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# 1                   **Iron affects the biodegradation of natural dissolved organic matter**

2  
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## 15   **Key Points:**

- 16       • The association of Fe with DOM can stimulate bacterial growth and the biodegradation  
17       of DOM
- 18       • Insoluble Fe-precipitates on the bacterial cells can inhibit bacterial growth on DOM
- 19       • Fe associated with DOM reduces the bioavailability of P, which decreases bacterial  
20       growth on DOM

## 21   **Abstract**

22       Iron (Fe) may alter the biodegradation of dissolved organic matter (DOM), by associating  
23       with (DOM), phosphorus (P) and microbes. We isolated DOM and a bacterial community from

24 boreal lake water and examined bacterial growth on DOM in laboratory experiments. Fe was  
25 introduced either together with DOM (DOM-Fe) or into bacterial suspension, which led to the  
26 formation of insoluble Fe-precipitates on bacterial surfaces (Fe coating). In the latter case, the  
27 density of planktonic bacteria was an order of magnitude lower than that in the corresponding  
28 treatment without introduced Fe. The association of Fe with DOM decreased bacterial growth,  
29 respiration, and growth efficiency compared with DOM alone at the ambient concentration of  
30 dissolved P ( $0.16 \mu\text{mol L}^{-1}$ ), indicating that DOM-associated Fe limited the bioavailability of P.  
31 Under a high concentration ( $21 \mu\text{mol L}^{-1}$ ) of P, bacterial biomass and respiration were similar or  
32 several times higher in the treatment where DOM was associated with Fe than in a corresponding  
33 treatment without Fe. Based on the next generation sequencing of 16S rRNA genes, *Caulobacter*  
34 dominated bacterial communities grown on DOM-Fe. This study demonstrated that association  
35 of Fe with a bacterial surface or P reduce bacterial growth and the consumption of DOM. In  
36 contrast, DOM-Fe is bioavailable and bound Fe can even stimulate bacterial growth on DOM  
37 when P is not limiting.

38

## 39 **1 Introduction**

40 Dissolved organic matter (DOM) contains a large pool of reactive organic carbon forming a  
41 major source of energy and nutrients for heterotrophic bacteria. Iron (Fe) is the most abundant  
42 element on Earth and interferes with the biogeochemical cycles of many important elements such  
43 as carbon and phosphorus (P) [Cotner and Heath, 1990; Sarkkola *et al.*, 2013; Kritzberg *et al.*,  
44 2014; Weyhenmeyer *et al.*, 2014]. Global carbon cycling has been a topical issue during the last  
45 decades. Nevertheless, the links between carbon and other key elements in biogeochemical  
46 cycles have remained poorly quantified. Iron can have a more important role in carbon cycling in

47 boreal zone than presently understood. For example, it plays a key role in carbon sequestration in  
48 boreal lake sediments [Kortelainen *et al.*, 2004; von Wachenfeldt and Tranvik, 2008; Einola *et*  
49 *al.*, 2011]. Recently, the concentrations of both dissolved organic carbon (DOC) and Fe have  
50 increased in boreal rivers and streams [Kritzberg and Engström, 2012; Sarkkola *et al.*, 2013;  
51 Weyhenmeyer *et al.*, 2014] causing pressure to better understand links between DOC and Fe in  
52 the present and future conditions. To best of our knowledge, the role of Fe in the degradation of  
53 DOM has not been examined, although Fe can alter the biodegradation of organic matter (OM)  
54 in many ways, as exemplified below.

55 Insoluble Fe(oxy)hydroxides, like other mineral surfaces, can bind OM [Hedges and Oades,  
56 1997; Baldock and Skjemstad, 2000; Kaiser and Guggenberger, 2000; 2007; Chan *et al.*, 2011;  
57 Bennett *et al.*, 2014]. The adsorption of OM on mineral surfaces reduces the availability of OM  
58 for osmotrophic microbes (archaea, bacteria, and fungi), and can result in the long-term  
59 preservation of OM [Keil *et al.*, 1994; Hedges and Keil, 1995; White and Knowles, 2000;  
60 Rothman and Forney, 2007; Lalonde *et al.*, 2012].

61 Fe can form coordination complexes with DOM [Leenheer *et al.*, 1998; Sjöstedt *et al.*, 2013].  
62 For example, the potential binding sites of Fe in a hypothetical average molecule of Suwannee  
63 River fulvic acid (SRFA) include four carboxylic groups, one quinoid structure, two phenolic  
64 groups, and four carbonyls associated with esters and ketones [Leenheer *et al.*, 1998; Sjöstedt *et*  
65 *al.*, 2013]. According to the proton donating carboxyl and phenolic groups, SFRA contains 14.3  
66  $\mu\text{mol} [\text{mg C}]^{-1}$  of potential binding sites for Fe [Leenheer *et al.*, 1998; Ritchie and Perdue, 2003;  
67 Sjöstedt *et al.*, 2013; Xiao *et al.*, 2013]. Not all the potential binding sites are occupied by Fe in  
68 freshwaters, but loadings of up to ca. 2  $\mu\text{mol Fe} [\text{mg C}]^{-1}$  have been reported [Neubauer *et al.*,  
69 2013a]. In the environment, DOM consists of thousands of molecules, which range from

70 dissolved single compounds to colloids [*Gustafsson and Gschwend, 1997; Wagner et al., 2015*].  
71 Similarly, the species of Fe range from simple ions to colloids [*Boyd and Ellwood, 2010;*  
72 *Sjöstedt et al., 2013*). Due to poor solubility of many inorganic Fe species, the association of Fe  
73 with DOM is crucial for the solubility of Fe [*Shapiro, 1964; Gustafsson and Gschwend, 1997;*  
74 *Boyd and Ellwood, 2010*). These associations ranging from monomeric Fe-DOM complexes to  
75 colloidal assemblages of DOM and Fe are called collectively as “DOM-Fe” in this study. The  
76 biodegradation of organic component in DOM-Fe is poorly known [*Boudot et al., 1989; White*  
77 *and Knowles, 2000; Nancharaiah et al., 2006*].

78 Fe can indirectly alter the biodegradation of OM by influencing the speciation and  
79 availability of P. In soils and sediments, Fe reduces the availability of P by binding it to insoluble  
80 non-available forms [*Heiberg et al., 2012; Baken et al., 2015*]. Fe can associate with DOM and  
81 absorb phosphate also in water column [*Francko and Heath, 1982; Steinberg and Baltes, 1984;*  
82 *Cotner and Heath, 1990; De Haan et al., 1990; Sundman et al. 2016*]. A tight association  
83 between Fe and P reduces the bioavailability of P and can potentially limit the activity of  
84 decomposers e.g., in lakes where the concentration of P is low [*Karlsson et al., 2001; Vidal et*  
85 *al., 2011*].

86 The proton donating functional groups (carboxyl, hydroxyl, sulfhydryl, and phosphoryl) on  
87 the bacterial cell surface can bind Fe [*Fein et al., 1997; Pokrovsky et al., 2008; Yee et al., 2004*].  
88 Such binding can transfer and immobilize microbes on solid surfaces when Fe forms insoluble  
89 precipitates [*Liu et al., 2015*], e.g., during the oxidation of dissolved Fe(II) to  
90 Fe(III)(oxy)hydroxides along with an increasing redox potential [*Hatamie et al., 2016*]. An  
91 extensive accumulation of Fe on the cell surfaces (Fe coating; [*Franzblau et al., 2016*]) or

92 embedding under a layer of Fe(III)(oxy)hydroxides can be expected to reduce the metabolic  
93 activity of decomposers and the biodegradation of OM.

94 Besides the possible negative effects of Fe on the biodegradation of OM, Fe can also  
95 stimulate the decomposition of OM. Fe associated with quinone moieties of DOM can cause  
96 abiotic oxidation-reduction reactions, which, for instance, convert the reduced hydroquinones  
97 through semiquinone radicals to oxidized quinones [Yuan *et al.*, 2016]. Such reactions can be  
98 sources of reactive oxygen (O<sub>2</sub>) species and lead to the breakage of aromatic rings [Miller *et al.*,  
99 2013; Comba *et al.*, 2015; Yuan *et al.*, 2016]. This ring cleavage can produce volatile  
100 hydrocarbons, CO<sub>2</sub>, and organic acids such as oxalic acid [Pracht *et al.*, 2001; Studenroth *et al.*,  
101 2013; Comba *et al.*, 2015]. These reactions can take place abiotically between the reduced and  
102 oxidized forms of natural organic matter and those of Fe (FeII/III). Thus, the abiotic reactions of  
103 Fe can facilitate decomposition of OM and produce substrates for microbes.

104 Fe also facilitates the decomposition of OM through the active metabolism of microbes.  
105 Brown rot fungi and an ectomycorrhizal fungus, *Paxillus involutus*, use Fe in a biochemical  
106 Fenton reaction to break down particulate organic matter in wood and soil, respectively [Arantes  
107 *et al.*, 2012; Rineau *et al.*, 2012]. These fungi secrete reducing components that convert Fe(III) to  
108 Fe(II) and dioxygen through superoxide to hydroxyl peroxide to yield the two reactants of the  
109 Fenton reaction [Arantes *et al.*, 2012]. The Fenton reaction produces hydroxyl radicals that can  
110 break down organic matter non-selectively.

111 Heterotrophic bacteria can use extracellular enzymes to break down molecules that are too  
112 large (>600 g mol<sup>-1</sup>) for direct uptake [Arnosti, 2004]. This enzymatic catalysis is selective and  
113 primarily limited to the hydrolysis of predictable biopolymers [Arnosti, 2004]. In many  
114 freshwaters, the bulk DOM is dominated by high molecular mass (>600 g mol<sup>-1</sup>) heterogeneous

115 humic substances that also include non-hydrolysable bonds [Tranvik, 1988; Arnosti, 2004].  
116 Although much of freshwater DOM is too large for direct uptake or non-hydrolysable by  
117 extracellular enzymes of bacterioplankton, typically a half of the bulk DOC is lost in <2.5 years  
118 in Swedish lakes [Algesten *et al.*, 2003]. Flocculation may direct DOM into sediments and solar  
119 radiation can break down a part of the DOM, but these processes cannot alone explain the loss of  
120 DOM that is apparently refractory to biodegradation in lakes or in long laboratory incubations  
121 [Vähätalo and Wetzel, 2008; Vähätalo *et al.*, 2010; Koehler *et al.*, 2012]. Therefore, it is possible  
122 that the decomposition of DOM in freshwaters may also include other dark reactions, e.g.,  
123 catalyzed by Fe abiotically or through active metabolism by bacteria.

124 The communities of bacteria in bacterioplankton contain hundreds of species with their  
125 specific metabolic functions [Taipale *et al.*, 2011]. If bacteria are able to utilize Fe to break down  
126 DOM extracellularly, it is likely that this functional trait is limited to certain species or groups of  
127 species. Such species may possibly mediate extracellular redox reactions of Fe through organic  
128 or inorganic (e.g., superoxide) redox shuttles [Melton *et al.*, 2014] and/or have extracellular  
129 oxidoreductases [Diaz *et al.*, 2013]. Although bacteria may have potential mechanisms for  
130 extracellular decomposition of DOM with the help of Fe [Diaz *et al.*, 2013; Melton *et al.*, 2014],  
131 such Fe-stimulated decomposition of DOM and the species of bacteria involved has not been  
132 reported.

133 The laboratory experiments of this study assessed the role of Fe on the biodegradation of  
134 natural DOM extracted from a humic lake using a community of bacterioplankton from the same  
135 lake. In the experiments, Fe was introduced in several different ways and had a possibility to  
136 associate with DOM, P, and the cell surface of bacteria to form insoluble Fe(oxy)hydroxides. We

137 examined the growth and respiration of a bacterial community on DOM up to 28 days and  
138 identified genus-level changes in the composition of bacterial community along the experiments.

## 139 **2 Materials and Methods**

### 140 2.1 Sampling and DOM extraction

141 Surface water samples were collected from humic Lake Valkea-Kotinen (61°14'N,  
142 25°04'E), a pristine headwater lake in southern Finland mainly surrounded by coniferous forest  
143 [Vähätalo *et al.*, 1999; Arvola *et al.*, 2010]. In the acidic (pH 5.4) surface water of Lake Valkea-  
144 Kotinen, the concentrations are 945  $\mu\text{mol L}^{-1}$  for DOC, 5  $\mu\text{mol L}^{-1}$  for total Fe, and 0.16  $\mu\text{mol}$   
145  $\text{L}^{-1}$  for dissolved P [Keskitalo *et al.*, 1998; Vähätalo *et al.*, 2003; Einola *et al.*, 2011]. The  
146 majority of DOM (75%) consists of humic substances and has a high molecular mass [Vogt *et*  
147 *al.*, 2004]. The weighted average molecular mass of DOM is 1130  $\text{g mol}^{-1}$  according to mass  
148 spectrometry and ca. 4000  $\text{g mol}^{-1}$  according to size exclusion chromatography [Vogt *et al.*,  
149 2004].

150 A water sample collected on 26 October 2012 was immediately filtered through a 0.45- $\mu\text{m}$   
151 filter (AcroPak<sup>TM</sup> 1000 capsule, Pall). On the following day, the water sample was further  
152 filtered through a 0.2- $\mu\text{m}$  filter (Sartobran 300 sterile capsule, Sartorius Stedim) and acidified to  
153 pH ~2 with 37% HCl (Titrisol®, Merck). The acidified water sample was stored in the dark at 11  
154 °C, and NaF was added to the final concentration of 0.01  $\text{mol L}^{-1}$  before solid-phase extraction  
155 (SPE) of DOM according to Dittmar *et al.* [2008]. Fluoride ions were expected to exchange Fe  
156 from their DOM ligands and reduce the Fe content of the extracted DOM [Gao and Zepp, 1998].  
157 In order to examine the extraction efficiency of DOC and the removal efficiency of Fe, a small  
158 aliquot of extracted DOM was re-dissolved in Milli-Q water. The extraction efficiency of DOC



159 was 76% and the removal efficiency of Fe was 97% according to analytical measurements  
160 described in 2.3.1.

