**Vertical distribution of Fe and Fe(III)-reducing bacteria in
the sediments of Lake Donghu, China**

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1 **Vertical distribution of Fe and Fe(III)-reducing bacteria in the sediments of Lake**

2 **Donghu, China**

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Abstract: In lake sediments, Fe is the most versatile element and the redox cycling of Fe has a wide influence on the biogeochemical cycling of organic and inorganic substances. The aim of the present study was to analyze the vertical distribution of Fe and Fe(III)-reducing bacteria (FeRB) in the surface sediment (30 cm) of Lake Donghu, China. At the three sites we surveyed, FeRB and Fe(II)-oxidizing bacteria (FeOB) coexisted in anoxic sediments. *Geobacter*-related FeRB accounted for 5–31% of the total *Bacteria*, while *Gallionella*-related FeOB accounted only 0.1–1.3%. A significant correlation between the relative abundance of poorly crystalline Fe and *Geobacter* spp. suggested that poorly crystalline Fe favored microbial Fe(III) reduction. Poorly crystalline Fe and *Geobacter* spp. were significantly associated with solid-phase Fe(II) and total inorganic phosphorus (TIP) levels. Pore water Fe(II) concentrations negatively correlated with NO_3^- at all sites. We concluded that *Geobacter* spp. were abundant in the sediments of Lake Donghu and the redox of Fe might participate in the cycling of N and P in sediments. These observations provided insight into the roles of microbial Fe cycling in lake sediments.

Keywords: Vertical distribution; Fe reduction; Poorly crystalline Fe; Fe(III)-reducing bacteria; Lake Donghu

Introduction

Sediment is an important compartment of lake ecosystems with complex chemical constituents (organic and inorganic) and microbial biomass (Tamaki et al. 2005; Wobus et al. 2003). The oxidation of organic matter with the reduction of Fe(III) is one of the most important redox reactions in anoxic environments (Bongoua-Devisme et al. 2013; Hori et al. 2010). Wang et al. (2011) have confirmed that Fe(II)-oxidizing bacteria (FeOB) are ubiquitous in wetland sediments. Fe(III) oxides, such as poorly crystalline ferrihydrite produced by FeOB, were shown to be suitable for dissimilatory Fe(III)-reducing bacteria (FeRB) (Blothe and Roden 2009; Straub et al. 1998).

Microbial Fe(III) reduction contributes significantly to Fe redox cycling in different environments (Bongoua-Devisme et al. 2012; Slobodkin et al. 1999; Thamdrup 2000). In natural sediments, Fe(III) oxides can be microbiologically reduced by strictly anaerobic or facultative FeRB using Fe(III) oxides as external electron acceptors (Lovley et al. 2004; Luef et al. 2013). FeRB are of wide interest because of their impacts on natural environments (Bongoua-Devisme et al. 2013; Chen et al. 2003; Islam et al. 2004). *Geobacter* spp. are important model microorganism for bioremediation (Akob et al. 2008; Anderson et al. 2003; Qian et al. 2011) because of their diverse respiratory capabilities, conferred in part by multicomponent and branched electron transport systems (Shi et al. 2007). Microbial Fe reduction has an extremely broad range of influences on the sediment and pore water geochemistry in surface sediment environments (Hori et al. 2010; Slobodkin et

50 al. 1999).

51 Lake Donghu (30°33'N, 114°23'E) is a shallow freshwater lake about 33 km²
52 located in Wuhan, China. As the largest urban lake in China, Lake Donghu is
53 extremely and increasingly polluted by human activities over the past 50 years, such
54 as the construction of roadway dikes and the discharge of residential and industrial
55 wastewater, which have led to eutrophication (Liu et al. 2006; Xie and Liu 2001).
56 Changes in Fe redox cycling are linked to carbon and energy flow, as well as to the
57 behavior of various inorganic compounds of N, S and P in sediments (Blothe and
58 Roden 2009; Sørensen 1982). The vertical distribution of Fe and FeRB in different
59 trophic sediments of Lake Donghu was assessed in the present study by chemical
60 analysis and real time quantitative PCR (qPCR), providing further insight into the
61 biogeochemical processes of lake sediments.

