

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

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1	Iron isotope fractionation during bio- and photo-degradation
2	of organo-ferric colloids in boreal humic waters
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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 $LMW_{< 1 kDa}$ during photolysis
30	Bio-and photodegradation of organo-ferric colloids can produce very large, from -2.5 to $+3.2\%$
31	$\delta^{57}$ Fe isotopic variations in boreal waters
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#### 41 ABSTRACT

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

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#### 1. Introduction

Despite broad use of Fe isotopes for tracing various large-scale, long-term processes at the 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 shortterm processes such as bio- and photo-degradation of organic matter that binds Fe in freshwater remain poorly understood. This is especially true for organic carbon and Fe-rich boreal waters, that, 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 other hand, strongly contribute to Fe and other micro-nutrient export by rivers to the coastal productive zones<sup>13,14</sup>. 67 The majority of dissolved ( $< 0.22 \mu m$ ) Fe and other metals in boreal waters are present in the form of organic and organo-mineral colloids  $(1 \text{ nm} - 0.22 \mu\text{m})$  whose transport and bioavailability are 68 dramatically different from inorganic ions or simple organic complexes<sup>15</sup>. Two main processes 69 responsible for fluxes and residence time of organic C and metal colloids in boreal high-latitude aquatic 70 environments are heterotrophic bacterial respiration (degradation of DOM) and photolysis<sup>16</sup>. Colloidal 71 transformation often occurs along the hydrological continuum of interconnected soil waters, mires, 72 streams and large oligotrophic lakes<sup>17</sup>. During this transformation, induced mainly by aquatic 73 74 microorganisms and sunlight irradiation, the organic and organo-ferric colloids are subjected to i) 75 change of dominant source and molecular weight from allochthonous large-size humic and fulvic molecules to small size autochthonous organic ligands<sup>17</sup>, ii) degradation of DOC into CO<sub>2</sub> or 76 intracellular uptake of DOC by bacterioplankton and liberation of Fe(III) ions, leading to Fe 77 oxy(hydr)oxide precipitation<sup>18,19</sup>, and *iii*) enrichment in low molecular weight organic ligands and their 78 complexes with metals<sup>20</sup>. 79

In contrast to numerous works devoted to microbiological<sup>21</sup> and photochemical<sup>22</sup> 80 81 mineralization of dissolved organic carbon (DOC), nitrogen and phosphorus, the bacterial and sunlight-induced transformations of Fe-rich colloids in inland waters have been little studied<sup>23,24</sup>. The 82 transformation of colloidal Fe in boreal waters, including bogs, lakes and rivers<sup>25</sup> has been studied 83 under the metabolic action of heterotrophic soil and aquatic bacteria<sup>18,26-27</sup> and sunlight<sup>28</sup>. In these 84 85 works we demonstrated that the biodegradation of organo-ferric colloids by heterotrophic bacteria consists of *i*) element uptake inside the cells; *ii*) element adsorption at the cell surface, and *iii*) Fe 86 87 hydroxide precipitation leading to scavenging of associated trace metals. During both biodegradation 88 and photolysis of natural DOM. Fe plays a pivotal role in controlling the fate of trace elements (TE). 89 This control occurs via i) formation of insoluble Fe(III) hydroxides that coprecipitate other trace metals<sup>28</sup>, and *ii*) generation of low molecular weight (LMW) organic ligands that bind Fe, thus 90 competing with strong organic complexes of other metals<sup>19,20,28</sup>. 91

92	The Fe isotope approach is an efficient tool for deciphering elementary processes involving
93	microbes and aqueous solutions <sup>29-33</sup> , DOM - Fe <sup>34</sup> and mineral - Fe <sup>35,36</sup> interaction including colloids <sup>3.6</sup>
94	<sup>37</sup> . The latter study demonstrated an unusual enrichment of heavier Fe isotopes in the LMW (< 1-10
95	kDa) fraction relative to remaining colloidal fraction (by up to +3 to +4 ‰ in $\delta^{57}$ Fe) and suggested
96	that bio- and/or photo-transformation of colloids may be responsible for heavy Fe isotope signatures
97	in LMW fraction of boreal waters. However, the photo-oxidation of organo-ferric colloids removes Fe
98	in the form of Fe(III) hydroxides <sup>19,23,38</sup> which can enrich the remaining solution in lighter Fe isotopes,
99	consistent with numerous laboratory experiments on Fe hydroxide precipitation from Fe(II)-bearing
100	aqueous solution <sup>39</sup> . Whereas for $Fe^{3+}(aq) \rightarrow Fe(III)_{solid}$ (hematite) reaction at equilibrium, a negligible
101	fractionation of -0.15±0.30 ‰ ( $\Delta^{57}$ Fe) was reported <sup>40</sup> , the Fe <sup>2+</sup> (aq) $\rightarrow$ Fe(III) <sub>solid</sub> (ferrihydrite) reaction
102	has $\Delta^{57}$ Fe of +4.8±0.15 ‰ <sup>39</sup> . Other experiments producing goethite reported a fractionation factor of
103	+1.7±0.14‰, corresponding to the Fe(III) <sub>solid</sub> being enriched in heavier isotopes <sup>41</sup> . As for the
104	bacterially-induced colloid transformation, two main processes controlling the isotope fractionation
105	during metal-live cell interaction are 1) fast adsorption of heavier Fe isotope on the cell surfaces <sup>30,31</sup> ,
106	and 2) long-term assimilation of heavier $\frac{Fe}{Fe}$ isotopes as recently shown for phytoplankton <sup>42</sup> and
107	magnetotactic bacteria <sup>33</sup> . Note however that, in contrast to the fairly good knowledge of isotope
108	fractionation of ionic metals and their organic complexes interaction with microbial cells, virtually
109	nothing is known on colloidal metal – cell interaction reactions. The present study is aimed at the
110	quantification of Fe isotope signatures during photo- and bio-degradation of DOM-Fe complexes in
111	high latitude boreal waters. We hypothesized preferential removal of heavier Fe isotopes by live cell
112	adsorption and assimilation and Fe hydroxide precipitation in the course of bio- and photodegradation
113	of organo-ferric colloids. We further tested the possibility of formation of isotopically heavy Fe in
114	$LMW_{< 1 kDa}$ fraction of river waters, as it was put forward in a previous study <sup>37</sup> . To test these
115	hypotheses, we selected a stream and a fen water, two dominant types of Fe-rich freshwaters of the
116	subarctic. By choosing two main processes controlling the fate of organo-ferric colloids (bio- and
117	photo-degradation) and two representative examples of boreal Fe- and DOM-rich surface waters, we

- 118 were capable to reveal the main factors that govern Fe isotope behavior on a short-term (daily) scale
- 119 in large territory of subarctic landscapes.
- 120

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

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

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

140 The experiments included on-site photodegradation of sterile-filtered (< 0.22  $\mu$ m) 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 form 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 $g_{wet}$ L <sup>-1</sup> . Note
150	that the amount of Fe originated from bacteria addition does not exceed 1 $\mu$ g L <sup>-1</sup> for this concentration
151	of biomass <sup>27</sup> . The flasks were shaken at 25±0.5°C and aerated via Biosilico® ventilation porous caps
152	during 4 days. Aliquots of homogeneous suspension were sampled after 0, 1, 8, 22, 30, 50 and 100 h
153	after the addition of bacteria and filtered through $0.22 \mu m$ membrane. All the experiments were run in
154	duplicates. The control experiment was sterile filtered (< $0.22 \ \mu m$ ) biomass-free stream and fen water
155	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 $(0.22 \mu m, 250 \mu m)$
160	mL volume). Filtered fluids were transferred into 270-mL sterilized quartz flasks, filled with 10% air
161	headspace and covered by porous sterile stoppers. The dark control of quartz flasks was identical to
162	the light samples except that the reactors were wrapped in Al foil. Both dark and light reactors were
163	run in duplicates and they were placed on flat surface at the border of the lake (66°17'04"N,
164	30°52'05"E). The flasks were exposed to direct unshaded sunlight from July 9 to July 20 during
165	essentially anticyclonic weather. The daytime duration in this period was between 22 and 20.5 hours.
166	The temperature of the experimental reactors followed the diurnal cycle and was equal to $18 \pm 5^{\circ}$ C
167	over 250 h of exposure, which was within the range of actual water temperature in stream and fen
168	during the month of July <sup>17,37</sup> . The quartz reactors were sampled after 0, 100 and 200 h of exposure of
169	the fen water (ZPBL) and after 0, 110 and 250 h of exposure of the stream (KAR-1). For each sampling

