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

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

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Key messages:

Adsorption of heavy Fe isotopes on heterotrophic bacteria cell surface

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

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

Generation of isotopically light Fe(II) in $LMW_{< 1\ kDa}$ during photolysis

Bio-and photodegradation of organo-ferric colloids can produce very large, from -2.5 to +3.2‰ $\delta^{57}Fe$ isotopic variations in boreal waters

ABSTRACT

Bio-degradation and photolysis of dissolved organic matter (DOM) in boreal high-latitude waters are the two main factors controlling aquatic fluxes and residence time of carbon but also metal nutrients associated with DOM such as Fe. The DOM is usually present in the form of organic and organo-mineral colloids that also account for the majority of dissolved Fe. Here we use the stable Fe isotope 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 onto heterotrophic bacteria *P. aureofaciens* produced enrichment in +0.4‰ (δ^{57}Fe) in the heavier isotopes of the cell surface relative to the remaining solution. In contrast, long-term assimilation of Fe by live cells yielded preferential incorporation of lighter isotopes into the cells (-0.7‰ relative to aqueous solution). The sunlight-induced oxidation of Fe(II) in fen water and coagulation of organo-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 organo-ferric colloids, occurring within a few days of exposure time, can produce several per mil isotopic excursions in shallow lentic and lothic inland waters of high latitude boreal regions. Considerable daily scale variations of Fe isotopic composition should therefore be taken into account during interpretation of riverine flux of Fe isotope to the ocean or tracing weathering processes using Fe isotopes in surface waters at high latitudes.

1. Introduction

Despite broad use of Fe isotopes for tracing various large-scale, long-term processes at the Earth's surface[1,2], notably in river[3-8] or lake[9,10] waters, the fractionation of Fe isotopes during short-term 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_2 emissions and storage[11] and from the other hand, strongly contribute to Fe and other micro-nutrient export by rivers to the coastal productive zones[13,14].

The majority of dissolved (< 0.22 µm) Fe and other metals in boreal waters are present in the form of organic and organo-mineral colloids (1 nm - 0.22 µm) whose transport and bioavailability are dramatically different from inorganic ions or simple organic complexes[15]. Two main processes responsible for fluxes and residence time of organic C and metal colloids in boreal high-latitude aquatic environments are heterotrophic bacterial respiration (degradation of DOM) and photolysis[16]. Colloidal transformation often occurs along the hydrological continuum of interconnected soil waters, mires, streams and large oligotrophic lakes[17]. During this transformation, induced mainly by aquatic microorganisms and sunlight irradiation, the organic and organo-ferric colloids are subjected to *i*) change of dominant source and molecular weight from allochthonous large-size humic and fulvic molecules to small size autochthonous organic ligands[17], *ii*) degradation of DOC into CO_2 or intracellular uptake of DOC by bacterioplankton and liberation of Fe(III) ions, leading to Fe oxy(hydr)oxide precipitation[18,19], and *iii*) enrichment in low molecular weight organic ligands and their complexes with metals[20].

In contrast to numerous works devoted to microbiological[21] and photochemical[22] 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[23,24]. The transformation of colloidal Fe in boreal waters, including bogs, lakes and rivers[25] has been studied under the metabolic action of heterotrophic soil and aquatic bacteria[18,26-27] and sunlight[28]. In these 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 hydroxide precipitation leading to scavenging of associated trace metals. During both biodegradation and photolysis of natural DOM, Fe plays a pivotal role in controlling the fate of trace elements (TE). This control occurs via *i*) formation of insoluble Fe(III) hydroxides that coprecipitate other trace metals[28], and *ii*) generation of low molecular weight (LMW) organic ligands that bind Fe, thus competing with strong organic complexes of other metals[19,20,28].

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

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

2. Materials and methods

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 January is -13 °C, and +15 °C in July; average annual precipitation ranges between 450 and 550 mm y^{-1}. Our study area is in the most elevated part of Karelia, within a landscape of tectonic denudation hills, plateaus and ridges with an average altitude of 300-400 m, with separate insulated massifs. Predominant soils are illuvial-humic and illuvial-ferruginous-humic podzols. Coniferous forest (pine 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[17,37]. Within the hydrological continuum created by glacial processes around 8-10 thousand years ago, the water and soluble compounds travel from the ombrotrophic peat mire zone downstream the river towards a large oligotrophic lake. At the lake coast, there are minerotrophic fens located in depressions that receive their water via shallow groundwater and soil flux[43].

A small stream and a coastal fen were collected in July 2015, during summer baseflow period. The Palojoki stream (watershed area = 32 km², bedrock of granites, gneisses, syenites, and syenite-diorites of the early-Proterozoic and late-Archean, covered by glacial Quaternary deposits under podzol soils[43]) was sampled in the middle course of the flow (sample KAR-1[17]). The fen adjacent to the western coast of the Tsipringa Lake had an area of 1.19 km^2 and is underlain by Early-Archean biotite granito-gneisses (sample ZPBL[17]).

The experiments included on-site photodegradation of sterile-filtered (< 0.22 µm) stream and fen water in quartz reactors and laboratory microcosm experiments in the presence of 1 g_{wet}/L of *Pseudomonas aureofaciens*, highly abundant culturable bacteria isolated form podzol soils[44]. The experimental scheme described in previous works from our group[27,28], is shown in **Fig. 1**. The

biodegradation experiments with stream and fen waters were performed after 6 months storage of sterile-filtered samples in the refrigerator. Freshly-collected bacteria at the stationary growth stage were rinsed in 0.85% NaCl and allowed to starve in nutrient-free 0.85% NaCl solution during 5 days. The cells were rinsed again 3 times before the experiment, concentrated in a mother suspension and added to sterile, acid-washed polypropylene flasks with stream or fen water (typically 2 mL of bacterial suspension to 200 mL of water) to provide the wet biomass concentration of 1 g_{wet} L^{-1}. Note that the amount of Fe originated from bacteria addition does not exceed 1 µg L^{-1} for this concentration of biomass[27]. The flasks were shaken at 25±0.5°C and aerated via Biosilico® ventilation porous caps during 4 days. Aliquots of homogeneous suspension were sampled after 0, 1, 8, 22, 30, 50 and 100 h after the addition of bacteria and filtered through 0.22 µm membrane. All the experiments were run in duplicates. The control experiment was sterile filtered (< 0.22 µm) biomass-free stream and fen water and it was processed exactly as bacterial samples.