## 161 2.2 Experimental design and procedures

162 The experiments briefly described in Tables 1–2 and Fig. 1 were designed to address the  
163 following study questions:

- 164 1) “Fe” – Can Fe influence the biodegradation of DOM?
- 165 2) “DOM-Fe” – Is DOM-Fe bioavailable?
- 166 3) “DOM-Fe low/high P” – Does the bacterial growth on DOM-Fe depend on the concentration  
167 of P?
- 168 4) “Fe coating” – Can precipitation of Fe on the bacterial cell surface affect the biodegradation of  
169 DOM?

170 In this section, we first describe the features common to all experiments and then present the  
171 details of each experiment. Iron(III) sulfate hydrate ( $\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$ , 399.88 g mol<sup>-1</sup>, AnalaR)  
172 and iron(II) sulfate hydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 278.01 g mol<sup>-1</sup>, Sigma-Aldrich) were used as Fe  
173 sources. The  $\beta$ -glycerophosphate disodium salt hydrate (GlyP,  $\text{C}_3\text{H}_{17}\text{Na}_2\text{O}_6\text{P} \cdot 5\text{--}6\text{H}_2\text{O}$ , 216.04 g  
174 mol<sup>-1</sup>, VWR International) was used as a source of P for bacteria. All chemicals used in the  
175 experiment were >98% pure, and all solutions were prepared in deionized water (Milli-Q,  
176 Millipore).

177 An indigenous grazer-free bacterial community was isolated and used as an identical  
178 inoculum of the bacterial community in the consecutive experiments. Bacteria present in the  
179 collected lake water sample were first passed through a 0.8- $\mu\text{m}$  polycarbonate filter  
180 (Cyclopore<sup>TM</sup> track etched membrane, Whatman) and then diluted 100 times with <0.2  $\mu\text{m}$   
181 filtered lake water. This diluted bacterial community was cultured in the dark at room

182 temperature for 4 days, after which the bacterial suspension was divided into 50-mL aliquots and  
183 stored at  $-20\text{ }^{\circ}\text{C}$ . Before each experiment, an aliquot of frozen bacterial suspension was thawed  
184 at  $30\text{ }^{\circ}\text{C}$  in a water bath for 15 min and was introduced as a bacterial inoculum (10% vol/vol in  
185 “Fe” and 5% vol/vol in the other experiments).

186 For the experimental bioassays, DOM, Fe, nutrients, artificial lake water (ALW), and  
187 bacteria were introduced in different concentrations, combinations and ways (Fig. 1; Tables  
188 1–2). DOM was introduced as a stock solution ( $50\text{ mg L}^{-1}$ ), which was prepared by dissolving  
189 the extracted DOM in Milli-Q water and filtering the solution through a  $0.2\text{-}\mu\text{m}$  filter (Supor<sup>®</sup>-  
190 200, Pall). The concentration of introduced DOM was adjusted to  $20\text{ mg L}^{-1}$  ( $= 948\text{ }\mu\text{mol C L}^{-1}$ ),  
191 matching the concentration in the study lake [Vähätalo *et al.*, 2003; Einola *et al.*, 2011] or in  
192 Finnish boreal lakes in general [Kortelainen, 1993]. An acidic (pH 1) stock solution ( $0.5\text{ mmol}$   
193  $\text{L}^{-1}\text{ Fe}_2(\text{SO}_4)_3\cdot n\text{H}_2\text{O}$  in  $0.1\text{ mol L}^{-1}\text{ HCl}$  (Titrisol<sup>®</sup>, Merck) was used as a source of Fe(III) in all  
194 experiments with introduced Fe(III). The stock solution of ALW was prepared according to  
195 Kester *et al.* [1967] and was added to form the final inorganic ion concentrations presented in  
196 Table 1 and an ionic strength of  $0.87$  (calculated as  $\text{mmol L}^{-1}$ ), which is close to the average  
197 ionic strength of Finnish river waters ( $0.8$ , [Xiao *et al.*, 2015]). The nutrients N and P were  
198 introduced as separate solutions of  $\text{NH}_4\text{Cl}$  and GlyP, respectively (Table 1). GlyP was selected as  
199 a source of P instead of inorganic phosphate, which can effectively complex with Fe(III) into  
200 insoluble precipitates [Francis and Dodge, 1993]. GlyP is one of the few organophosphates that  
201 can be transported across the cell membrane, forming a source of P but not serving a carbon  
202 substrate [Schweizer *et al.*, 1982]. An isolated identical bacterial community indigenous to Lake  
203 Valkea-Kotinen was used as a bacterial inoculum. Finally, the volume of bioassays was adjusted  
204 to 100 mL with Milli-Q water. Biodegradation of DOM was assessed as bacterial growth

205 determined as the bacterial density and the consumption of dissolved O<sub>2</sub> in bioassays extending  
206 up to 25 days in the dark at 22 °C.

207 The “Fe” experiment (Fig. 1) assessed the growth of bacteria on DOM in the presence and  
208 absence of introduced Fe(III). DOM, Fe(III), ALW, nutrients, and bacteria were mixed together  
209 at pH ~2 and titrated to pH 7 with 1 mol L<sup>-1</sup> NaOH using a Titrette® bottle-top burette (Brand  
210 GMBH, Germany). During the titration, Fe(III) had the possibility to associate with DOM, GlyP,  
211 the salts of ALW, and/or bacteria. The “Fe” experiment included two concentrations of Fe: no  
212 introduced Fe (DOM alone treatment) and 130 μmol L<sup>-1</sup> Fe(III) (DOM+130Fe treatment; Fig. 1  
213 and Table 2). The treatment containing DOM alone was calculated to contain a low (<0.43 μmol  
214 L<sup>-1</sup>) concentration of Fe that originated from the bacterial inoculum (0.25 μmol L<sup>-1</sup>) and  
215 extracted DOM (0.17 μmol L<sup>-1</sup>; Table 2).

216 The “DOM-Fe” experiment addressed the bioavailability of DOM-Fe. DOM-Fe was created  
217 before the introduction of bacteria, ALW, and nutrients (Fig. 1). The concentration of Gly-P was  
218 adjusted to 21 μmol L<sup>-1</sup> (high P, Table 2), which is two orders of magnitude higher than the  
219 concentration of dissolved P in the study lake. To create DOM-Fe, a stock solution of Fe(III) was  
220 introduced to an acidic (pH ~2) stock solution of DOM and then slowly titrated to pH ~7 (Fig.  
221 1). During the titration, the proton donating sites of DOM became available to bind Fe(III), and  
222 DOM-Fe(III) was formed. Three concentrations of Fe(III) were used: no introduced Fe (DOM  
223 alone), 20 μmol L<sup>-1</sup> Fe (DOM-20Fe), and 80 μmol L<sup>-1</sup> Fe (DOM-80Fe; Table 2). The loadings  
224 of Fe(III) on DOM ranged from 1 μmol Fe [mg]<sup>-1</sup> to 4 μmol Fe [mg]<sup>-1</sup> when calculated as the  
225 ratio of the introduced μmol Fe to the mass of DOM (20 mg L<sup>-1</sup>) used in the experiments (Table  
226 2). The corresponding loading in the collected water was 0.22 μmol Fe [mg]<sup>-1</sup> based on our  
227 analytical measurements for Fe (4.98 μmol L<sup>-1</sup>) and DOC (945 μmol L<sup>-1</sup>) explained in 2.3.1, and

228 assuming a 50% carbon content in the mass of DOM. The content of potential binding sites for  
229 Fe was estimated at  $3.7 \mu\text{mol} [\text{mg}]^{-1}$  as the content of proton-donating carboxyl and hydroxyl  
230 groups in the reverse-osmosis-extracted DOM from our study lake [Vogt *et al.*, 2004;  
231 unpublished data]. The introduced concentrations of Fe(III) resulted in a partial (DOM-20Fe) or  
232 a full (DOM-80Fe) occupancy of potential binding sites on DOM. The actual complexation of  
233 Fe(III) to DOM was not evaluated, but no visible precipitates were observed. The DOM-Fe(III)  
234 created for the experiments may include oligomers of Fe(III) stabilized by DOM in addition to  
235 true complexes between Fe(III) and DOM molecules.

236 The “DOM-Fe high/low P” experiment addressed the role of P in the biodegradation of  
237 DOM-Fe using two concentrations of P (21 and  $0.16 \mu\text{mol L}^{-1}$ ). The low concentration of P  
238 matched the concentration of dissolved P in the study lake [Vähätalo *et al.*, 2003] or in oligo-  
239 mesotrophic lakes in general [Wetzel, 2001]. In “DOM-Fe high/low P”, DOM-Fe was created as  
240 in “DOM-Fe” experiments, but only one concentration of Fe ( $60 \mu\text{mol L}^{-1}$ ) was used (Fig. 1,  
241 Table 2).

242 The “Fe coating” experiment aimed to associate Fe on the surface structures of bacterial  
243 cells and immobilize some of the cells into insoluble precipitates (Fig. 1, Table 2). This  
244 experiment used a  $0.5 \text{ mmol L}^{-1}$  stock solution of Fe(II) prepared by dissolving  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in  
245  $0.1 \text{ mol L}^{-1}$  HCl. The acidic (pH 2) solution of Fe(II) was slowly titrated to  $\text{pH } 5.0 \pm 0.1$  and  
246 incubated for 1 h to oxidize part of Fe(II) to Fe(III) [Ferris *et al.*, 1987]. The bacterial inoculum  
247 was introduced as a ligand for Fe, and the pH of the mixture was titrated to 7. Finally, the rest of  
248 ingredients (DOM, ALW, and nutrients) were introduced as in the other experiments, except that  
249 two different concentrations of P were used ( $0.16 \mu\text{mol L}^{-1}$  in #5,  $21 \mu\text{mol L}^{-1}$  in #7, Table 2).

250 The “No Fe” experiment consisted of control treatments without introduced Fe. The growth  
251 of bacteria on the introduced inorganic salts was tested in treatment #1. In #1, acidified MQ  
252 water was titrated to pH 7, after which it received ALW, bacteria, and nutrients, including a low  
253 concentration of GlyP, but no DOM. Treatment #2 evaluated whether bacteria can use GlyP as a  
254 source of carbon. Treatment #2 was prepared similarly to #1, except that a high concentration of  
255 GlyP ( $21 \mu\text{mol L}^{-1}$ ) was used. Treatments #3 and #4 evaluated the growth of bacteria on DOM  
256 without introduced Fe at a low and high concentration of GlyP, respectively. For #3 and #4, an  
257 acidic solution of DOM was first titrated to pH 7 and then received ALW, nutrients, and bacteria,  
258 as in the “DOM-Fe” and “DOM-Fe low/high P” experiments.

## 259 2.3 Analytical methods

### 260 2.3.1 Fe and DOC measurements

261 Fe samples were preserved by adding 50  $\mu\text{L}$  super-purity nitric acid (Romil) to 10 mL of  
262 samples. Fe concentrations were determined using inductively coupled plasma mass  
263 spectroscopy (Elan Dynamic Reaction Cell II, Perkin-Elmer Sciex). DOC samples were acidified  
264 to pH~2 with  $1 \text{ mol L}^{-1}$  HCl and measured with a total organic carbon analyzer (TOC-V<sub>CPN</sub>,  
265 Shimadzu). The inorganic carbon was purged (by acidifying and bubbling) following a high  
266 temperature ( $670 \text{ }^\circ\text{C}$ ) catalytic combustion oxidation of organic carbon into  $\text{CO}_2$ , which was  
267 detected a by an infrared analyzer. The standard solutions for DOC measurement were prepared  
268 from potassium hydrogen phthalate (VWR chemicals) dissolving in Milli-Q water. Milli-Q water  
269 was used as blank [Benner and Strom, 1993].

### 270 2.3.2 Bacterial counting

271 Bacterial samples were collected after shaking the culture flasks, except in the “Fe coating”  
272 experiment. These treatments included obvious precipitates, and only supernatant was collected

273 from the flasks without shaking. Collected bacterial samples (1 mL) were fixed with  
274 paraformaldehyde (1% final concentration) and glutaraldehyde (0.05% final concentration),  
275 incubated for 10 min in the dark and stored at  $-86\text{ }^{\circ}\text{C}$  in an ultra-low temperature freezer  
276 (Thermo Scientific Forma) [Marie *et al.*, 1996].

277 Bacterial densities were measured by flow cytometry (LSR II, BD Biosciences, USA)  
278 [Gasol and Del Giorgio, 2000]. Bacterial samples were thawed at room temperature for 1 h, and  
279 stained with SYBR Green I (Sigma-Aldrich) for 10 min in the dark before measurement. A  
280 volume of 10  $\mu\text{L}$  reference beads with a known density (Countbright<sup>TM</sup> absolute counting beads,  
281 Life Technologies<sup>TM</sup>, Invitrogen) was added to each 1 mL sample to relate the number of  
282 detected bacterial cells to the volume. Bacterial densities ( $\text{cells L}^{-1}$ ) were converted to bacterial  
283 biomass ( $\mu\text{mol C L}^{-1}$ ) using a carbon content of 30 fg C cell<sup>-1</sup> [Fukuda *et al.*, 1998].

