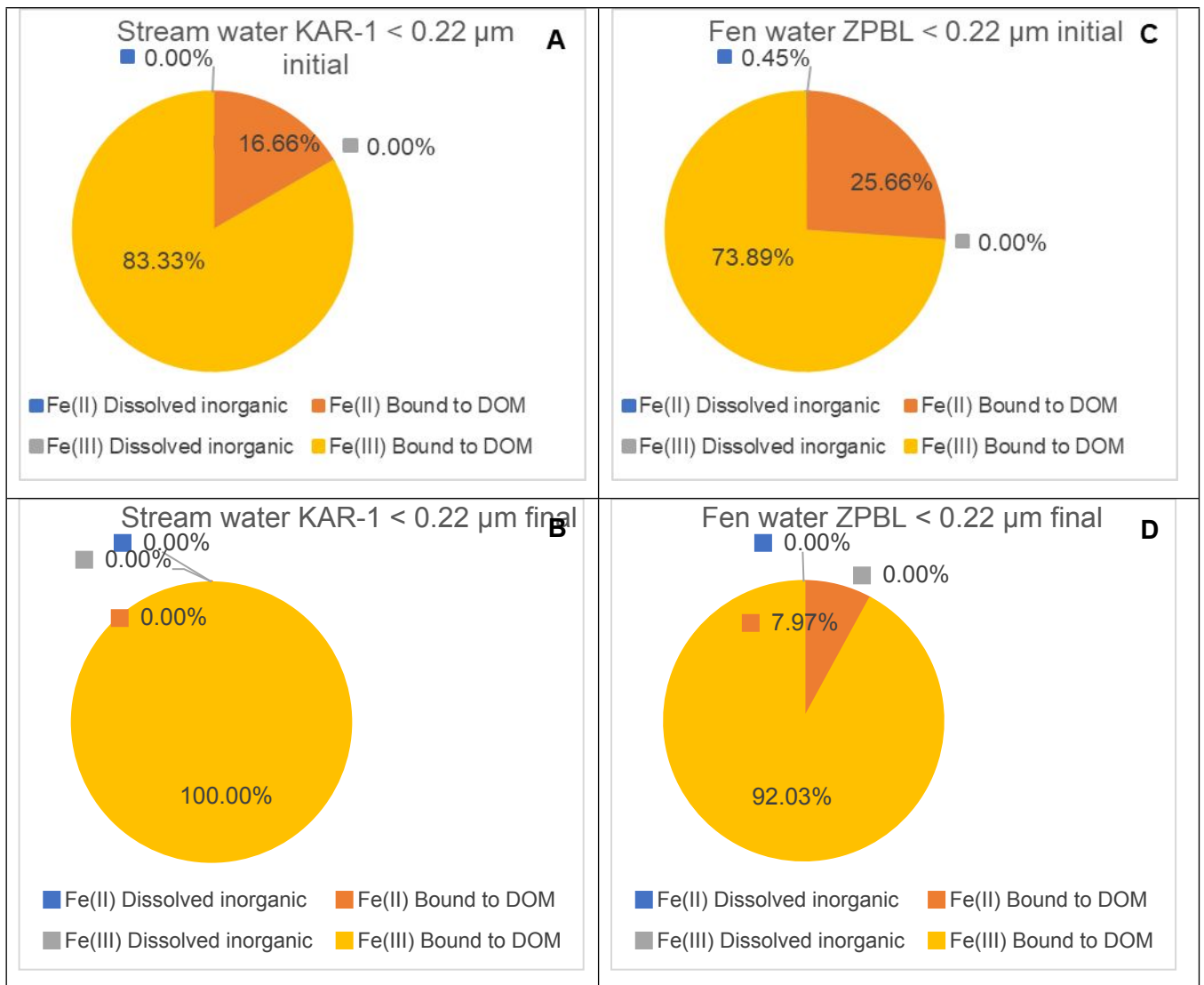
<b>Bio-degradation control reactors</b>											
hrs	Fen water (ZPBL) < 0.22 $\mu\text{m}$					hrs	Stream water (KAR-1) < 0.22 $\mu\text{m}$				
	pH	DOC, mg L <sup>-1</sup>	Fe, $\mu\text{g L}^{-1}$	Fe(II), $\mu\text{g L}^{-1}$	$\delta^{57}\text{Fe}$ $\pm 2$ SE, ‰		pH	DOC, mg L <sup>-1</sup>	Fe, $\mu\text{g L}^{-1}$	Fe(II), $\mu\text{g L}^{-1}$	$\delta^{57}\text{Fe}$ $\pm 2$ SE, ‰
0	4.9	37.4	2470 $\pm$ 45	645 $\pm$ 20	1.06 $\pm$ 0.05	0	6.6	12.4	180 $\pm$ 10	30 $\pm$ 20	2.87 $\pm$ 0.07
8	4.9	35.2	2339 $\pm$ 30	no data	no data	8	6.6	12.2	173 $\pm$ 10	20 $\pm$ 10	no data
22	5.0	37.7	2335 $\pm$ 30	576 $\pm$ 60	1.05 $\pm$ 0.07	22	6.6	12.7	171 $\pm$ 10	no data	2.94 $\pm$ 0.09
30	5.1	37.0	2335 $\pm$ 30	no data	1.06 $\pm$ 0.03	50	6.8	12.5	164 $\pm$ 10	30 $\pm$ 20	2.93 $\pm$ 0.12
50	5.1	36.9	2295 $\pm$ 30	643 $\pm$ 10	1 $\pm$ 0.13	100	6.9	12.1	160 $\pm$ 10	<LOQ*	no data
100	5.1	36.5	2242 $\pm$ 30	627 $\pm$ 10	0.99 $\pm$ 0.14						
<b>Sunlight exposure control reactors</b>											