170	the whole reactor was sacrificed. The samples were immediately filtered through 0.22 µm Sartorius
171	single-used filter into pre-cleaned polypropylene vials. These waters were then acidified using bi-
172	distilled HNO <sub>3</sub> for trace metals analysis or directly used, without acidification, for DOC, UV <sub>254 nm</sub> ,
173	DIC and anions determinations. The $0.22 \mu$ m filtrate from quartz reactors was additionally ultrafiltered
174	through 1 and 10 kDa regenerated cellulose single-use filters with an Amicon 8400 frontal
175	Ultrafiltration unit (continuously stirred 400-ml polycarbonate cell maintained under 1.5–2 atm
176	pressure). Details of ultrafiltration procedure in humic boreal waters of North Karelia and discussion
177	of possible artifacts are presented elsewhere <sup>17,37,43</sup> . In these experiments, we consider the behavior of
178	Fe in <0.22 $\mu$ m and <10 kDa fractions of the stream water and in <0.22 $\mu$ m, <10 and <1 kDa fractions
179	of the fen water.
180	All experiments including controls were run in duplicates (two independent reactors) and both
181	solution duplicates were processed for isotope analyses. Analyses of DOC and Fe concentration in
182	various filtered and ultrafiltered fractions were performed using Shimadzu TOC-VCSN and Agilent
183	ICP MS, following procedures described previously <sup>27,28</sup> . The DOC was measured with an uncertainty
184	of 3% and a detection limit of 0.1 mg L <sup>-1</sup> . The total dissolved Fe represents the sum of Fe(II) and
185	Fe(III) in the $< 0.22 \ \mu$ m fraction. The uncertainty of Fe concentration measurements ranged from 5 to
186	10% (1SD). The international geostandard SLRS-5 (Riverine Water Reference Material for Trace
187	Metals certified by the National Research Council of Canada) measured each 20 samples was used to
188	check the validity and reproducibility of Fe analysis. Good agreements were found between the
189	replicate measurements of Fe in SLRS-5 and the certified values (relative difference < 20% SD on the
190	repeated measurements).
191	Assessment of Fe(II) and Fe(III) concentrations was performed using the conventional ferrozine
192	method <sup>45</sup> . The efficiency of this method in organic-rich tropical waters was further demonstrated in
193	Rio Negro <sup>46</sup> . Because of possible interferences from DOM in humic boreal waters, the ferrozine
194	method was used employing a standard addition technique. This allowed to achieve a detection limit
195	of 15 $\mu$ g L <sup>-1</sup> and an uncertainty from 10-20% at 15 < Fe(II) <100 $\mu$ g L <sup>-1</sup> to 5-10% at Fe(II) > 100 $\mu$ g

196  $L^{-1}$ . Further, we verified the stability of Fe(II) in oxygenated humic waters (pH = 4.6 to 6.5; DOC =

197 20 to 50 mg  $L^{-1}$ ) using peat and moss leachate (similar to main allochthonous DOM in studied sites<sup>47</sup>).

198 The Fe(II) concentration remained stable within  $\pm 10\%$  over 24-180 h of exposure.

199 The Fe(III) and Fe(II) complexation with organic ligands and solution saturation degree with 200 respect to secondary Fe hydroxide in the course of experiment were calculated using Visual MINTEQ<sup>48</sup>, in conjunction with a NICA-Donnan humic ion bonding model, as described previously<sup>17</sup>. 201 202 We considered carboxylic and phenolic complexes of fulvic acids with  $Fe^{2+}$  and  $Fe^{3+}$  ions, as well as 203 Fe(II) weak (electrostatic) interaction with fulvic acids. 204 For Fe isotope analysis, filtered and acidified water samples were evaporated in the clean 205 laboratory. After acid digestion of the residue using a mixture of HCl and HNO<sub>3</sub>, Fe was purified via anion exchange chromatography with HCl using Bio Rad AG1 X4 resin, 200–400 mesh to remove all 206 matrix elements<sup>37</sup>. For this, we used thermoretractable Teflon columns with an internal diameter of 4 207 mm<sup>8</sup>. The resins were conditioned using 6 M of HCl prior to the sample loading in 0.5 ml of 6 M HCl. 208 209 The matrix species were eluted in 3 ml of the same acid and, subsequently, Fe was quantitatively eluted with 2 ml of 0.05 M HCl. The purified Fe solutions were evaporated at 120°C. After evaporation, 210 211 purified Fe samples were redissolved in a 0.05 M HCl solution that was used for the MC-ICP-MS 212 analysis. Iron and the internal standard, Ni, were set to concentration producing signals of ca. 40 V of <sup>56</sup>Fe and 20V for <sup>60</sup>Ni. Iron isotope measurements were performed at the CNRS-INSU National MC-213 214 ICP-MS facility in Lyon using a Thermo Electron Neptune Plus and at GET-CNRS in Toulouse using a Thermo Electron Neptune MC-ICP-MS (Bremen, Germany)<sup>49</sup>. All analyses are reported in the delta 215 notation relative to the IRMM-014 standard, expressed as  $\delta^{57}$ Fe, which represents the deviation in per 216 217 mil relative to the reference material:

218 
$$\delta^{57} \text{Fe} (\%) = \left(\frac{({}^{57}Fe/{}^{54}Fe)_{sample}}{({}^{57}Fe/{}^{54}Fe)_{IRMM 14}} - 1\right) *1000$$

219 We also obtained  $\delta^{56}$ Fe values but, since the relationships between  $\delta^{56}$ Fe and  $\delta^{57}$ Fe of the 220 samples plot on a single mass fractionation line ( $\delta^{57}$ Fe = 1.466 ×  $\delta^{57}$ Fe + 0.005, R<sup>2</sup> = 0.9995, p < 10<sup>-1</sup>

221	<sup>4</sup> ), only $\delta^{57}$ Fe values are discussed in this paper. Data quality was checked by the analysis of our in-
222	house hematite standard every 5 samples in the analytical sequence. Our mean value of $0.771\pm0.047\%$
223	(2SD) for this standard obtained between Lyon and Toulouse from pooling 51 individual analyses by
224	groups of 6 was consistent with the values of $0.766 \pm 0.072\%$ reported previously <sup>8</sup> .
225	
226	3. Results and Discussion
227	3.1. Iron in the $< 0.22 \ \mu m$ fraction during biodegradation experiments: Heavier isotopes
228	adsorption onto and lighter isotopes uptake by live cells of P. aureofaciens
229	The studied surface waters were oxygenated, organic- and Fe-rich (pH = 7.1, DOC = $12 \text{ mg L}^{-1}$
230	<sup>1</sup> , Fe = 208 $\mu$ g L <sup>-1</sup> in KAR-1; pH = 5.4, DOC = 38.7 mg L <sup>-1</sup> , and Fe = 4310 $\mu$ g L <sup>-1</sup> in ZPBL). The
231	stream water contained ~24% of Fe(II) and the fen contained ~20% of Fe(II); the LMW <sub>&lt;1 kDa</sub> fraction
232	of DOC was sizably higher in the stream compared to the fen (77 and 29%, respectively). The
233	experimental results are reported in Table 1 whereas those of the control experiments are listed in
234	Table S1. The pH value increased by 0.6 and 1.2 unit over 4 days of <i>P. aureofaciens</i> reaction with
235	stream water and fen water, respectively (Table 1). There was no sizeable DOC decrease over the first
236	1 h of reaction. The long-term removal $(1 - 100 \text{ h})$ of DOC by <i>P. aureofaciens</i> was not pronounced in
237	the stream water but was high in the fen water (-33±5%).
238	Iron exhibited initial adsorption after 1 h of reaction as it is seen from the difference between

the stream and the fen water control and bacterial suspension at the beginning of experiment (**Fig. 2 A** and **B and Table 1**). The proportions of fast adsorption (0-1 h) and assimilation/coagulation (1-93 h) relative to total Fe removal in the experiments were respectively equal to 35% and 37% in the stream water and 30% and 25% in the fen water. In the latter, the concentration of divalent Fe decreased almost 3-fold over the first day of reaction relative to control (**Fig. 2 C**). No sizeable change of Fe(II) in stream water could be assessed because the Fe(II) concentration in KAR-1 (not shown) was  $30\pm 20$  $\mu g/L$  (~16% of total dissolved Fe), with a quantification limit of Fe(II) by this method of 15  $\mu g/L^{45}$ .

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 Fe(III)-fulvic acid (FA) complexes. The Fe(II) bound to FA fully disappeared in the stream and 248 249 decreased by a factor of 3 in the fen waters (Fig. S1). Specifically, the Fe(III) complex with carboxylic 250 groups of FA degraded 4-fold in both fen and stream water, whereas phenolic complex of Fe(III) remained stable (Table S2). The proportion of Fe(II) complex with carboxylates of FA increased 251 252 whereas Fe(II)-weak electrostatic complex completely disappeared. This is consistent with highly 253 reactive behavior of dissolved Fe(II) in biodegradation experiments. 254 The second  $< 0.22 \,\mu$ m filtration step in the laboratory of the control water samples showed that the 6 months storage in the fridge did not affect stream water chemical composition but it induced a 255 decrease of Fe concentration in the fen water from 4310 µg/L to 2470 µg/L, accompanied by a change 256 in  $\delta^{57}$ Fe from 0.25±0.06‰ to 1.06±0.04‰. A strong  $\delta^{57}$ Fe increase from 1.59±0.05‰ to 2.87±0.07‰ 257 also resulted from the storage for the stream water (Table 1 and S1). This change could be linked to 258 259 some Fe(OH)<sub>3</sub> precipitation due to either coagulation of Fe(III)-rich organic colloids or Fe(II) oxidation. To preserve at least the Fe isotopic composition of DOC-rich waters over several months, 260 immediate freezing after filtration in the field on the sampling site is preferable<sup>50</sup>, but this is not always 261 possible for logistical reasons. 262