62 **Materials and Methods**

63 **Study site and sampling**

64

65 The locations of the sample sites in Lake Donghu are indicated in Fig. 1. Site 1
66 (30°33'1.70"N, 114°21'25.85"E) is near a densely populated area. Site 2
67 (30°33'2.20"N, 114°22'28.99"E) is at the center of the lake, and Site 3 (30°33'12.53"N,
68 114°23'35.31"E) is beside a scenic area. Table 1 provides all measured environmental
69 conditions of bottom water of the sampling sites. So Sites 1, 2 and 3 represent three
70 different distinct trophic states (Liu et al. 2006). Sediment cores were collected by a
71 sediment columnar dredge (6 cm diameter, 60 cm length) which can obtain the

sediment without disturbance. Sampling conditions (temperature, dissolved oxygen and pH) were assessed *in situ* using an YSI ProODO™ handheld dissolved oxygen meter (USA). The sediment cores were cut into six parts with the following sampling intervals: 0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 cm and sealed *in situ* and carefully transported to the laboratory at 4°C immediately.

Fresh sediment samples were centrifuged at $5000 \times g$ for 10 min for pore water. Pore water samples for chemical analysis were filtered through glass microfiber filters (Whatman, England). The sediments were collected. For chemical analyses of sediment samples, samples were transferred into glass bottles and stored at 4°C. For molecular analyses of sediment samples, the sediments were placed into sterile test tubes and stored at –20°C.

Chemical analysis

Pore water samples were further subdivided into two subsamples: one subsample was analyzed by ion chromatography (Metrohm, Switzerland) for SO_4^{2-} , NO_3^- and PO_4^{3-} immediately, and the second subsample was acidified with 1 mL of 0.5 M HCl to prevent precipitation of Fe (Azzoni et al. 2005), and then measured for Fe(II) in triplicate using the 2,2,-bipyridyl assay (Moss and Mellon 1942).

Total organic carbon (TOC) of sediment was measured using an Elementar Vario TOC system (China). Total inorganic phosphorus (TIP) was sequentially extracted from 2 g sediment with 20 mL of 1.0 M MgCl_2 (pH 7.5) for 2 h and 20 mL of 0.5 M HCl for 1 h (Azzoni et al. 2005). Total phosphorus (TP) was extracted from 2 g fresh sediment with 40 mL of 3.5 M HCl for 16 h. All extracts were analyzed for P by

spectrophotometric determination at 700 nm (Ruban et al. 2001). Total organic phosphorus (TOP) was calculated as the difference between TP and TIP. Solid-phase Fe(II) concentrations were determined using a modified 2,2,-bipyridyl spectrophotometric method (Moss and Mellon 1942) after digestion with 25 mL of 0.5 M HCl for 1 h (Gault et al. 2011; Weiss et al. 2004).

To measure the forms and availability of Fe, Fe fractions among the solid-phase compartments were investigated with sequential extractions. We used a five-step sequential extraction procedure that was slightly modified from Weiss et al. (2004). Briefly, about 2 g sediments were sequentially extracted by 1.0 M MgCl₂ (pH 5), 0.1 M sodium pyrophosphate, 0.2 M acid ammonium oxalate (pH 3), dithionite-citrate-bicarbonate solution (containing 0.03 M Na₃C₆H₅O₇·2H₂O, 0.125 M NaHCO₃ and 0.06 M Na₂S₂O₄) and concentrated nitric acid. Specifically, exchangeable Fe (Fe_{ex}) was extracted with 15 mL of 1.0 M MgCl₂ for 1 h (Heron et al. 1994). Organically bound fractions were extracted with 40 mL of 0.1 M sodium pyrophosphate for 16 h (Parfitt et al. 1988). Poorly crystalline or amorphous Fe (Fe_{poor}) was extracted in the dark with 40 mL of 0.2 M acid ammonium oxalate for 4 h (Phillips and Lovley, 1987). Strongly crystalline Fe (Fe_{cry}) was extracted with 40 mL of dithionite-citrate-bicarbonate solution for 16 h (Darke et al. 1994). Finally, residual Fe (Fe_{re}) was extracted with 20 mL of concentrated nitric acid for 2 h. All extracts were analyzed for Fe by colorimetry at 520 nm (Moss and Mellon 1942).