### 284 2.3.3 Bacterial respiration

285 Bacterial respiration (BR) was estimated as the consumption of dissolved  $\text{O}_2$  measured with  
286 needle-type  $\text{O}_2$  microsensor optodes (PreSens GmbH, Regenbunrg) [Warkentin *et al.*, 2007] at 15-  
287 min intervals. The aliquots were closed in biological  $\text{O}_2$  demand bottles incubated in dark  
288 conditions in a water bath maintained at  $20\text{ }^{\circ}\text{C}$  with a thermostat (Lauda Ecoline Staredition  
289 RE112, Germany). An optode was inserted into the sample via a hole drilled through the ground-  
290 glass stopper and sealed with parafilm. The drift of the instrument defining the detection limit for  
291 BR was measured in three blank experiments in which Milli-Q water was incubated for 300  
292 hours under conditions identical to the respiration measurements. During the blank experiments,  
293 the apparent decline in  $\text{O}_2$  was  $1.5 \pm 0.5\ \mu\text{mol L}^{-1} (300\ \text{h})^{-1}$  (mean  $\pm$  sd,  $n = 3$ ). The decline in  
294 the concentration of  $\text{O}_2$  was converted into an increase in the concentration of  $\text{CO}_2$ , assuming a  
295 1:1 molar ratio between the consumed  $\text{O}_2$  and the produced  $\text{CO}_2$  in BR. This respiratory quotient

296 is similar to earlier studies, which have used values ranging from 0.82 to 1.2 [*Søndergaard and*  
297 *Middelboe, 1995; Del Giorgio and Cole, 1998; Cory et al., 2014*]. The temporal trend in  
298 accumulated CO<sub>2</sub> (μmol C L<sup>-1</sup>) was determined by a polynomial fitting to the measurements  
299 using R-language and the smooth spline function from the R package “stats” [*R Core Team,*  
300 2014].

#### 301 2.3.4 Bacterial growth efficiency

302 The bacterial growth efficiency (BGE) was calculated by dividing the increase in bacterial  
303 biomass (BP) by the sum of the increase in bacterial biomass and bacterial respiration (BR):  
304  $BGE = (BP)/(BP+BR)$  [*Del Giorgio and Cole, 1998*]. The BGE in #5 may be an underestimate,  
305 because bacteria immobilized with precipitates were not included in BP although they possibly  
306 contributed to BR.

#### 307 2.3.5 16S rRNA sequencing

308 Bacterial samples (1.5 mL) were collected on day 28 of the simultaneous “No Fe”, “Fe  
309 coating”, and “DOM-Fe high/low P” experiments. The samples were centrifuged at 14000 rpm  
310 for 30 min and the bacterial cell pellets were frozen at -20 °C for later extraction. Bacterial DNA  
311 was extracted using Quick Extract DNA Extraction Solution (Epicentre) according to the  
312 manufacturer’s instructions. From the extracts, the V1–V2 region of the 16S rRNA gene was  
313 amplified using the universal bacterial primer pair 27F (5’-  
314 AGAGAGTTTGATCMTGGCTCAG-3’) and 338r (TGCTGCCTCCCGTAGGAGT). A 30 μL  
315 PCR reaction contained 15 μL 1×Dream Taq Master mix (Fermentas), 0.3 μmol L<sup>-1</sup> of each  
316 primer, and 2 μL of the DNA sample. PCR amplification was carried out on a CFX96  
317 thermocycler (Biorad) with 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s; 52 °C for 30  
318 s; and 72 °C for 180 s, and a final elongation step of 5 min at 72 °C. Successful PCR products

319 were re-amplified for 9 cycles with the same primer pair, but including adaptor A (5'–  
320 CCATCTCATCCCTGCGTGTCTCCGAC–3') and unique 10–12-bp-long barcodes at the  
321 beginning of the forward primer and P1\_338r (5'–CCTCTCTATGGGCAGTCGGTGAT  
322 TGCTGCCTCCCGTAGGAGT–3') as the reverse primer to allow Ion Torrent sequencing and  
323 assignment to specific samples. PCR products were cleaned using the Agencourt AMPure XP  
324 magnetic beads purification system (Beckman Coulter) and quantified using the Qubit dsDNA  
325 HS Assay Kit (Invitrogen). Amplicons were subsequently combined in equimolar concentrations  
326 for sequencing. The product was then seeded into an Ion PGM Template OT2 reaction following  
327 the manufacturer's instructions (Life Technologies). Templated beads were enriched using the  
328 Ion OneTouch ES system and sequencing libraries were loaded on an Ion 314 Chip and  
329 sequenced using the Ion PGM Sequencing 400 Kit. Sequences were analyzed using Mothur  
330 software packages [*Boyle and Edmond, 1977*]. Sequences shorter than 200 bp or which  
331 contained >2 ambiguities and a maximum of 8 homopolymers were removed. Unique sequences  
332 were identified and aligned using the Silva bacteria database. Loosely aligned and chimeric  
333 sequences were removed before taxonomic classification. After quality filtering, 174700 reads  
334 were obtained, with an average of 8735 reads per sample (min = 3804, max = 11201).  
335 Taxonomic assignment of the OTUs was carried out using the ribosomal database project (RDP)  
336 reference database (trainset 9\_032012) with a confidence threshold of 80%. The sequences were  
337 added to the European Nucleotide Archive (ENA) under submission number PRJEB8364.  
338 Similarities of the microbial communities were analyzed by cluster analysis using the Sorensen  
339 similarity index and the PC-ORD 6.0 software package (MjM Software Design).



## 340 2.4 Statistical analyses

341 The design of “DOM-Fe” experiment included three replicated incubations for each  
342 treatment, which allows to test differences between replicates. The differences were estimated  
343 using standard deviation (SD) and shown as standard error bars in Fig. 2b. The designs of other  
344 four experiments only included single incubation for each treatment, and therefore we were not  
345 able to test differences between true replicates. However, we calculated the coefficient of  
346 variation (CV) for each treatment of “DOM-Fe” experiment, which was used for estimating the  
347 SD of bacterial production determinations in “No Fe”, “Fe coating”, and “DOM-Fe low/high P”  
348 experiments.

349 The statistical differences between treatments were tested with paired t-test using two-tailed  
350 distributions, which was performed using Microsoft Excel 2013. The level of significance was  
351 set at  $p = 0.05$ .

## 352 **3 Results**

### 353 3.1 Effects of Fe on bacterial density

354 In the “Fe” experiment (Fig. 1, Tables 1–2), the introduction of Fe(III) (DOM+130Fe)  
355 significantly reduced the density of bacteria growing on DOM compared with the corresponding  
356 treatment without Fe (DOM alone; paired t-test,  $t = 4.47$ ,  $df = 9$ ,  $p = 0.0016$ ; Fig. 2a). In this  
357 experiment, Fe was potentially able to associate with DOM to form DOM-Fe, with GlyP to form  
358 P-Fe, and/or with bacteria to form bacteria-Fe (Fig. 1, Table 2), and any of these complexes  
359 could potentially have been responsible for the reduction in bacterial density (Fig. 2a).

#### 360 3.1.1 Effects of DOM-Fe on bacterial density

361 The “DOM-Fe” experiment was designed to generate DOM-Fe (Fig. 1, Table 1–2), which  
362 had no consistent negative effect on bacterial density (Fig. 2b). From day 4 to day 12, the

363 bacterial densities of the DOM-80Fe treatment were significantly higher than in the DOM alone  
364 treatment (paired t-test,  $t = -6.98$ ,  $df = 7$ ,  $p = 0.0002$ ). This experiment revealed that when Fe  
365 was associated with DOM, it did not reduce the bacterial density, but to some extent even  
366 stimulated bacterial growth.

367 The potential stimulatory role of DOM-Fe was further tested in the “DOM-Fe high/low P”  
368 experiment (Fig. 1, Tables 1–2), in which the growth of bacteria was consistently highest with a  
369 maximum density of  $13 \pm 0.7 \times 10^9$  cells  $L^{-1}$  in treatment #9 with DOM-Fe and  $21 \mu\text{mol } L^{-1}$   
370 GlyP (Fig. 3a). In the corresponding treatment #4 without Fe, the maximum density of bacteria  
371 was an order of magnitude lower ( $2.4 \pm 0.4 \times 10^9$  cells  $L^{-1}$ ) than in treatment #9 with DOM-Fe.  
372 These results indicate that DOM-Fe was able to stimulate bacterial growth when the  
373 concentration of GlyP was high (Figs 2 and 3a).

374 When the experiments were carried out with a low concentration of GlyP representative of  
375 dissolved P in Lake Valkea-Kotinen ( $0.16 \mu\text{mol } L^{-1}$  in #3 and #8), DOM-Fe reduced bacterial  
376 densities (max  $0.4 \pm 0.02 \times 10^9$  cells  $L^{-1}$ ; #8) to lower than one-third of those in the  
377 corresponding treatment without Fe (max  $1.5 \pm 0.3 \times 10^9$  cells  $L^{-1}$ ; #3, Fig. 3a). The bacterial  
378 growth was consistently higher in the high ( $21 \mu\text{mol } L^{-1}$ , #4 and #9) than in the low ( $0.16 \mu\text{mol}$   
379  $L^{-1}$ , #3 and #8) concentration of GlyP (Fig. 3a), indicating that a high concentration of GlyP  
380 stimulated the growth of bacteria.

381 Because bacterial densities were elevated in the high concentration of GlyP (treatments #9  
382 and #4; Fig. 3a), we tested whether bacteria can use GlyP as a carbon source (treatments #1 and  
383 #2, Fig. 1). Bacteria reached a density that was less than two orders of magnitude lower in ALW  
384 with  $0.16 \mu\text{mol } L^{-1}$  GlyP ( $0.06 \pm 0.01 \times 10^9$  cells  $L^{-1}$  in #1) or with  $21 \mu\text{mol } L^{-1}$  GlyP ( $0.02 \pm$   
385  $0.004 \times 10^9$  cells  $L^{-1}$  in #2) than in the corresponding treatments with DOM (#3 or #4,

386 respectively; Fig. 3b). These results indicate that GlyP or other salts in ALW (Table 1) were  
387 negligible sources of organic carbon for bacteria, and GlyP acted as a source of P.

### 388 3.1.2 Effect of Fe coating on bacterial density

389 When bacteria were coated with Fe (“Fe coating”, #5–#7; Fig. 1, Table 2), obvious  
390 precipitates were observed after a few hours of incubation. The planktonic bacterial density  
391 measured above the precipitates reached  $0.04 \pm 0.002 \times 10^9$  cells  $L^{-1}$  in treatment #5 with low P  
392 and  $0.19 \pm 0.009 \times 10^9$  cells  $L^{-1}$  in #7 with high P (Fig. 3b). No obvious increase in planktonic  
393 bacterial numbers was found in treatment #6 without DOM (Fig. 3). The results of the “Fe  
394 coating” experiments support the earlier findings: GlyP was not a source of carbon (#2, #6), but a  
395 source of P and stimulated the growth of bacteria on DOM (Figs 3a and 3b). When Fe associated  
396 with bacteria (#5 and #7), the bacterial densities were orders of magnitude lower than in the  
397 DOM-Fe treatments (#8 and #9, Fig. 3a and 3b).

398 These results demonstrate that the growth of bacteria was dependent on the type of Fe  
399 association. Fe associated with bacterial cells (Fe coating) or with P reduced bacterial densities,  
400 but DOM-Fe in the presence of high P even increased the bacterial densities (Figs 1–3).

### 401 3.2 Effects of Fe on bacterial respiration

402 We selected four treatments, namely #3 (DOM alone), #5 (Fe coating with DOM), #8  
403 (DOM-Fe with low P), and #9 (DOM-Fe with high P), to follow the cumulative bacterial  
404 respiration ( $\mu\text{mol C L}^{-1}$ ) during the incubations (Fig. 4). After a lag phase of several days, the  
405 cumulative bacterial respiration was eventually highest and lowest in treatments #9 and #5,  
406 respectively (Fig. 4), in agreement with the bacterial densities among the selected treatments  
407 (Fig. 3). In contrast to the bacterial densities (Fig. 3), the cumulative bacterial respiration was

408 higher on DOM with Fe (#8) than without Fe (#3; paired t-test,  $t = -52.6$ ,  $df = 1538$ ,  $p = 0$ ; Fig.  
409 4), indicating that Fe also changed the metabolic performance of bacteria.

### 410 3.3 Effects of Fe on BGE

411 The metabolic performance was estimated as the BGE (Table 3) by dividing the  
412 accumulated planktonic bacterial biomass (mol C; Fig. 3) by the estimated bacterial carbon  
413 demand. The latter was calculated as the sum of the planktonic bacterial biomass (mol C; Fig. 3)  
414 and bacterial respiration (mol C; Fig. 4), which also includes the potential respiration of bacteria  
415 in the precipitated Fe-oxyhydroxides and the potential oxidation of Fe(II) by O<sub>2</sub> in treatment #5  
416 (Fig. 4). The BGEs were highest in treatment #9 (DOM-Fe and 21  $\mu\text{mol L}^{-1}$  GlyP) followed by  
417 treatment #3 (DOM alone, Table 3). The BGE was very low in treatment #8, DOM-Fe with a  
418 low concentration of P, as well in treatment #5, in which bacteria were coated with Fe (Table 3).

