Author(s): Xiao, Yihua; Hoikkala, Laura; Kasurinen, Ville; Tiirola, Marja; Kortelainen, Pirkko; Vähätalo, Anssi

Title: The effect of iron on the biodegradation of natural dissolved organic matter

Year: 2016

Version:

Please cite the original version:

Xiao, Y., Hoikkala, L., Kasurinen, V., Tiirola, M., Kortelainen, P., & Vähätalo, A. (2016). The effect of iron on the biodegradation of natural dissolved organic matter. Journal of Geophysical Research G: Biogeosciences, 121(10), 2544-2561. https://doi.org/10.1002/2016JG003394

Iron affects the biodegradation of natural dissolved organic matter

Yi-Hua Xiao[1, 2]*, Laura Hoikkala[3], Ville Kasurinen[4], Marja Tiirola[2], Pirkko Kortelainen[5], Anssi V. Vähätalo[2]

[1]Department of Environmental Sciences, University of Helsinki, Viikinkaari 1, P.O. Box 65, FI-00014 Helsinki, Finland.

[2]Department of Biological and Environmental Science, University of Jyväskylä, Survontie 9, P.O. Box 35, FI-40014 Jyväskylä, Finland.

[3]Helsinki University Centre for Environment, Viikinkaari 2, P.O. Box 65, FI-00014 Helsinki, Finland.

[4]Department of Forest Sciences, University of Helsinki, Latokartanonkaari 7, P.O. Box 27, FI-00014 Helsinki, Finland.

[5]Finnish Environment Institute, P.O. Box 140, FI-00251 Helsinki, Finland.

*Corresponding author: Yi-Hua Xiao (yihuaxiao2010@gmail.com)

Key Points:

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

Abstract

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

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

1 Introduction

Dissolved organic matter (DOM) contains a large pool of reactive organic carbon forming a major source of energy and nutrients for heterotrophic bacteria. Iron (Fe) is the most abundant element on Earth and interferes with the biogeochemical cycles of many important elements such as carbon and phosphorus (P) [*Cotner and Heath*, 1990; *Sarkkola et al.*, 2013; *Kritzberg et al.*, 2014; *Weyhenmeyer et al.*, 2014]. Global carbon cycling has been a topical issue during the last decades. Nevertheless, the links between carbon and other key elements in biogeochemical 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 μmol $[mg\ C]^{-1}$ 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 μmol Fe $[mg\ C]^{-1}$ have been reported [*Neubauer et al.*,
69 2013a]. In the environment, DOM consists of thousands of molecules, which range from

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

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

The proton donating functional groups (carboxyl, hydroxyl, sulfhydryl, and phosphoryl) on the bacterial cell surface can bind Fe [*Fein et al.*, 1997; *Pokrovsky et al.*, 2008; *Yee et al.*, 2004]. Such binding can transfer and immobilize microbes on solid surfaces when Fe forms insoluble precipitates [*Liu et al.*, 2015], e.g., during the oxidation of dissolved Fe(II) to Fe(III)(oxy)hydroxides along with an increasing redox potential [*Hatamie et al*., 2016]. An 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 hydroquines
97 through semiquinone radicals to oxidized quinones [*Yuan et al.*, 2016]. Such reactions can be
98 sources of reactive oxygen (O_2) 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_2, 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 covert 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^{-1}) 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^{-1}) 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