hrs	Fen water (ZPBL) < 0.22 $\mu\text{m}$					hrs	Stream water (KAR-1) < 0.22 $\mu\text{m}$				
0	5.4	38.7	4310 $\pm$ 100	840 $\pm$ 50	0.25 $\pm$ 0.07	0	7.2	11.9	208 $\pm$ 20	50 $\pm$ 20	1.59 $\pm$ 0.05
100	5.4	35.3	3630 $\pm$ 50	1010 $\pm$ 70	0.36 $\pm$ 0.14	110	7.0	11.5	210 $\pm$ 30	80 $\pm$ 10	1.59 $\pm$ 0.02
200	5.3	37.0	3580 $\pm$ 50	780 $\pm$ 70	0.47 $\pm$ 0.03	250	6.8	11.8	160 $\pm$ 30	46 $\pm$ 10	1.82 $\pm$ 0.60
hrs	Fen water (ZPBL) < 10 kDa					hrs	Stream water (KAR-1) < 10 kDa				
0	5.3	26.5	1125 $\pm$ 50	620 $\pm$ 20	0.19 $\pm$ 0.02	0	6.8	10.2	35 $\pm$ 5	20 $\pm$ 10	2.93 $\pm$ 0.06
100	5.4	25.2	1230 $\pm$ 50	570 $\pm$ 70	0.68 $\pm$ 0.01	110	7.1	8.3	40 $\pm$ 5	25 $\pm$ 10	2.86 $\pm$ 0.25
200	5.4	22.2	1010 $\pm$ 50	480 $\pm$ 70	0.73 $\pm$ 0.04	250	6.8	8.4	20 $\pm$ 5	15 $\pm$ 10	2.85 $\pm$ 0.38
hrs	Fen water (ZPBL) < 1 kDa					* Limit of quantification					
0	5.6	10.6	200 $\pm$ 20	70 $\pm$ 10	-0.18 $\pm$ 0.03						
100	5.8	5.3	122 $\pm$ 20	60 $\pm$ 10	0.60 $\pm$ 0.07						
200	5.7	9.0	182 $\pm$ 20	110 $\pm$ 10	0.43 $\pm$ 0.07						

