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

Yi-Hua Xiao1,2, Laura Hoikkala3, Ville Kasurinen4, Marja Tiirila3, Pirkko Kortelainen5, and Anssi V. Vähätalo2

1Department of Environmental Sciences, University of Helsinki, Helsinki, Finland, 2Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland, 3Helsinki University Centre for Environment, Helsinki, Finland, 4Department of Forest Sciences, University of Helsinki, Helsinki, Finland, 5Finnish Environment Institute, Helsinki, Finland

Abstract Iron (Fe) may alter the biodegradation of dissolved organic matter (DOM), by interacting 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 reduces 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; Kitzberg 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. Fe can have a more important role in carbon cycling than presently understood. For example, it plays a key role in carbon sequestration in lake sediments (Kortelainen et al., 2004; von Wachenfeldt and Tranvik, 2008; Einola et al., 2011). Recently, the concentrations of both dissolved organic carbon (DOC) and Fe have increased at least in boreal rivers and streams (Kitzberg and Ekström, 2012; Sarkkola et al., 2013; Weyhenmeyer et al., 2014) causing pressure to better understand links between DOC and Fe in the present and future conditions. To the best of our knowledge, the role of Fe in the degradation of DOM has not been examined, although Fe can alter the biodegradation of organic matter (OM) in many ways, as exemplified below.

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

Fe can form coordination complexes with DOM (Leenheer et al., 1998; Sjöstedt et al., 2013). For example, the potential binding sites of Fe in a hypothetical average molecule of Suwannee River fulvic acid (SRFA) include four carboxylic groups, one quinoid structure, two phenolic groups, and four carboxyls associated with esters and ketones (Leenheer et al., 1998; Sjöstedt et al., 2013). According to the proton-donating carboxyl and phenolic groups, SRFA contains 14.3 μmol [mg C]⁻¹ of potential binding sites for Fe (Leenheer et al., 1998; Ritchie...
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 nonavailable 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 Hoan 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 embedding under a layer of Fe(III)(oxy)hydroxides can be expected to reduce the metabolic activity of decomposers and the biodegradation of OM.

Besides the possible negative effects of Fe on the biodegradation of OM, Fe can also stimulate the decompositional activity of OM. Fe associated with quinine moieties of DOM can cause abiotic oxidation-reduction reactions, which, for instance, convert the reduced hydroquinones through semiquinone radicals to oxidized quinones [Yuan et al., 2016]. Such reactions can be sources of reactive oxygen (O2) species and lead to the breakage of aromatic rings [Miller et al., 2013; Comba et al., 2015; Yuan et al., 2016]. This ring cleavage can produce volatile hydrocarbons, CO2, and organic acids such as oxalic acid [Pracht et al., 2001; Studenroth et al., 2013; Comba et al., 2015]. These reactions can take place abiotically between the reduced and oxidized forms of natural OM and those of Fe (FeII/III). Thus, the abiotic reactions of Fe can facilitate decomposition of OM and produce substrates for microbes.

Fe also facilitates the decomposition of OM through the active metabolism of microbes. Brown rot fungi and an ectomycorrhizal fungus, Paxillus involutus, use Fe in a biochemical Fenton reaction to break down particulate OM in wood and soil, respectively [Arantes et al., 2012; Rineau et al., 2012]. These fungi secrete reducing components that covert Fe(III) to Fe(II) and dioxigen through superoxide to hydrogen peroxide to yield the two reactants of the Fenton reaction [Arantes et al., 2012]. The Fenton reaction produces hydroxyl radicals that can break down OM nonselectively.

Heterotrophic bacteria can use extracellular enzymes to break down molecules that are too large (>600 g mol−1) for direct uptake [Arnosti, 2004]. This enzymatic catalysis is selective and primarily limited to the hydrolysis of predictable biopolymers [Arnosti, 2004]. In many freshwaters, the bulk DOM is dominated by high molecular mass (>600 g mol−1) heterogeneous humic substances that also include nonhydrolysable bonds [Tranvik, 1988; Arnosti, 2004]. Although much of freshwater DOM is too large for direct uptake or nonhydrolysable by extracellular enzymes of bacterioplankton, typically a half of the bulk DOC is lost in <2.5 years in Swedish lakes [Algesten et al., 2003]. Flocculation may direct DOM into sediments, and solar radiation can break down a part of the DOM, but these processes cannot alone explain the loss of DOM that is apparently refractory to biodegradation in lakes or in long laboratory incubations [Vähätalo and Wetzel, 2008; Vähätalo et al., 2010; Koehler et al., 2012]. Therefore, it is possible that the decomposition of DOM in freshwaters may also include other dark reactions, e.g., catalyzed by Fe abiotically or through active metabolism by bacteria.