For photo-degradation experiments, the stream and fen waters were collected in pre-cleaned polypropylene jars and stored in dark cold place (6±2°C) prior the experiments. The waters were processed on-site, within 2 h after sampling, in the field-constructed clean laboratory[37]. Sterile filtration was performed using single-used Sartorius polystyrene vacuum filtration units (0.22 µm, 250 mL volume). Filtered fluids were transferred into 270-mL sterilized quartz flasks, filled with 10% air headspace and covered by porous sterile stoppers. The dark control of quartz flasks was identical to the light samples except that the reactors were wrapped in Al foil. Both dark and light reactors were run in duplicates and they were placed on flat surface at the border of the lake (66°17'04"N, 30°52'05"E). The flasks were exposed to direct unshaded sunlight from July 9 to July 20 during essentially anticyclonic weather. The daytime duration in this period was between 22 and 20.5 hours. The temperature of the experimental reactors followed the diurnal cycle and was equal to 18 ± 5°C over 250 h of exposure, which was within the range of actual water temperature in stream and fen during the month of July[17,37]. The quartz reactors were sampled after 0, 100 and 200 h of exposure of the fen water (ZPBL) and after 0, 110 and 250 h of exposure of the stream (KAR-1). For each sampling,

the whole reactor was sacrificed. The samples were immediately filtered through 0.22 µm Sartorius single-used filter into pre-cleaned polypropylene vials. These waters were then acidified using bi-distilled HNO_3 for trace metals analysis or directly used, without acidification, for DOC, $UV_{254\ nm}$, DIC and anions determinations. The 0.22 µm filtrate from quartz reactors was additionally ultrafiltered through 1 and 10 kDa regenerated cellulose single-use filters with an Amicon 8400 frontal Ultrafiltration unit (continuously stirred 400-ml polycarbonate cell maintained under 1.5–2 atm pressure). Details of ultrafiltration procedure in humic boreal waters of North Karelia and discussion of possible artifacts are presented elsewhere[17,37,43]. In these experiments, we consider the behavior of Fe in <0.22 µm and <10 kDa fractions of the stream water and in <0.22 µm, <10 and <1 kDa fractions of the fen water.

All experiments including controls were run in duplicates (two independent reactors) and both solution duplicates were processed for isotope analyses. Analyses of DOC and Fe concentration in various filtered and ultrafiltered fractions were performed using Shimadzu TOC-VCSN and Agilent ICP MS, following procedures described previously[27,28]. The DOC was measured with an uncertainty of 3% and a detection limit of 0.1 mg L^{-1}. The total dissolved Fe represents the sum of Fe(II) and Fe(III) in the < 0.22 µm fraction. The uncertainty of Fe concentration measurements ranged from 5 to 10% (1SD). The international geostandard SLRS-5 (Riverine Water Reference Material for Trace Metals certified by the National Research Council of Canada) measured each 20 samples was used to check the validity and reproducibility of Fe analysis. Good agreements were found between the replicate measurements of Fe in SLRS-5 and the certified values (relative difference < 20% SD on the repeated measurements).

Assessment of Fe(II) and Fe(III) concentrations was performed using the conventional ferrozine method[45]. The efficiency of this method in organic-rich tropical waters was further demonstrated in Rio Negro[46]. Because of possible interferences from DOM in humic boreal waters, the ferrozine method was used employing a standard addition technique. This allowed to achieve a detection limit of 15 µg L^{-1} and an uncertainty from 10-20% at 15 < Fe(II) <100 µg L^{-1} to 5-10% at Fe(II) > 100 µg

L^{-1}. Further, we verified the stability of Fe(II) in oxygenated humic waters (pH = 4.6 to 6.5; DOC = 20 to 50 mg L^{-1}) using peat and moss leachate (similar to main allochthonous DOM in studied sites[47]). The Fe(II) concentration remained stable within ±10% over 24-180 h of exposure.

The Fe(III) and Fe(II) complexation with organic ligands and solution saturation degree with respect to secondary Fe hydroxide in the course of experiment were calculated using Visual MINTEQ[48], in conjunction with a NICA-Donnan humic ion bonding model, as described previously[17]. We considered carboxylic and phenolic complexes of fulvic acids with Fe^{2+} and Fe^{3+} ions, as well as Fe(II) weak (electrostatic) interaction with fulvic acids.

For Fe isotope analysis, filtered and acidified water samples were evaporated in the clean laboratory. After acid digestion of the residue using a mixture of HCl and HNO_3, Fe was purified via anion exchange chromatography with HCl using Bio Rad AG1 X4 resin, 200–400 mesh to remove all matrix elements[37]. For this, we used thermoretractable Teflon columns with an internal diameter of 4 mm[8]. The resins were conditioned using 6 M of HCl prior to the sample loading in 0.5 ml of 6 M HCl. 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, purified Fe samples were redissolved in a 0.05 M HCl solution that was used for the MC-ICP-MS analysis. Iron and the internal standard, Ni, were set to concentration producing signals of ca. 40 V of ^{56}Fe and 20V for ^{60}Ni. Iron isotope measurements were performed at the CNRS-INSU National MC-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)[49]. All analyses are reported in the delta notation relative to the IRMM-014 standard, expressed as $\delta^{57}Fe$, which represents the deviation in per mil relative to the reference material:

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

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

[4]), only δ^{57}Fe values are discussed in this paper. Data quality was checked by the analysis of our in-house hematite standard every 5 samples in the analytical sequence. Our mean value of 0.771±0.047‰ (2SD) for this standard obtained between Lyon and Toulouse from pooling 51 individual analyses by groups of 6 was consistent with the values of 0.766±0.072‰ reported previously[8].

3. Results and Discussion

3.1. Iron in the < 0.22 µm fraction during biodegradation experiments: Heavier isotopes adsorption onto and lighter isotopes uptake by live cells of P. aureofaciens

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

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±20 µg/L (~16% of total dissolved Fe), with a quantification limit of Fe(II) by this method of 15 µg/L[45].

All initial solutions were undersaturated with respect to Fe-bearing minerals. Calculated 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 decreased by a factor of 3 in the fen waters (**Fig. S1**). Specifically, the Fe(III) complex with carboxylic 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 whereas Fe(II)-weak electrostatic complex completely disappeared. This is consistent with highly reactive behavior of dissolved Fe(II) in biodegradation experiments.