DNA extraction and qPCR

Microbial DNA was extracted using EZNA soil DNA kits D5625-01 (Omega,

116 USA) according to the manufacturer's instructions. Extracted genomic DNA was
117 detected by electrophoresis in an 1% agarose gel in $0.5 \times$ Tris-borate-EDTA (TBE)
118 buffer and stored at -20°C until use.

119 Quantitative analysis was conducted on the 16S rRNA gene fragments of total
120 *Bacteria*, *Gallionella*-related FeOB, and *Geobacter*-related FeRB. All primers
121 summarized in Table 2 were synthesized by Tianyi huiyuan Biotechnology Company
122 (Wuhan, China) and diluted to a 10 mmol/L concentration stored at 4°C .

123 The amplification of qPCR was carried out using a real time PCR detector
124 (Bio-Rad, USA) in the conditions described in Table 2 using SYBR Green as the
125 signal dye. qPCR amplification was performed in 10 μL reaction volumes containing
126 5 μL iTaq universal SYBR Green Supermix (Bio-Rad, USA), 3.5 μL water, 0.5 μL of
127 each primer and 0.5 μL DNA template (sample DNA or plasmid DNA for standard
128 curves). A final melting curve analysis was performed to check the specificity of the
129 amplification reaction. A no-template control, which contains all components except
130 the template, was included in triplicate in all qPCR experiments. Cycle thresholds
131 were determined by comparing with the standard curves constructed using a 10-fold
132 serial dilution (10^8 to 10^4 copies per μL) of the newly extracted plasmids containing
133 the corresponding gene fragments. Standard curves were checked for validity by r^2
134 value and amplification efficiency (Table 2). All qPCRs were performed in triplicate.

135 As standards for qPCR, plasmids were used to carry 16S rRNA gene fragments
136 of *Bacteria* (GenBank accession number KP714253), *Gallionella* spp. (GenBank
137 accession number KP714251) and *Geobacter* spp. (GenBank accession number

138 KP714257), respectively. Briefly, PCR amplification products were analyzed by 1%
139 agarose gel electrophoresis to check for the specificity of amplification. After
140 confirmation by agarose gel electrophoresis, the PCR products were cloned into
141 vector pMD18-T (Takara, Japan) and then transformed into the host *Escherichia coli*
142 cells (DH 5 α , Takara, Japan) according to the manufacturer's instructions. Positive
143 clones were separated. Plasmid DNA was extracted using AxyPrep plasmid miniprep
144 kits (Axygen, China). Circular plasmids were digested with *Aat* II (Takara, Japan).
145 Targeted clones were sequenced and the phylogeny was checked in the GenBank with
146 BLAST.

147 Cell numbers per g dry sediment were calculated from the qPCR 16S rRNA gene
148 copy numbers considering the average rRNA operon numbers of the respective taxa
149 (*Bacteria*, *Gallionella* spp. and *Geobacter* spp.) as listed in the rRNA Operon Copy
150 Number Database (Stoddard et al. 2014). The NCBI Genome site was used to estimate
151 16S rRNA gene copy number for *Bacteria* as 3.99 copies, *Gallionella* spp. as 2.5
152 copies and *Geobacter* spp. as 2.67 copies.