The communities of bacteria in bacterioplankton contain hundreds of species with their specific metabolic functions [Taipale et al., 2011]. If bacteria are able to utilize Fe to break down DOM extracellularly, it is
The laboratory experiments of this study assessed the role of Fe on the biodegradation of natural DOM extracted from a humic lake using a community of bacterioplankton from the same lake. In the experiments, Fe was introduced in several different ways and had a possibility to associate with DOM, P, or the cell surface of bacteria to form insoluble Fe(oxy)hydroxides [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 redissolved in Milli-Q water. The extraction efficiency of DOC was 76%, and the removal efficiency of Fe was 97% according to analytical measurements described in section 2.3.1.

2.2. Experimental Design and Procedures

The experiments briefly described in Tables 1 and 2 and Figure 1 were designed to address the following study questions:

1. “Fe”—Can Fe influence the biodegradation of DOM?
2. “DOM-Fe”—Is DOM-Fe bioavailable?
3. “DOM-Fe low/high P”—Does the bacterial growth on DOM-Fe depend on the concentration of P?
4. “Fe coating”—Can precipitation of Fe on the bacterial cell surface affect the biodegradation of DOM?

In this section, we first describe the features common to all experiments and then present the details of each experiment. Iron(III) sulfate hydrate ($\text{Fe}_2\text{(SO}_4\text{)}_3 \cdot n\text{H}_2\text{O}$, 399.88 g mol$^{-1}$, AnalaR) and iron(II) sulfate hydrate (Fe$^{2+}$SO$\text{}_4$ and H$_2$O, 159.69 g mol$^{-1}$, AnalaR) are likely that this functional trait is limited to certain species or groups of species. Such species may possibly mediate extracellular redox reactions of Fe through organic or inorganic (e.g., superoxide) redox shuttles [Melton et al., 2014] and/or have extracellular oxidoreductases [Diaz et al., 2013]. Although bacteria may have potential mechanisms for extracellular decomposition of DOM with the help of Fe [Melton et al., 2014], such Fe-stimulated decomposition of DOM and the species of bacteria involved has not been reported.
(FeSO₄ · 7H₂O, 278.01 g mol⁻¹, Sigma-Aldrich) were used as Fe sources. The β-glycerophosphate disodium salt hydrate (GlyP, C₃H₁₇Na₂O₆P · 5–6H₂O, 216.04 g mol⁻¹, VWR International) was used as a source of P for bacteria. All chemicals used in the experiment were >98% pure, and all solutions were prepared in deionized water (Milli-Q, Millipore).

Table 2. The Concentrations of DOM, Fe, and GlyP in Each Treatment of the Experiments

| “Experiment” With Treatments | DOM (μmol C L⁻¹) | Fe (μmol L⁻¹) | GlyP (μmol L⁻¹) |
|------------------------------|------------------|--------------|----------------|
| DOM alone                    | 948              | (0.42)⁴      | 21             |
| DOM + 130Fe                  | 948              | 130          | 21             |
| “DOM-Fe”                     |                  |              |                |
| DOM alone                    | 948              | (0.42)⁴      | 21             |
| DOM-20Fe                     | 948              | 20           | 21             |
| DOM-80Fe                     | 948              | 80           | 21             |
| “No Fe”                      |                  |              |                |
| #1 No DOM + low P³           | 948              | (0.25)⁵      | 0.16           |
| #2 No DOM + high P³          | 948              | (0.25)⁵      | 21             |
| #3 DOM + low P⁴              | 948              | (0.42)⁴      | 0.16           |
| #4 DOM + high P              | 948              | (0.42)⁴      | 21             |
| “Fe Coating”                 |                  |              |                |
| #5 (Fe coating) + DOM + low P | 948              | 60           | 0.16           |
| #6 (Fe coating) + No DOM + high P³ | 948              | 60           | 21             |
| #7 (Fe coating) + DOM + high P  | 948              | 60           | 21             |
| “DOM-Fe High/Low P³”         |                  |              |                |
| #8 (DOM-Fe) + low P          | 948              | 60           | 0.16           |
| #9 (DOM-Fe) + high P         | 948              | 60           | 21             |