### 419 3.4 Effects of Fe on the bacterial community composition

420 The bacterial inoculum primarily consisted of *Betaproteobacteria* (mean  $\pm$  SE, 67.0%  $\pm$   
421 1.4%) mostly affiliated to the genera *Duganella*, *Polynucleobacter*, and *Undibacterium*, and of  
422 *Alphaproteobacteria* (12.9%  $\pm$  0.3%), including the genus *Novosphingobium* (Figs 5 and 6,  
423 Table S1). In the treatments without DOM and no significant growth (treatments #1 and #2), or  
424 when Fe was coated on the bacterial surface (treatments #5–#7), these remained the major  
425 classes together with *Actinobacteria* (Table S1). If DOM was added without Fe (treatments #3  
426 and #4) or as DOM-Fe with high P (treatment #9), the community became dominated by  
427 *Alphaproteobacteria*, and especially by the genus *Caulobacter* (Figs 5 and 6, Table S1). In these  
428 treatments, the frequency of *Caulobacter*-associated sequences increased from 1% to 95% (Fig.  
429 6). In the “No Fe” experiment with DOM and high P (#4), *Caulobacter*-associated sequences  
430 (34% of sequences) were also accompanied by other *Alphaproteobacteria* (*Sphingomonas* and

431 *Bradyrhizobium*) and *Betaproteobacteria* (*Burkholderia* and *Sediminibacterium*) (Fig. 6 and  
432 Table S1). Altogether, the experimental treatments changed the initial composition of the  
433 bacterial community towards the dominance of *Caulobacter*, which was also primarily  
434 responsible for highest bacterial biomass in the DOM-Fe treatment with high P.

#### 435 **4 Discussion**

436 Our experimental results demonstrate that Fe has multiple and contrasting effects on  
437 bacterial growth, respiration, growth efficiency, and the composition of the bacterial community.  
438 Table 4 summarizes our main findings and outlines the division of the discussion into four  
439 sections.

440 4.1 Fe coating and the formation of particulate Fe(oxy)hydroxide reduces bacterial growth and  
441 the consumption of DOM

442 Many functional groups on the surface of bacteria, e.g., carboxylic, hydroxyl, and  
443 phosphoryl, can bind Fe [Beveridge and Murray, 1980; Ferris *et al.*, 1987; González *et al.*,  
444 2014]. At the beginning of our “Fe coating” experiments, the concentration of these binding sites  
445 was  $6.0 \times 10^{-7}$  mmol L<sup>-1</sup> when calculated from the initial bacterial biomass of 0.08 μmol C L<sup>-1</sup>  
446 and  $3.1 \times 10^{-4}$  moles of proton-donating surface sites per gram of cells reported by *Ha et al.*  
447 [2010]. The concentration of Fe used in the “Fe coating” experiment was eight orders of  
448 magnitude higher than the available binding sites on the surface of bacteria, and must have  
449 resulted in an extensive coating of the bacterial surface structures with Fe. The extensive  
450 occupation of the surface binding sites by Fe can be expected to impair the normal functioning of  
451 cell surfaces (e.g., the transport of solutes across cell membranes), which may partly explain the  
452 low growth and respiration in the experiments with Fe-coated bacteria.

453 In the Fe coating experiments of the present study, red-brown precipitates were observed  
454 after a few hours of incubation. These precipitates adsorbed and immobilized bacteria, because  
455 the initial densities of planktonic bacteria in the Fe coating treatments were only a fraction of  
456 those observed in the other treatments (Fig. 3). The growth of immobilized bacteria is expected  
457 to be limited, for example, because Fe(oxy)hydroxides effectively adsorb negatively charged  
458 ions such as DOM [Riedel *et al.*, 2012; Riedel *et al.*, 2013] and limit the substrate availability for  
459 bacteria trapped in Fe(oxy)hydroxides. In the environment, the regrowth of bacteria or an  
460 inflow/import of new bacteria likely compensates the immobilization of bacterioplankton into  
461 Fe(oxy)hydroxides. Therefore, in most environments, the immobilization of bacteria into  
462 Fe(III)(oxy)hydroxides has likely only low impact on the biodegradation of DOM.

463 The results of the Fe coating experiments additionally demonstrated that despite the  
464 extensive coating and immobilization of the bacterial inoculum at the beginning of experiments,  
465 some bacteria were able to grow as planktonic forms (treatments #7 in Fig. 3b). However, their  
466 densities did not reach the levels found in the other DOM treatments (treatments #3, #4, #8, and  
467 #9 in Fig. 3a), suggesting that Fe precipitates also reduced the availability of substrates for the  
468 planktonic bacteria. The precipitated Fe(oxy)hydroxides can adsorb DOM from the water  
469 column and convert it into a particulate form [Boyle and Edmond, 1977; Riedel *et al.*, 2012;  
470 Riedel *et al.*, 2013; Swenson *et al.*, 2015]. Such adsorption of OM by particulate  
471 Fe(oxy)hydroxides may lead to the long-term preservation of OM in marine [Lalonde *et al.*,  
472 2012] and freshwater sediments [Kortelainen *et al.*, 2004; Einola *et al.*, 2011].

473 It is also possible that in our “Fe coating” experiments, the Fe(oxy)hydroxides adsorbed a  
474 part of GlyP and reduced the availability of P to planktonic bacteria. The adsorption of P by  
475 Fe(oxy)hydroxides in oxic sediments is the primary mechanism limiting the bioavailability of P,

476 which regulates the overall productivity of lakes [Wetzel, 2001], because osmotrophic organisms  
477 such as bacteria or phytoplankton poorly utilize P bound to particulate Fe(oxy)hydroxides. Thus,  
478 the immobilization of P into solid Fe(oxy)hydroxides may reduce the bioavailability of P,  
479 bacterial growth, and the biodegradation of DOM.

#### 480 4.2 Fe associated with DOM binds P and reduces bacterial growth

481 In the present study, bacterial growth on DOM was significantly higher in the high  
482 concentration of P compared to the low P concentration, as earlier observed in our study lake  
483 [Vähätalo *et al.*, 2003]. Bacteria from Lake Valkea-Kotinen reached a higher biomass in  
484 hypolimnetic water with an elevated concentration of P than in epilimnetic water depleted in P  
485 [Vähätalo *et al.*, 2003]. The availability of P has also been observed to limit the growth of  
486 bacterioplankton in other lakes with a high concentration of DOM [Karlsson *et al.*, 2001; Vidal  
487 *et al.*, 2011]. Bacterioplankton requires high amounts of P in its biomass, with a typical C-to-P  
488 ratio of 45 [Goldman *et al.*, 1987]. According to this ratio, the low concentration of P ( $0.16 \mu\text{mol}$   
489  $\text{L}^{-1}$ ) can support  $7.2 \mu\text{mol C L}^{-1}$  of bacterial biomass, which is close to the observed biomass of  
490 bacteria ( $3.6 \mu\text{mol C L}^{-1}$ ) grown on DOM in the low P treatment (treatment #3 in Fig. 3), but  
491 lower than  $33 \mu\text{mol C L}^{-1}$  found in the high P treatment #9 (Fig. 3). The results from the present  
492 study and earlier investigations, as well as the stoichiometric calculations, indicate that the  
493 maximum bacterial biomass in this study was limited by the availability of P in the treatments in  
494 which an environmentally relevant low concentration of P was used.

495 In our study, DOM-Fe reduced the growth of bacteria when the concentration of P was low.  
496 Fe(III) associated with DOM can bind phosphate to form DOM-Fe(III)-P associations [Francko  
497 and Heath, 1982; De Haan *et al.*, 1990; Sundman *et al.*, 2016]. The photochemical release of P  
498 from the DOM of our study lake indicates that DOM-Fe(III)-P are present in Lake Valkea-

499 Kotinen and can bind ca. 0.03–0.05  $\mu\text{mol P L}^{-1}$  [Vähätalo *et al.*, 2003]. In the present study, we  
500 selected organic phosphate (GlyP) as a source of P to avoid the strong complexation between  
501 Fe(III) and inorganic phosphate. In GlyP, one oxygen atom of phosphate forms an ester bond  
502 with glycerol, but three other oxygen atoms of phosphate can potentially form a coordination  
503 bond with Fe(III). Thus, it is likely that 60  $\mu\text{mol L}^{-1}$  DOM-Fe(III) bound a part of 0.16  $\mu\text{mol L}^{-1}$   
504 GlyP into DOM-Fe(III)-P-Gly associations, where P and Gly refer to phosphate and glycerol  
505 moieties of GlyP, respectively. The formation of DOM-Fe(III)-P-Gly can be expected to reduce  
506 the availability of P, which was probably the primary reason for the reduced bacterial growth in  
507 the treatments, in which the availability of P already limited the growth of bacteria without  
508 introduced Fe(III). Fe has been shown to play a key role in regulating the availability of P in both  
509 sediment and soils [Ekholm and Lehtoranta, 2011; Heiberg *et al.*, 2012; Baken *et al.*, 2015], but  
510 our results indicate that DOM associated species of Fe can also regulate the availability of P to  
511 bacteria and reduce the biodegradation of DOM in the water column.

512 Despite the suspected reduced bioavailability of P due to DOM-Fe(III)-P-Gly, bacteria did  
513 grow on DOM, but their growth was delayed and associated with a marked consumption of O<sub>2</sub>  
514 (treatment #8 versus #3 in Figs 3 and 4). These results indicate that bacteria were able to  
515 assimilate P (and DOM) from DOM-Fe(III)-P-Gly, but at an additional metabolic cost. The  
516 photochemical and microbial reduction of Fe(III) in DOM-Fe(III)-P can break apart DOM, Fe(II)  
517 and P [Francko and Heath, 1982; Cotner and Heath, 1990; Schröder *et al.*, 2003]). Therefore, it  
518 is possible that microbes in our study retrieved P by reducing the Fe(III) in DOM-Fe(III)-P-Gly.

#### 519 4.3 DOM-Fe(III) is bioavailable

520 According to our study, the association of Fe(III) with DOM does not reduce microbial  
521 growth on DOM compared to DOM without Fe when P is not limiting microbial growth. In



522 agreement with our results, aerobic microbes such as *Pseudomonas*, a community of microbes  
523 from soil or activated sludge, can degrade bidentate (or non-specified) complexes between citrate  
524 and Fe(III) with similar or reduced rates compared to uncomplexed citric acid [Boudot *et al.*,  
525 1989; Francis and Dodge, 1993; Nancharaiah *et al.*, 2006]. These findings also apply to fulvic  
526 acid associated with Fe(III) [Boudot *et al.*, 1989]. The biodegradation rates of citrate or fulvic  
527 acids markedly decrease when Fe(III) is introduced at amounts exceeding the binding capacity of  
528 organic ligands and the substrates for microbes are adsorbed or immobilized into solid  
529 Fe(oxy)hydroxides ([Boudot *et al.*, 1989]; treatments #5, #6, and #7 of the present study). Our  
530 study, together with others [Boudot *et al.*, 1989; Francis and Dodge, 1993; Nancharaiah *et al.*,  
531 2006], indicates that the association of Fe with DOM does not reduce the bioavailability of DOM  
532 as long as DOM is not precipitated and adsorbed into insoluble metal (oxy)hydroxides.

533       Although some bacteria can take up a few specific organic Fe complexes (siderophore-Fe  
534 and heme-Fe [Ma *et al.*, 2009]), little is known about the mechanism for the uptake of organic  
535 moiety from DOM-Fe(III). When Fe is bound on a microbial substrate, the uptake of substrate  
536 with Fe is expected to be blocked or reduced, because the Fe atom is nearly two times larger than  
537 those of carbon, oxygen, and nitrogen, the common atoms in bacterial substrates [Pyykkö, 2015].  
538 Therefore, Fe is likely extracellularly removed from a DOM-Fe(III) prior to the transport of the  
539 DOM substrate into the cytoplasm. Extracellular reduction of DOM-associated Fe(III) followed  
540 by the release of Fe(II) is a mechanism for Fe acquisition in cyanobacteria (e.g., *Synechocystis*  
541 *sp.*) [Kranzler *et al.*, 2011; Lis *et al.*, 2015]. This mechanism can be also used for the uptake of  
542 DOM substrate from DOM-Fe(III). Outer-membrane *c*-type cytochromes and pilin (in bacteria  
543 that lack *c*-cytochromes) are crucial for transferring electrons to extracellular electron acceptors,  
544 such as Fe(III) oxides, in soils and sediments [Richardson, 2000; Reguera *et al.*, 2005]. In

545 cyanobacteria, pilin (e.g., PilA1) facilitates the donation of electrons to external electron  
546 acceptors, such as DOM-Fe(III) and Fe oxides [*Lamb et al.*, 2014]. Pili may also have  
547 contributed to the bacterial consumption of DOM-Fe in our experiments, because *Caulobacter*  
548 can have pili [*Skerker and Shapiro*, 2000].

#### 549 4.4 DOM-Fe(III) stimulates the growth of bacteria

550 In some treatments of our study with a high concentration of Fe, the association of Fe(III)  
551 with DOM increased microbial growth, respiration, and growth efficiency on DOM. This  
552 increase may result from purely abiotic Fe-catalyzed reactions [*Pracht et al.*, 2001; *Studenroth et*  
553 *al.*, 2013; *Comba et al.*, 2015] and/or involve the active metabolism of microbes, as described  
554 earlier for fungi [*Arantes et al.*, 2012; *Rineau et al.*, 2012]. Abiotic reactions may already have  
555 broken quinone-type parts of our DOM into small organic acids [*Studenroth et al.*, 2013; *Comba*  
556 *et al.*, 2015] during the preparation of DOM-Fe under acidic starting conditions. This was  
557 possibly seen as elevated O<sub>2</sub> consumption during the first four days of incubation in the Fe(III)  
558 supplied treatments compared to the DOM alone treatment (Fig. 4). Therefore, it is possible that  
559 the abiotic formation of oxalic, maleic, fumaric, and malonic acids found earlier in soils  
560 [*Studenroth et al.*, 2013] may also take place in an aquatic environment rich in iron and the  
561 humic type of DOM.