The second < 0.22 µm 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 decrease of Fe concentration in the fen water from 4310 µg/L to 2470 µg/L, accompanied by a change in δ^{57}Fe from 0.25±0.06‰ to 1.06±0.04‰. A strong δ^{57}Fe increase from 1.59±0.05‰ to 2.87±0.07‰ also resulted from the storage for the stream water (Table 1 and S1). This change could be linked to some $Fe(OH)_3$ 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, immediate freezing after filtration in the field on the sampling site is preferable[50], but this is not always possible for logistical reasons.

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

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

The interaction of stream water with heterotrophic bacterium produced first, a drop in $\delta^{57}Fe$ of 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 $\Delta^{57}Fe_{cell\text{-}solution}$ of -0.7‰. This implies removal of lighter Fe isotopes due to their preferential intracellular uptake. After an initial drop in the stream water $\delta^{57}Fe$, the long-term removal of lighter Fe isotopes by live bacteria produced ca 0.7‰ increase in $\delta^{57}Fe$ over the first 20 h of reaction that remained constant to 50 h (**Fig. 3A**). This strongly supports biological mechanism of lighter Fe isotopes uptake inside cells rather than metabolically-induced $Fe(OH)_3$ precipitation. In the latter case, the solid phase would be enriched in heavy isotope ($\Delta^{57}Fe_{FeOOH\text{-}Fe(aq)}$ = +1.5 to +4-5‰)[32,53,54] as also supported by natural observations[9,53]. The present result is at variance with previous Fe assimilation by magnetotactic bacteria[33] or phytoplankton[42] that inferred a heavier Fe isotope uptake inside the cells due to a combined Fe oxidation. However, the Fe(III)-DOM complexes in solution retain heavy isotope as it is known for $Fe^{3+}(H_2O)_6$ –Fe(III) chelate (DFO-B): the organic complexes are enriched in heavy isotopes with an isotopic offset of +0.90±0.23 ‰[55], and overall, there is a strong positive correlation between Fe fractionation factors and the Fe-binding affinity of the ligands[56]. According to vMinteq calculation, the majority of Fe in our experimental solutions was bound to phenolic complexes with

297 fulvic acids (Fig. S1, Table S2). Therefore, we suggest that strong complexation of isotopically heavy
298 Fe(III) with DOM prevents the intracellular uptake of heavier Fe isotopes.

299 In the fen water, we observed a similar δ^{57}Fe evolution during biodegradation experiments,
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
302 heavy isotope (<0.1‰) and a weak assimilation of light isotopes $\Delta^{57}Fe_{cell\text{-}solution} \leq 0.1‰$ over first 40
303 h (**Fig. 3 B**). Relative to the sterile control, a decrease in δ^{57}Fe at the beginning of experiment signified
304 preferential adsorption of heavy isotopes with $\Delta^{57}Fe_{cell\text{-}solution}$ = +0.2±0.1‰. Over 100 h of reaction,
305 light Fe isotopes were taken up by the cells as the δ^{57}Fe of solution increased from +0.86±0.1‰ to
306 +1.05±0.05‰.

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 µg/L) at otherwise similar bacterial concentration ($1g_{wet}$/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

313 *3.2. Iron concentrations and isotopic signatures evolution during photodegradation of organo-ferric*
314 *colloids: Removal of heavier isotopes due to Fe(III) hydroxide formation and appearance of*
315 *isotopically light Fe(II) in the LMW fraction*

316 There was no pH variation in the course of photodegradation experiments: within the uncertainty
317 of duplicates (< 0.1-0.2 pH unit), the pH in the dark control and the light experiment was identical and
318 equal to 7.0±0.2 and 5.3±0.1 in the stream and fen water, respectively (Table 1 and S1). The DOC
319 concentration in <0.22 µm fraction of stream and fen water decreased by 30 and 50%, respectively,
320 over 200 to 250 h of photodegradation experiment (Table 1). There was a good correlation between
321 ultraviolet absorbency ($UV_{254\ nm}$) and Fe concentration in the fen water (R^2 = 0.928) which was absent
322 in the stream water in the course of experiment (R^2 = 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 µm and <10 kDa fractions (from 50 to 15 µg/L and from 20 µg/L to < limit of quantification, respectively, **Fig. 4 B**).

The removal of Fe during fen water exposure to sunlight was strong in the ≤ 0.22 µm fraction (~ -75% of initial concentration) and did not occur in the ≤ 10 kDa fraction (≤7% of the initial concentration, **Fig. 4 D and Table 1**). A notable increase in total Fe concentration (ca. 210%) in the $LMW_{<1\ kDa}$ fraction was observed over 200 h of exposure (**Fig. 4 D**). The divalent Fe concentration decreased by 50% in the ≤ 0.22 µm fraction but strongly increased (~500%) in the ≤ 1 kDa fraction (**Fig. 4 E and Table 1**). Over 196 h of irradiation, the Fe(II) proportion increased from 20 to 50% in the ≤ 0.22 µm fraction and from 35 to 55% in the < 1 kDa fraction. The ≤10 kDa fraction did not demonstrate any difference in Fe(II) concentration between the sunlight-irradiated samples and the dark control.

During sunlight exposure, the ≤ 0.22 µm fraction of the fen water became depleted in Fe as the molar ratio of C_{org}:Fe increased from 50 to 100. This trend mainly stems from more efficient Fe removal compared to DOC (Table 1). The change of this ratio in the stream water was within the experimental uncertainty. The C_{org}:Fe ratio in the fen water colloids (1 kDa – 0.22 µm) did not change in the course of photo-degradation, from 31 at the beginning of experiment to 32 after 193 h of sunlight exposure. In the $LMW_{<1kDa}$ fraction of fen water, the C_{org}:Fe ratio decreased from 247 to 126.