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

2 Materials and Methods

2.1 Sampling and DOM extraction

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

A water sample collected on 26 October 2012 was immediately filtered through a 0.45-μm filter (AcroPakTM 1000 capsule, Pall). On the following day, the water sample was further filtered through a 0.2-μm filter (Sartobran 300 sterile capsule, Sartorius Stedim) and acidified to pH ~2 with 37% HCl (Titrisol®, Merck). The acidified water sample was stored in the dark at 11 °C, and NaF was added to the final concentration of 0.01 mol L–1 before solid-phase extraction (SPE) of DOM according to *Dittmar et al.* [2008]. Fluoride ions were expected to exchange Fe from their DOM ligands and reduce the Fe content of the extracted DOM [*Gao and Zepp*, 1998]. In order to examine the extraction efficiency of DOC and the removal efficiency of Fe, a small 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 ($Fe_2(SO_4)_3 \cdot nH_2O$, 399.88 g mol^{-1}, AnalaR)
172 and iron(II) sulfate hydrate ($FeSO_4 \cdot 7H_2O$, 278.01 g mol^{-1}, Sigma-Aldrich) were used as Fe
173 sources. The β-glycerophosphate disodium salt hydrate (GlyP, $C_3H_{17}Na_2O_6P \cdot 5–6H_2O$, 216.04 g
174 mol^{-1}, 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-μm polycarbonate filter
180 (Cyclopore^{TM} track etched membrane, Whatman) and then diluted 100 times with <0.2 μm
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 °C. Before each experiment, an aliquot of frozen bacterial suspension was thawed
184 at 30 °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 mg L^{-1}), which was prepared by dissolving
189 the extracted DOM in Milli-Q water and filtering the solution through a 0.2-µm filter (Supor®-
190 200, Pall). The concentration of introduced DOM was adjusted to 20 mg L^{-1} (= 948 µmol 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 mmol
193 L^{-1} $Fe_2(SO_4)_3 \cdot nH_2O$ in 0.1 mol L^{-1} HCl (Titrisol®, 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 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 NH_4Cl 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_2 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^{-1} NaOH using a Titrette® bottletop 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^{-1} 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^{-1}) concentration of Fe that originated from the bacterial inoculum (0.25 μmol L^{-1}) and
215 extracted DOM (0.17 μmol L^{-1}; 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^{-1} (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^{-1} Fe (DOM-20Fe), and 80 μmol L^{-1} Fe (DOM-80Fe; Table 2). The loadings
224 of Fe(III) on DOM ranged from 1 μmol Fe $[mg]^{-1}$ to 4 μmol Fe $[mg]^{-1}$ when calculated as the
225 ratio of the introduced μmol Fe to the mass of DOM (20 mg L^{-1}) used in the experiments (Table
226 2). The corresponding loading in the collected water was 0.22 μmol Fe $[mg]^{-1}$ based on our
227 analytical measurements for Fe (4.98 μmol L^{-1}) and DOC (945 μmol L^{-1}) explained in 2.3.1, and

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

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

The "Fe coating" experiment aimed to associate Fe on the surface structures of bacterial cells and immobilize some of the cells into insoluble precipitates (Fig. 1, Table 2). This experiment used a 0.5 mmol L^{-1} stock solution of Fe(II) prepared by dissolving $FeSO_4$ $7H_2O$ in 0.1 mol L^{-1} HCl. The acidic (pH 2) solution of Fe(II) was slowly titrated to pH 5.0 ± 0.1 and incubated for 1 h to oxidize part of Fe(II) to Fe(III) [*Ferris et al.*, 1987]. The bacterial inoculum was introduced as a ligand for Fe, and the pH of the mixture was titrated to 7. Finally, the rest of ingredients (DOM, ALW, and nutrients) were introduced as in the other experiments, except that two different concentrations of P were used (0.16 μmol L^{-1} in #5, 21 μmol L^{-1} in #7, Table 2).

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

2.3 Analytical methods

2.3.1 Fe and DOC measurements

Fe samples were preserved by adding 50 μL super-purity nitric acid (Romil) to 10 mL of samples. Fe concentrations were determined using inductively coupled plasma mass spectroscopy (Elan Dynamic Reaction Cell II, Perkin-Elmer Sciex). DOC samples were acidified to pH~2 with 1 mol L^{-1} HCl and measured with a total organic carbon analyzer (TOC-V_{CPN}, Shimadzu). The inorganic carbon was purged (by acidifying and bubbling) following a high temperature (670 °C) catalytic combustion oxidation of organic carbon into CO_2, which was detected a by an infrared analyzer. The standard solutions for DOC measurement were prepared from potassium hydrogen phthalate (VWR chemicals) dissolving in Milli-Q water. Milli-Q water was used as blank [*Benner and Strom*, 1993].

2.3.2 Bacterial counting

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

from the flasks without shaking. Collected bacterial samples (1 mL) were fixed with paraformaldehyde (1% final concentration) and glutaraldehyde (0.05% final concentration), incubated for 10 min in the dark and stored at –86 °C in an ultra-low temperature freezer (Thermo Scientific Forma) [*Marie et al.*, 1996].

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

2.3.3 Bacterial respiration

Bacterial respiration (BR) was estimated as the consumption of dissolved O_2 measured with needle-type O_2 microsensor optodes (PreSens GmbH, Regenburg) [*Warkentin et al.*, 2007] at 15-min intervals. The aliquots were closed in biological O_2 demand bottles incubated in dark conditions in a water bath maintained at 20 °C with a thermostat (Lauda Ecoline Staredition RE112, Germany). An optode was inserted into the sample via a hole drilled through the ground-glass stopper and sealed with parafilm. The drift of the instrument defining the detection limit for BR was measured in three blank experiments in which Milli-Q water was incubated for 300 hours under conditions identical to the respiration measurements. During the blank experiments, the apparent decline in O_2 was 1.5 ± 0.5 μmol L^{-1} $(300\ h)^{-1}$ (mean ± sd, n = 3). The decline in the concentration of O_2 was converted into an increase in the concentration of CO_2, assuming a 1:1 molar ratio between the consumed O_2 and the produced 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_2 (μmol C L^{-1}) 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^{-1} 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