Although the fractionation of Fe between bacterial cells and organo-ferric colloids has not been 263 studied previously, the adsorption of metal cations such as  $Zn^{2+}$  at the microorganism cell surface is 264 known to favor the heavier isotopes<sup>51</sup> and both  $Fe^{2+}$  and  $Fe^{3+}$  ions follow this rule<sup>30,31</sup>. The heavier Fe 265 266 isotopes are preferentially adsorbed onto solid surfaces during equilibrium isotope fractionation processes<sup>36</sup>. Experiments that lasted 11 days with anaerobic phototrophic Fe-oxidizing, aerobic 267 neutrophilic Fe-oxidizing, and heterotrophic Fe-reducing bacteria demonstrated that metabolically-268 269 produced hydrous ferric oxide (HFO) exhibited heavier isotopic composition than the initial Fe(II), with a fractionation factor  $(\Delta^{57}\text{Fe})$  of  $+2.2\pm0.3\%^{52}$ . Further, depending on the phytoplankton species 270 271 and the composition of the culture medium, the cell surface was found to be enriched in heavier

isotopes by  $\pm 2.4\pm 0.6$  % to  $\pm 2.9\pm 0.1$  % for Fe<sup>2+</sup> containing solution and by  $\pm 0.4\pm 0.2$  to  $\pm 1.0\pm 0.2$  % 272 for  $Fe^{3+}$  solution<sup>30</sup>. More recent measurements of Fe isotope fractionation during Fe adsorption onto 273 phytoplankton cells quantitatively confirmed these data<sup>31</sup>. The value  $\Delta^{57}$ Fe<sub>cell-solution</sub> of  $\pm 0.5 \pm 0.1\%$  for 274 the river water inferred from the present study suggests essentially  $Fe^{3+}$  ion adsorption onto P. 275 *aurefaciens* cell surfaces, presumably forming Fe(III)-phosphoryl complexes<sup>44</sup>. Note however that the 276 277 competition between surface organic moieties and aqueous organic complexes for heavy Fe isotopes 278 can decrease the magnitude of isotopic offset in humic waters compared to experimental solutions with 279 low DOC. At present, the isotopic offset for adsorption of organo-ferric colloids onto organic surfaces 280 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  $\delta^{57}$ Fe of 282 ca 0.4‰ in solution due to adsorption of heavier isotopes on the cell surface, and then a gradual increase in aqueous  $\delta^{57}$ Fe from +2.5±0.1‰ to +3.2±0.1‰ between 1 and 24 h (Fig. 3 A) yielding 283  $\Delta^{57}$ Fe<sub>cell-solution</sub> of -0.7%. This implies removal of lighter Fe isotopes due to their preferential 284 intracellular uptake. After an initial drop in the stream water  $\delta^{57}$ Fe, the long-term removal of lighter 285 Fe isotopes by live bacteria produced ca 0.7% increase in  $\delta^{57}$ Fe over the first 20 h of reaction that 286 287 remained constant to 50 h (Fig. 3A). This strongly supports biological mechanism of lighter Fe 288 isotopes uptake inside cells rather than metabolically-induced Fe(OH)<sub>3</sub> precipitation. In the latter case, the solid phase would be enriched in heavy isotope  $(\Delta^{57}\text{Fe}_{\text{FeOOH-Fe}(aq)} = +1.5 \text{ to } +4-5\%)^{32,53,54}$  as also 289 supported by natural observations<sup>9,53</sup>. The present result is at variance with previous Fe assimilation 290 by magnetotactic bacteria<sup>33</sup> or phytoplankton<sup>42</sup> that inferred a heavier Fe isotope uptake inside the cells 291 292 due to a combined Fe oxidation. However, the Fe(III)-DOM complexes in solution retain heavy isotope as it is known for Fe<sup>3+</sup>(H<sub>2</sub>O)<sub>6</sub> –Fe(III) chelate (DFO-B): the organic complexes are enriched in heavy 293 isotopes with an isotopic offset of  $\pm 0.90 \pm 0.23 \ \text{\%}^{55}$ , and overall, there is a strong positive correlation 294 between Fe fractionation factors and the Fe-binding affinity of the ligands<sup>56</sup>. According to vMinteq 295 296 calculation, the majority of Fe in our experimental solutions was bound to phenolic complexes with fulvic acids (Fig. S1, Table S2). Therefore, we suggest that strong complexation of isotopically heavy
Fe(III) with DOM prevents the intracellular uptake of heavier Fe isotopes.

In the fen water, we observed a similar  $\delta^{57}$ Fe evolution during biodegradation experiments, 299 300 though with smaller variations. The isotopic composition of dissolved Fe did not appreciably change 301 during *P. aureofaciens* short-term interaction with fen water: we observed only weak adsorption of heavy isotope (<0.1‰) and a weak assimilation of light isotopes  $\Delta^{57}$ Fe<sub>cell-solution</sub>  $\leq 0.1$ ‰ over first 40 302 h (**Fig. 3 B**). Relative to the sterile control, a decrease in  $\delta^{57}$ Fe at the beginning of experiment signified 303 preferential adsorption of heavy isotopes with  $\Delta^{57}$ Fe<sub>cell-solution</sub> =  $\pm 0.2 \pm 0.1\%$ . Over 100 h of reaction, 304 305 light Fe isotopes were taken up by the cells as the  $\delta^{57}$ Fe of solution increased from  $\pm 0.86 \pm 0.1\%$  to 306  $+1.05\pm0.05\%$ .

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

- 312
- 3.2. Iron concentrations and isotopic signatures evolution during photodegradation of organo-ferric
  colloids: Removal of heavier isotopes due to Fe(III) hydroxide formation and appearance of
  isotopically light Fe(II) in the LMW fraction

There was no pH variation in the course of photodegradation experiments: within the uncertainty of duplicates (< 0.1-0.2 pH unit), the pH in the dark control and the light experiment was identical and equal to  $7.0\pm0.2$  and  $5.3\pm0.1$  in the stream and fen water, respectively (Table 1 and S1). The DOC concentration in <0.22 µm fraction of stream and fen water decreased by 30 and 50%, respectively, over 200 to 250 h of photodegradation experiment (Table 1). There was a good correlation between ultraviolet absorbency (UV<sub>254 nm</sub>) and Fe concentration in the fen water (R<sup>2</sup> = 0.928) which was absent in the stream water in the course of experiment (R<sup>2</sup> = 0.19). In the stream water, Fe concentration remained generally constant within the uncertainties over 250 h of exposure to sunlight, relative to the dark control, and no formation of flocculent material was noted (**Fig. 4 A**). There was 50 to 80% removal of Fe(II), between 110 and 250 h of exposure, which occurred for both <0.22  $\mu$ m and <10 kDa fractions (from 50 to 15  $\mu$ g/L and from 20  $\mu$ g/L to < limit of quantification, respectively, **Fig. 4 B**).

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

337 During sunlight exposure, the  $\leq 0.22 \,\mu$ m fraction of the fen water became depleted in Fe as the 338 molar ratio of C<sub>org</sub>:Fe increased from 50 to 100. This trend mainly stems from more efficient Fe 339 removal compared to DOC (Table 1). The change of this ratio in the stream water was within the 340 experimental uncertainty. The C<sub>org</sub>:Fe ratio in the fen water colloids (1 kDa – 0.22 µm) did not change 341 in the course of photo-degradation, from 31 at the beginning of experiment to 32 after 193 h of sunlight 342 exposure. In the LMW<sub><1kDa</sub> fraction of fen water, the C<sub>org</sub>:Fe ratio decreased from 247 to 126.

All size fractions of the initial stream solution and the LMW<sub><1 kDa</sub> fraction of the fen water were undersaturated with respect to Fe-bearing minerals. The initial <  $0.22 \,\mu$ m fen water was supersaturated with respect to goethite, lepidocrocite and magnetite (Saturation Indexes = 1.3, 0.62, and 3.6, respectively). The speciation calculation using vMinteq also demonstrated that Fe(III) is fully complexed with DOM (**Fig. S2**). Therefore, the removal of Fe in the form of particulate Fe hydroxide could be only due to liberation of part of Fe from organic complexes after photolytic degradation of

349 colloidal DOM that stabilized Fe (III) polymers in solutions. Calculated Fe(II) and Fe(III) speciation 350 in the fen water before and after photodegradation experiment demonstrated an appearance of  $\sim 2\%$  of inorganic Fe(II) and approx. 2-fold increase of Fe(II) bound to DOM, in both  $< 0.22 \mu m$  and < 1 kDa351 352 size fraction (Fig. S2). In the stream water, there was a 3-fold decrease of Fe(II) bound to DOM. Specifically, the Fe(III) complex with carboxylic groups of FA decreased and increased 3-fold in fen 353 354 and stream waters, respectively (Table S3). Phenolic complexes of Fe(III) which accounted for 355 >93...99% of all Fe, remained constant in the course of experiment. In the fen water, the proportion 356 of Fe(II)-weak electrostatic complex strongly decreased (by a factor of 4.5 and 1.5 in  $< 0.22 \mu m$  and < 1 kDa fractions, respectively) whereas Fe(II) complex with FA carboxylates increased in < 0.22 µm 357 fraction and stayed constant in < 1 kDa fraction (**Table S3**). According to vMinteq predictions,  $4.5 \times 10^{-1}$ 358 359 <sup>5</sup> M of ferrihydrite should could have precipitated from the fen water over 200 h of solar irradiation. 360 This is equivalent to  $\sim 2250 \,\mu g/L$  of dissolved Fe concentration decrease, which is comparable to the 361 range encountered in the experiment (ca. 3400 µg/L, see Fig. 4 D and Table 1).