562 A steep increase in O<sub>2</sub> consumption by bacteria growing on DOM-Fe with a high  
563 concentration of P after 12 days of incubation (Fig. 4) was not likely supported by the abiotic  
564 formation of bioavailable carbon during the formation of DOM-Fe, but rather indicates the active  
565 metabolism of bacteria to obtain carbon from DOM-Fe. In brown rot fungi, the utilization of Fe  
566 in the biochemical Fenton reaction requires large investments in the form of organic compounds  
567 or/and enzymes secreted in wood [*Arantes et al.*, 2012]. Brown rot fungi first secrete small

568 molecular mass organic acids (e.g., oxalic acid) to acidify the external milieu next to hyphae and  
569 solubilize Fe(III), and then also reduced quinones such as 2,3-dimethoxyhydroquinone to reduce  
570 Fe(III) to Fe(II) for the biochemical Fenton reaction [Arantes *et al.*, 2012]. The biochemical  
571 Fenton reaction is profitable, as it facilitates access to cellulose, the primary carbon source of  
572 fungi in wood. It is notable that the organic carbon content per unit volume is four orders of  
573 magnitude higher than in a typical solution of DOM in freshwater. The active metabolism of  
574 bacterioplankton for the utilization of DOM-Fe is probably different, simpler, and less costly  
575 than the biochemical Fenton used by brown rot fungi.

576 Bacteria may produce superoxide to utilize DOM-Fe. Numerous bacterial phyla, such as  
577 *Alphaproteobacteria*, including *Caulobacter*, can mediate single-electron transfer from their  
578 intracellular metabolites such as NAD(P)H to dioxygen at their cell surface and thus generate  
579 superoxide [Rose, 2012; Diaz *et al.*, 2013]. In circumneutral waters, the half-life of superoxide  
580 ranges from tens of seconds to hours [Rose, 2012], which allows it to diffuse away from the cells  
581 into the external milieu to reach DOM-Fe within the 50  $\mu\text{m}$  to 500  $\mu\text{m}$  range [Fenchel, 2002].  
582 Superoxide can reduce DOM-associated Fe(III) to Fe(II), which can be oxidized back to Fe(III)  
583 primarily by  $\text{O}_2$  [Fujii *et al.*, 2008]. The oxidation of Fe(II) converts  $\text{O}_2$  to superoxide, which can  
584 be further converted to  $\text{H}_2\text{O}_2$  [Fujii *et al.*, 2008].  $\text{H}_2\text{O}_2$  can react with Fe(II) and produce highly  
585 reactive hydroxyl radicals (Fenton reaction, [Rose, 2012]). These hydroxyl radicals can  
586 transform DOM into bioavailable substrates [Goldstone *et al.*, 2002]. As the generation of  $\text{H}_2\text{O}_2$   
587 requires the acidic form of superoxide,  $\text{HOO}^\bullet$  ( $\text{pK}_a = 4.8$ , [Rose, 2012]), the formation of  $\text{H}_2\text{O}_2$   
588 was presumably not as effective in our experiments (pH 7) as it can be in acidic conditions.

589 It is possible that oxidation-reduction reactions of Fe associated with DOM and initiated by  
590 microbial superoxide lead to the breakdown of organic matter through mechanisms different

591 from the Fenton chemistry. For example, Fe catalyzes the breakage of the aromatic ring of  
592 catechol or related derivatives in dioxygenase enzymes, which are also found in *Caulobacter*  
593 [Orville *et al.*, 1997; Bugg and Winfield, 1998]. However, dioxygenase enzymes are located in  
594 the cytoplasm [Arras *et al.*, 1998], which is an unlikely site for the enzymatic cleavage of DOM-  
595 Fe(III). The same cleavage reaction can also take place without enzymes through a Fe(III)-  
596 semiquinone-superoxide complex [Bugg and Winfield, 1998]. Fe(III) forms complexes  
597 preferentially with aromatic moieties of DOM [Fujii *et al.*, 2014], indicating the close  
598 association of Fe(III) with the quinonoid structures in DOM. When such complexes react with  
599 superoxide, the oxidation of DOM and breakage of the quinonoid ring catalyzed by Fe(III) may  
600 take place [Bugg and Winfield, 1998].

601 The electron donating (or accepting) capacity linked to quinonoid structures is about 0.6  
602  $\mu\text{mol} [\text{mg}]^{-1}$  in Nordic Lake DOM [Aeschbacher *et al.*, 2012]. Assuming the same capacity for  
603 our DOM, the concentration of electron donating (or accepting) group was  $12 \mu\text{mol L}^{-1}$  in our  
604 experiments. The related concentration of quinonoid structures can be estimated as  $6 \mu\text{mol L}^{-1}$   
605 assuming two electron donating (or accepting) sites for each quinonoid structure. A complete  
606 breakage of quinonoid structures into bioavailable low molecular weight aliphatic (carbonyl)  
607 compounds can release  $36 \mu\text{mol L}^{-1}$  carbon, accounting for 6 carbons per quinonoid structure.  
608 This could explain the enhanced microbial metabolism of *Caulobacter* in treatment #9. It is  
609 notable that quinonoid structures constitute only a small part of the total aromatic content of  
610 humic substances, which is, however, a potential source of new quinonoids through many types  
611 of oxidative reactions [Aeschbacher *et al.*, 2012].

## 612 4.5 Environmental relevance and conclusions

613 The processes examined in this study, the associations of Fe with DOM, P, or cell surfaces  
614 and the precipitation of Fe into insoluble forms, are common processes in numerous  
615 environments (see *Xiao et al.* [2013]). The gradients of pH, redox potential and ionic strength in  
616 soils and sediments as well as aquatic systems in their vicinity are the hotspots for the formation  
617 of DOM-Fe [*Boyle and Edmond*, 1977; *Riedel et al.*, 2012 and 2013; *Neubauer et al.*, 2013b;  
618 *Xiao et al.*, 2013]. If in these instances the loading of Fe exceeds the binding capacity of DOM,  
619 insoluble precipitates of Fe(III)(oxy)hydroxides will form [*Nierop et al.*, 2002]. When the  
620 precipitates of Fe(III)(oxy)hydroxides are associated with DOM and/or P [*Blomqvist et al.*, 2004;  
621 *Helms et al.*, 2013; *Angelico et al.*, 2014], they direct the bioavailable forms of OM and P into  
622 poorly bioavailable particulate forms and decrease the overall biodegradation of OM ([*Boudot et*  
623 *al.*, 1989], this study). Earlier studies indicate that this decrease in biodegradation can be so  
624 extensive, that it leads into a long term preservation of OM [*Kortelainen et al.*, 2004; *Einola et*  
625 *al.*, 2011; *Lalonde et al.*, 2012]. Thus, Fe has a clear negative impact on biodegradation of DOM  
626 when it converts dissolved and bioavailable forms of DOM and P into poorly bioavailable  
627 particulate forms associated to Fe(III)(oxy)hydroxides.

628 Although Fe associated to DOM can be expected to reduce the availability of organic  
629 component in DOM-Fe, this seems not to be the case [*Boudot et al.*, 1989; *Francis and Dodge*,  
630 1993; *Nancharaiah et al.*, 2006; this study]. Even the microbial mechanisms for removing Fe  
631 from DOM-Fe are poorly known, it is good to remember that DOM-Fe has existed in the Earth  
632 as long as there has been organic matter and posed a challenge to microbial evolution since the  
633 origin of life. Our study suggests that the association of Fe with DOM can increase the  
634 biodegradation of DOM by bacterioplankton. The mechanism for this increase is likely different

635 than described earlier for fungi growing on solid substrates [Arantes *et al.*, 2012; Rineau *et al.*,  
636 2012], and may involve abiotic and/or biochemical redox reactions of Fe [Pracht *et al.*, 2001;  
637 Studenroth *et al.*, 2013; Comba *et al.*, 2015]. In our study, a single genus *Caulobacter* dominated  
638 the bacterial communities when the biodegradation of DOM was stimulated by Fe. It is possible  
639 that the active metabolisms of *Caulobacter* (e.g., superoxide produced by extracellular  
640 oxidoreductases [Diaz *et al.*, 2013] can promote extracellular non-selective degradation of  
641 humic-like DOM. Such mechanism would have a high environmental relevance, since humic-  
642 like DOM dominates the pool of DOM in many soils, sediments, fresh and coastal waters. Due to  
643 its heterogenous composition, the extracellular enzymatic hydrolysis of humic-like DOM is poor  
644 [Arnosti, 2004] but its intense absorption of solar radiation makes it sensitive for photochemical  
645 degradation [Vähätalo *et al.*, 2000]. Photodegradation takes place only on sunlit solid surfaces  
646 [Vähätalo *et al.*, 1998] or in a shallow stratum of surface waters [Salonen and Vähätalo, 1994;  
647 Vähätalo *et al.*, 2000]. Fe-stimulated biodegradation of DOM can target also such humic-like  
648 DOM that remains below the sunlit surfaces in the dark. In aquatic environments, Fe-stimulated  
649 biodegradation of DOM is expected to be most intensive at sites with high concentration of  
650 DOM.-Fe. Such sites include low-order humic-rich streams and lakes with low concentrations of  
651  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  hydrologically closely connected to soils and sediments that act as sources of Fe.

652 The selective removal of Fe from water column with increasing residence time of water  
653 [Köhler *et al.*, 2013; Weyhenmeyer *et al.*, 2014] decreases the contribution of Fe to the  
654 biodegradation of DOM. The additional selective removal of Fe in marine waters [Sholkovitz,  
655 1976] reduces the concentration of Fe to very low (nM) level [Boyd and Ellwood, 2010], which  
656 contrasts to the orders of magnitude higher concentrations in freshwaters (e.g., a mean of 29  $\mu\text{M}$   
657 in Finnish rivers, [Xiao *et al.*, 2015]). In the deep dark ocean, the turnover time of chromophoric

658 DOM (a tracer of Fe-poor humic-like DOM) is 634 years [Catalá *et al.*, 2015], while the  
659 turnover time for bulk organic carbon (dominated by humic-like and Fe-rich DOM) in Swedish  
660 lakes is 2.5 years [Algesten *et al.*, 2003; Weyhenmeyer *et al.*, 2014]. These turnover times are not  
661 directly comparable, because the turnover time of OC in Swedish lakes includes sedimentation  
662 and photodegradation although the latter explains <10% of OC loss [Koehler *et al.*, 2014]. The  
663 comparison nevertheless indicates that the biodegradation rates of humic-like DOM are  
664 considerably faster in Fe-rich freshwaters than in Fe-poor marine waters [Algesten *et al.*, 2003;  
665 Catalá *et al.*, 2015]. Our study suggests that Fe associated with DOM can stimulate the  
666 biodegradation of humic-like DOM in Fe-rich freshwaters.

667

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678 Nucleotide Archive (ENA) under submission number PRJEB8364.

679 **Tables**

680 Table 1. Composition of inorganic ions in the bioassays.

Chemical	Final concentration ( $\mu\text{mol L}^{-1}$ )
<i>Artificial lake water (ALW)</i>	
NaCl	699
Na <sub>2</sub> SO <sub>4</sub>	48.2
KCl	15.5
NaHCO <sub>3</sub>	3.99
KBr	1.41
H <sub>3</sub> BO <sub>3</sub>	0.72
NaF	0.12
MgCl <sub>2</sub> ·6H <sub>2</sub> O	4.17
CaCl <sub>2</sub> ·2H <sub>2</sub> O	4.17
<i>Nutrient</i>	
NH <sub>4</sub> Cl	208

681



682 Table 2. The concentrations of DOM, Fe, and GlyP in each treatment of the experiments. All  
 683 treatments included ALW (Table 1) and bacterial inoculum introduced in the sequence shown in  
 684 Fig. 1.

“Experiment” with treatments	DOM ( $\mu\text{mol C L}^{-1}$ )	Fe ( $\mu\text{mol L}^{-1}$ )	GlyP ( $\mu\text{mol L}^{-1}$ )
<b>“Fe”</b>			
DOM alone	948	(0.42)*	21
DOM+130Fe	948	130	21
<b>“DOM-Fe”</b>			
DOM alone	948	(0.42)*	21
DOM-20Fe	948	20	21
DOM-80Fe	948	80	21
<b>“No Fe”</b>			
#1 No DOM+low P	–	(0.25)*	0.16
#2 No DOM+high P	–	(0.25)*	21
#3 DOM+low P	948	(0.42)*	0.16
#4 DOM+high P	948	(0.42)*	21
<b>“Fe coating”</b>			
#5 (Fe coating)+DOM+low P	948	60	0.16
#6 (Fe coating)+No DOM+high P	–	60	21
#7 (Fe coating)+DOM+high P	948	60	21
<b>“DOM-Fe high/low P”</b>			
#8 (DOM-Fe)+low P	948	60	0.16
#9 (DOM-Fe)+high P	948	60	21

685 –, no addition of extracted DOM.

686 \* Fe concentrations in parentheses indicate no addition of Fe, and the Fe is from the introduced original bacterial  
 687 inoculum ( $0.25 \mu\text{mol L}^{-1}$ ) and extracted DOM ( $0.17 \mu\text{mol L}^{-1}$ ). “Fe” and “DOM-Fe” experiments were carried out  
 688 consecutively, but “No Fe”, “Fe coating” and “DOM-Fe high/low P” experiments were conducted simultaneously.

689 Table 3. Bacterial growth efficiencies (BGE) determined for days 7 and 11 of treatments #3, #5,  
 690 #8, and #9. Determinations used the bacterial biomass (Fig. 3) and accumulated cumulative  
 691 bacterial respiration (Fig. 4).

Time	Treatments			
	#3 DOM+low P	#5† (Fe coating)+DOM+low P	#8 (DOM-Fe)+low P	#9 (DOM-Fe)+high P
7 d	21.1%	0.05%	-0.02%*	63.4%
11 d	17.5%	0.38%	0.26%	36.8%

692 \* A negative BGE refers to a decline in the bacterial biomass during the bioassay.