were re-amplified for 9 cycles with the same primer pair, but including adaptor A (5'–CCATCTCATCCCTGCGTGTCTCCGAC–3') and unique 10−12-bp-long barcodes at the beginning of the forward primer and P1_338r (5'–CCTCTCTATGGGCAGTCGGTGAT TGCTGCCTCCCGTAGGAGT–3') as the reverse primer to allow Ion Torrent sequencing and assignment to specific samples. PCR products were cleaned using the Agencourt AMPure XP magnetic beads purification system (Beckman Coulter) and quantified using the Qubit dsDNA HS Assay Kit (Invitrogen). Amplicons were subsequently combined in equimolar concentrations for sequencing. The product was then seeded into an Ion PGM Template OT2 reaction following the manufacturer's instructions (Life Technologies). Templated beads were enriched using the Ion OneTouch ES system and sequencing libraries were loaded on an Ion 314 Chip and sequenced using the Ion PGM Sequencing 400 Kit. Sequences were analyzed using Mothur software packages [*Boyle and Edmond*, 1977]. Sequences shorter than 200 bp or which contained >2 ambiguities and a maximum of 8 homopolymers were removed. Unique sequences were identified and aligned using the Silva bacteria database. Loosely aligned and chimeric sequences were removed before taxonomic classification. After quality filtering, 174700 reads were obtained, with an average of 8735 reads per sample (min = 3804, max = 11201). Taxonomic assignment of the OTUs was carried out using the ribosomal database project (RDP) reference database (trainset 9_032012) with a confidence threshold of 80%. The sequences were added to the European Nucleotide Archive (ENA) under submission number PRJEB8364. Similarities of the microbial communities were analyzed by cluster analysis using the Sorensen 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 µmol 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 µmol 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 µmol L^{-1}, #4 and #9) than in the low (0.16 µmol
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 µmol L^{-1} GlyP ($0.06 \pm 0.01 \times 10^9$ cells L^{-1} in #1) or with 21 µmol L^{-1} GlyP ($0.02 \pm$
385 0.004×10^9 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 (μmol 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

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

3.3 Effects of Fe on BGE

The metabolic performance was estimated as the BGE (Table 3) by dividing the accumulated planktonic bacterial biomass (mol C; Fig. 3) by the estimated bacterial carbon demand. The latter was calculated as the sum of the planktonic bacterial biomass (mol C; Fig. 3) and bacterial respiration (mol C; Fig. 4), which also includes the potential respiration of bacteria in the precipitated Fe-oxyhydroxides and the potential oxidation of Fe(II) by O_2 in treatment #5 (Fig. 4). The BGEs were highest in treatment #9 (DOM-Fe and 21 µmol L^{-1} GlyP) followed by treatment #3 (DOM alone, Table 3). The BGE was very low in treatment #8, DOM-Fe with a low concentration of P, as well in treatment #5, in which bacteria were coated with Fe (Table 3).

3.4 Effects of Fe on the bacterial community composition

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

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

4 Discussion

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

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

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

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

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

It is also possible that in our "Fe coating" experiments, the Fe(oxy)hydroxides adsorbed a part of GlyP and reduced the availability of P to planktonic bacteria. The adsorption of P by 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 µmol
489 L^{-1}) can support 7.2 µmol C L^{-1} of bacterial biomass, which is close to the observed biomass of
490 bacteria (3.6 µmol C L^{-1}) grown on DOM in the low P treatment (treatment #3 in Fig. 3), but
491 lower than 33 µmol 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-

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

Despite the suspected reduced bioavailability of P due to DOM-Fe(III)-P-Gly, bacteria did grow on DOM, but their growth was delayed and associated with a marked consumption of O_2 (treatment #8 versus #3 in Figs 3 and 4). These results indicate that bacteria were able to assimilate P (and DOM) from DOM-Fe(III)-P-Gly, but at an additional metabolic cost. The photochemical and microbial reduction of Fe(III) in DOM-Fe(III)-P can break apart DOM, Fe(II) and P [*Francko and Heath*, 1982; *Cotner and Heath*, 1990; *Schröder et al.*, 2003]). Therefore, it is possible that microbes in our study retrieved P by reducing the Fe(III) in DOM-Fe(III)-P-Gly.