All treatments included ALW (Table 1) and bacterial inoculum introduced in the sequence shown in Figure 1. No addition of extracted DOM. Fe concentrations in parentheses indicate no addition of Fe, and the Fe is from the introduced original bacterial inoculum (0.25 μmol L⁻¹) and extracted DOM (0.17 μmol L⁻¹). Fe and DOM-Fe experiments were carried out consecutively, but No Fe, Fe coating, and DOM-Fe high/low P experiments were conducted simultaneously.

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 NH₄Cl and glycerophosphate (GlyP; Tables 1 and 2). ALW is the solution of inorganic ions (Table 1). In the experiments marked with asterisk, some treatments did not receive Fe(III) or DOM (Table 2).
An indigenous grazer-free bacterial community was isolated and used as an identical inoculum of the bacterial community in the consecutive experiments. Bacteria present in the collected lake water sample were first passed through a 0.8 μm polycarbonate filter (Cyclopore® track-etched membrane, Whatman) and then diluted 100 times with <0.2 μm filtered lake water. This diluted bacterial community was cultured in the dark at room temperature for 4 days, after which the bacterial suspension was divided into 50 mL aliquots and stored at −20°C. Before each experiment, an aliquot of frozen bacterial suspension was thawed at 30°C in a water bath for 15 min and was introduced as a bacterial inoculum (10% vol/vol in “Fe” and 5% vol/vol in the other experiments).

For the experimental bioassays, DOM, Fe, nutrients, artificial lake water (ALW), and bacteria were introduced in different concentrations, combinations, and ways (Figure 1 and Tables 1 and 2). DOM was introduced as a stock solution (50 mg L⁻¹), which was prepared by dissolving the extracted DOM in Milli-Q water and filtering the solution through a 0.2 μm filter (Supor®-200, Pall). The concentration of introduced DOM was adjusted to 20 mg L⁻¹ (=948 μmol C L⁻¹), matching the concentration in the study lake [Vähätalo et al., 2003; Einola et al., 2011] or in Finnish boreal lakes in general [Kortelainen, 1993]. An acidic (pH 1) stock solution (0.5 mmol L⁻¹ Fe₂(SO₄)₃ · nH₂O in 0.1 mol L⁻¹ HCl (Titrisol®, Merck) was used as a source of Fe(III) in all experiments with introduced Fe(III). The stock solution of ALW was prepared according to Kester et al. [1967] and was added to form the final inorganic ion concentrations presented in Table 1 and an ionic strength of 0.87 (calculated as mmol L⁻¹), which is close to the average ionic strength of Finnish river waters (0.8) [Xiao et al., 2015]. The nutrients N and P were introduced as separate solutions of NH₄Cl and GlyP, respectively (Table 1). GlyP was selected as a source of P instead of inorganic phosphate, which can effectively complex with Fe(III) into insoluble precipitates [Francis and Dodge, 1993]. GlyP is one of the few organophosphates that can be transported across the cell membrane, forming a source of P but not serving a carbon substrate [Schweizer et al., 1982]. An isolated identical bacterial community indigenous to Lake Valkea-Kotinen was used as a bacterial inoculum. Finally, the volume of bioassays was adjusted to 100 mL with Milli-Q water. Biodegradation of DOM was assessed as bacterial growth determined as the bacterial density and the consumption of dissolved O₂ in bioassays extending up to 25 days in the dark at 22°C.