All size fractions of the initial stream solution and the $LMW_{<1\ kDa}$ fraction of the fen water were undersaturated with respect to Fe-bearing minerals. The initial < 0.22 µm 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

colloidal DOM that stabilized Fe (III) polymers in solutions. Calculated Fe(II) and Fe(III) speciation in the fen water before and after photodegradation experiment demonstrated an appearance of ~2% of inorganic Fe(II) and approx. 2-fold increase of Fe(II) bound to DOM, in both < 0.22 µm and < 1 kDa 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 and stream waters, respectively (**Table S3**). Phenolic complexes of Fe(III) which accounted for >93…99% of all Fe, remained constant in the course of experiment. In the fen water, the proportion of Fe(II)-weak electrostatic complex strongly decreased (by a factor of 4.5 and 1.5 in < 0.22 µm and < 1 kDa fractions, respectively) whereas Fe(II) complex with FA carboxylates increased in < 0.22 µm fraction and stayed constant in < 1 kDa fraction (**Table S3**). According to vMinteq predictions, 4.5×10^{-5} M of ferrihydrite should could have precipitated from the fen water over 200 h of solar irradiation. This is equivalent to ~2250 µg/L of dissolved Fe concentration decrease, which is comparable to the 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 < 0.22 µm fraction of dark control and sun-light irradiated quartz reactors (**Fig. 5A**). The δ^{57}Fe value of the < 10 kDa fraction decreased from +2.93±0.05‰ to +1.92±0.1‰ after first 110 h of exposure and then remained constant. Note that the initial < 10 kDa fraction of stream water was ca. 1.5 ‰ isotopically heavier than the < 0.22 µm fraction, in agreement with previous measurement of Fe isotopic composition in colloids of Northern Karelian streams[37].

The isotopic ratio in the dark control of fen water remained stable at δ^{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»[50]. In contrast to dark controls, sunlight irradiation of the fen water strongly impacted δ^{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 δ^{57}Fe in the <0.22 µm and the

374 <1-10 kDa fraction, respectively. Over the next 100 h, the δ^{57}Fe further decreased by 1.5 ‰ in the
375 <0.22 µm fraction and stabilized at δ^{57}Fe = -2.2±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 µm fraction (**Fig. 4A** and **5A**), in general agreement with stability of
378 boreal high latitude metal concentration in riverwaters with respect to sunlight irradiation[28,57]. After
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
382 which are enriched in heavy isotopes[55]. For example, aquatic prokaryotes produce the LMW (0.3 – 1
383 kDa) Fe(III) siderophores[58]. These light sensitive, presumably aromatic Fe complexes represent a
384 small iron fraction of overall $Fe_{<10\ kDa}$ pool, but our results indicate that it exhibits a very high δ^{57}Fe
385 value. The isotopic signature of $LMW_{<1\text{-}10\ kDa}$ Fe poor, C-rich fraction of Karelian waters reaches
386 +4.2‰[37]. We hypothesize that these isotopically heavy Fe-organic complexes have low residence time
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 <0.22 µm fraction and all filtrates and ultrafiltrates became strongly impoverished in heavy
390 isotopes (**Fig. 4 D, 5 B**). The removal of Fe followed that of $SUVA_{254}$ decrease[28], suggesting that the
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
395 sunlight oxidation of organo-ferric colloids observed in this study (+2.3±0.1‰) is generally consistent
396 with known fractionation of Fe between Fe(III) hydroxide and Fe(II) solution: ($\Delta^{57}Fe_{FeOOH\text{-}Fe(aq)}$ =
397 +2.3±0.3‰, ref. 52-54).

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

generated in the < 1 kDa fraction is enriched in light isotopes compared with initial Fe(III) in colloids, thus producing overall negative $\delta^{57}Fe$ value in $LMW_{< 1\ kDa}$ after irradiation. Assuming an equilibrium fractionation factor between Fe(II) and Fe(III) of -4.3‰[59] and considering the starting water $\delta^{57}Fe$ close to 0‰ (**Fig. 5B**), the 55% of Fe(II) in < 1 kDa fraction (**Fig. 4 F**) provides 0.55×(-4.3‰) = -2.4‰ of $\delta^{57}Fe$, in full agreement with values shown in **Fig. 5 B**. This <0.22 µm fraction comprises two Fe pools: (1) Fe remaining after $Fe^{2+}(aq) \rightarrow Fe(OH)_3$ and after $Fe^{3+}(colloidal) \rightarrow Fe(OH)_3$ precipitation, and (2) isotopically light Fe^{2+} produced in the LMW fraction. It is important to note that the photolysis of organo-ferric colloids which represents 80% of Fe in <0.22 µm fraction, removes Fe(III) in the form of $Fe(OH)_3$ hydroxides corresponding to $\Delta^{57}Fe_{Fe(III)hydroxide-Fe(aq)}$ of 2.4±0.1‰, **Fig 5B**. The observed enrichment of solid phase in heavier isotopes is therefore similar to $Fe^{2+}(aq) \rightarrow Fe(OH)_3$ reaction ($\Delta^{57}Fe_{Fe(III)hydroxide-Fe(aq)}$ = 2.4 ‰[52]) rather than to $Fe^{3+}(aq) \rightarrow Fe(OH)_3$ reaction ($\Delta^{57}Fe_{Fe(III)hydroxide-Fe(aq)}$ = -0.9 ‰[60]). Note here that in the photolysis experiments, the loss of dissolved Fe and change of Fe isotope fractionation are mainly interpreted as precipitation of hydrous Fe(III)-oxides. We therefore neglect Fe(II) sorption onto hydrous Fe(III)-oxides and subsequent atom (isotope) exchange between Fe(II) and hydrous Fe(III)-oxides. These processes are responsible for substantial Fe isotope fractionation in a number of organic-free systems[36, 61-64]. In humic waters, both remaining $Fe(II)_{aq}$ ions and >FeOOH surface sites of newly precipitated Fe(III) hydroxides are likely to be bound to carboxylic and phenolic groups of fulvic acids. These surface organic - Fe complexes interfere with Fe(II)-Fe(III) hydroxide isotope exchange which would therefore require special investigation. Further, in order to fully account for short-term variation of Fe concentration, speciation and isotope ratios, one has to consider both dissolved and particulate Fe fracions, as well as isotopic composition of both Fe(II) and Fe(III) species.

We observed quite large differences in the degree of Fe concentrations and Fe isotopic composition during photolysis of the fen and the stream water. These differences may stem from the combination of two main factors controlling the degree of DOM and Fe photo-reactivity in natural 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 DOM[65,66] and the DOM photobleaching is enhanced by elevated Fe concentration via the photo-Fenton effect below pH 6[67]. Overall, in natural settings, one may expect large variation of Fe and DOM photo-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[68], whereas acidic DOC-rich waters from a subtropical swamp under UV irradiation demonstrated 10 times more efficient removal of Fe relative to DOC[69]. As such, depending on the lithological context of peatlands (i.e., acidic (felsic) or carbonate (sedimentary) rocks), the degree of Fe chemical and isotopic composition change under sunlight may be dramatically different.