4.3 DOM-Fe(III) is bioavailable

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

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

Although some bacteria can take up a few specific organic Fe complexes (siderophore-Fe and heme-Fe [*Ma et al.*, 2009]), little is known about the mechanism for the uptake of organic moiety from DOM-Fe(III). When Fe is bound on a microbial substrate, the uptake of substrate with Fe is expected to be blocked or reduced, because the Fe atom is nearly two times larger than those of carbon, oxygen, and nitrogen, the common atoms in bacterial substrates [*Pyykkö*, 2015]. Therefore, Fe is likely extracellularly removed from a DOM-Fe(III) prior to the transport of the DOM substrate into the cytoplasm. Extracellular reduction of DOM-associated Fe(III) followed by the release of Fe(II) is a mechanism for Fe acquisition in cyanobacteria (e.g., *Synechocystis sp.*) [*Kranzler et al.*, 2011; *Lis et al.*, 2015]. This mechanism can be also used for the uptake of DOM substrate from DOM-Fe(III). Outer-membrane *c*-type cytochromes and pilin (in bacteria that lack *c*-cytochromes) are crucial for transferring electrons to extracellular electron acceptors, 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_2 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_2 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 μm to 500 μm 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 O_2 [*Fujii et al*., 2008]. The oxidation of Fe(II) converts O_2 to superoxide, which can
584 be further converted to H_2O_2 [*Fujii et al*., 2008]. H_2O_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 H_2O_2
587 requires the acidic form of superoxide, $HOO^{\bullet}$ (p*K*a = 4.8, [*Rose*, 2012]), the formation of H_2O_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 μmol $[mg]^{-1}$ 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 μmol L^{-1} in our
604 experiments. The related concentration of quinonoid structures can be estimated as 6 μmol 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 μmol 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 coverts 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 Ca^{2+} and 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 μM
657 in Finnish rivers, [*Xiao et al.*, 2015]). In the deep dark ocean, the turnover time of chromophoric

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

Acknowledgments

This work was funded by the Finnish Cultural Foundation (103757-47394) and Kone Foundation (35-3243), Finland, and by Academy of Finland grant 260797 and European Research Council (ERC) Consolidator grant 615146 for M. Tiirola. We thank Timo Sara-Aho for the measurement of water Fe concentrations and Sukithar Kochappi Rajan for the help in bioinformatics. We also thank Prof. Rolf D. Vogt (University of Oslo) and Prof. Schmitt-Kopplin Philippe (German Research Center for Environmental Health) for providing the carboxyl and hydroxyl group data from Lake Valkea-Kotinen. The data used are presented in the figures, tables, and supporting information which are available from the corresponding author upon request (yihuaxiao2010@gmail.com). The sequences have been added to the European Nucleotide Archive (ENA) under submission number PRJEB8364.

679 **Tables**

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

Chemical	Final concentration ($\mu mol\ L^{-1}$)
Artificial lake water (ALW)	
NaCl	699
Na_2SO_4	48.2
KCl	15.5
$NaHCO_3$	3.99
KBr	1.41
H_3BO_3	0.72
NaF	0.12
$MgCl_2 \cdot 6H_2O$	4.17
$CaCl_2 \cdot 2H_2O$	4.17
Nutrient	
NH_4Cl	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 (μmol C L^{-1})	Fe (μmol L^{-1})	GlyP (μmol L^{-1})
"Fe"			
DOM alone	948	(0.42)*	21
DOM+130Fe	948	130	21
"DOM-Fe"			
DOM alone	948	(0.42)*	21
DOM-20Fe	948	20	21
DOM-80Fe	948	80	21
"No Fe"			
#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
"Fe coating"			
#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
"DOM-Fe high/low P"			
#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 μmol L^{-1}) and extracted DOM (0.17 μmol 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 (**section 4.1**).	[*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 (**section 4.2**).	[*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 (**section 4.3**).	[*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 (**section 4.4**).	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

Figures captions

Figure 1. The preparation of experiments to address the study questions. The preparation of experiments shows the sequence of introduction of solutes and bacteria to acidified (pH 2) Milli-Q water during the titration (with NaOH). Blue and orange colors indicate that "Fe" and "DOM-Fe" experiments were carried out consecutively. The green color indicates that "No Fe", "Fe coating" and "DOM-Fe high/low P" were carried out simultaneously. Nutrients refer to the solutions of NH_4Cl and glycerophosphate (GlyP; Tables 1–2). ALW is the solution of inorganic ions (Table 1). In the experiments marked with *, some treatments did not receive Fe(III) or DOM (Table 2).

Figure 2. Bacterial density and biomass in the "Fe" (a) and "DOM-Fe" (b) experiments. (a) The "Fe" experiment included two treatments: DOM alone and DOM with 130 µmol L^{-1} Fe. (b) The "DOM-Fe" experiment included three treatments: DOM alone, DOM-20Fe (DOM with 20 µmol L^{-1} Fe), and DOM-80Fe (DOM with 80 µmol L^{-1} Fe). Standard error bars in panel b were calculated from three replicated incubations. The experimental design is presented in Fig. 1 and Tables 1–2.

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

References

Aeschbacher, M., C. Graf, R. P. Schwarzenbach, and M. Sander (2012), Antioxidant properties of humic substances, *Enviorn. Sci. Technol*., *46*, 4916-4925.

Algesten, G., S. Sobek, A.-K. Bergström, A. Ågren, L. J. Tranvik, and M. Jansson (2003), Role of lakes for organic carbon cycling in boreal zone, *Global Change Biol.*, *10*, 141-147, doi: 10.1046/j.1529-8817.2003.00721.x.