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

The “DOM-Fe” experiment addressed the bioavailability of DOM-Fe. DOM-Fe was created before the introduction of bacteria, ALW, and nutrients (Figure 1). The concentration of Gly-P was adjusted to 21 μmol L⁻¹ (high P; Table 2). The loading of Fe(III) on DOM ranged from 1 μmol Fe (mg DOM)⁻¹ to 4 μmol Fe (mg DOM)⁻¹ when calculated as the ratio of the introduced micromole Fe to the mass of DOM (20 mg L⁻¹) used in the experiments (Table 2). The corresponding loading in the collected water was 0.22 μmol Fe (mg DOM)⁻¹ based on our analytical measurements for Fe (4.98 μmol L⁻¹) and DOC (945 μmol L⁻¹) explained in section 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 DOM)⁻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 oligomesotrophic 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 (Figure 1 and 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 (Figure 1 and Table 2). This experiment used a 0.5 mmol L⁻¹ stock solution of Fe(II) prepared by dissolving FeSO₄·7H₂O 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 #6 and #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 Milli-Q 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 superpurify 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-VCPN, Shimadzu). The inorganic carbon was purged (by acidifying and bubbling) following a high-temperature (670°C) catalytic combustion oxidation of organic carbon into CO₂, which was detected by an infrared analyzer. The standard solutions for DOC measurement were prepared from potassium hydrogen phthalate (VWR chemicals) dissolved in Milli-Q water [Aarnos et al., 2012].

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 ultralow-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 of water. 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 O₂ measured with needle-type O₂ microsensor optodes (PreSens GmbH, Regensburg) [Warkentin et al., 2007] at 15 min intervals. The aliquots were closed in biological O₂ 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 h under conditions identical to the respiration measurements. During the blank experiments, the apparent decline in O₂ was 1.5 ± 0.5 μmol L⁻¹ (300 h)⁻¹ (mean ± SD, n = 3). The decline in the concentration of O₂ was converted into an increase in the concentration of CO₂, assuming a 1:1 molar ratio between the consumed O₂ and the produced CO₂ in BR. This respiratory quotient is similar to earlier studies, which have
used values ranging from 0.82 to 1.2 [Søndergaard and Middelboe, 1995; Del Giorgio and Cole, 1998; Cory et al., 2014]. The temporal trend in accumulated CO₂ (μmol C L⁻¹) was determined by a polynomial fitting to the measurements using R-language and the smooth spline function from the R package “stats” [R Core Team, 2014].

2.3.4. Bacterial Growth Efficiency

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

2.3.5. 16S rRNA Sequencing

Bacterial samples (1.5 mL) were collected on day 28 of the simultaneous “No Fe”, “Fe coating”, and “DOM-Fe high/low P” experiments. The samples were centrifuged at 14,000 rpm for 30 min, and the bacterial cell pellets were frozen at −20°C for later extraction. Bacterial DNA was extracted using Quick Extract DNA Extraction Solution (Epicentre) according to the manufacturer’s instructions. From the extracts, the V1–V2 region of the 16S rRNA gene was amplified using the universal bacterial primer pair 27 F (5′-AGAGAGTTTGTACMTGCTCAG-3′) and 338r (TGCTGCCCTCCCGTAGGAGT). A 30 μL PCR reaction contained 15 μL 1 × Dream Taq Master mix (Fermentas), 0.3 μmol L⁻¹ of each primer, and 2 μL of the DNA sample. PCR amplification was carried out on a CFX96 thermocycler (Biorad) with 98°C for 30 s, followed by 35 cycles of 98°C for 10 s; 52°C for 30 s; and 72°C for 180 s and a final elongation step of 5 min at 72°C. Successful PCR products were reamplified for nine cycles with the same primer pair but including adaptor A (5′-CCATCTCATCCTCCGTGCTCCGAC-3′) and unique 10 – 12-bp-long barcodes at the beginning of the forward primer and P1_338r (5′-CTCTCTATGGGCAGTGAGT CCATCTCATCCTCGGTCTCCGAC-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 greater than two ambiguities and a maximum of eight 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, 174,700 reads were obtained, with an average of 8735 reads per sample (min = 3804, max = 11,201). Taxonomic assignment of the operational taxonomic units (OTUs) was carried out using the ribosomal database project 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 Sørensen similarity index and the PC-ORD 6.0 software package (MJM Software Design).