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

The isotopic ratio in the dark control of fen water remained stable at  $\delta^{57}$ Fe = 0.2±0.3‰, except in the LMW<1 kDa fraction, where a +0.6‰ increase was observed, related to to the Fe and DOC loss (Table S1). Similar effect was reported in the organic-rich Negro River where it was attributed to the loss of isotopically light «heteroaggregates»<sup>50</sup>. In contrast to dark controls, sunlight irradiation of the fen water strongly impacted  $\delta^{57}$ Fe in all 3 fractions, <0.22 µm, <10 kDa and <1 kDa (**Fig. 5 B**). Over first 100 h of reaction, there was sizeable, from 1 to 2‰, decrease of  $\delta^{57}$ Fe in the <0.22 µm and the

#### <1-10 kDa fraction, respectively. Over the next 100 h, the $\delta^{57}$ Fe further decreased by 1.5 ‰ in the

 $375 = -2.2 \pm 0.22 \mu m$  fraction and stabilized at  $\delta^{57}$ Fe =  $-2.2 \pm 0.2\%$  in the <1 and <10 kDa fractions.

376 Sunlight exposure of stream water did not produce any sizable change of Fe concentration and 377 isotopic signature in the  $< 0.22 \mu m$  fraction (Fig. 4A and 5A), in general agreement with stability of boreal high latitude metal concentration in riverwaters with respect to sunlight irradiation<sup>28,57</sup>. After 378 379 100 h of sunlight irradiation, the < 10 kDa fraction of stream water became 1.5‰ lighter compared to 380 the initial value or the dark control (Fig. 5A). A plausible explanation for this isotopic pattern invokes 381 the presence of strong low molecular weight (< 10 kDa) Fe(III)-organic ligand (chelate) complexes which are enriched in heavy isotopes<sup>55</sup>. For example, aquatic prokaryotes produce the LMW (0.3 – 1) 382 383 kDa) Fe(III) siderophores<sup>58</sup>. These light sensitive, presumably aromatic Fe complexes represent a small iron fraction of overall Fe<sub><10 kDa</sub> pool, but our results indicate that it exhibits a very high  $\delta^{57}$ Fe 384 385 value. The isotopic signature of LMW<sub><1-10 kDa</sub> Fe poor, C-rich fraction of Karelian waters reaches +4.2‰<sup>37</sup>. We hypothesize that these isotopically heavy Fe-organic complexes have low residence time 386 387 in the river channel and are produced due to periphyton or plankton metabolic activity.

388 The Fe in the fen water was strongly sensitive to sunlight irradiation as > 70% Fe was removed 389 from the  $\leq 0.22 \,\mu$ m fraction and all filtrates and ultrafiltrates became strongly impoverished in heavy isotopes (Fig. 4 D, 5 B). The removal of Fe followed that of SUVA<sub>254</sub> decrease<sup>28</sup>, suggesting that the 390 391 majority of Fe that is removed from the fen water was bound to aromatic (colored) organic carbon. 392 This is also confirmed by the dominance of Fe(III)-phenolic groups of FA in Fe speciation (**Table S3**). 393 It is possible that the photolysis of Fe-DOM complexes liberates ionic Fe which could be removed 394 from solution in the form of isotopically heavy Fe hydroxides. The removal of heavy isotopes by sunlight oxidation of organo-ferric colloids observed in this study ( $\pm 2.3\pm 0.1\%$ ) is generally consistent 395 396 with known fractionation of Fe between Fe(III) hydroxide and Fe(II) solution: ( $\Delta^{57}$ Fe<sub>FeOOH-Fe(aq)</sub> = +2.3±0.3‰, ref. 52-54). 397

In contrast to what was hypothesized in our earlier work on these waters<sup>37</sup>, no enrichment in heavy isotopes of LMW fraction was observed during photolysis of humic waters. Here we suggest that Fe(II)

400	generated in the < 1 kDa fraction is enriched in light isotopes compared with initial Fe(III) in colloids,
401	thus producing overall negative $\delta^{57}$ Fe value in LMW <sub>&lt;1 kDa</sub> after irradiation. Assuming an equilibrium
402	fractionation factor between Fe(II) and Fe(III) of -4.3‰ <sup>59</sup> and considering the starting water $\delta^{57}$ Fe
403	close to 0‰ (Fig. 5B), the 55% of Fe(II) in < 1 kDa fraction (Fig. 4 F) provides $0.55 \times (-4.3\%) = -10^{-10}$
404	2.4‰ of $\delta^{57}$ Fe, in full agreement with values shown in <b>Fig. 5 B</b> . This <0.22 µm fraction comprises two
405	Fe pools: (1) Fe remaining after $Fe^{2+}(aq) \rightarrow Fe(OH)_3$ and after $Fe^{3+}(colloidal) \rightarrow Fe(OH)_3$
406	precipitation, and (2) isotopically light $Fe^{2+}$ produced in the LMW fraction. It is important to note that
407	the photolysis of organo-ferric colloids which represents 80% of Fe in $<0.22 \ \mu m$ fraction, removes
408	Fe(III) in the form of Fe(OH) <sub>3</sub> hydroxides corresponding to $\Delta^{57}$ Fe <sub>Fe(III)hydroxide-Fe(aq)</sub> of 2.4±0.1‰, Fig
409	<b>5B</b> . The observed enrichment of solid phase in heavier isotopes is therefore similar to $Fe^{2+}(aq) \rightarrow$
410	$Fe(OH)_3$ reaction $(\Delta^{57}Fe_{Fe(III)hydroxide-Fe(aq)} = 2.4 \%^{52})$ rather than to $Fe^{3+}(aq) \rightarrow Fe(OH)_3$ reaction
411	$(\Delta^{57}\text{Fe}_{\text{Fe(III)hydroxide-Fe(aq)}} = -0.9 ^{60})$ . Note here that in the photolysis experiments, the loss of dissolved
412	Fe and change of Fe isotope fractionation are mainly interpreted as precipitation of hydrous Fe(III)-
413	oxides. We therefore neglect Fe(II) sorption onto hydrous Fe(III)-oxides and subsequent atom
414	(isotope) exchange between Fe(II) and hydrous Fe(III)-oxides. These processes are responsible for
415	substantial Fe isotope fractionation in a number of organic-free systems <sup>36, 61-64</sup> . In humic waters, both
416	remaining Fe(II) <sub>aq</sub> ions and >FeOOH surface sites of newly precipitated Fe(III) hydroxides are likely
417	to be bound to carboxylic and phenolic groups of fulvic acids. These surface organic - Fe complexes
418	interfere with Fe(II)-Fe(III) hydroxide isotope exchange which would therefore require special
419	investigation. Further, in order to fully account for short-term variation of Fe concentration, speciation
420	and isotope ratios, one has to consider both dissolved and particulate Fe fracions, as well as isotopic
421	composition of both Fe(II) and Fe(III) species.
422	We observed quite large differences in the degree of Fe concentrations and Fe isotopic
423	composition during photolysis of the fen and the stream water. These differences may stem from the
424	combination of two main factors controlling the degree of DOM and Fe photo-reactivity in natural

425 waters: the fen water is a 1.5 unit of pH lower than the stream and has a factor of 30 higher in Fe

concentration. The water acidity is known to exert a strong positive effect on the photolysis of 426 DOM<sup>65,66</sup> and the DOM photobleaching is enhanced by elevated Fe concentration via the photo-Fenton 427 effect below pH 6<sup>67</sup>. Overall, in natural settings, one may expect large variation of Fe and DOM photo-428 429 liability depending on the environmental context. Thus, slightly alkaline (pH = 8) surface water from a temperate peatland in China exhibited quite higher photo-stability of Fe<sup>68</sup>, whereas acidic DOC-rich 430 431 waters from a subtropical swamp under UV irradiation demonstrated 10 times more efficient removal 432 of Fe relative to  $DOC^{69}$ . As such, depending on the lithological context of peatlands (i.e., acidic (felsic)) 433 or carbonate (sedimentary) rocks), the degree of Fe chemical and isotopic composition change under 434 sunlight may be dramatically different.

435

436 3.3. Complexity of Fe isotope fractionation in boreal humic waters and implication for inland
437 waters Fe isotope budget

438 We hypothesize several processes responsible for chemical removal and isotopic redistribution of 439 Fe among different colloidal pools in DOM- and Fe-rich stream and fen water, shown schematically 440 in **Fig. 6.** High molecular size ( $\frac{10 \text{ kDa} - 0.45 \text{ }\mu\text{m}}{0.45 \text{ }\mu\text{m}}$ ) organo-ferric colloids representing the majority of dissolved ( $<0.22 \mu m$ ) Fe<sup>17,37</sup> are stabilized by organic ligands originating from topsoil and bog peat 441 442 leaching. In addition, a small fraction of Fe(III) could be linked to highly specific, LMW<sub><1-10 kDa</sub> ligands 443 having a short residence time in the river channel; these complexes could be produced via in-stream 444 plankton and periphyton activity. In the fen water, a sizeable fraction of Fe is in the form of Fe(II) 445 inorganic and organic complexes<sup>70</sup>. In the river water, the HMW colloids are subjected to biologically-446 controlled transformations via i) adsorption onto surfaces of aquatic bacteria, favoring heavier isotopes to the cells, with an overall  $\Delta^{57}$ Fe<sub>cell ads-solution</sub> = +0.4±0.1‰, and *ii*) assimilation by live bacteria, 447 favoring lighter Fe isotopes to the cells with  $\Delta^{57}$ Fe<sub>cell incorp-solution</sub> = -0.7±0.1‰. On a short-term scale 448 449 (hours), the humic waters, once placed in contact with bacteria, are therefore becoming enriched in 450 lighter Fe isotopes, followed, on a long-term scale (days), by enrichment in heavier Fe isotopes. 451 Considering available data on preferential heavy isotope adsorption onto phytoplanktonic and 452 peryphytic cyanobacteria inhabiting natural waters (from  $\pm 0.4$  to  $\pm 2.9\%$ , ref. 30), and neglecting 453 Rayleigh distillation processes given that we are dealing with open systems (hydrological continuum, 454 ref. 17), the overall magnitude of diurnal variation of dissolved (< 0.45 µm)  $\delta^{57}$ Fe in small streams and 455 stagnant surface waters in the presence of common soil bacteria may range from  $\pm 2.7\%$  to  $\pm 0.7\%$ . 456 This greatly exceeds the range of Fe isotopic excursions in various sediments and in all possible 457 bedrocks<sup>71</sup>.