Angelico, R., A. Ceglie, J.-Z., He, Y.-R. Liu, G. Palumbo, and C. Colombo (2014), Particle size, charge and colloidal stability of humic acids coprecipitated with Ferrihydrite, *Chemosphere*, *99*, 239-247, doi: 10.1016/j.chemosphere.2013.10.092.

Arantes, V., J. Jellison, and B. Goodell (2012), Peculiarities of brown-rot fungi and biochemical Fenton reaction with regard to their potential as a model for bioprocessing biomass, *Appl. Microbiol. Biotechnol.*, *94*(2), 323-338, doi: 10.1007/s00253-012-3954-y.

Arnosti, C. (2004), Speed bumps and barricades in the carbon cycle: substrate structural effects on carbon cycling, *Mar. Chem.*, *92*, 263-273, doi:10.1016/j.marchem.2004.06.030.

Arras, T., J. Schirawski, and G. Unden (1998), Availability of O_2 as a substrate in the cytoplasm of bacteria under aerobic and microaerobic conditions, *J. Bacteriol.*, *180*(8), 2133-2136.

Arvola, L., M. Rask, J. Ruuhijarvi, T. Tulonen, J. Vuorenmaa, T. Ruoho-Airola, and J. Tulonen (2010), Long-term patterns in pH and colour in small acidic boreal lakes of varying hydrological and landscape settings, *Biogeochemistry*, *101*(1-3), 269-279, doi:10.1007/s10533-010-9473-y.

Baken, S., M. Verbeeck, D. Verheyen, J. Diels, and E. Smolders (2015), Phosphorus losses from agricultural land to natural waters are reduced by immobilization in iron-rich sediments of drainage ditches, *Water Res.*, *71*, 160-170, doi:10.1016/j.watres.2015.01.008.

Baldock, J. A., and J. O. Skjemstad (2000), Role of the soil matrix and minerals in protecting natural organic materials against biological attack, *Org. Geochem.*, *31*(7-8), 697-710, doi:10.1016/S0146-6380(00)00049-8.

Benner, R., and M. Strom (1993), A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation, *Mar. Chem.*, *41*(1-3): 153-160, doi: 10.1016/0304-4203(93)90113-3.

Bennett, S. A., B. M. Toner, R. Barco, and K. J. Edwards (2014), Carbon adsorption onto FE oxyhydroxide stalks produced by a lithotrophic iron-oxidizing bacteria, *Geobiology*, *12*, 146-156, doi: 10.1111/gbi.12074.

Beveridge, T. J., and R. G. E. Murray (1980), Sites of metal deposition in the cell wall of *Bacillus subtilis*, *J. Bacteriol.*, *141*(2), 876-887.

Blomqvist, S., A. Gunnars, and R. Elmgren (2004), Why the limiting nutrient differs between temperate coastal seas and freshwater lakes: A matter of salt, *Limnol. Oceanogr.*, *49*(6), 2236-2241.

Boudot, J. P., A. B. H. Brahim, R. Steiman, and F. Seiglemurandi (1989), Biodegradation of synthetic organo-metallic complexes of iron and aluminium with selected metal to carbon ratios, *Soil Biol. Biochem.*, *21*(7), 961-966, doi:10.1016/0038-0717(89)90088-6.

Boyd, P. W., and M. J. Ellwood (2010), The biogeochemical cycle of iron in the ocean, *Nat. Geosci.*, *3*, 675-682, doi:10.1038/ngeo964.

Boyle, E. A., and J. M. Edmond (1977), The mechanism of iron removal in estuaries, *Geochim. 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. A*ppl. 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 heterogneous 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 xomposites during the addition of Fe(II)aq, *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.

Heiberg, L., C. B. Koch, C. Kjaergaard, H. S. Jensen, and H. C. B. Hansen (2012), Vivianite precipitation and phosphate sorption following iron reduction in anoxic soils, *J. Environ. Qual.*, *41*(3), 938-949, doi:10.2134/jeq2011.0067.

Helms, J. R., J. Mao, K. Schmidt-Rohr, H. Abdulla, and K. Mopper (2013), Photochemical flocculation of terrestrial dissolved organic matter and iron, *Geochim. Cosmochim. Acta,,121*, 398-413, doi: 10.1016/j.gca.2013.07.025.

Kaiser, K., and G. Guggenberger (2000), The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils, *Org. Geochem.*, *31*(7-8), 711-725, doi:10.1016/S0146-6380(00)00046-2.

Kaiser, K., and G. Guggenberger (2007), Sorptive stabilization of organic matter by microporous goethite: sorption into small pores vs. surface complexation, *Eur. J. Soil Sci.*, *58*(1), 45-59, doi:10.1111/j.136502389.2006.00799.x.

Karlsson, J., A. Jonsson, and M. Jansson (2001), Bacterioplankton production in lakes along an altitude gradient in the subarctic north of Sweden, *Microb. Ecol.*, *42*(3), 372-382, doi:10.1007/s00248-001-0009-9.