153 **Statistical analysis**

154 Quantitative data is expressed as the mean $\pm$ SD. One way analysis of variance
155 followed by *post-hoc* least significant difference testing was used to determine the
156 variations among different sites. Statistical significance was set at the $P < 0.05$ level.
157 Non-parametric Spearman's correlation analysis was performed in Statistical Product
158 and Service Solutions (SPSS, USA) software to uncover quantitative associations
159 among variables.

160 **Results**

161 **Pore water characteristics**

162

163 Pore water geochemical analysis of lake sediment cores collected from Lake
164 Donghu, revealed that the sediments were anoxic and highly reduced throughout the
165 first 30 cm (Fig. 2). The gradients of pore water SO_4^{2-} , NO_3^- , PO_4^{3-} and Fe(II) related
166 obviously to the trophic state of sediments. In general, SO_4^{2-} and NO_3^- concentrations
167 decreased with depth, while PO_4^{3-} and Fe(II) concentrations increased with depth.
168 The maximum contents of Fe(II) in pore water were 1 mg/L at Sites 1 and 2, whereas
169 at Site 3 was 0.5 mg/L.

170 **Sediment chemistry and sequential Fe fractionation**

171

172 The gradients of TOC, TOP, TIP and Fe(II) (Fig. 3) in sediments were also
173 obviously related to the trophic states. The sediment TOC followed a similar gradient
174 of Sites 1 and 2, with TOC content up to 30 g/kg and decreased steeply. TOC levels of
175 Site 3 did not change much with depth and were < 10 g/kg. The concentrations of TP
176 (TOP and TIP) of Sites 1 and 2 were significantly higher than that of Site 3 ($P < 0.01$).
177 In general, TIP decreased with depth, while TOP fluctuated with depth. The highest
178 concentrations of Fe(II) were measured in 25–30 cm sampling interval.

179

180 The sediment was particularly rich in Fe (25.48 ± 1.56 g/kg) and the spatial
181 variability was primarily related to depth. There were various types of Fe oxides (Fig.

4), ranging from exchangeable structures to highly crystalline oxide forms. Exchangeable Fe was very low in the sediment. Organic Fe and poorly crystalline Fe decreased with depth. Fe mainly existed as poorly crystalline Fe and crystalline Fe.

Quantification of bacterial abundance using qPCR analyses

Group specific qPCR with genomic DNA as template was used to estimate the relative abundance of known FeRB and FeOB in anoxic sediments (Fig. 5). The total number of *Bacteria* ranged from 1.11×10^9 to 2.05×10^{10} cells /g dry sediment. Total *Bacteria* populations were significantly higher in the surface sediment (0–5 cm) than at other levels of all sites, and were influenced significantly by depth ($P < 0.05$). The number of *Geobacter* spp. ranged from 2.33×10^8 to 1.36×10^9 cells /g dry sediment and represented an important component of the bacterial community of Lake Donghu and accounted for 5–31% of total *Bacteria*. *Gallionella* spp. were also detected in all sediments and the cell numbers ranged from 1.27×10^7 to 3.33×10^7 /g dry sediment, accounting for only 0.1–1.3% of total *Bacteria*. *Geobacter* spp. consistently outnumbered *Gallionella* spp. throughout the sediment profiles.

Discussion

In this study, *Geobacter*-related FeRB in sediment samples characterized by depth were investigated to obtain information on the spatial distribution of *Geobacter* spp. and the relationships between Fe forms and FeRB in sediment. Vertical distribution of Fe in sediment depends upon both abiotic factors and microbial processes. *Geobacter* spp. and *Gallionella* spp. were detected in each site, which

suggested that Fe(II) oxidation and Fe(III) reduction were simultaneous within the sediments. Weiss et al. (2003) believed that poorly crystalline Fe could continually regenerated through the action of FeOB. It is thought that poorly crystalline forms of Fe are the major products of both abiotic and biotic Fe oxidation in lake sediments, which may be further crystallized by other sediment components such as organic matter (Straub et al. 1998; Thamdrup 2000). Our results also showed a significant correlation between the percentage of poorly crystalline Fe and *Gallionella* spp. (Table 3).