458 Further, photolysis of DOM and Fe-DOM complexes in surface waters, which operates at the 459 time scale comparable to water and solute residence time in these waters (1-10 days), is capable to 460 dramatically enrich in lighter Fe isotopes (by 1.5 to 2.5‰) of both dissolved ( $<0.22 \mu m$ ) and LMW<sub><1</sub>-461 10 kDa water fraction. We suggest that the two major processes of organo-ferric colloid transformation 462 under sunlight in natural waters include: (i) degradation of the organic part of colloids (1 kDa - 0.22) 463 µm) and production of low molecular weight (< 1 kDa) organic ligands including carboxylic and 464 aromatic chelates capable of strong binding of Fe ions; (ii) coagulation and precipitation of Fe-rich oxy(hydroxide) after the solar radiation-induced removal of stabilizing organic matter. 465

466 The enigmatic enrichment of the LMW fraction (<1-10 kDa) of the stream water in heavy isotopes<sup>37</sup> is therefore most likely linked to strong, presumably aromatic, isotopically heavy Fe(III)-467 468 organic complexes. These compounds have low residence time and are produced in the river channel 469 due to periphyton or plankton metabolic activity. In addition, sunlight irradiation of subarctic humic waters may produce 10-fold increase in aliphatic and aromatic carbonic acids<sup>72</sup> capable to bind both 470 Fe(II) and Fe(III). Altogether, bio- and photo-degradation of dissolved Fe in river, stream and bog 471 waters can produce from -2 to +3  $\% \delta^{57}$ Fe variation on time scales of a few days. Because this time 472 473 is generally shorter than the water residence time in surface waters, considering of Fe isotopic signature 474 of rivers and streams as a conservative value inherited from soils and using it for tracing the sources 475 and weathering regime on watersheds are not warranted at short time- and length-scales. Therefore, 476 naturally-induced variations in biological activity (switching from heterogenic bacteria uptake to 477 adsorption onto aquatic phototrophs) and sunlight illumination can modify the overall Fe isotopic

- 478 signatures of surface waters by several permil. Moreover, when considering the processes responsible
- 479 for Fe chemical and isotopic transformation in organic-rich streams, one has to assess both dissolved
- 480 (<0.45 or  $<0.22 \mu m$ ) and LMW<sub>< 1-10 kDa</sub> fractions of metal, since their isotopic signatures and photo-
- 481 susceptibility may be dramatically (by 1 to 3‰) different.
- 482

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- 488

#### 489 **References**

(1) Beard, B.L.; Johnson, C.M.; Von Damm, K.L.; Poulson, R.L. Iron isotope constraints on Fe
cycling and mass balance in oxygenated Earth oceans. *Geology* 2003, *31*, 629-632.

(2) Labatut, M.; Lacan, F.; Pradoux, C.; Chmeleff, J.; Radic, A.; Murray, J.W.; Poitrasson, F.;
Johansen, A.M.; Thil, F. Iron sources and dissolved-particulate interactions in the seawater of the
Western Equatorial Pacific, iron isotope perspectives. *Global Biogeochem. Cycles* 2014, 28, 10441065.

496 (3) Ingri, J.; Malinovsky, D.; Rodushkin, I.; Baxter, D.C.; Widerlund, A.; Andersson, P.;
497 Gustafsson, O.; Forsling, W.; Ohlander, B. Iron isotope fractionation in river colloidal matter. *Earth*498 *Planet. Sci. Lett.* 2006, 245 (3-4), 792-798.

(4) Ingri, J.; Conrad, S.; Lidman, F.; Nordblad, F.; Engstrom, E.; Rodushkin, I.; Porcelli, D. Iron
isotope pathways in the boreal landscape: Role of the riparian zone. *Geochim. Cosmochim. Acta* 2018, 239, 49-60.

502 (5) Escoube, R.; Rouxel, O.; Pokrovsky, O.S.; Schroth, A.; Holmes, R.M.; Donard, O.F.X. Iron
503 isotope systematics in Arctic rivers. *Comptes Rendus Geoscience* 2015, *347* (7-8), 377-385; DOI
504 10.1016/j.crte.2015.04.005.

(6) Conrad, S.; Ingri, J.; Gelting, J.; Nordblad, F.; Engström, E.; Rodushkin, I.; Andersson, P.S.;
Porcelli, D.; Gustafsson, Ö.; Semiletov, I.; Öhlander, B. Distribution of Fe isotopes in particles and
colloids in the salinity gradient along the Lena River plume, Laptev Sea. *Biogeosciences*, 2019, 16,
1305-1319, https://doi.org/10.5194/bg-16-1305-2019.

- (7) Chen, J.B.; Busigny, V.; Gaillardet, J.; Louvat, P.; Wang, Y.N. Iron isotopes in the Seine River
  (France): Natural versus anthropogenic sources. *Geochim. Cosmochim. Acta* 2014, *128*, 128-143.
- 511 (8) Poitrasson, F.; Vieira, L.C.; Seyler, P.; dos Santos Pinheiro, G.M.; Mulholland, D.S.; Bonnet,
- 512 M.P.; Martinez, J.M.; Lima, B.A.; Allard, T.; Boaventura, G.R.; Chmeleff, J.; Dantas, E.; Guyot, J.L.; 513 Mancini, L.; Pimentel, M.M.; Santos, R.V.; Sondag, F.; Vauchel, P. Iron isotope composition of the
- 513 Mancini, L.; Pimentel, M.M.; Santos, R.V.; Sondag, F.; Vauchel, P. Iron isotope composi 514 bulk waters and sediments from the Amazon River Basin. *Chem. Geol.* **2014**, *377*, 1-11.
- 515 (9) Song, L.; Liu, C.-Q.; Wang, Z.-L.; Zhu X.; Teng, Y.; Liang, L.; Tang, S.; Li, J. Iron isotope 516 fractionation during biogeochemical cycle: information from suspended particulate matter (SPM) in
- 517 Aha Lake and its tributaries, Guizhou, China. *Chem. Geol.* **2011**, 280 (1–2), 170–179.
- 518 (10) Busigny, V.; Planavsky, N.J.; Jezequel, D.; Crowe, S.; Louvat, P.; Moureau, J.; Viollier, E.;
- Lyons, T.W. Iron isotopes in an Archean ocean analogue. *Geochim. Cosmochim. Acta* 2014, *133*, 443462.