Keil, R. G., D. B. Montlucon, F. G. Prahl, and J. I. Hedges (1994), Sorptive Preservation of Labile Organic-Matter in Marine-Sediments, *Nature*, *370*(6490), 549-552, doi:10.1038/370549a0.

Keskitalo, J., K. Salonen, and A.-L. Holopainen (1998), Long-term fluctuation in environmental conditions, plankton and macrophytes in a humic lake, Valkea-Kotinen, *Boreal Environ. Res.*, *3*, 251-262.

Kester, D. R., I. W. Duedall, D. N. Connors, and R. M. Pytkowic (1967), Preparation of Artificial Seawater, *Limnol. Oceanogr.*, *12*(1), 176-179.

Koehler, B., T. Landelius, G. A. Weyhenmeyer, N. Machida, and L. J. Tranvik (2014), Sunlight-induced carbon dioxide emissions from inland waters, *Global Biogeochem. Cycles*, *28*, 696-711, doi: 10.1002/2014GB004850.

Koehler, B., E. von Wachenfeldt, D. Kothawala, and L. J. Tranvik (2012), Reactivity continuum of dissolved organic carbon decomposition in lake water, *J. Geophys. Res. Biogeosci.*, *117*, G01024, doi: 10.1029/2011JG001793.

Kortelainen, P. (1993), Content of total organic carbon in Finnish lakes and its relationship to catchment characteristics, *Can. J. Fish. Aquat. Sci.*, *50*(7), 1477-1483.

Kortelainen, P., H. Pajunen, M. Rantakari, and M. Saarnisto (2004), A large carbon pool and small sink in boreal Holocene lake sediments, *Global Change Biol.*, *10*(10), 1648-1653, doi:10.1111/j.1365-2486.2004.00848.x.

Kranzler, C., H. Lis, Y. Shaked, and N. Keren (2011), The role of reduction in iron uptake processes in a unicellular, planktonic cyanobacterium, *Environ. Microbiol.*, *13*(11), 2990-2999, doi:10.1111/j.1462-2920.2011.02572.x.

Kritzberg E. S., and S. M. Ekström (2012), Increasing iron concentrations in surface waters - a factor behind brownification? *Biogeosciences 9*, 1465-1478, doi: 10.5194/bg-9-1465-2012.

Kritzberg, E. S., A. B. Villanueva, M. Jung, and H. E. Reader (2014), Importance of Boreal Rivers in Providing Iron to Marine Waters, *PloS One*, *9*(9), doi:10.1371/journal.pone.0107500.

Köhler, S., D. Kothawala, M. N. Futter, O. Liungman, and L. Tranvik (2013), In-Lake processes offset increased terrestrial inputs of dissolved organic carbon and color to lakes, *PloS One*, *8*(8), e70598, doi: 10.1371/journal.pone.0070598.

Lalonde, K., A. Mucci, A. Ouellet, and Y. Gelinas (2012), Preservation of organic matter in sediments promoted by iron, *Nature*, *483*(7388), 198-200, doi:10.1038/nature10855.

Lamb, J. J., R. E. Hill, J. J. Eaton-Rye, and M. F. Hohmann-Marriott (2014), Functional Role of PilA in Iron Acquisition in the Cyanobacterium Synechocystis sp PCC 6803, *Plos One*, *9*(8), doi:10.1371/journal.pone.0105761.

Leenheer, J. A., G. K. Brown, P. MacCarthy, and S. E. Cabaniss (1998), Models of metal binding structures in fulvic acid from the Suwannee River, Georgia, *Environ. Sci. Technol.*, *32*(16), 2410-2416, doi:10.1021/Es9708979.

Lis, H., Y. Shaked, C. Kranzler, N. Keren, and F. M. M. Morel (2015), Iron bioavailability to phytoplankton: an empirical approach, *ISME Journal*, *9*(4), 1003-1013, doi:10.1038/ismej.2014.199.

Liu, Z., H. Wang, J. Li, Z. Hong, and R. Xu (2015), Adhesion of *Escherichia coli* and *Bacillus subtilis* to amorphous Fe and Al hydroxides and their effects on the surface charges of the hydroxides, *J Soils Sediments*, *15*(11), 2293-2303, doi: 10.1007/s11368-015-1147-x.

Ma, Z., F. E. Jacobsen, and D. P. Giedroc (2009), Coordination Chemistry of Bacterial Metal Transport and Sensing, *Chem. Rev.*, *109*(10), 4644-4681, doi:10.1021/cr900077w.

Marie, D., D. Vaulot, and F. Partensky (1996), Application of the novel nucleic acid dyes YOYO-1, YO-PRO-1, and PicoGreen for flow cytometric analysis of marine prokaryotes, *Appl. Environ. Microb.*, *62*(5), 1649-1655.