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

We hypothesize several processes responsible for chemical removal and isotopic redistribution of Fe among different colloidal pools in DOM- and Fe-rich stream and fen water, shown schematically in **Fig. 6.** High molecular size (10 kDa – 0.45 µm) organo-ferric colloids representing the majority of dissolved (<0.22 µm) Fe[17,37] are stabilized by organic ligands originating from topsoil and bog peat leaching. In addition, a small fraction of Fe(III) could be linked to highly specific, $LMW_{<1\text{-}10\,kDa}$ ligands having a short residence time in the river channel; these complexes could be produced via in-stream plankton and periphyton activity. In the fen water, a sizeable fraction of Fe is in the form of Fe(II) inorganic and organic complexes[70]. In the river water, the HMW colloids are subjected to biologically-controlled transformations via *i*) adsorption onto surfaces of aquatic bacteria, favoring heavier isotopes to the cells, with an overall $\Delta^{57}Fe_{cell\ ads\text{-}solution}$ = +0.4±0.1‰, and *ii*) assimilation by live bacteria, favoring lighter Fe isotopes to the cells with $\Delta^{57}Fe_{cell\ incorp\text{-}solution}$ = -0.7±0.1‰. On a short-term scale (hours), the humic waters, once placed in contact with bacteria, are therefore becoming enriched in lighter Fe isotopes, followed, on a long-term scale (days), by enrichment in heavier Fe isotopes. Considering available data on preferential heavy isotope adsorption onto phytoplanktonic and

peryphytic cyanobacteria inhabiting natural waters (from +0.4 to +2.9‰, ref. 30), and neglecting Rayleigh distillation processes given that we are dealing with open systems (hydrological continuum, ref. 17), the overall magnitude of diurnal variation of dissolved (< 0.45 μm) δ^{57}Fe in small streams and stagnant surface waters in the presence of common soil bacteria may range from +2.7‰ to +0.7‰. This greatly exceeds the range of Fe isotopic excursions in various sediments and in all possible bedrocks[71].

Further, photolysis of DOM and Fe-DOM complexes in surface waters, which operates at the time scale comparable to water and solute residence time in these waters (1-10 days), is capable to dramatically enrich in lighter Fe isotopes (by 1.5 to 2.5‰) of both dissolved (<0.22 μm) and $LMW_{< 1\text{-}10\ kDa}$ water fraction. We suggest that the two major processes of organo-ferric colloid transformation under sunlight in natural waters include: (*i*) degradation of the organic part of colloids (1 kDa – 0.22 μm) and production of low molecular weight (< 1 kDa) organic ligands including carboxylic and 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.

The enigmatic enrichment of the LMW fraction (<1-10 kDa) of the stream water in heavy isotopes[37] is therefore most likely linked to strong, presumably aromatic, isotopically heavy Fe(III)-organic complexes. These compounds have low residence time and are produced in the river channel 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[72] capable to bind both Fe(II) and Fe(III). Altogether, bio- and photo-degradation of dissolved Fe in river, stream and bog waters can produce from -2 to +3 ‰ δ^{57}Fe variation on time scales of a few days. Because this time is generally shorter than the water residence time in surface waters, considering of Fe isotopic signature of rivers and streams as a conservative value inherited from soils and using it for tracing the sources and weathering regime on watersheds are not warranted at short time- and length-scales. Therefore, naturally-induced variations in biological activity (switching from heterogenic bacteria uptake to adsorption onto aquatic phototrophs) and sunlight illumination can modify the overall Fe isotopic

signatures of surface waters by several permil. Moreover, when considering the processes responsible for Fe chemical and isotopic transformation in organic-rich streams, one has to assess both dissolved (<0.45 or <0.22 µm) and $LMW_{< 1\text{-}10\ kDa}$ fractions of metal, since their isotopic signatures and photo-susceptibility may be dramatically (by 1 to 3‰) different.

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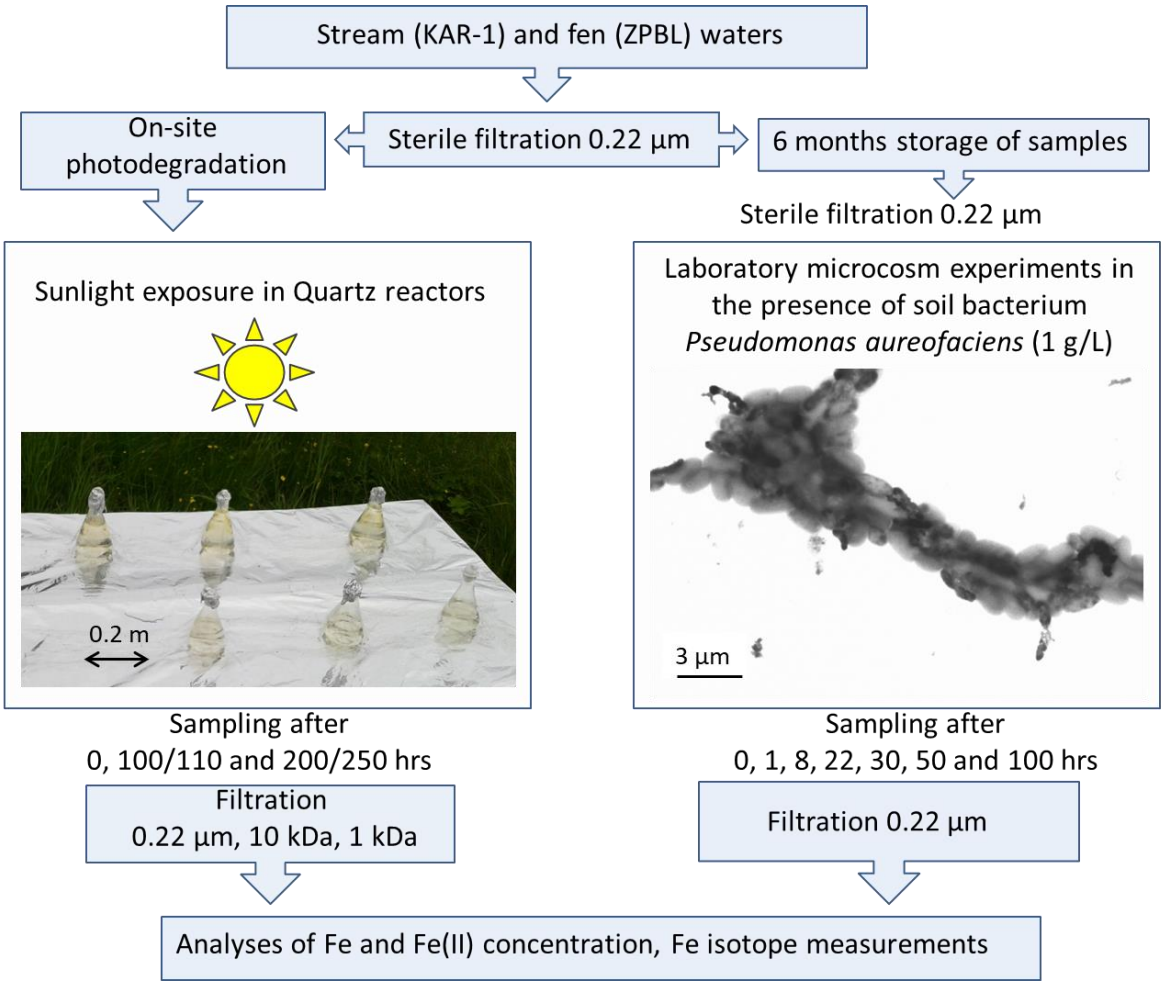
Table 1. Measured pH, DOC, Fe concentrations and isotopic ratios, relative to IRMM-14, during bio- and photo-degradation experiments. Note that the DOC in biodegradation experiments of stream water slightly increased due to cell lysis and exometabolite production. The standard error was calculated using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