521 (11) Serikova, S.; Pokrovsky, O.S.; Ala-aho, P.; Kazantsev, V.; Kirpotin, S.; Kopysov, S.; Krickov, 522 I.; Laudon, H.; Manasypov, R.M.; Shirokova, L.S.; Soulsby, C.; Tetzlaff, D.; Karlsson, J. High riverine 523 CO<sub>2</sub> emissions at the permafrost boundary of Western Siberia. *Nature Geoscience* **2018**, *11*, 825-829. 524 (12) Serikova, S., Pokrovsky, O.S., Laudon, H., Krickov, I.V., Lim, A.G., Manasypov, R.M., 525 Karlsson, J. High carbon emissions from thermokarst lakes of Western Siberia. Nature Comm., 2019, 526 10, Art No 1552. https://doi.org/10.1038/s41467-019-09592-1. 527 (13) Kritzberg, E.S.; Villanueva, A.B.; Jung, M.; Reader, H.E. Importance of boreal rivers in 528 providing iron to marine waters. PLoS ONE 2014, 9, e107500, DOI 10.1371/journal.pone.0107500. 529 (14) Hirst, C.; Kutscher, L; Murphy, M; Shaw, S.; Burke, I.T.; Kaulich, B.; Maximov, T.; 530 Pokrovsky, O.S.; Mörth, C.-M; Andersson, P.S.; Porcelli, D. Characterization and stability of Fe-531 bearing particles in the Lena River catchment, NE Russia. Geochim. Cosmochim. Acta 2017, 213, 553-532 573. 533 (15) Hassellov, M.; Von Der Kammer, F. Iron oxides as geochemical nanovectors for metal 534 transport in soil-river systems. *Elements* **2008**, *4* (6), 401–406. 535 (16) Vonk, J.E.; Tank, S.E.; Bowden, W.B.; Laurion, I.; Vincent, W.F.; Alekseychik, P.; Amyot, 536 M.; Billet, M.F.; Canário, J.; Cory, R.M.; Deshpande, B.N.; Helbig, M.; Jammet, M.; Karlsson, J.; 537 Larouche, J.; MacMillan, G.; Rautio, M.; Walter Anthony, K.M.; Wickland, K.P. Reviews and 538 Syntheses: Effects of permafrost thaw on Arctic aquatic ecosystems. Biogeosciences 2015, 12, 7129-539 7167. 540 (17) Ilina, S.M.; Lapitsky, S.A.; Alekhin, Y.V.; Viers, J.; Benedetti, M.; Pokrovsky, O.S. 541 Speciation, size fractionation and transport of trace element in the continuum soil water – mire – lake - river - large oligotrophic lake of a subarctic watershed. Aquat. Geochem. 2016, 22 (1), 65-95. 542 543 (18) Shirokova, L.S.; Bredoire, R.; Rolls, J.-L.; Pokrovsky, O.S. Moss and peat leachate 544 degradability by heterotrophic bacteria: fate of organic carbon and trace metals. Geomicrobiology J. 545 2017, 34 (8), 641-655. 546 (19) Kopacek, J.; Klementova, S.; Norton, S.A. Photochemical production of ionic and particulate 547 aluminum and iron in lakes. Environ. Sci. Technol. 2005, 39, 3656–3662. 548 (20) Shiller, A.M.; Duan, S.; van Erp, P.; Bianchi, T.S. Photo-oxidation of dissolved organic 549 matter in river water and its effect on trace element speciation. *Limnol. Oceanography* **2006**, *51*(4), 550 1716-1728 551 (21) Mann, P.J.; Sobczak, W.V.; LaRue, M.M.; Bulygina, E.; Davydova, A.; Vonk, J.E.; Schade, 552 J.; Davydov, S.; Zimov, N.; Holmes, R.M.; Spencer, R.G.M. Evidence for key enzymatic controls on 553 metabolism of Arctic river organic matter. Global Change Biol. 2014, 20 (4), 1089-1100. 554 (22) Vähätalo, A.V.; Salonen, K.; Münster, U.; Järvinen, M.; Wetzel, R.G. Photochemical 555 transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Acta 556 Hvdrobiol. 2003, 156, 287-314. (23) Kelton, N.; Molot, L.A.; Dillon, P.J. Effect of ultraviolet and visible radiation on iron lability 557 558 in boreal and artificial waters. Aquat. Sci. 2007, 69, 86-95. 559 (24) Garg, S.; Ito, H.; Rose, A.L.; Waite, T.D. Mechanism and kinetics of dark iron redox transformations in acidic natural organic matter solutions. Environ. Sci. Technol. 2013, 47, 1861-1867. 560 (25) Blazevic, A.;, Orlowska, E.; Kandioller, W.; Jirsa, F.; Keppler, B.K.; Tafili-Krueziu, M.; 561 Linert, W.; Krachler, R.F.; Krachler, R.; Rompel, A. Photoreduction of terrigenous Fe-humic 562 substances leads to bioavailable iron in oceans. Angew. Chem. Int. Ed. 2016, 55, 6417-6422. 563 564 (26) Oleinikova, O.V.; Shirokova, L.S.; Drozdova, O.Yu.; Lapitskiy, S.A.; Pokrovsky, O.S. Low 565 biodegradability of dissolved organic matter and trace metals from subarctic waters. Sci. Total Env. 2018, 618, 174-187. 566 567 (27) Oleinikova, O.V.; Shirokova, L.S.; Gérard, E.; Drozdova, O.Yu; Lapitskiy, S.A.; Bychkov, A.Yu; 568 Pokrovsky, O.S. Transformation of organo-ferric peat colloids by a heterotrophic bacterium. Geochim. 569 Cosmochim. Acta 2017, 205, 313-330. 570 (28) Oleinikova, O.V.; Drozdova, O.Yu; Lapitskiy, S.A.; Demin, V.V.; Bychkov, A.Yu; 571 Pokrovsky, O.S. Dissolved organic matter degradation by sunlight coagulates organo-mineral colloids

- and produces low-molecular weight fraction of metals in boreal humic waters. *Geochim. Cosmochim. Acta* 2017, *211*, 97-114.
- 574 (29) Beard, B.L.; Johnson, C.M.; Cox, L.; Sun, H.; Nealson, K.H.; Aguilar, C. Iron isotope
  575 biosignatures. *Science* 1999, *285* (5435), 1889-1892.

576 (30) Mulholland, D.S.; Poitrasson, F.; Shirokova, L.S.; Gonzalez, A.; Pokrovsky, O.S.; 577 Boaventura, G.R.; Vieira, L.C. Iron isotope fractionation during Fe(II) and Fe(III) adsorption on 578 cyanobacteria. *Chem. Geol.* **2015**, *400*, 24-33.

(31) Swanner, E.D.; Bayer, T.; Wu, W.; Hao, L.; Obst, M.; Sundman, A.; Byrne, J.M.; Michel, F.M.;
Kleinhanns, I.C.; Kappler, A.; Schoenberg, R. Iron isotope fractionation during Fe(II) oxidation
mediated by the oxygen-producing marine cyanobacterium *Synechococcus* PCC 7002. *Environ. Sci. Technol.* 2017, *51* (9), 4897-4906.

- (32) Swanner, E.D.; Wu, W.; Schoenberg, R.; Byrne, J.; Michel, F.M.; Pan, Y.; Kappler, A.
  Fractionation of Fe isotopes during Fe(II) oxidation by a marine photoferrotroph is controlled by the
  formation of organic Fe-complexes and colloidal Fe fractions. *Geochim. Cosmochim. Acta* 2015, *165*, 44-61.
- (33) Amor, M.; Busigny, V.; Louvat, P.; Tharaud, M.; Gelabert, A.; Cartigny, P.; Carlut, J.;
  Isambert, A.; Durand-Dubief, M.; Ona-Nguema, G.; Alphandery, E.; Chebbi, I.; Guyot, F. Iron uptake
  and magnetite biomineralization in the magnetotactic bacterium Magnetospirillum magneticum strain
  AMB-1: An iron isotope study. *Geochim. Cosmochim. Acta* 2018, *232*, 225-243.
- (34) Zhou, Z.; Latta, D.E.; Noor, N.; Thompson, A.; Borch, T.; Scherer, M.M. Fe(II)-catalyzed
  transformation of organic matter-ferrihydrite coprecipitates: A closer look using Fe isotopes. *Environ. Sci. Technol.* 2018, *52*, 11142-11150.
- (35) Brantley, S.L.; Liermann, L.J.; Guynn, R.L.; Anbar, A.; Icopini, G.A.; Barling, J. Fe isotopic
  fractionation during mineral dissolution with and without bacteria. *Geochim. Cosmochim. Acta* 2004,
  68, 3189-3204.
- (36) Crosby, H.A.; Johnson, C.M.; Roden, E.E.; Beard, B.L. Coupled Fe(II)-Fe(III) electron and
  atom exchange as a mechanism for Fe isotope fractionation during dissimilatory iron oxide reduction. *Environ. Sci. Technol.* 2005, *39*, 6698–6704.
- (37) Ilina, S.M.; Poitrasson, F.; Lapitskiy, S.A.; Alekhin, Yu.V.; Viers, J.; Pokrovsky, O.S. Extreme
  iron isotope fractionation between colloids and particles of boreal and temperate organic-rich waters. *Geochim. Cosmochim. Acta* 2013, *101*, 96-111.
- (38) Kopácek, J.; Maresova, M.; Norton, S.A.; Porcal, P.; Vesely, J. Photochemical source of metals
  for sediments. *Environ. Sci. Technol.* 2006, 40, 4455–4459.
- 605 (39) Wu, L.L.; Beard, B.L.; Roden, E.E.; Johnson, C.M. Stable iron isotope fractionation between 606 aqueous Fe(II) and hydrous ferric oxide. *Environ. Sci. Technol.* **2011**, *45* (5), 1847-1852.
- 607 (40) Skulan, J.L.; Beard, B.L.; Johnson, C.M. Kinetic and equilibrium Fe isotope fractionation 608 between aqueous Fe(III) and hematite. *Geochim. Cosmochim. Acta* **2002**, *66*, 2995–3015.
- (41) Frierdich, A.J.; Beard, B.L.; Reddy, T.R.; Scherer, M.M.; Johnson, C.M. Iron isotope
  fractionation between aqueous Fe(II) and goethite revisited: New insights based on a multi-direction
  approach to equilibrium and isotopic exchange rate modification. *Geochim. Cosmochim. Acta* 2014, *139*, 383-398.
- (42) Sun, R.Y.; Wang, B.L. Iron isotope fractionation during uptake of ferrous ion by phytoplankton. *Chem. Geol.* 2018, 481, 65-73.
- (43) Vasyukova, E.V.; Pokrovsky, O.S.; Viers, J., Oliva, P., Dupré, B., Martin, F., Candadaup, F. Trace
  elements in organic- and iron-rich surficial fluids of the boreal zone: Assessing colloidal forms via dialysis
  and ultrafiltration. *Geochim. Cosmochim. Acta* 2010, 74, 449-468.
- 618 (44) González, A.G.; Pokrovsky, O.S.; Jiminez-Villacorta, F.; Shirokova, L.S.; Santana-Casiano, J.M.;
- González-Davila, M.; Emnova, E.E. Iron adsorption onto soil and aquatic bacteria: XAS structural study. *Chem. Geol.* 2014, *372*, 32-45
- (45) Viollier, E.; Inglett, P.W.; Hunter, K.; Roychoudhury, A.N.; Van Cappellen, P. The ferrozine
  method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl. Geochem.* 2000, *15* (6), 785790.