2.4. Statistical Analyses

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

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

3. Results

3.1. Effects of Fe on Bacterial Density

In the “Fe” experiment (Figure 1 and Tables 1 and 2), the introduction of Fe(III) (DOM + 130Fe) significantly reduced the density of bacteria growing on DOM compared with the corresponding treatment without Fe
In this experiment, Fe was potentially able to associate with DOM to form DOM-Fe, with GlyP to form P-Fe, and/or with bacteria to form bacteria-Fe (Figure 1 and Table 2), and any of these complexes could potentially have been responsible for the reduction in bacterial density (Figure 2a).

### 3.1.1. Effects of DOM-Fe on Bacterial Density

The "DOM-Fe" experiment was designed to generate DOM-Fe (Figure 1 and Tables 1 and 2), which had no consistent negative effect on bacterial density (Figure 2b). From day 4 to day 12, the bacterial densities of the DOM-80Fe treatment were significantly higher than in the DOM alone treatment (paired t test, \( t = -6.98 \), d.f. = 7, \( p < 0.001 \)). This experiment revealed that when Fe was associated with DOM, it did not reduce the bacterial density but to some extent even stimulated bacterial growth.

The potential stimulatory role of DOM-Fe was further tested in the "DOM-Fe high/low P" experiment (Figure 1 and Tables 1 and 2), in which the growth of bacteria was consistently highest with a maximum density of 13 ± 0.7 × 10⁹ cells L⁻¹ in treatment #9 with DOM-Fe and 21 μmol L⁻¹ GlyP (Figure 3a). In the corresponding treatment #4 without Fe, the maximum density of bacteria was an order of magnitude lower (2.4 ± 0.4 × 10⁹ cells L⁻¹) than in treatment #9 with DOM-Fe. These results indicate that DOM-Fe was able to stimulate bacterial growth when the concentration of GlyP was high (Figures 2b and 3a).

When the experiments were carried out with a low concentration of GlyP representative of dissolved P in Lake Valkea-Kotinen (0.16 μmol L⁻¹ in #3 and #8), DOM-Fe reduced bacterial densities (max 0.4 ± 0.02 × 10⁹ cells L⁻¹) to lower than one third of those in the corresponding treatment without Fe (max 1.5 ± 0.3 × 10⁹ cells L⁻¹; #3; Figure 3a). The bacterial growth was consistently higher in the high (21 μmol L⁻¹, #4 and #9) than in the low (0.16 μmol L⁻¹, #3 and #8) concentration of GlyP (Figure 3a), indicating that a high concentration of GlyP stimulated the growth of bacteria.

Because bacterial densities were elevated in the high concentration of GlyP (treatments #9 and #4; Figure 3a), we tested whether bacteria can use GlyP as a carbon source (treatments #1 and #2; Figure 1). Bacteria reached a density that was less than 2 orders of magnitude lower in ALW with 0.16 μmol L⁻¹ GlyP (0.06 ± 0.01 × 10⁹ cells L⁻¹ in #1) or with 21 μmol L⁻¹ GlyP (0.02 ± 0.004 × 10⁹ cells L⁻¹ in #2) than in the corresponding treatments with DOM (#3 or #4, respectively; Figure 3b). These results indicate that GlyP or other salts in ALW (Table 1) were negligible sources of organic carbon for bacteria, and GlyP acted as a source of P.

### 3.1.2. Effect of Fe Coating on Bacterial Density

When bacteria were coated with Fe ("Fe coating", #5 – #7; Figure 1 and Table 2), obvious precipitates were observed after a few hours of incubation. The planktonic bacterial density measured above the precipitates reached 0.04 ± 0.002 × 10⁹ cells L⁻¹ in treatment #5 with low P and 0.19 ± 0.009 × 10⁹ cells L⁻¹ in #7 with high P (Figure 3b). No obvious increase in planktonic bacterial numbers was found in treatment #6 without (DOM alone; paired t test, \( t = 4.47 \), d.f. = 9, \( p = 0.002 \); Figure 2a). In this experiment, Fe was potentially able to associate with DOM to form DOM-Fe, with GlyP to form P-Fe, and/or with bacteria to form bacteria-Fe (Figure 1 and Table 2), and any of these complexes could potentially have been responsible for the reduction in bacterial density (Figure 2a).
DOM (Figure 3). The results of the “Fe coating” experiments support the earlier findings: GlyP was not a source of carbon (#2 and #6) but a source of P it stimulated the growth of bacteria on DOM (Figures 3a and 3b). When Fe was associated with bacteria (#5 and #7), the bacterial densities were orders of magnitude lower than in the DOM-Fe treatments (#8 and #9; Figures 3a and 3b).