Bio-degradation (1 g_{wet}/L of *Pseudomonas aureofaciens*)											
hrs	**Fen water ZPBL <0.22 µm**					**hrs**	**Stream water KAR-1 <0.22 µm**				
	pH	**DOC, mg L^{-1}**	**Fe, µg L^{-1}**	**Fe(II), µg L^{-1}**	**δ^{57}Fe ±2 SE, ‰**		**pH**	**DOC, mg L^{-1}**	**Fe, µg L^{-1}**	**Fe(II), µg L^{-1}**	**δ^{57}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						
Sunlight exposure											
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						



745

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 µm filtration after sampling. In
752 photodegradation experiments, the stream water was processed for the < 0.22 µm and the <
753 10 kDa filtration and the fen water was filtered through 0.22 µm, 10 kDa and 1 kDa.

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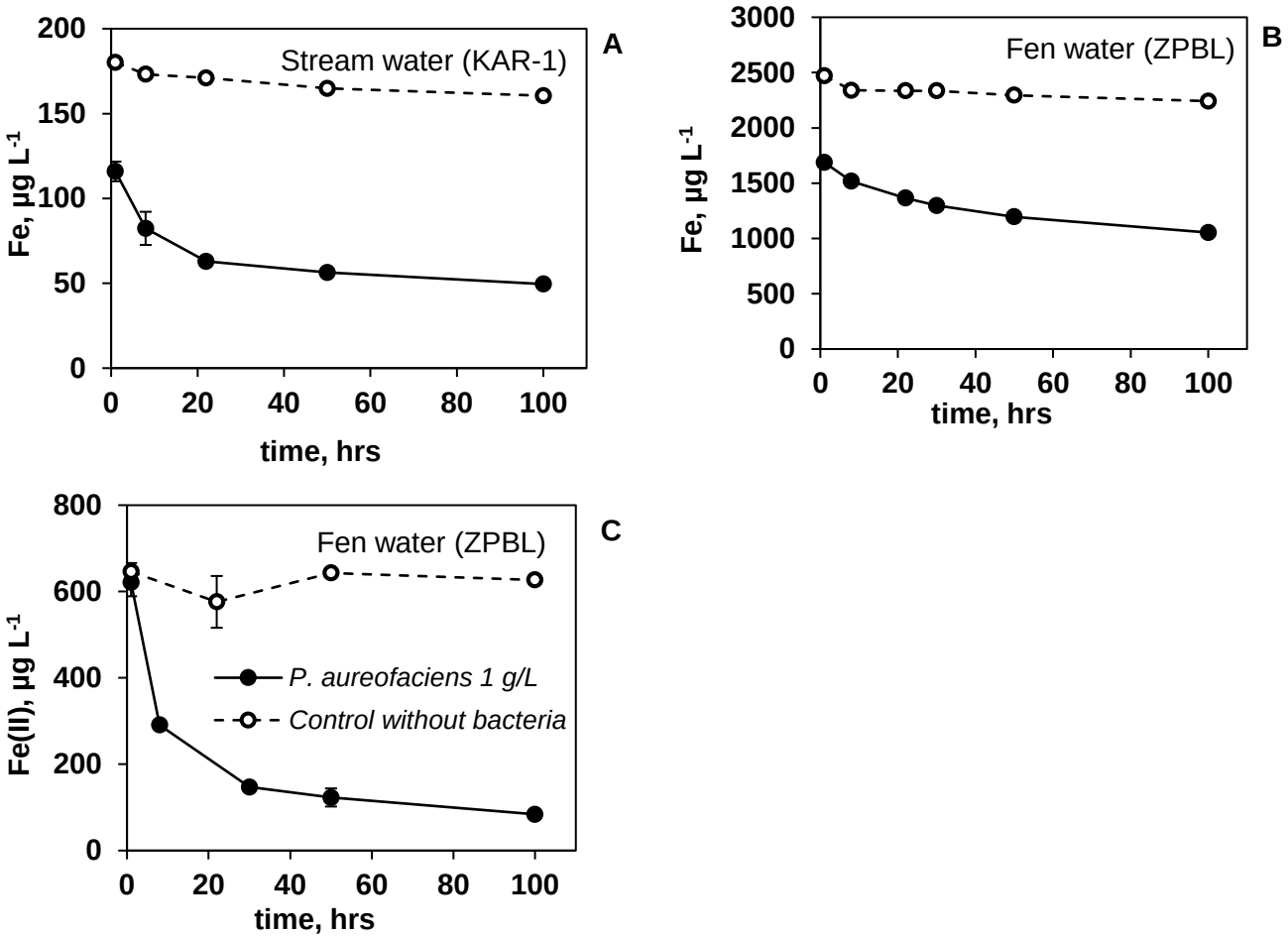


Fig. 2. Evolution of total dissolved Fe concentration during biodegradation experiments of stream (**A**) and fen (**B**) water. **(C):** Fe(II) concentration evolution in the fen water during biodegradation. The error bars of biotic experiments represent 1 SD of duplicates. In most cases, they are smaller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