624 (46) Mulholland, D.S.; Poitrasson, F.; Boaventura, G.R.; Allard, T.; Vieira, L.C.; Santos, R.V.; 625 Mancini, L.; Seyler, P. Insights into iron sources and pathways in the Amazon River provided by 626 isotopic and spectroscopic studies. Geochim. Cosmochim. Acta 2015, 150, 142-159. 627 (47) Ilina, S.M.; Drozdova, O.Y.; Lapitsky, S.A.; Alekhin, Yu.V.; Demin, V.V.; Zavgorodnaya, 628 Yu.A.; Shirokova, L.S.; Viers, J.; Pokrovsky, O.S. Size fractionation and optical properties of 629 dissolved organic matter in the continuum soil solution-bog-river and terminal lake of a boreal 630 watershed. Organic Geochem. 2014, 66, 14-24. 631 (48) Gustafsson, J. Visual MINTEQ ver. 3.1. http://vminteq.lwr.kth.se, 2014, assessed 8.02.2019. 632 (49) Poitrasson F.; Freydier, R. Heavy iron isotope composition of granites determined by high 633 resolution MC-ICP-MS. Chem. Geol. 2005, 222, 132-147. 634 (50) Mulholland, D.S.; Poitrasson, F.; Boaventura, G.R. Effect of different water storage procedures 635 on the dissolved Fe concentration and isotopic composition of chemically contrasted waters from the 636 Amazon River Basin. Rapid Comm. Mass Spectr. 2015, 29, 2102-2108. 637 (51) Kafantaris, F.C.A.; Borrok, D.M. Zinc isotope fractionation during surface adsorption and 638 intracellular incorporation by bacteria. Chem. Geol. 2014, 366, 42-51. 639 (52) Croal, L.R.; Johnson, C.M.; Beard, B.L.; Newman, D.K. Iron isotope fractionation by Fe(II)-640 oxidizing photoautotrophic bacteria. Geochim. Cosmochim. Acta 2004, 68, 1227-1242. 641 (53) Bullen, T.D.; White, A.F.; Childs, C.W.; Vivit, D.V.; Schulz, M.S. Demonstration of 642 significant abiotic iron isotope fractionation in nature. Geology **2001**, *29*, 699-702. 643 (54) Beard, B.L.; Handler, R.M.; Scherer, M.M.; Wu, L.; Czaja, A.D.; Heimann, A.; Johnson, C.M. 644 Iron isotope fractionation between aqueous ferrous iron and goethite. Earth Planet. Sci. Lett. 2010, 645 295 (1-2), 241-250. 646 (55) Dideriksen, K.; Baker, J.A.; Stipp, S.L.S. Equilibrium Fe isotope fractionation between 647 inorganic aqueous Fe(III) and the siderophore complex, Fe(III)-desferrioxamine B. Earth Planet. Sci. 648 Lett. 2008, 269, 280-290. 649 (56) Morgan, J. L.; Wasylenki, L. E.; Nuester, J.; Anbar, A. D. Fe isotope fractionation during 650 equilibration of Fe-organic complexes. Environ. Sci. Technol. 2010, 44, 6095-6101. 651 (57) Chupakova, A.A.; Chupakov, A.V.; Neverova, N.V.; Shirokova, L.S.; Pokrovsky, O.S. 652 Photodegradation of river dissolved organic matter and trace metals in the largest European Arctic 653 estuary. Sci. Total Environ. 2018, 622-623, 1343-1352. 654 (58) Barbeau, K.; Rue, E.; Bruland, K.; Butler A. Photochemical cycling of iron in the surface ocean 655 mediated by microbial iron (III)-binding ligands. *Nature* **2001**, *413*, 409–413. 656 (59) Welch, S.A.; Beard, B.L.; Johnson, C.M.; Braterman, P.S. Kinetic and equilibrium Fe isotope 657 fractionation between aqueous Fe(II) and Fe(III). Geochim. Cosmochim. Acta 2003, 67(22), 4231-658 4250. 659 (60) Balci, N.; Bullen, T.D.; Witte-Lien, K.; Shanks, W.C.; Motelica, M.; Mandernack, K.W. Iron isotope fractionation during microbially stimulated Fe(II) oxidation and Fe(III) precipitation. 660 661 Geochim. Cosmochim. Acta 2006, 70, 622–639. 662 (61) Teutsch, N.; Von Gunten, U.; Porcelli, D.; Cirpka, O.A.; Halliday, A.N. Adsorption as a cause for iron isotope fractionation in reduced groundwater. Geochim. Cosmochim. Acta 2005, 69(17), 4175-663 664 4185. 665 (62) Johnson, C.M.; Beard, B.L.; Roden, E.E.; Newman, D.K.; Nealson, K.H. Isotopic constraints 666 on biogeochemical cycling of Fe. Rev. Mineral. Geochem. 2004, 55, 359-408. 667 (63) Reddy, T.R.; Frierdich, A.J.;, Beard, B.L.; Johnson, C.M. The effect of pH on stable iron isotope exchange and fractionation between aqueous Fe(II) and goethite. Chem. Geol. 2015, 397, 118-668 669 127. 670 (64) Handler, R.M.; Beard, B.L.; Johnson, C.M.; Scherer, M.M. Atom exchange between 671 aqueous Fe(II) and goethite: an Fe isotope tracer study. Environ. Sci. Technol. 2009, 43(4), 1102-1107. 672 (65) Voelker, B.M.; Sulzberger, B. Effects of fulvic acid on Fe(II) oxidation by hydrogen peroxide. 673 674 Environ. Sci. Technol. 1996, 30, 1106-1114.

- (66) Anesio, A.M.; Granéli, W. Increased photoreactivity of DOC by acidification: Implication for the carbon cycle in humic lakes. Limnol. Oceanogr. 2003, 48, 735-744. (67) Gao, H.; Zepp, R.G. Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeastern United States. Environ. Sci. Technol. 1998, 32, 2940-2946. (68) Wang, Y.; Xiang, W.; Yang, W.; Yan, S.; Bao, Z.; Liu, Y. Photo-stability of iron-phenolic complexes derived from peatland upon irradiation in waters under simulated sunlight. Chem. Geol. 2018, 485, 14-23. (69) Helms, J.R.; Mao, J.; Schmidt-Rohr, K.; Abdulla, H.; Mopper, K. Photochemical flocculation of terrestrial dissolved organic matter and iron. Geochim. Cosmochim. Acta 2013, 121, 398-413. (70) Catrouillet, C.; Davranche, M.; Dia, A.; Bouhnik-Le Coz, M.; Marsac, R.; Pourret, O.; Gruau, G. Geochemical modeling of Fe(II) binding to humic and fulvic acids. Chemical Geol. 2014, 372, 109-118. (71) Dauphas, N.; John, S.G.; Rouxel, O. Iron isotope systematics. Rev. Mineral. Geochem. 2017, 82, 415-510. (72) Drozdova, O.Yu.; Ilina, S.M.; Lapitsky, S.A. The transformation of dissolved organic matter in the soil water - bog - stream - terinal lake continuum of a boreal watershed (Northern Karelia), in: Pokrovsky, O.S., Shirokova, L.S., eds. Dissolved Organic Matter (DOM): Properties, Applications and Behavior, Nova Publishers, N.Y., 2017, pp. 115-133.

## **Table 1.** Measured pH, DOC, Fe concentrations and isotopic ratios, relative to IRMM-14, during bioand photo-degradation experiments. Note that the DOC in biodegradation experiments of stream water slightly increased due to cell lysis and exometabolite production. The standard error was calculated using the Student's t factor: $SE = (t \times SD)/\sqrt{N}$ , where N is the number of measurements.