These results demonstrate that the growth of bacteria was dependent on the type of Fe association. Fe associated with bacterial cells (Fe coating) or with P reduced bacterial densities, but DOM-Fe in the presence of high P even increased the bacterial densities (Figures 2–3).

3.2. Effects of Fe on Bacterial Respiration

We selected four treatments, namely, #3 (DOM alone), #5 (Fe coating with DOM), #8 (DOM-Fe with low P), and #9 (DOM-Fe with high P), to follow the cumulative bacterial respiration (μmol C L⁻¹) during the incubations (Figure 4). After a lag phase of several days, the cumulative bacterial respiration was eventually highest and lowest in treatments #9 and #5, respectively (Figure 4), in agreement with the bacterial densities among the selected treatments (Figure 3). In contrast to the bacterial densities (Figure 3), the cumulative bacterial respiration was higher on DOM with Fe (#8) than without Fe (#3; paired t test, t = -52.6, d.f. = 1538, p < 0.001; Figure 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; Figure 3) by the estimated bacterial carbon demand. The latter was calculated as the sum of the planktonic bacterial biomass (mol C; Figure 3) and bacterial respiration (mol C; Figure 4), which also includes the potential respiration of bacteria in the precipitated Fe oxyhydroxides and the potential oxidation of Fe(II) by O₂ in treatment #5 (Figure 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 (Figures 5 and 6 and Table S1 in the supporting information). 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 (Figures 5 and 6 and Table S1). In these treatments, the frequency of Caulobacter-associated sequences increased from 1% to 95% (Figure 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) (Figure 6 and Table S1). Altogether, the experimental treatments changed the initial composition of the bacterial community toward 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. Reduction of Bacterial Growth and the Consumption of DOM by Fe Coating and the Formation of Particulate Fe(oxy)Hydroxide

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;
At the beginning of our Fe coating experiments, the concentration of these binding sites was $6.0 \times 10^{-10}$ mol 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 8 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 (Figure 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, 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(III)(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.

### 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      | Boyle and Edmond [1977], Riedel et al. [2012, 2013], and González et al. [2014]     |
|    bacterial growth and the consumption of DOM (section 4.1).               |                                                                                       |
| 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], and Sundman et al. [2016] |
| 3   DOM-Fe is bioavailable (section 4.3).                                   | Boudot et al. [1989], Francis and Dodge [1993], and 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], and enzyme-like reactions [Bugg and Winfield, 1998] |
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 Figure 3b). However, their densities did not reach the levels found in the other DOM treatments (treatments #3, #4, #8, and #9 in Figure 3a), suggesting that Fe precipitates also reduced the availability of substrates for the planktonic bacteria. The precipitated Fe(oxo)hydroxides can adsorb DOM from the water column and convert it into a particulate form [Boyle and Edmond, 1977; Riedel et al., 2012, 2013; Swenson et al., 2015]. Such adsorption of OM by particulate Fe(oxo)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(oxo)hydroxides adsorbed a part of GlyP and reduced the availability of P to planktonic bacteria. The adsorption of P by Fe(oxo)hydroxides in oxic sediments is the primary mechanism limiting the bioavailability of P, which regulates the overall productivity of lakes [Wetzel, 2001], because osmotrophic organisms such as bacteria or phytoplankton poorly utilize P bound to particulate Fe(oxo)hydroxides. Thus, the immobilization of P into solid Fe(oxo)hydroxides may reduce the bioavailability of P, bacterial growth, and the biodegradation of DOM.