Geobacter-related FeRB populations accounted for a major portion (5–31%) of the total *Bacteria*, while *Gallionella*-related FeOB were only 0.1–1.3%. Therefore, Fe(III) reduction is a major phenomenon and microbial Fe(III) reduction was a major biogeochemical process in anoxic sediments (Himmelheber et al. 2009; Sobolev and Roden 2002). Results from sequential extractions indicated that Fe fractions mainly existed as poorly crystalline Fe and crystalline Fe. Poorly crystalline Fe oxides readily function as electron acceptors for FeRB (Lovley et al. 2004; Weber et al. 2006). A significant correlation between the percentage of poorly crystalline Fe and *Geobacter* spp. (Table 3) suggested that poorly crystalline Fe favored microbial reduction, as identified in previous studies (Hu et al. 2014; Roden and Wetzel 2002; Weiss et al. 2004). The percentage of poorly crystalline Fe was higher than that of crystalline Fe. In contrast to crystalline Fe oxides reduction, which rarely exceed 20% of the total Fe(III) present (Roden and Urrutia 2002; Weiss et al. 2004), poorly crystalline Fe is subject to almost all dissolution (Thamdrup 2000).

Another factor contributing to the large difference in the vertical distribution of *Geobacter* spp. may be the higher levels of carbon in sediments. Previous studies of microbial abundance in sediments indicated that those with larger amounts of carbon generally had a larger microbial population (Carr et al. 2013; Dong et al. 2014; Hardison et al. 2013). The overall microbial abundance in sediments ultimately depends on the availability of suitable nutrients and energy sources (Carr et al. 2013). In this study, a positive correlation was observed between total *Bacteria* and TOC (Table 3). The process of Fe reduction needs organic carbon (Chen et al. 2003; Gächter and Müller 2003) or H₂ (Ehrlich and Newman 2009) as electron donor. There was no significant correlation between *Geobacter* spp. and TOC in Lake Donghu sediments. Bongoua-Devisme *et al.* (2012) found very low organic carbon contents (1.7–1.8 g/kg) were sufficient for the development of bacterial population diversity and affected the activity of FeRB significantly. These data imply that the TOC in the sediment of Lake Donghu is sufficient to provide enough electron donors for *Geobacter*.

Fe is perhaps the most versatile element, and the redox cycling of Fe exerts a wide influence on the biogeochemical cycling of N, S and P in sediment environments (Azzoni et al. 2005; Weber et al. 2006; Wilms et al. 2007). Pore water and sediment profiles of Fe geochemistry also showed active Fe(III) reduction at depth. The dissolved Fe(II) concentrations in pore water increased with depth (0–30 cm), providing evidence that Fe(III) reduction was taking place in anoxic sediments. NO₃[−] concentrations were significantly negatively correlated with pore water Fe(II) at all

248 sites (Table 4). Previous studies had showed the reoxidation of Fe(II) is not limited to
249 O₂ in anoxic sediments (Melton et al. 2012; Roden et al. 2004; Straub et al. 1996).
250 Fe(II) can also be oxidized with NO₃⁻ as the electron acceptor (Oshiki et al. 2013;
251 Straub et al. 2001). Microbial catalyzed NO₃⁻-dependent Fe(II) oxidation has the
252 potential to accelerate the dynamic of microbially mediated Fe redox cycling in
253 anoxic sediments (Straub et al. 1996; Weber et al. 2006). Furthermore, Weber et al.
254 (2006) proposed that microbial anoxic iron cycling by *Geobacter* spp. was capable to
255 participate in both dissimilatory Fe(III) reduction and NO₃⁻-dependent Fe(II)
256 oxidation. Coby et al. (2011) also found *Geobacter* participated in nitrate-dependent
257 Fe(II) oxidation in the cycling cultures. The decline in SO₄²⁻ concentrations were
258 observed with depth, suggesting the existence of microbial SO₄²⁻ reduction (Gault et
259 al. 2011). Sulfur-reducing bacteria may have contributed to reduce Fe(III) indirectly
260 (Straub and Schink 2004). In particular, the concentrations of SO₄²⁻ correlated with
261 Fe(II) at Site 1, but no significantly correlation at Sites 2 and 3. The sampling
262 intervals over which Fe(II) concentrations in pore water increased (15–30 cm) were
263 characterized by near-constant SO₄²⁻ concentrations, suggesting the absence of
264 significant SO₄²⁻ reduction at Sites 2 and 3 (Lin et al. 2007).