			Bio-	degradation	<b>n</b> $(1 g_{wet}/L of L)$	Pseudo	monas	aureofacie	ns)		
	<mark>Fen</mark> water ZPBL <mark>&lt;</mark> 0.22 μm						Stream water KAR-1 <mark>&lt;</mark> 0.22 μm				
hrs	pН	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, ‰	hrs	рН	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*< td=""><td>2.54±0.06</td></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*< td=""><td>2.68±0.16</td></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*< td=""><td>3.21±0.15</td></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*< td=""><td>3.26±0.13</td></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*< td=""><td>no data</td></loq*<>	no data
100	6.1	25.2	1054±5	84±5	1.04±0.04						
					Sunlight e	xposur	e				
hrs	<mark>Fen</mark> water ZPBL <mark>&lt;</mark> 0.22 μm			hrs	Stream water KAR-1 <mark>&lt;</mark> 0.22 μm						
	~ .			0.40 . 50	0.25+0.07	0	7.2	11.0	208 + 20	50.20	1 50+0 05
0	5.4	38.7	4310±100	840±50	$0.25\pm0.07$	0	1.2	11.7	200120	$50\pm 20$	1.39±0.03
0 100	5.4 5.1	38.7 20.3	4310±100 1840±400	840±50 770±170	-0.8±0.38	110	6.8	10.4	193±25	$50\pm 20$ 60±25	1.75±0.2
0 100 200	5.4 5.1 5.3	38.7 20.3 18.7	4310±100 1840±400 915±30	840±50 770±170 450±10	-0.8±0.38 -2.63±0.03	110 250	6.8 6.7	10.4 8.2	193±25 175±25	$30\pm 20$ $60\pm 25$ $15\pm 5$	1.75±0.2 2.1±0.12
0 100 200 hrs	5.4       5.1       5.3	38.7 20.3 18.7	4310±100 1840±400 915±30 en water ZP	840±50 770±170 450±10 BL <10 kDa	-0.8±0.38 -2.63±0.03	110 250 hrs	6.8 6.7	11.9 10.4 8.2 Stream	193±25 175±25 water KA	60±25 15±5 <b>R-1 &lt;10 I</b>	1.75±0.2 2.1±0.12
0 100 200 hrs 0	5.4 5.1 5.3 5.3	38.7 20.3 18.7 <b>Fo</b> 26.5	4310±100 1840±400 915±30 en water ZP 1125±50	840±50 770±170 450±10 BL <10 kDa 620±20	-0.8±0.38 -2.63±0.03	110 250 hrs 0	7.2       6.8       6.7       6.8	11.9 10.4 8.2 Stream 10.2	$\frac{193\pm25}{175\pm25}$ water KA 35±5	50±20 60±25 15±5 <b>R-1 &lt;10 I</b> 20±10	1.35±0.03 1.75±0.2 2.1±0.12 <b>kDa</b> 2.93±0.06
0 100 200 hrs 0 100	5.4 5.1 5.3 5.3 5.2	38.7 20.3 18.7 <b>F</b> 26.5 18.7	4310±100 1840±400 915±30 en water ZP 1125±50 1000±50	840±50 770±170 450±10 BL <10 kDa 620±20 510±20	0.25±0.07 -0.8±0.38 -2.63±0.03 0.19±0.02 -1.98±0.13	110 250 hrs 0 110	7.2       6.8       6.7       6.8       6.9	11.9 10.4 8.2 Stream 10.2 10.0	$\frac{256\pm26}{193\pm25}$ $\frac{193\pm25}{175\pm25}$ water KA $\frac{35\pm5}{49\pm10}$	30±20       60±25       15±5 <b>R-1 &lt;10 I</b> 20±10       30±20	1.35±0.03 1.75±0.2 2.1±0.12 <b>xDa</b> 2.93±0.06 1.95±0.06
0 100 200 hrs 0 100 200	5.4 5.1 5.3 5.3 5.2 5.3	38.7 20.3 18.7 <b>F</b> 26.5 18.7 17.9	4310±100 1840±400 915±30 en water ZP 1125±50 1000±50 945±50	840±50 770±170 450±10 BL ≤10 kDa 620±20 510±20 470±30	-0.8±0.38 -2.63±0.03 0.19±0.02 -1.98±0.13 -2.49±0.15	110 250 hrs 0 110 250	7.2         6.8         6.7         6.8         6.9         6.8	11.9 10.4 8.2 <b>Stream</b> 10.2 10.0 8.1	$   \begin{array}{r}     203 \pm 20 \\     193 \pm 25 \\     175 \pm 25 \\     \hline     \textbf{water KA} \\     35 \pm 5 \\     49 \pm 10 \\     14 \pm 2   \end{array} $	$50\pm 20$ $60\pm 25$ $15\pm 5$ <b>R-1 &lt;10 I</b> $20\pm 10$ $30\pm 20$ $< LOQ^*$	$\begin{array}{c} 1.35 \pm 0.03 \\ \hline 1.75 \pm 0.2 \\ \hline 2.1 \pm 0.12 \\ \hline \textbf{xDa} \\ \hline 2.93 \pm 0.06 \\ \hline 1.95 \pm 0.06 \\ \hline 1.89 \pm 0.07 \end{array}$
0 100 200 <b>hrs</b> 100 200 <b>hrs</b>	5.4         5.1         5.3         5.3         5.2         5.3	38.7 20.3 18.7 <b>F</b> 26.5 18.7 17.9 <b>F</b>	4310±100 1840±400 915±30 en water ZP 1125±50 1000±50 945±50 en water ZP	840±50 770±170 450±10 BL ≤10 kDa 620±20 510±20 470±30 BL ≤1 kDa	-0.8±0.07 -0.8±0.38 -2.63±0.03 0.19±0.02 -1.98±0.13 -2.49±0.15	0 110 250 hrs 0 110 250 * Lim	7.2         6.8         6.7         6.8         6.9         6.8         6.9         6.8         it of qu	11.9 10.4 8.2 <b>Stream</b> 10.2 10.0 8.1 mantificatio	$   \begin{array}{r}     203 \pm 20 \\     193 \pm 25 \\     175 \pm 25 \\     \hline     xvater KA \\     35 \pm 5 \\     49 \pm 10 \\     14 \pm 2 \\     n   \end{array} $	30±20 60±25 15±5 <b>R-1 &lt;10 I</b> 20±10 30±20 < LOQ*	$1.35\pm0.03$ $1.75\pm0.2$ $2.1\pm0.12$ <b>xDa</b> $2.93\pm0.06$ $1.95\pm0.06$ $1.89\pm0.07$
0 100 200 <b>hrs</b> 0 100 200 <b>hrs</b> 0	5.4         5.1         5.3         5.3         5.2         5.3         5.3         5.6	38.7 20.3 18.7 26.5 18.7 17.9 <b>F</b> 10.6	4310±100 1840±400 915±30 en water ZP 1125±50 1000±50 945±50 en water ZP 200±20	840±50 770±170 450±10 BL <10 kDa 620±20 510±20 470±30 BL <1 kDa 70±10	0.23±0.07 -0.8±0.38 -2.63±0.03 0.19±0.02 -1.98±0.13 -2.49±0.15 -0.18±0.03	110 250 hrs 0 110 250 * Lim	7.2         6.8         6.7         6.8         6.9         6.8         6.9         6.8         it of quite	11.9 10.4 8.2 <b>Stream</b> 10.2 10.0 8.1 mantificatio	$ \frac{193\pm25}{175\pm25} $ water KA $ \frac{35\pm5}{49\pm10} $ 14±2 n	50±20 60±25 15±5 <b>R-1 ≤10 I</b> 20±10 30±20 < LOQ*	1.35±0.03 1.75±0.2 2.1±0.12 <b>xDa</b> 2.93±0.06 1.95±0.06 1.89±0.07
0 100 200 <b>hrs</b> 100 200 <b>hrs</b> 0 100	5.4 5.1 5.3 5.3 5.2 5.3 5.6 5.3	38.7 20.3 18.7 26.5 18.7 17.9 <b>F</b> 10.6 11.5	4310±100 1840±400 915±30 en water ZP 1125±50 1000±50 945±50 en water ZP 200±20 560±30	840±50 770±170 450±10 BL <10 kDa 620±20 510±20 470±30 BL <1 kDa 70±10 300±100	0.23±0.07 -0.8±0.38 -2.63±0.03 0.19±0.02 -1.98±0.13 -2.49±0.15 -0.18±0.03 -2.34±0.11	0 110 250 hrs 0 110 250 * Lim	7.2         6.8         6.7         6.8         6.9         6.8         6.9         6.8         it of quite	11.9 10.4 8.2 <b>Stream</b> 10.2 10.0 8.1 mantificatio	$\frac{203\pm20}{193\pm25}$ $\frac{193\pm25}{175\pm25}$ water KA $\frac{35\pm5}{49\pm10}$ $14\pm2$ n	50±20 60±25 15±5 <b>R-1 &lt;10 I</b> 20±10 30±20 < LOQ*	1.35±0.03 1.75±0.2 2.1±0.12 xDa 2.93±0.06 1.95±0.06 1.89±0.07



746	Fig. 1. Conceptual scheme of experiments (run in duplicates): a photo of quartz reactors exposed to
747	sunlight and a Transmission Electron Microscopy (TEM) image of bacterial cells with
748	precipitated Fe hydroxides. The photodegradation experiments were performed on-site,
749	immediately after water sampling and sterile filtration. The biodegradation experiments
750	required sterile laboratory environments and were run after 6 months of water storage. The
751	biodegradation experiments included only $< 0.22$ $\mu m$ filtration after sampling. In
752	photodegradation experiments, the stream water was processed for the $< 0.22 \ \mu m$ and the $<$
753	10 kDa filtration and the fen water was filtered through 0.22 µm, 10 kDa and 1 kDa.



Fig. 2. Evolution of total dissolved Fe concentration during biodegradation experiments of stream (A)
and fen (B) water. (C): Fe(II) concentration evolution in the fen water during biodegradation.
The error bars of biotic experiments represent 1 SD of duplicates. In most cases, they are
smaller than the symbol size. Bacterial experiments are shown by solid circles and bacteriafree control is represented by open circles for all three panels as indicated in (C).



Fig. 3. Evolution of isotopic ratios 8<sup>57</sup>Fe during biodegradation experiments of stream (A) and fen (B) water. Note log scale for time axis.
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**Fig. 4.** Evolution of Fe<sub>tot</sub> and Fe(II) concentration during photodegradation experiments of stream (**A**, **B**, **C**) and fen (**D**, **E** and **F**) water in quartz reactors. Note a drop in Fe<sub>tot</sub> and Fe(II) concentration of the  $< 0.22 \,\mu$ m dark control at 200 and 250 h not observed in previously stored samples in biodegradation experiments (Fig. 2), which may be due to partial coagulation of freshly sampled natural water upon storage.



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

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- biodegradation (right) of organic and organo-ferric colloids (1 kDa 0.22 μm). Live heterotrophic *P*.
- 802 *aureofaciens* bacteria adsorb heavier and assimilate lighter Fe isotopes. Sunlight irradiation
- 803 generates isotopically light Fe(II) in low molecular weight (< 1 kDa) fraction and produces heavy
- 804 isotope enrichment in particulate fraction relative to total dissolved (< 0.22 µm) form. After bio- or
- 805 photo-degradation of organic matter which constitutes organo-ferric colloids, the liberation of Fe(III)
- 806 ions and precipitation of Fe(III) hydroxide occur. This removes heavier isotopes from solution into
- 807 the solid phase. Altogether, bio-and photodegradation of organo-ferric colloids can produce very Fe)
- 808 large, from -2 to +3‰ isotopic variations ( $\delta^{57}$ Fe) in boreal humic waters.
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