Melton, E. D., E. D. Swanner, S. Behrens, C. Schmidt, and A. Kappler (2014), The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle, *Nature Rev.*, 12, 797-808, doi: 10.1038/nrmicro3347.

Miller, C. J., A. L. Rose, and T. D. Waite (2013), Hydroxyl radical production by H_2O_2-mediated oxidation of Fe(II) complexed by Suwannee river fulvic acid under circumneutral freshwater conditions, *Environ. Sci. Technol.*, *47*(2), 829-835, doi: 10.1021/es303876h.

Nancharaiah, Y. V., N. Schwarzenbeck, T. V. K. Mohan, S. V. Narasimhan, P. A. Wilderer, and V. P. Venugopalan (2006), Biodegradation of nitrilotriacetic acid (NTA) and ferric-NTA complex by aerobic microbial granules, *Water Res.*, *40*(8), 1539-1546, doi:10.1016/j.watres.2006.02.006.

Neubauer, E., S. J. Köhler, F. von der Kammer, H. Laudon, and T. Hofmann (2013a), Effects of pH and Stream order on iron and arsenic speciation in boreal catchments, *Environ. Sci. Technol.*, *47*(13), 7120-7128, doi: 10.1021/es401193j.

Neubauer, E., W. D. C. Schenkeveld, K. L. Plathe, C. Rentenberger, F. von der Kammer, S. M. Kraemer, and T. Hofmann (2013b), The influence of pH on iron speciation in podzol extracts: Iron complexes with natural organic matter, and iron mineral nanoparticles, *Sci. Total Environ.*, *461*, 108-116, doi:10.1016/j.scitotenv.2013.04.076.

Nierop, K. G. J., B. Jansen, and J. M. Verstraten (2002), Dissolved organic matter, aluminium and iron interactions: precipitation induced by metal/carbon ratio, pH and competition, *Sci. Total Environ.*, *300*, 201-211.

Orville, A. M., J. D. Lipscomb, and D. H. Ohlendorf (1997), Crystal structures of substrate and substrate analog complexes of protocatechuate 3,4-dioxygenase: Endogenous Fe^{3+} ligand displacement in response to substrate binding, *Biochemistry*, *36*(33), 10052-10066, doi:10.1021/bi970469f.

Pokrovsky, O. S., R. E. Martinez, S. V. Golubev, E. I. Kompantseva, and L. S. Shirokova (2008), Adsorption of metals and protons on Gloeocapsa sp cyanobacteria: A surface speciation 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.

Sjöstedt, C., I. Persson, D. Hesterberg, D. B. Kleja, H. Borg, and J. P. Gustafsson (2013), Iron speciation in soft-water lakes and soils as determined by EXAFS spectroscopy and geochemical modelling, *Geochim. Cosmochim. Acta*, *105*, 172-186, doi:10.1016/j.gca.2012.11.035.

Skerker, J. M., and L. Shapiro (2000), Identification and cell cycle control of a novel pilus system in Caulobacter crescentus, *Embo Journal*, *19*(13), 3223-3234, doi:10.1093/emboj/19.13.3223.

Søndergaard, M., and M. Middelboe (1995), A cross-system analysis of labile dissolved organic carbon, *Mar. Ecol. Prog. Ser.*, *118*(1-3), 283-294, doi:10.3354/meps118283.

Steinberg, C., and G. F. Baltes (1984), Influence of metal compounds on fulvic acid/molybdenum blue reactive phosphate associations, *Arch. Hydrobiol.*, *100*(1), 61-71.

Studenroth, S., S. G. Huber, K. Kotte, and H. F. Schoeler (2013), Natural Abiotic Formation of Oxalic Acid in Soils: Results from Aromatic Model Compounds and Soil Samples, *Environ. Sci. Technol.*, *47*(3): 1323-1329, doi: 10.1021/es304208a.

Sundman, A., T. Karlsson, S. Sjöberg, and P. Persson (2016), Impact of iron-organic matter complexes on aqueous phosphate concentrations, *Chem. Geol.*, *426*, 109-117, doi: 10.1016/j.chemgeo.2016.02.008.

Swenson, T. L., B. P. Bowen, P. S. Nico, and T. R. Northen (2015), Competitive sorption of microbial metabolites on an iron oxide mineral, *Soil Biol. Biochem.*, *90*, 34-41, doi: 10.1016/j.soilbio.2015.07.022.

Taipale, S., P. Kankaala, M. W. Hahn, R. I. Jones, and M. Tiirola (2011), Methane-oxidizing and photoautotrophic bacteria are major producers in a humic lake with a large anoxic hypolimnion, *Aqual. Microb. Ecol.*, *64*, 81-95, doi: 10.3354/ame01512.

Tranvik, L. J. (1988), Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content, *Microbial. Ecol.*, *16*(3), 311-322.