4.2. Poor Bioavailability of P in DOM-Fe

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

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

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

4.4. Stimulation of Bacterial Growth by DOM-Fe(III)

In some treatments of our study with a high concentration of Fe, the association of Fe(III) with DOM increased microbial growth, respiration, and growth efficiency on DOM. This increase may result from purely abiotic Fe-catalyzed reactions [Pracht et al., 2001; Studenroth et al., 2013; Comba et al., 2015] and/or involve the active metabolism of microbes, as described earlier for fungi [Arantes et al., 2012; Rineau et al., 2012]. Abiotic reactions may already have broken quinone-type parts of our DOM into small organic acids [Studenroth et al., 2013; Comba et al., 2015] during the preparation of DOM-Fe under acidic starting conditions. This was possibly seen as elevated O2 consumption during the first 4 days of incubation in the Fe(III) supplied treatments compared to the DOM alone treatment (Figure 4). Therefore, it is possible that the abiotic formation of oxalic, maleic, fumaric, and malonic acids found earlier in soils [Studenroth et al., 2013] may also take place in an aquatic environment rich in Fe and the humic type of DOM.

A steep increase in O2 consumption by bacteria growing on DOM-Fe with a high concentration of P after 12 days of incubation (Figure 4) was not likely supported by the abiotic formation of bioavailable carbon during the formation of DOM-Fe but rather indicates the active metabolism of bacteria to obtain carbon from DOM-Fe. In brown rot fungi, the utilization of Fe in the biochemical Fenton reaction requires large investments in the form of organic compounds or/and enzymes secreted in wood [Arantes et al., 2012]. Brown rot fungi first secrete small molecular mass organic acids (e.g., oxalic acid) to acidify the external milieu next to hyphae and solubilize Fe(III) and then also reduced quinones such as 2,3-dimethoxyhydroquinone to reduce Fe(III) to Fe(II) for the biochemical Fenton reaction [Arantes et al., 2012]. The biochemical Fenton reaction is profitable, as it facilitates access to cellulose, the primary carbon source of fungi in wood. It is notable that the organic carbon content per unit volume is 4 orders of magnitude higher in wood than in a typical solution of DOM in freshwater. The active metabolism of bacterioplankton for the utilization of DOM-Fe is probably different, simpler, and less costly than the biochemical Fenton used by brown rot fungi.
Bacteria may produce superoxide to utilize DOM-Fe. Numerous bacterial phyla, such as Alphaproteobacteria, including Caulobacter, can mediate single-electron transfer from their intracellular metabolites such as NAD (P)H to dioxygen at their cell surface and thus generate superoxide [Rose, 2012; Diaz et al., 2013]. In circumneutral waters, the half-life of superoxide ranges from tens of seconds to hours [Rose, 2012], which allows it to diffuse away from the cells into the external milieu to reach DOM-Fe within the 50 μm to 500 μm range [Fenchel, 2002]. Superoxide can reduce DOM-associated Fe(III) to Fe(II), which can be oxidized back to Fe(III) primarily by O2 [Fujii et al., 2008]. The oxidation of Fe(II) converts O2 to superoxide, which can be further converted to H2O2 [Fujii et al., 2008]. H2O2 can react with Fe(II) and produce highly reactive hydroxyl radicals (Fenton reaction) [Rose, 2012]. These hydroxyl radicals can transform DOM into bioavailable substrates [Goldstone et al., 2002]. As the generation of H2O2 requires the acidic form of superoxide, HOO⁻ (pKa = 4.8) [Rose, 2012], the formation of H2O2 was presumably not as effective in our experiments (pH 7) as it can be in acidic conditions.

It is possible that oxidation-reduction reactions of Fe associated with DOM and initiated by microbial superoxide lead to the breakdown of OM through mechanisms different from the Fenton chemistry. For example, Fe catalyzes the breakage of the aromatic ring of catechol or related derivatives in dioxygenase enzymes, which are also found in Caulobacter [Orville et al, 1997; Bugg and Winfield, 1998]. However, dioxygenase enzymes are located in the cytoplasm [Arras et al, 1998], which is an unlikely site for the enzymatic cleavage of DOM-Fe(III). The same cleavage reaction can also take place without enzymes through a Fe(III)-semiquinone-superoxide complex [Bugg and Winfield, 1998]. Fe(III) forms complexes preferentially with aromatic moieties of DOM [Fujii et al, 2014], indicating the close association of Fe(III) with the quinonoid structures in DOM. When such complexes react with superoxide, the oxidation of DOM and breakage of the quinonoid ring catalyzed by Fe(III) may take place [Bugg and Winfield, 1998].