265 The biogeochemical reactions of Fe affect the availability and mobility of
266 sediment P (Azzoni et al. 2005; Ribeiro et al. 2014; Rozan et al. 2002). Our results
267 also showed a significant positive correlation between dissolved Fe(II) and PO₄³⁻ at
268 Sites 1 and 2 (Table 4). Fe oxide containing substrates are excellent sorbents for P
269 (Gomez et al. 1999; Rentz et al. 2009). The number of *Geobacter* spp. was

significantly associated with TIP (Table 3). Poorly crystalline Fe is primarily associated with TIP (Table 3), this was similar to observations in earlier research (Anschutz et al. 1998). Microbial Fe(III) reduction and the subsequent Fe(II) produced in reducing sediments affect inorganic P released to pore water (Azzoni et al. 2005). We concluded that higher levels of poorly crystalline Fe might bind inorganic P in sediment. Thus, any alteration to sediment Fe oxides, such as the vertical distribution of Fe oxides in sediment, had important implications for sediment geochemistry.

Conclusion

Vertical distribution of Fe and FeRB in sediments of Lake Donghu was investigated in this study. Various types of Fe oxides coexisted in sediments. FeRB and FeOB were detected at each sampling site, which suggested that Fe(II) oxidation and Fe(III) reduction were simultaneous within the sediments. *Geobacter* spp. populations were abundant, while *Gallionella* spp. accounted for only 0.1–1.3% of the total *Bacteria* in the sediments of Lake Donghu. The spatial distribution of *Geobacter* spp. correlated significantly with poorly crystalline Fe, which reflected that poorly crystalline Fe was labile with respect to microbial reduction. Pore water and sediment profiles of Fe geochemistry also showed active Fe(III) reduction at depth. Pore water Fe(II) concentrations significantly negatively correlated with NO_3^- at all sites. Poorly crystalline Fe and *Geobacter* spp. were significantly associated with Fe(II) and TIP levels. Results from this work suggested that the vertical distributions of Fe and FeRB might participate in the cycle of N and P in anoxic sediments of Lake

292 Donghu. These observations provide insight into the role of microbial Fe cycling in
293 lake sediments.

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463 hypereutrophic lake. The Scientific World J. 1: 337-356.
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465

466 **Fig. 1.** Location of the sampling sites at Lake Donghu

467

468 **Fig. 2.** Vertical distribution of pore water SO_4^{2-} , NO_3^- , PO_4^{3-} and Fe(II) by sample
469 depth

470

471 **Fig. 3.** Vertical distribution of sediment total organic carbon (TOC), total organic
472 phosphorus (TOP), total inorganic phosphorus (TIP) and Fe(II) by sample depth

473

474 **Fig. 4.** Vertical distribution of sediment Fe fractions by sample depth. Fe_{ex} ,
475 exchangeable Fe; Fe_{org} , organic Fe; Fe_{poor} , poorly crystalline Fe; Fe_{cry} , crystalline Fe

476

477 **Fig. 5.** Vertical distribution of total number of total *Bacteria*, *Gallionella* spp. and
478 *Geobacter* spp. by sample depth