693 † The biomass of bacteria associated with precipitates is not included in BP and thus BGE calculated as  
 694  $BP/(BP+BR)$  is possibly underestimated.

695 Table 4. The conclusions based on our results and references supporting them.

	Conclusions	Supporting references
1	Fe coating and the formation of particulate Fe(oxy)hydroxide reduce bacterial growth and the consumption of DOM ( <b>section 4.1</b> ).	[Boyle and Edmond, 1977; Riedel et al., 2012; Riedel et al., 2013; González et al., 2014]
2	Fe associated with DOM binds P and limits bacterial growth ( <b>section 4.2</b> ).	[Francko and Heath, 1982; De Haan et al., 1990; Karlsson et al., 2001; Vähätalo et al., 2003; Vidal et al., 2011; Sundman et al., 2016]
3	DOM-Fe is bioavailable ( <b>section 4.3</b> ).	[Boudot et al., 1989; Francis and Dodge, 1993; Nancharaiah et al., 2006]
4	Fe associated with DOM can stimulate bacterial growth and the biodegradation of DOM ( <b>section 4.4</b> ).	Abiotic [Pracht et al., 2001; Studenroth et al., 2013; Comba et al., 2015]; biochemical Fenton [Arantes et al., 2012; Rineau et al., 2012]; superoxide mediated [Rose, 2012; Diaz et al., 2013]; enzyme-like reactions [Bugg and Winfield, 1998]

696

697 **Figures captions**

698 Figure 1. The preparation of experiments to address the study questions. The preparation of  
699 experiments shows the sequence of introduction of solutes and bacteria to acidified (pH 2) Milli-  
700 Q water during the titration (with NaOH). Blue and orange colors indicate that “Fe” and “DOM-  
701 Fe” experiments were carried out consecutively. The green color indicates that “No Fe”, “Fe  
702 coating” and “DOM-Fe high/low P” were carried out simultaneously. Nutrients refer to the  
703 solutions of NH<sub>4</sub>Cl and glycerophosphate (GlyP; Tables 1–2). ALW is the solution of inorganic  
704 ions (Table 1). In the experiments marked with \*, some treatments did not receive Fe(III) or  
705 DOM (Table 2).

706 Figure 2. Bacterial density and biomass in the “Fe” (a) and “DOM-Fe” (b) experiments. (a) The  
707 “Fe” experiment included two treatments: DOM alone and DOM with 130 μmol L<sup>-1</sup> Fe. (b) The  
708 “DOM-Fe” experiment included three treatments: DOM alone, DOM-20Fe (DOM with 20 μmol  
709 L<sup>-1</sup> Fe), and DOM-80Fe (DOM with 80 μmol L<sup>-1</sup> Fe). Standard error bars in panel b were  
710 calculated from three replicated incubations. The experimental design is presented in Fig. 1 and  
711 Tables 1–2.

712 Figure 3. Bacterial density and biomass in (a) the “DOM-Fe low/high P” and (b) “Fe coating”  
713 experiments with their corresponding “No Fe” controls. Panel (a) shows the experiments (#3–4,  
714 8–9) that addressed the impact of P and the association of Fe with DOM on the bacterial growth.  
715 Panel (b) shows the experiments that addressed bacterial growth when coated with Fe (#5–7) or  
716 when grown in artificial lake water without DOM and Fe (#1–2). For clarity, the panels have  
717 different scales. Standard error bars represent the typical variability of three replicated  
718 incubations in the “DOM-Fe” experiment (Fig. 2). The experimental design for the treatments is  
719 presented in Fig. 1 and Tables 1–2.

720 Figure 4. Cumulative bacterial respiration during treatments #3, #5, #8, and #9 explained in  
721 Table 2 and Fig. 1. Milli-Q represents the blank and shows apparent respiration in ion-exchanged  
722 water without introduced bacteria.

723 Figure 5. Dendrogram of Sorensen cluster analysis and the log-transformed percentage of  
724 sequences (range 0–98%) assigned at the subclass level in the original inoculum (triplicates) and  
725 on day 28 of treatments #1–#9 (in duplicate, except #9). The average standard error for duplicate  
726 (or triplicate) samples was 1.2% when calculated for the class *Alphaproteobacteria*. The  
727 percentage of the sequences in main bacterial phyla and genus-level assignments are shown in  
728 supplementary data Table S1. Treatments #1–#9 are explained in Fig. 1 and Table 2.

729 Figure 6. Taxonomic classification of the 16S rRNA gene sequences in the original inoculum  
730 and treatments #3, #4, #8, and #9 on day 28. Genus-level percentage assignments of sequences  
731 (Table S1) are shown in each pie. *Caulobacter* (red slice) represented <1% of bacterial  
732 community in both original inoculum and #8, but dominated in treatments #3, #4, and #9 after  
733 incubation. The pie area of treatments #3, #4, #8, and #9 was plotted according to the cube root  
734 of the bacterial biomass (BP). The treatments are explained in Fig. 1 and Table 2. Results are  
735 shown in genus level in Table S1.

736

737 **References**

- 738 Aeschbacher, M., C. Graf, R. P. Schwarzenbach, and M. Sander (2012), Antioxidant properties  
739 of humic substances, *Environ. Sci. Technol.*, *46*, 4916-4925.
- 740 Algesten, G., S. Sobek, A.-K. Bergström, A. Ågren, L. J. Tranvik, and M. Jansson (2003), Role  
741 of lakes for organic carbon cycling in boreal zone, *Global Change Biol.*, *10*, 141-147, doi:  
742 10.1046/j.1529-8817.2003.00721.x.
- 743 Angelico, R., A. Ceglie, J.-Z., He, Y.-R. Liu, G. Palumbo, and C. Colombo (2014), Particle size,  
744 charge and colloidal stability of humic acids coprecipitated with Ferrihydrite, *Chemosphere*,  
745 *99*, 239-247, doi: 10.1016/j.chemosphere.2013.10.092.
- 746 Arantes, V., J. Jellison, and B. Goodell (2012), Peculiarities of brown-rot fungi and biochemical  
747 Fenton reaction with regard to their potential as a model for bioprocessing biomass, *Appl.*  
748 *Microbiol. Biotechnol.*, *94*(2), 323-338, doi: 10.1007/s00253-012-3954-y.
- 749 Arnosti, C. (2004), Speed bumps and barricades in the carbon cycle: substrate structural effects  
750 on carbon cycling, *Mar. Chem.*, *92*, 263-273, doi:10.1016/j.marchem.2004.06.030.
- 751 Arras, T., J. Schirawski, and G. Unden (1998), Availability of O<sub>2</sub> as a substrate in the cytoplasm  
752 of bacteria under aerobic and microaerobic conditions, *J. Bacteriol.*, *180*(8), 2133-2136.
- 753 Arvola, L., M. Rask, J. Ruuhijarvi, T. Tulonen, J. Vuorenmaa, T. Ruoho-Airola, and J. Tulonen  
754 (2010), Long-term patterns in pH and colour in small acidic boreal lakes of varying  
755 hydrological and landscape settings, *Biogeochemistry*, *101*(1-3), 269-279,  
756 doi:10.1007/s10533-010-9473-y.
- 757 Baken, S., M. Verbeeck, D. Verheyen, J. Diels, and E. Smolders (2015), Phosphorus losses from  
758 agricultural land to natural waters are reduced by immobilization in iron-rich sediments of  
759 drainage ditches, *Water Res.*, *71*, 160-170, doi:10.1016/j.watres.2015.01.008.
- 760 Baldock, J. A., and J. O. Skjemstad (2000), Role of the soil matrix and minerals in protecting  
761 natural organic materials against biological attack, *Org. Geochem.*, *31*(7-8), 697-710,  
762 doi:10.1016/S0146-6380(00)00049-8.
- 763 Benner, R., and M. Strom (1993), A critical evaluation of the analytical blank associated with  
764 DOC measurements by high-temperature catalytic oxidation, *Mar. Chem.*, *41*(1-3): 153-160,  
765 doi: 10.1016/0304-4203(93)90113-3.
- 766 Bennett, S. A., B. M. Toner, R. Barco, and K. J. Edwards (2014), Carbon adsorption onto FE  
767 oxyhydroxide stalks produced by a lithotrophic iron-oxidizing bacteria, *Geobiology*, *12*,  
768 146-156, doi: 10.1111/gbi.12074.
- 769 Beveridge, T. J., and R. G. E. Murray (1980), Sites of metal deposition in the cell wall of  
770 *Bacillus subtilis*, *J. Bacteriol.*, *141*(2), 876-887.
- 771 Blomqvist, S., A. Gunnars, and R. Elmgren (2004), Why the limiting nutrient differs between  
772 temperate coastal seas and freshwater lakes: A matter of salt, *Limnol. Oceanogr.*, *49*(6),  
773 2236-2241.
- 774 Boudot, J. P., A. B. H. Brahim, R. Steiman, and F. Seiglemurandi (1989), Biodegradation of  
775 synthetic organo-metallic complexes of iron and aluminium with selected metal to carbon  
776 ratios, *Soil Biol. Biochem.*, *21*(7), 961-966, doi:10.1016/0038-0717(89)90088-6.
- 777 Boyd, P. W., and M. J. Ellwood (2010), The biogeochemical cycle of iron in the ocean, *Nat.*  
778 *Geosci.*, *3*, 675-682, doi:10.1038/ngeo964.
- 779 Boyle, E. A., and J. M. Edmond (1977), The mechanism of iron removal in estuaries, *Geochim.*  
780 *Cosmochim. Acta*, *41*, 1313-1324.

- 781 Bugg, T. D. H., and C. J. Winfield (1998), Enzymatic cleavage of aromatic rings: mechanistic  
 782 aspects of the catechol dioxygenases and later enzymes of bacterial oxidative cleavage  
 783 pathways, *Natural Product Reports*, *15*(5), 513-530.
- 784 Catalá, T. S.. et al. (2015), Water mass age and aging driving chromophoric dissolved organic  
 785 matter in the dark global ocean, *Global Biogeochem. Cycles*, *29*, 917–934,  
 786 doi:10.1002/2014GB005048.
- 787 Chan, C. S., S. C. Fakra, D. Emerson, E. J. Fleming, and K. J. Edwards (2011), Lithotrophic  
 788 iron-oxidizing bacteria produce organic stalks to control mineral growth: implications for  
 789 biosignature formation, *ISME Journal*, *5*, 717-727, doi: 10.1038/ismej.2010.173.
- 790 Comba, P., M. Kerscher, T., Krause, and H. F. Schloer (2015), Iron-catalysed oxidation and  
 791 halogenation of organic matter in nature, *Environ. Chem.*, *12*(4), 381-395, doi:  
 792 10.1071/EN14240.
- 793 Cory, R. M., C. P. Ward, B. C. Crump, and G. W. Kling (2014), Sunlight controls water column  
 794 processing of carbon in arctic fresh waters, *Science*, *345*(6199), 925-928,  
 795 doi:10.1126/science.1253119.
- 796 Cotner, J. B., and R. T. Heath (1990), Iron redox effects on photosensitive phosphorus release  
 797 from dissolved humic materials, *Limnol. Oceanogr.*, *35*(5), 1175-1181.
- 798 Cotta, M.A., 1992. Interaction of ruminal bacteria in the production and utilization of  
 799 maltooligosaccharides from starch. *Appl. Environ. Microbiol.*, *58*(1), 48-54.
- 800 De Haan, H., R. I. Jones, and K. Salonen (1990), Abiotic transformations of iron and phosphate  
 801 in humic lake water revealed by double-isotope labeling and gel filtration,  
 802 *Limnol.Oceanogr.*, *35*(2), 491-497.
- 803 Del Giorgio, P. A., and J. J. Cole (1998), Bacterial growth efficiency in natural aquatic systems,  
 804 *Annu. Rev. Ecol. Syst.*, *29*, 503-541, doi:10.1146/annurev.ecolsys.29.1.503.
- 805 Diaz, J. M., C. M. Hansel, B. M. Voelker, C. M. Mendes, P. F. Andeer, and T. Zhang (2013),  
 806 Widespread Production of Extracellular Superoxide by Heterotrophic Bacteria, *Science*,  
 807 *340*(6137), 1223-1226, doi:10.1126/science.1237331.
- 808 Dittmar, T., B. Koch, N. Hertkorn, and G. Kattner (2008), A simple and efficient method for the  
 809 solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater, *Limnol.*  
 810 *Oceanogr.-Meth.*, *6*, 230-235.
- 811 Einola, E., M. Rantakari, P. Kankaala, P. Kortelainen, A. Ojala, H. Pajunen, S. Makela, and L.  
 812 Arvola (2011), Carbon pools and fluxes in a chain of five boreal lakes: A dry and wet year  
 813 comparison, *J.Geophys. Res.-Biogeo.*, *116*, doi:10.1029/2010jg001636.
- 814 Ekholm, P. and Lehtoranta, J. (2011), Does control of soil erosion inhibit aquatic eutrophication?  
 815 *J. Environ. Manage.*, *93* (1), 140-146.
- 816 Fenchel, T. (2002), Microbial behavior in a heterogeneous world, *Science*, *296*(5570), 1068-1071,  
 817 doi: 10.1126/science.1070118.
- 818 Fein, J. B., C. J. Daughney, N. Yee, and T. A. Davis (1997), A chemical equilibrium model for  
 819 metal adsorption onto bacterial surfaces, *Geochim. Cosmochim. Acta*, *61*(16), 3319-3328,  
 820 doi:10.1016/S0016-7037(97)00166-X.
- 821 Ferris, F. G., W. S. Fyfe, and T. J. Beveridge (1987), Bacteria as nucleation sites for authigenic  
 822 minerals in a metal-contaminated lake sediment, *Chem. Geol.*, *63*(3-4), 225-232,  
 823 doi:10.1016/0009-2541(87)90165-3.
- 824 Francis, A. J., and C. J. Dodge (1993), Influence of Complex Structure on the Biodegradation of  
 825 Iron-Citrate Complexes, *Appl. Environ. Microb.*, *59*(1), 109-113.