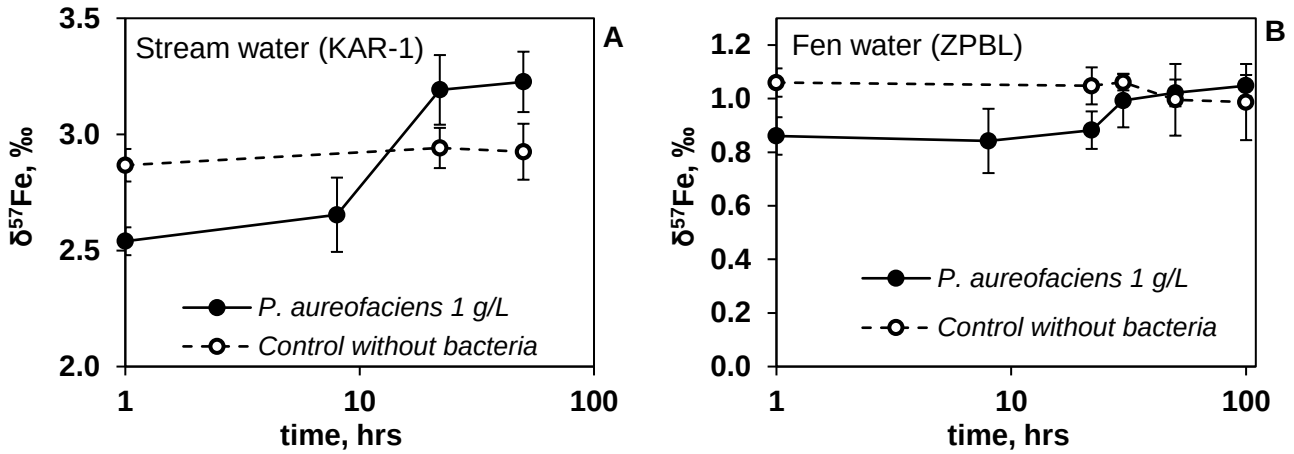
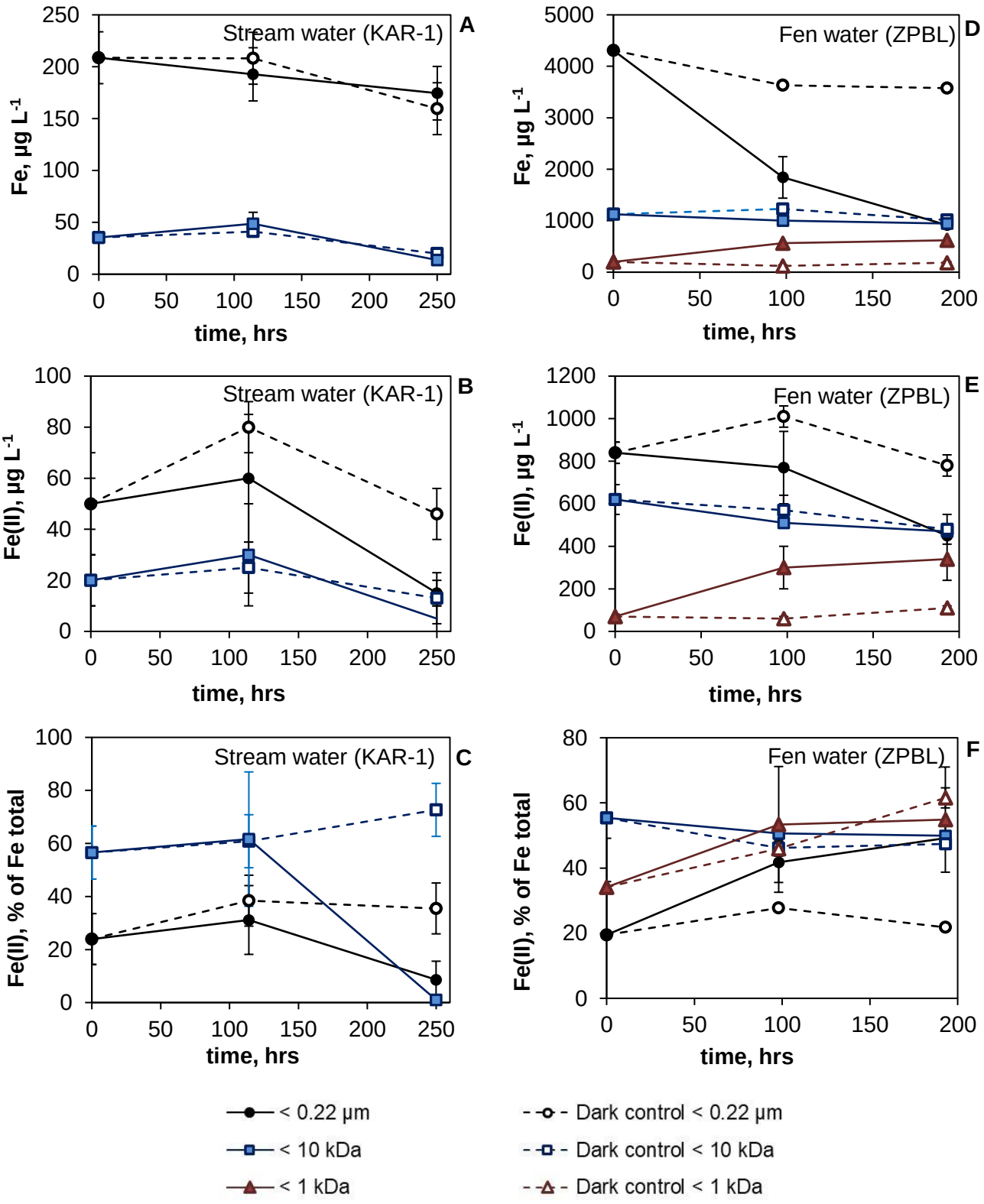


Fig. 3. Evolution of isotopic ratios δ^{57}Fe during biodegradation experiments of stream (A) and fen (B) water. Note log scale for time axis..



780 **Fig. 4.** Evolution of Fe_{tot} and Fe(II) concentration during photodegradation experiments of
781 stream (**A, B, C**) and fen (**D, E and F**) water in quartz reactors. Note a drop in Fe_{tot} and Fe(II)
782 concentration of the < 0.22 µm dark control at 200 and 250 h not observed in previously stored samples
783 in biodegradation experiments (Fig. 2), which may be due to partial coagulation of freshly sampled
784 natural water upon storage.