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

4.5. Environmental Relevance and Conclusions

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

Although Fe associated to DOM can be expected to reduce the availability of organic component in DOM-Fe, this seems not to be the case [Boudot et al., 1989; Francis and Dodge, 1993; Nanchenaih et al., 2006, this study]. Even the microbial mechanisms for removing Fe from DOM-Fe are poorly known, it is good to remember that DOM-Fe has existed in the Earth as long as there has been OM and posed a challenge to microbial evolution since the origin of life. Our study suggests that the association of Fe with DOM can increase the biodegradation of DOM by bacterioplankton. The mechanism for this increase is likely different than
described earlier for fungi growing on solid substrates [Arantes et al., 2012; Rineau et al., 2012] and may involve abiotic and/or biochemical redox reactions of Fe [Pracht et al., 2001; Studenroth et al., 2013; Comba et al., 2015].

In our study, a single genus Caulobacter dominated the bacterial communities when the biodegradation of DOM was stimulated by Fe. It is possible that the active metabolisms of Caulobacter (e.g., superoxide produced by extracellular oxidoreductases) [Diaz et al., 2013] can promote extracellular nonselective degradation of humic-like DOM. Such mechanism would have a high environmental relevance, since humic-like DOM dominates the pool of DOM in many soils, sediments, and fresh and coastal waters. Due to its heterogenous composition, the extracellular enzymatic hydrolysis of humic-like DOM is poor [Arnosti, 2004] but its intense absorption of solar radiation makes it sensitive for photochemical degradation [Vähätalo et al., 2000]. Photodegradation takes place only on sunlit solid surfaces [Vähätalo et al., 1998] or in a shallow stratum of surface waters [Salonen and Vähätalo, 1994; Vähätalo et al., 2000]. Fe-stimulated biodegradation of DOM can target also such humic-like DOM that remains below the sunlit surfaces in the dark. In aquatic environments, Fe-stimulated biodegradation of DOM is expected to be most intensive at sites with high concentration of DOM-Fe. Such sites include low-order humic-rich streams and lakes with low concentrations of Ca²⁺ and Mg²⁺ hydrologically closely connected to soils and sediments that act as sources of Fe.

The selective removal of Fe from water column with increasing residence time of water [Köhler et al., 2013; Weyhenmeyer et al., 2014] decreases the contribution of Fe to the biodegradation of DOM. The additional selective removal of Fe in marine waters [Sholkovitz, 1976] reduces the concentration of Fe to very low (nmol L⁻¹) level [Boyd and Ellwood, 2010], which contrasts to the orders of magnitude higher concentrations in freshwaters (e.g., a mean of 29 nmol L⁻¹ 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 [Catáld 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; Catáld et al., 2015; Catáld et al., 2016]. Our study suggests that Fe associated with DOM can stimulate the biodegradation of humic-like DOM in Fe-rich freshwaters.
Vidal, L. O., W. Graneli, C. B. Daniel, L. Heiberg, and F. Roland (2011), Carbon and phosphorus regulating bacterial metabolism in oligotrophic

Vähätalo, A. V., M. Salkinoja-Salonen, P. Taalas, and K. Salonen (2000), Spectrum of the quantum yield for photochemical mineralization of

Sholkovitz, E. (1976), Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater, Geochim. Cosmochim. Acta, 40, 831–845, doi:10.1016/0016-7037(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.

Schweizer, H., M. Argast, and W. Boos (1982), Characteristics of a binding protein-dependent transport system for sn-glycerol-3-phosphate in

Schröder, I., E. Johnson, and S. de Vries (2003), Microbial ferric iron reductases,

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.

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. Microbiol., 73(21), 6722–6729, doi:10.1128/Aem.00405-07.

Wetzel, R. G. (2001), The phosphorus cycle, in

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.

Xia, 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.

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

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.