- 826 Francko, D. A., and R. T. Heath (1982), UV-sensitive complex phosphorus: Association with  
827 dissolved humic material and iron in a bog lake, *Limnol. Oceanogr.*, *27*(3), 564-569.
- 828 Franzblau, R. E., C. J. Daughney, P. J. Swedlund, C. G. Weisener, M. Moreau, B. Johannessen,  
829 and S. L. Harmer (2016), Cu(II) removal by *Anoxybacillus flavithermus*-iron oxide  
830 xocomposites during the addition of Fe(II)<sub>aq</sub>, *Geochim. Cosmochim. Acta*, *172*, 139-158, doi:  
831 1.1016/j.gca.2015.09.031.
- 832 Fujii, M., A. Imaoka, C. Yoshimura, and T. D. Waite (2014), Effects of Molecular Composition  
833 of Natural Organic Matter on Ferric Iron Complexation at Circumneutral pH, *Environ. Sci.*  
834 *Technol.*, *48*(8), 4414-4424, doi:10.1021/es405496b.
- 835 Fujii, M., H. Ito, A. L. Rose, T. D. Waite, and T. Omura (2008), Superoxide-mediated Fe(II)  
836 formation from organically complexed Fe(III) in coastal waters, *Geochim. Cosmochim.*  
837 *Acta*, *72*(24), 6079-6089, doi:10.1016/j.gca.2008.09.029.
- 838 Fukuda, R., H. Ogawa, T. Nagata, and I. Koike (1998), Direct determination of carbon and  
839 nitrogen contents of natural bacterial assemblages in marine environments, *Appl. Environ.*  
840 *Microb.*, *64*(9), 3352-3358.
- 841 Gao, H. Z., and R. G. Zepp (1998), Factors influencing photoreactions of dissolved organic  
842 matter in a coastal river of the southeastern United States, *Environ. Sci. Technol.*, *32*(19),  
843 2940-2946, doi:10.1021/es9803660.
- 844 Gasol, J. M., and P. A. Del Giorgio (2000), Using flow cytometry for counting natural planktonic  
845 bacteria and understanding the structure of planktonic bacterial communities, *Scientia*  
846 *Marina*, *64*(2), 197-224.
- 847 Goldman, J. C., D. A. Caron, and M. R. Dennett (1987), Regulation of gross growth efficiency  
848 and ammonium regeneration in bacteria by substrate C : N ratio, *Limnol. Oceanogr.*, *32*(6),  
849 1239-1252.
- 850 Goldstone, J. V., M. J. Pullin, S. Bertilsson, and B. M. Voelker (2002), Reactions of hydroxyl  
851 radical with humic substances: Bleaching, mineralization, and production of bioavailable  
852 carbon substrates, *Environ. Sci. Technol.*, *36*(3), 364-372, doi:10.1021/es0109646.
- 853 González, A. G., O. S. Pokrovsky, F. Jimenez-Villacorta, L. S. Shirokova, J. M. Santana-  
854 Casiano, M. Gonzalez-Davila, and E. E. Emnova (2014), Iron adsorption onto soil and  
855 aquatic bacteria: XAS structural study, *Chem. Geol.*, *372*, 32-45,  
856 doi:10.1016/j.chemgeo.2014.02.013.
- 857 Gustafsson, Ö., and P. M. Gschwend (1997), Aquatic colloid: Concepts, definitions, and current  
858 challenges, *Limnol. Oceanogr.*, *42*(3), 519-528.
- 859 Ha, J., A. Gelabert, A. M. Spormann, and G. E. Brown Jr. (2010), Role of extracellular  
860 polymeric substances in metal ion complexation on *Shewanella oneidensis*: Batch uptake,  
861 thermodynamic modeling, ATR-FTIR, and EXAFS study, *Geochim. Cosmochim. Acta*,  
862 *74*(1), 1-15, doi:10.1016/j.gca.2009.06.031.
- 863 Hatamie, A., H. Parham, B. Zargar, and Z. Heidari (2016), Evaluating magnetic nano-ferrofluid  
864 as a novel coagulant for surface water treatment, *J. Mol. Liq.*, *219*, 694-702, doi:  
865 10.1016/j.molliq.2016.04.020.
- 866 Hedges, J. I., and R. G. Keil (1995), Sedimentary organic matter preservation: an assessment and  
867 speculative synthesis, *Mar. Chem.*, *49*(2-3), 81-115, doi:10.1016/0304-4203(95)00008-f.
- 868 Hedges, J. I., and J. M. Oades (1997), Comparative organic geochemistries of soils and marine  
869 sediments, *Org. Geochem.*, *27*(7-8), 319-361, doi:10.1016/S0146-6380(97)00056-9.



- 870 Heiberg, L., C. B. Koch, C. Kjaergaard, H. S. Jensen, and H. C. B. Hansen (2012), Vivianite  
871 precipitation and phosphate sorption following iron reduction in anoxic soils, *J. Environ.*  
872 *Qual.*, *41*(3), 938-949, doi:10.2134/jeq2011.0067.
- 873 Helms, J. R., J. Mao, K. Schmidt-Rohr, H. Abdulla, and K. Mopper (2013), Photochemical  
874 flocculation of terrestrial dissolved organic matter and iron, *Geochim. Cosmochim.*  
875 *Acta.*, *121*, 398-413, doi: 10.1016/j.gca.2013.07.025.
- 876 Kaiser, K., and G. Guggenberger (2000), The role of DOM sorption to mineral surfaces in the  
877 preservation of organic matter in soils, *Org. Geochem.*, *31*(7-8), 711-725,  
878 doi:10.1016/S0146-6380(00)00046-2.
- 879 Kaiser, K., and G. Guggenberger (2007), Sorptive stabilization of organic matter by microporous  
880 goethite: sorption into small pores vs. surface complexation, *Eur. J. Soil Sci.*, *58*(1), 45-59,  
881 doi:10.1111/j.136502389.2006.00799.x.
- 882 Karlsson, J., A. Jonsson, and M. Jansson (2001), Bacterioplankton production in lakes along an  
883 altitude gradient in the subarctic north of Sweden, *Microb. Ecol.*, *42*(3), 372-382,  
884 doi:10.1007/s00248-001-0009-9.
- 885 Keil, R. G., D. B. Montlucon, F. G. Prahl, and J. I. Hedges (1994), Sorptive Preservation of  
886 Labile Organic-Matter in Marine-Sediments, *Nature*, *370*(6490), 549-552,  
887 doi:10.1038/370549a0.
- 888 Keskitalo, J., K. Salonen, and A.-L. Holopainen (1998), Long-term fluctuation in environmental  
889 conditions, plankton and macrophytes in a humic lake, Valkea-Kotinen, *Boreal Environ.*  
890 *Res.*, *3*, 251-262.
- 891 Kester, D. R., I. W. Duedall, D. N. Connors, and R. M. Pytkowic (1967), Preparation of  
892 Artificial Seawater, *Limnol. Oceanogr.*, *12*(1), 176-179.
- 893 Koehler, B., T. Landelius, G. A. Weyhenmeyer, N. Machida, and L. J. Tranvik (2014), Sunlight-  
894 induced carbon dioxide emissions from inland waters, *Global Biogeochem. Cycles*, *28*, 696-  
895 711, doi: 10.1002/2014GB004850.
- 896 Koehler, B., E. von Wachenfeldt, D. Kothawala, and L. J. Tranvik (2012), Reactivity continuum  
897 of dissolved organic carbon decomposition in lake water, *J. Geophys. Res. Biogeosci.*, *117*,  
898 G01024, doi: 10.1029/2011JG001793.
- 899 Kortelainen, P. (1993), Content of total organic carbon in Finnish lakes and its relationship to  
900 catchment characteristics, *Can. J. Fish. Aquat. Sci.*, *50*(7), 1477-1483.
- 901 Kortelainen, P., H. Pajunen, M. Rantakari, and M. Saarnisto (2004), A large carbon pool and  
902 small sink in boreal Holocene lake sediments, *Global Change Biol.*, *10*(10), 1648-1653,  
903 doi:10.1111/j.1365-2486.2004.00848.x.
- 904 Kranzler, C., H. Lis, Y. Shaked, and N. Keren (2011), The role of reduction in iron uptake  
905 processes in a unicellular, planktonic cyanobacterium, *Environ. Microbiol.*, *13*(11), 2990-  
906 2999, doi:10.1111/j.1462-2920.2011.02572.x.
- 907 Kritzberg E. S., and S. M. Ekström (2012), Increasing iron concentrations in surface waters - a  
908 factor behind brownification? *Biogeosciences* *9*, 1465-1478, doi: 10.5194/bg-9-1465-2012.
- 909 Kritzberg, E. S., A. B. Villanueva, M. Jung, and H. E. Reader (2014), Importance of Boreal  
910 Rivers in Providing Iron to Marine Waters, *PloS One*, *9*(9),  
911 doi:10.1371/journal.pone.0107500.
- 912 Köhler, S., D. Kothawala, M. N. Futter, O. Liungman, and L. Tranvik (2013), In-Lake processes  
913 offset increased terrestrial inputs of dissolved organic carbon and color to lakes, *PloS One*,  
914 *8*(8), e70598, doi: 10.1371/journal.pone.0070598.

- 915 Lalonde, K., A. Mucci, A. Ouellet, and Y. Gelinás (2012), Preservation of organic matter in  
916 sediments promoted by iron, *Nature*, 483(7388), 198-200, doi:10.1038/nature10855.
- 917 Lamb, J. J., R. E. Hill, J. J. Eaton-Rye, and M. F. Hohmann-Marriott (2014), Functional Role of  
918 PilA in Iron Acquisition in the Cyanobacterium *Synechocystis* sp PCC 6803, *Plos One*, 9(8),  
919 doi:10.1371/journal.pone.0105761.
- 920 Leenheer, J. A., G. K. Brown, P. MacCarthy, and S. E. Cabaniss (1998), Models of metal  
921 binding structures in fulvic acid from the Suwannee River, Georgia, *Environ. Sci. Technol.*,  
922 32(16), 2410-2416, doi:10.1021/Es9708979.
- 923 Lis, H., Y. Shaked, C. Kranzler, N. Keren, and F. M. M. Morel (2015), Iron bioavailability to  
924 phytoplankton: an empirical approach, *ISME Journal*, 9(4), 1003-1013,  
925 doi:10.1038/ismej.2014.199.
- 926 Liu, Z., H. Wang, J. Li, Z. Hong, and R. Xu (2015), Adhesion of *Escherichia coli* and *Bacillus*  
927 *subtilis* to amorphous Fe and Al hydroxides and their effects on the surface charges of the  
928 hydroxides, *J Soils Sediments*, 15(11), 2293-2303, doi: 10.1007/s11368-015-1147-x.
- 929 Ma, Z., F. E. Jacobsen, and D. P. Giedroc (2009), Coordination Chemistry of Bacterial Metal  
930 Transport and Sensing, *Chem. Rev.*, 109(10), 4644-4681, doi:10.1021/cr900077w.
- 931 Marie, D., D. Vaultot, and F. Partensky (1996), Application of the novel nucleic acid dyes  
932 YOYO-1, YO-PRO-1, and PicoGreen for flow cytometric analysis of marine prokaryotes,  
933 *Appl. Environ. Microb.*, 62(5), 1649-1655.
- 934 Melton, E. D., E. D. Swanner, S. Behrens, C. Schmidt, and A. Kappler (2014), The interplay of  
935 microbially mediated and abiotic reactions in the biogeochemical Fe cycle, *Nature Rev.*, 12,  
936 797-808, doi: 10.1038/nrmicro3347.
- 937 Miller, C. J., A. L. Rose, and T. D. Waite (2013), Hydroxyl radical production by H<sub>2</sub>O<sub>2</sub>-mediated  
938 oxidation of Fe(II) complexed by Suwannee river fulvic acid under circumneutral freshwater  
939 conditions, *Environ. Sci. Technol.*, 47(2), 829-835, doi: 10.1021/es303876h.
- 940 Nancharaiyah, Y. V., N. Schwarzenbeck, T. V. K. Mohan, S. V. Narasimhan, P. A. Wilderer, and  
941 V. P. Venugopalan (2006), Biodegradation of nitrilotriacetic acid (NTA) and ferric-NTA  
942 complex by aerobic microbial granules, *Water Res.*, 40(8), 1539-1546,  
943 doi:10.1016/j.watres.2006.02.006.
- 944 Neubauer, E., S. J. Köhler, F. von der Kammer, H. Laudon, and T. Hofmann (2013a), Effects of  
945 pH and Stream order on iron and arsenic speciation in boreal catchments, *Environ. Sci.*  
946 *Technol.*, 47(13), 7120-7128, doi: 10.1021/es401193j.
- 947 Neubauer, E., W. D. C. Schenkeveld, K. L. Plathe, C. Rentenberger, F. von der Kammer, S. M.  
948 Kraemer, and T. Hofmann (2013b), The influence of pH on iron speciation in podzol  
949 extracts: Iron complexes with natural organic matter, and iron mineral nanoparticles, *Sci.*  
950 *Total Environ.*, 461, 108-116, doi:10.1016/j.scitotenv.2013.04.076.
- 951 Nierop, K. G. J., B. Jansen, and J. M. Verstraten (2002), Dissolved organic matter, aluminium  
952 and iron interactions: precipitation induced by metal/carbon ratio, pH and competition, *Sci.*  
953 *Total Environ.*, 300, 201-211.
- 954 Orville, A. M., J. D. Lipscomb, and D. H. Ohlendorf (1997), Crystal structures of substrate and  
955 substrate analog complexes of protocatechuate 3,4-dioxygenase: Endogenous Fe<sup>3+</sup> ligand  
956 displacement in response to substrate binding, *Biochemistry*, 36(33), 10052-10066,  
957 doi:10.1021/bi970469f.
- 958 Pokrovsky, O. S., R. E. Martinez, S. V. Golubev, E. I. Kompantseva, and L. S. Shirokova (2008),  
959 Adsorption of metals and protons on *Gloeocapsa* sp cyanobacteria: A surface speciation  
960 approach, *Appl. Geochem.*, 23(9), 2574-2588, doi:10.1016/j.apgeochem.2008.05.007.