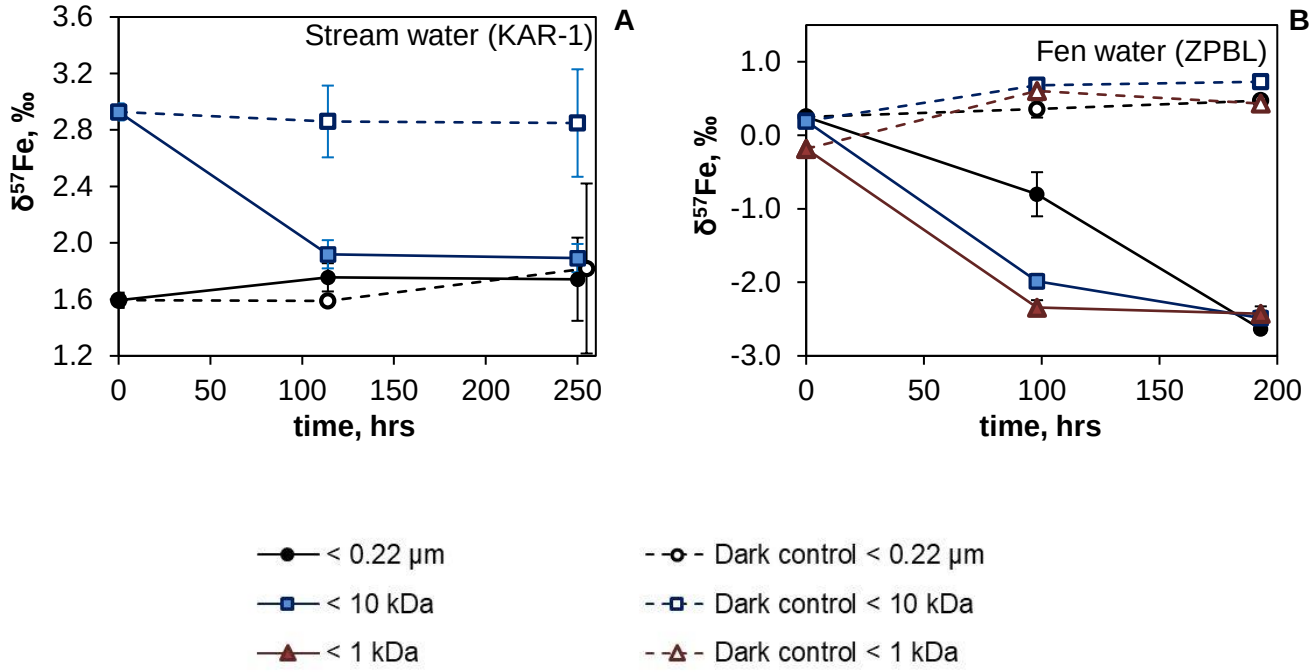


Fig 5. Evolution of isotopic ratios δ^{57}Fe during photodegradation experiments of stream (A) and fen (B) water. There is a clear decrease in δ^{57}Fe value in all fractions of photodegraded samples of fen water, but this decrease is only pronounced for the < 10 kDa fraction of stream water. Note that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

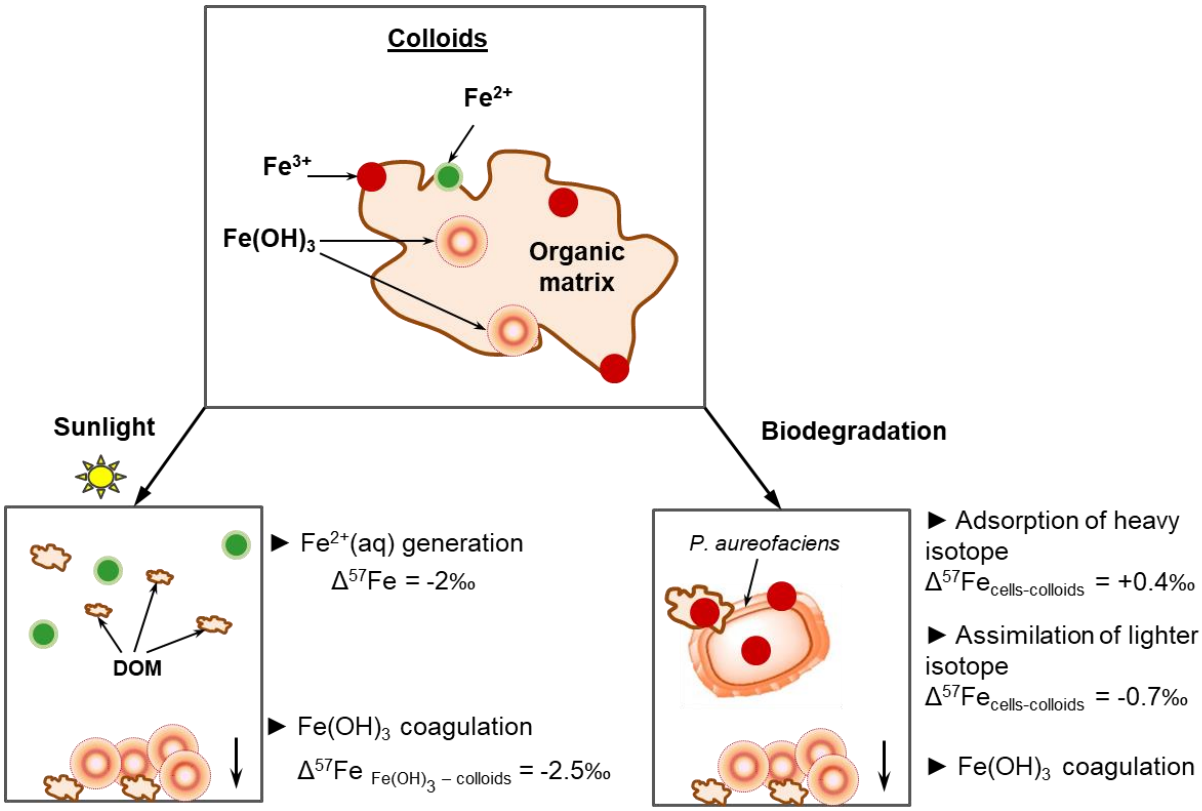


Fig. 6. Cartoon of Fe isotope fractionation under sunlight-induced transformation (left) and biodegradation (right) of organic and organo-ferric colloids (1 kDa - 0.22 µm). Live heterotrophic *P. aureofaciens* bacteria adsorb heavier and assimilate lighter Fe isotopes. Sunlight irradiation generates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