1 **Table 1.** Values of water quality in bottom waters from Lake Donghu

Site	T (°C)	pH	O ₂ (mg/L)	SO ₄ ²⁻ (mg/L)	NO ₃ ⁻ (mg/L)	PO ₄ ³⁻ (mg/L)
Site 1	23.4	8.23	3.22	5.27	0.30	1.68
Site 2	23.1	8.05	0.71	5.39	0.07	0.90
Site 3	23.2	8.16	2.21	4.99	0.01	0.68

2 **Table 2.** Conditions and primers for real time qPCR

Target group	Primer	Sequence (5'-3')	PCR cycling profile	<i>r</i> ² value	Amplification efficiency (%)	Reference
<i>Bacteria</i>	341F	CCTACGGGAGG	95°C/4 min,	0.988	99.4	(Hegler et al. 2012)
		CAGCAG	94°C/45 s,			
		GGACTACCAGG	65°C/20 s,			
	797R	GTATCTAATCCT	40 cycles			
<i>Gallionella</i> spp.	122F	ATATCGGAACAT	94°C/10min,	0.994	94.9	(Wang et al. 2009)
		GTCCGG	94°C/45s,			
		CTCTGGAAACTT	59°C/30s, 45			
	998R	CCTGAC	cycles			
<i>Geobacter</i> spp.	564F	CAAGTCGTACG	94°C/10	0.996	110.7	(Cummins et al. 2003)
		AGAAACATATC	min,			
		GAAGAGGATCG	94°C/30 s,			
	840R	TCTTTCCACGA	55.7°C/30 s,			
			40 cycles			

3 **Table 3.** Spearman correlation matrix between different sediment characteristics
4 determined

	TOC	TIP	TOP	Fe(II)	Fe _{ex}	Fe _{org}	Fe _{poor}	Fe _{cry}	<i>Gallionella</i>	<i>Geobacter</i>	<i>Bacteria</i>	Geo/Bac
TOC	1.000	.588*	.348	-.473*	.311	.631**	.652**	-.545*	.299	.402	.570*	-.669**
TIP		1.000	-.426	-.734**	-.287	.612**	.794**	-.437	.558*	.540*	.829**	-.775**
TOP			1.000	.141	.410	.075	-.059	-.065	.003	-.123	-.141	-.005
Fe(II)				1.000	.506*	-.595**	-.831**	.482*	-.573*	-.492*	-.721**	.715**
Fe _{ex}					1.000	.153	-.233	.028	-.216	-.272	-.286	.111
Fe _{org}						1.000	.787**	-.265	.567*	.362	.649**	-.792**
Fe _{poor}							1.000	-.319	.575*	.562*	.785**	-.810**
Fe _{cry}								1.000	-.253	-.472*	-.498*	.414
<i>Gallionella</i>									1.000	.459	.732**	-.699**
<i>Geobacter</i>										1.000	.827**	-.472*
<i>Bacteria</i>											1.000	-.839**
Geo/Bac												1.000

5 TOC, total organic carbon; TOP, total organic phosphorus; TIP, total inorganic phosphorus; Fe_{ex},
6 exchangeable Fe; Fe_{org}, organic Fe; Fe_{poor}, poorly crystalline Fe; Fe_{cry}, crystalline Fe; Geo/Bac, relative
7 abundance of *Geobacter* (% total *Bacteria*).
8 *, correlation is significant at the 0.05 level (2-tailed); **, correlation is significant at the 0.01 level
9 (2-tailed).
10 **Table 4.** Spearman correlation between pore water Fe(II) and other chemical
11 characteristics at the study sites

	$\text{Fe(II)} \times \text{NO}_3^-$	$\text{Fe(II)} \times \text{SO}_4^{2-}$	$\text{Fe(II)} \times \text{PO}_4^{3-}$
Site 1	-.886*	-.829*	.943**
Site 2	-.886*	-.657	.928**
Site 3	-.898*	-.577	.516

12 *, correlation is significant at the 0.05 level (2-tailed); **, correlation is significant at the 0.01 level

13 (2-tailed).

14

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