**Table S2.** Calculated speciation using vMINTEQ of Fe(II) and Fe(III) in < 0.22  $\mu\text{m}$  fraction of stream and fen water at the beginning (0 h) and after 100 h of biodegradation experiments. FA stands for Fulvic Acid.

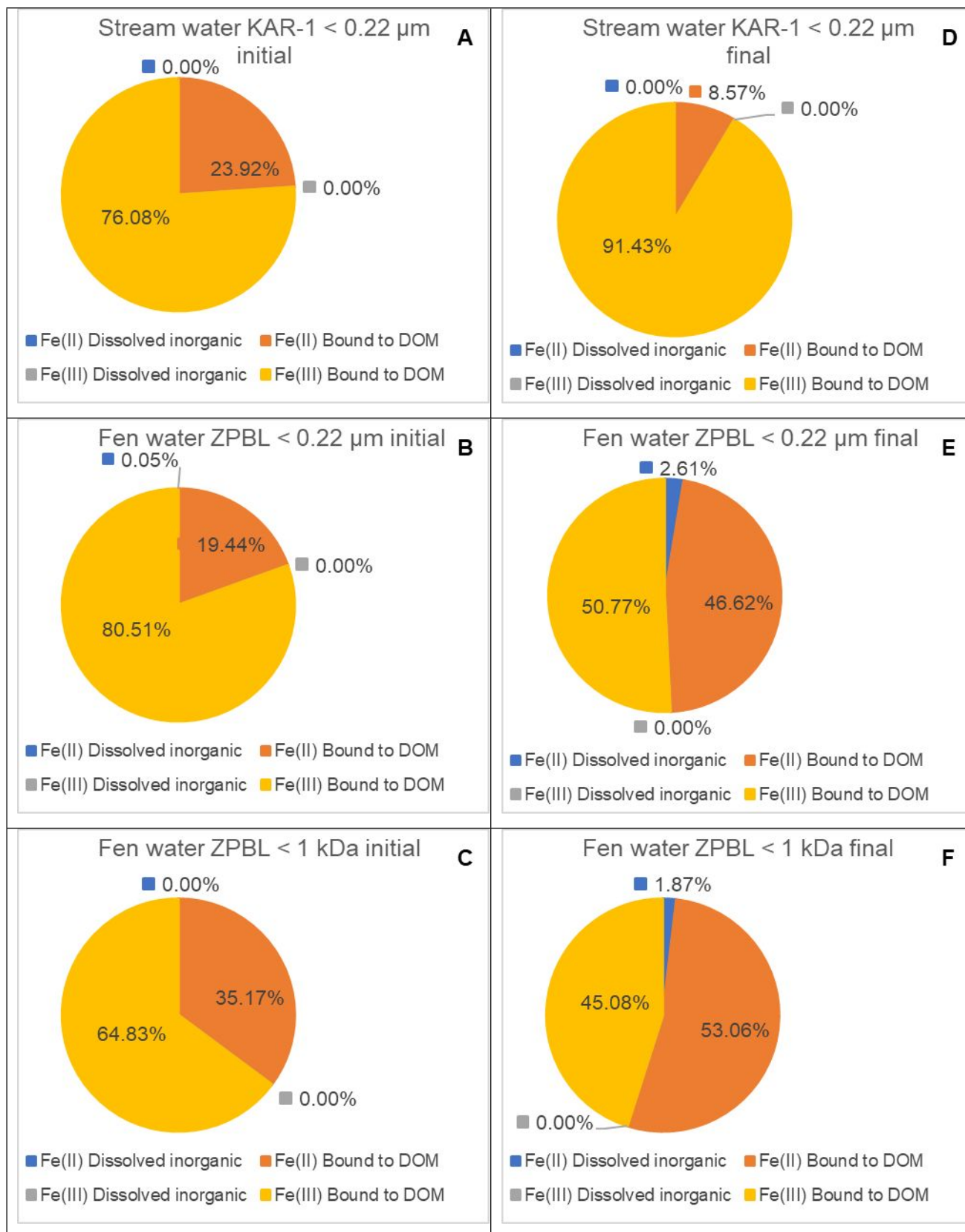
Iron	Species name	Fen water (ZPBL) < 0.22 $\mu\text{m}$		Stream water (KAR-1) < 0.22 $\mu\text{m}$	
		% of total Fe(II) or Fe(III) concentration			
		0 h	100 h	0 h	100 h
Fe(II)	Free ions	1.73	0	0	0
	Weakly (electrostatically) bound with FA	30.33	0.01	0	0
	Complex with FA's carboxylic groups	67.94	99.98	99.99	100.00
Fe(III)	Complex with FA's carboxylic groups	26.82	6.83	0.28	0.07
	Complex with FA's phenolic groups	73.18	93.17	99.72	99.93

**Table S3.** Speciation of Fe(II) and Fe(III) in < 0.22  $\mu\text{m}$  fraction of stream and in < 0.22  $\mu\text{m}$  and < 1 kDa fractions of fen water at the beginning (0 h) and after 200-250 h of sunlight exposure. FA stands for Fulvic Acid.

Iron	Species name	Fen water ZPBL < 0.22 $\mu\text{m}$		Fen water ZPBL < 1 kDa		Stream water KAR < 0.22 $\mu\text{m}$	
		% of total Fe(II) or Fe(III) concentration					
		0 h	200 h	0 h	200 h	0 h	250 h
Fe(II)	Free ions	0.26	3.40	0.01	3.40	0	0
	Weakly (electrostatically) bound with FA	56.53	12.33	18.46	12.33	0	0
	Complex with FA's carboxylic groups	43.21	84.26	81.53	84.26	99.99	100.00
Fe(III)	Complex with FA's carboxylic groups	6.60	2.20	6.60	2.20	0.11	0.32
	Complex with FA's phenolic groups	93.40	97.81	93.40	97.81	99.89	99.68



**Fig. S1.** Results of vMINTEQ speciation calculation for initial (0 h) and final (100 h) solutions during biodegradation experiments with stream (**A, B**) and fen (**C, D**) water.



**Fig. S2.** Results of vMINTEQ speciation calculation for initial (0 h) and final (200-250 h) solutions during photodegradation experiments with stream (A, D) and fen (B, C, E, F) waters.