Vähätalo, A. V., H. Aarnos, and S. Mantyniemi (2010), Biodegradability continuum and biodegradation kinetics of natural organic matter described by the beta distribution, *Biogeochemistry*, *100*(1-3), 227-240, doi: 10.1007/s10533-010-9419-4.

Vähätalo, A. V., M. Salkinoja-Salonen, P. Taalas, and K. Salonen (2000), Spectrum of the quantum yield for photochemical mineralization of dissolved organic carbon in a humic lake, *Linmol. Oceanogr.*, *45*(3), 664-676.

Vähätalo, A. V., K. Salonen, U. Munster, M. Jarvinen, and R. G. Wetzel (2003), Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake, *Arch. Hydrobiol.*, *156*(3), 287-314, doi:10.1127/0003-9136/2003/0156-0287.

Vähätalo, A. V., K. Salonen, M. Salkinoja-Salonen, and A. Hatakka (1999), Photochmical mineralization of synthetic lignin in lake water indicates enhanced turnover of aromatic organic matter undre solar rediation, *Biodegradation*, *10*, 415-420.

Vähätalo, A. V., M. Søndergaard, L. Schlüter, and S. Markager (1998), Impact of solar radiation on the decomposition of detrital leaves of eelgrass *Zostera marina*, *Mar. Ecol. Prog. Ser.*, *170*, 107-117.

Vähätalo, A. V., and R. G. Wetzel (2008), Long-term photochemical and microbial decomposition of wetland-derived dissolved organic matter with alteration of C-13 : C-12 mass ratio, *Limnol. Oceanogr.*, *53*(4), 1387-1392, doi: 10.4319/lo.2008.53.4.1387.

Vidal, L. O., W. Graneli, C. B. Daniel, L. Heiberg, and F. Roland (2011), Carbon and phosphorus regulating bacterial metabolism in oligotrophic boreal lakes, *J. Plankton Res.*, *33*(11), 1747-1756, doi:10.1093/plankt/fbr059.

Vogt, R. D., et al. (2004), Key site variables governing the functional characteristics of Dissolved Natural Organic Matter (DNOM) in Nordic forested catchments, *Aquat. Sci.*, *66*(2), 195-210, doi:10.1007/s00027-004-0710-0.

von Wachenfeldt, E., and L. J. Tranvik (2008), Sedimentation in boreal lakes - The role of flocculation of allochthonous dissolved organic matter in the water column, *Ecosystems*, *11*(5), 803-814, doi: 10.1007/s10021-008-9162-z.

Wagner, S., T. Riedel, J. Niggemann, A. V. Vähätalo, T. Dittmar, and R. Jaffé (2015), Linking the molecular signature of heteroatomic dissolved organic matter to watershed characteristics in world rivers, *Environ. Sci. Technol.*, *49*, 13798-13806, doi: 10.1021/acs.est.5b00525.

Warkentin, M., H. M. Freese, U. Karsten, and R. Schumann (2007), New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor spots, *Appl. Environ. Microb.*, *73*(21), 6722-6729, doi:10.1128/Aem.00405-07.

Wetzel, R. G. (2001), The phosphorus cycle, in *Limnology lake and river ecosystems*, edited, pp. 239-288, Academic press, USA.

Weyhenmeyer, G. A., Y. T. Prairie, and L. J. Tranvik (2014), Browning of Boreal Freshwaters Coupled to Carbon-Iron Interactions along the Aquatic Continuum, *Plos One*, *9*(2), doi:10.1371/journal.pone.0088104.

White, V. E., and C. J. Knowles (2000), Effect of metal complexation on the bioavailability of nitrilotriacetic acid to Chelatobacter heintzii ATCC 29600, *Arch. Microbiol.*, *173*(5-6), 373-382, doi:10.1007/s002030000157.

Xiao, Y.-H., A. Raike, H. Hartikainen, and A. V. Vahatalo (2015), Iron as a source of color in river waters, *Sci. Total Environ.*, *536*, 914-923, doi:10.1016/j.scitotenv.2015.06.092.

Xiao, Y.-H., T. Sara-Aho, H. Hartikainen, and A. V. Vähätalo (2013), Contribution of ferric iron to light absorption by chromophoric dissolved organic matter, *Limnol. Oceanogr.*, *58*(2), 653-662, doi:10.4319/lo.2013.58.2.0653.

Yee, N., L. G. Benning, V. R. Phoenix, and F. G. Ferris (2004), Characterization of metal-cyanobacteria sorption reactions: A combined macroscopic and infrared spectroscopic investigation, *Environ. Sci. Technol.*, *38*(3), 775-782, doi:10.1021/es0346680.

Yuan, X., J. A. Davis, and P. S. Nico (2016), Iron-Mediated Oxidation of Methoxyhydroquinone under Dark Conditions: Kinetic and Mechanistic Insights, *Environ. Sci. Technol.*, *50*(4), 1731-1740, doi: 10.1021/acs.est.5b03939.