- 961 Pracht, J., J. Boenigk, M. Isenbeck-Schroter, F., Keppler, and H. F. (2001), Abiotic Fe(III)  
962 induced mineralization of phenolic substances, *Chemosphere*, 44(4), 613-619, doi:  
963 10.1016/S0045-6535(00)00490-2.
- 964 Pyykkö, P. (2015), Additive covalent radii for single-, double-, and triple-bonded molecules and  
965 tetrahedrally bonded crystals: a summary, *J. Phys. Chem. A*, 119(11), 2326-2337,  
966 doi:10.1021/jp50658191.
- 967 R Core Team (2014), R: A language and environment for statistical computing, *R Foundation for*  
968 *Statistical Computing*, Vienna, Austria. URL: <http://www.R-project.org/>.
- 969 Reguera, G., K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen, and D. R. Lovley (2005),  
970 Extracellular electron transfer via microbial nanowires, *Nature*, 435(7045), 1098-1101,  
971 doi:10.1038/nature03661.
- 972 Richardson, D. J. (2000), Bacterial respiration: a flexible process for a changing environment,  
973 *Microbiology-Uk*, 146, 551-571.
- 974 Riedel, T., H. Biester, and T. Dittmar (2012), Molecular Fractionation of Dissolved Organic  
975 Matter with Metal Salts, *Environ. Sci. Technol.*, 46(8), 4419-4426, doi:10.1021/es203901u.
- 976 Riedel, T., D. Zak, H. Biester, and T. Dittmar (2013), Iron traps terrestrially derived dissolved  
977 organic matter at redox interfaces, *Proc. Natl. Acad. Sci. U. S. A.*, 110(25), 10101-10105,  
978 doi:10.1073/pnas.1221487110.
- 979 Rineau, F., D. Roth, F. Shah, M. Smits, T. Johansson, B. Canbäck, P. B. Olsen, P. Persson, M.  
980 N. Grell, E. Lindquist, I. V. Grigoriev, L. Lange, and A. Tunlid (2012), The ectomycorrhizal  
981 fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot  
982 mechanism involving Fenton chemistry, *Environ. Microbiol.*, 14(6), 1477-1487, doi:  
983 10.1111/j.1462-2920.2012.02736.x.
- 984 Ritchie, J. D., and E. M. Perdue (2003), Proton-binding study of standard and reference fulvic  
985 acids, humic acids, and natural organic matter, *Geochim. Cosmochim. Acta*, 67(1), 85-96.
- 986 Rose, A. L. (2012), The influence of extracellular superoxide on iron redox chemistry and  
987 bioavailability to aquatic microorganisms, *Frontiers in Microbiology*, 3,  
988 doi:10.3389/fmicb.2012.00124.
- 989 Rothman, D. H., and D. C. Forney (2007), Physical model for the decay and preservation of  
990 marine organic carbon, *Science*, 316(5829), 1325-1328, doi:10.1126/science.1138211.
- 991 Salonen, K., and A. V. Vähätalo (1994), Photochemical mineralisation of dissolved organic  
992 matter in Lake Skjervatjern, *Environ. Int.*, 20(3), 307-312.
- 993 Sarkkola, S., M. Nieminen, H. Koivusalo, A. Laurén, P. Kortelainen, T. Mattsson, M. Palviainen,  
994 S. Piirainen, M. Starr, and L. Finér, (2013), Iron concentrations are increasing in surface  
995 waters from forested headwater catchments in eastern Finland. *Sci. Total Environ.* 463-464:  
996 683-689.
- 997 Schröder, I., E. Johnson, and S. de Vries (2003), Microbial ferric iron reductases, *Fems*  
998 *Microbiol. Rev.*, 27(2-3), 427-447, doi:10.1016/s0168-6445(03)00043-3.
- 999 Schweizer, H., M. Argast, and W. Boos (1982), Characteristics of a binding protein-dependent  
1000 transport system for sn-Glycerol-3-Phosphate in *Escherichia Coli* that is part of the *pho*  
1001 regulon, *J. Bacteriol.*, 150(3), 1154-1163.
- 1002 Shapiro, J. (1964), Effect of yellow organic acids on iron and other metals in water, *J. Water*  
1003 *Works Assoc.*, 56, 1062-1082.
- 1004 Sholkovitz, E. (1976) Flocculation of dissolved organic and inorganic matter during the mixing  
1005 of river water and seawater. *Geochim. Cosmochim. Acta*, 40, 831-845. doi: 10.1016/0016-  
1006 7037(76)90035-1.

- 1007 Sjöstedt, C., I. Persson, D. Hesterberg, D. B. Kleja, H. Borg, and J. P. Gustafsson (2013), Iron  
 1008 speciation in soft-water lakes and soils as determined by EXAFS spectroscopy and  
 1009 geochemical modelling, *Geochim. Cosmochim. Acta*, *105*, 172-186,  
 1010 doi:10.1016/j.gca.2012.11.035.
- 1011 Skerker, J. M., and L. Shapiro (2000), Identification and cell cycle control of a novel pilus  
 1012 system in *Caulobacter crescentus*, *Embo Journal*, *19*(13), 3223-3234,  
 1013 doi:10.1093/emboj/19.13.3223.
- 1014 Søndergaard, M., and M. Middelboe (1995), A cross-system analysis of labile dissolved organic  
 1015 carbon, *Mar. Ecol. Prog. Ser.*, *118*(1-3), 283-294, doi:10.3354/meps118283.
- 1016 Steinberg, C., and G. F. Baltus (1984), Influence of metal compounds on fulvic  
 1017 acid/molybdenum blue reactive phosphate associations, *Arch. Hydrobiol.*, *100*(1), 61-71.
- 1018 Studenroth, S., S. G. Huber, K. Kotte, and H. F. Schoeler (2013), Natural Abiotic Formation of  
 1019 Oxalic Acid in Soils: Results from Aromatic Model Compounds and Soil Samples, *Environ.*  
 1020 *Sci. Technol.*, *47*(3): 1323-1329, doi: 10.1021/es304208a.
- 1021 Sundman, A., T. Karlsson, S. Sjöberg, and P. Persson (2016), Impact of iron-organic matter  
 1022 complexes on aqueous phosphate concentrations, *Chem. Geol.*, *426*, 109-117, doi:  
 1023 10.1016/j.chemgeo.2016.02.008.
- 1024 Swenson, T. L., B. P. Bowen, P. S. Nico, and T. R. Northen (2015), Competitive sorption of  
 1025 microbial metabolites on an iron oxide mineral, *Soil Biol. Biochem.*, *90*, 34-41, doi:  
 1026 10.1016/j.soilbio.2015.07.022.
- 1027 Taipale, S., P. Kankaala, M. W. Hahn, R. I. Jones, and M. Tirola (2011), Methane-oxidizing and  
 1028 photoautotrophic bacteria are major producers in a humic lake with a large anoxic  
 1029 hypolimnion, *Aquat. Microb. Ecol.*, *64*, 81-95, doi: 10.3354/ame01512.
- 1030 Tranvik, L. J. (1988), Availability of dissolved organic carbon for planktonic bacteria in  
 1031 oligotrophic lakes of differing humic content, *Microbial. Ecol.*, *16*(3), 311-322.
- 1032 Vähätalo, A. V., H. Aarnos, and S. Mantyniemi (2010), Biodegradability continuum and  
 1033 biodegradation kinetics of natural organic matter described by the beta distribution,  
 1034 *Biogeochemistry*, *100*(1-3), 227-240, doi: 10.1007/s10533-010-9419-4.
- 1035 Vähätalo, A. V., M. Salkinoja-Salonen, P. Taalas, and K. Salonen (2000), Spectrum of the  
 1036 quantum yield for photochemical mineralization of dissolved organic carbon in a humic  
 1037 lake, *Limnol. Oceanogr.*, *45*(3), 664-676.
- 1038 Vähätalo, A. V., K. Salonen, U. Munster, M. Jarvinen, and R. G. Wetzel (2003), Photochemical  
 1039 transformation of allochthonous organic matter provides bioavailable nutrients in a humic  
 1040 lake, *Arch. Hydrobiol.*, *156*(3), 287-314, doi:10.1127/0003-9136/2003/0156-0287.
- 1041 Vähätalo, A. V., K. Salonen, M. Salkinoja-Salonen, and A. Hatakka (1999), Photochemical  
 1042 mineralization of synthetic lignin in lake water indicates enhanced turnover of aromatic  
 1043 organic matter under solar radiation, *Biodegradation*, *10*, 415-420.
- 1044 Vähätalo, A. V., M. Søndergaard, L. Schlüter, and S. Markager (1998), Impact of solar radiation  
 1045 on the decomposition of detrital leaves of eelgrass *Zostera marina*, *Mar. Ecol. Prog. Ser.*,  
 1046 *170*, 107-117.
- 1047 Vähätalo, A. V., and R. G. Wetzel (2008), Long-term photochemical and microbial  
 1048 decomposition of wetland-derived dissolved organic matter with alteration of C-13 : C-12  
 1049 mass ratio, *Limnol. Oceanogr.*, *53*(4), 1387-1392, doi: 10.4319/lo.2008.53.4.1387.
- 1050 Vidal, L. O., W. Graneli, C. B. Daniel, L. Heiberg, and F. Roland (2011), Carbon and  
 1051 phosphorus regulating bacterial metabolism in oligotrophic boreal lakes, *J. Plankton Res.*,  
 1052 *33*(11), 1747-1756, doi:10.1093/plankt/fbr059.

- 1053 Vogt, R. D., et al. (2004), Key site variables governing the functional characteristics of  
1054 Dissolved Natural Organic Matter (DNOM) in Nordic forested catchments, *Aquat. Sci.*,  
1055 66(2), 195-210, doi:10.1007/s00027-004-0710-0.
- 1056 von Wachenfeldt, E., and L. J. Tranvik (2008), Sedimentation in boreal lakes - The role of  
1057 flocculation of allochthonous dissolved organic matter in the water column, *Ecosystems*,  
1058 11(5), 803-814, doi: 10.1007/s10021-008-9162-z.
- 1059 Wagner, S., T. Riedel, J. Niggemann, A. V. Vähätalo, T. Dittmar, and R. Jaffé (2015), Linking  
1060 the molecular signature of heteroatomic dissolved organic matter to watershed  
1061 characteristics in world rivers, *Environ. Sci. Technol.*, 49, 13798-13806, doi:  
1062 10.1021/acs.est.5b00525.
- 1063 Warkentin, M., H. M. Freese, U. Karsten, and R. Schumann (2007), New and fast method to  
1064 quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by  
1065 using optical oxygen sensor spots, *Appl. Environ. Microb.*, 73(21), 6722-6729,  
1066 doi:10.1128/Aem.00405-07.
- 1067 Wetzel, R. G. (2001), The phosphorus cycle, in *Limnology lake and river ecosystems*, edited, pp.  
1068 239-288, Academic press, USA.
- 1069 Weyhenmeyer, G. A., Y. T. Prairie, and L. J. Tranvik (2014), Browning of Boreal Freshwaters  
1070 Coupled to Carbon-Iron Interactions along the Aquatic Continuum, *Plos One*, 9(2),  
1071 doi:10.1371/journal.pone.0088104.
- 1072 White, V. E., and C. J. Knowles (2000), Effect of metal complexation on the bioavailability of  
1073 nitrilotriacetic acid to *Chelatobacter heintzii* ATCC 29600, *Arch. Microbiol.*, 173(5-6), 373-  
1074 382, doi:10.1007/s002030000157.
- 1075 Xiao, Y.-H., A. Raike, H. Hartikainen, and A. V. Vahatalo (2015), Iron as a source of color in  
1076 river waters, *Sci. Total Environ.*, 536, 914-923, doi:10.1016/j.scitotenv.2015.06.092.
- 1077 Xiao, Y.-H., T. Sara-Aho, H. Hartikainen, and A. V. Vähätalo (2013), Contribution of ferric iron  
1078 to light absorption by chromophoric dissolved organic matter, *Limnol. Oceanogr.*, 58(2),  
1079 653-662, doi:10.4319/lo.2013.58.2.0653.
- 1080 Yee, N., L. G. Benning, V. R. Phoenix, and F. G. Ferris (2004), Characterization of metal-  
1081 cyanobacteria sorption reactions: A combined macroscopic and infrared spectroscopic  
1082 investigation, *Environ. Sci. Technol.*, 38(3), 775-782, doi:10.1021/es0346680.
- 1083 Yuan, X., J. A. Davis, and P. S. Nico (2016), Iron-Mediated Oxidation of Methoxyhydroquinone  
1084 under Dark Conditions: Kinetic and Mechanistic Insights, *Environ. Sci. Technol.*, 50(4),  
1085 1731-1740, doi: 10.1021/acs.est.5b03939.