

Supplement of Biogeosciences Discuss., 12, 7705–7737, 2015
<http://www.biogeosciences-discuss.net/12/7705/2015/>
doi:10.5194/bgd-12-7705-2015-supplement
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Supplement of

Iron encrustations on filamentous algae colonized by *Gallionella*-related bacteria in a metal-polluted freshwater stream

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1. Supplemental Tables

Table S1. Tags, barcodes and forward primers used for pyrosequencing of the algae-bacterial communities.

Sample	Tag sequence	Barcode sequence	Forward primer sequence
Site O Aug, RNA	TCCTGCCT	AAAACGGG	GAGTTTGATCNTGGCTCAG
Site O Aug, DNA	TCTACGTT	AAAACGTT	GAGTTTGATCNTGGCTCAG
Site O Sep, RNA	GAACAGGA	AAAACACT	GAGTTTGATCNTGGCTCAG
Site O Sep, DNA	CTAGGATA	AAAACACG	GAGTTTGATCNTGGCTCAG
Site A Jul, RNA	CGATTGCT	AAAACGCA	GAGTTTGATCNTGGCTCAG
Site A Jul, DNA	TAATTGGA	AAAACGTA	GAGTTTGATCNTGGCTCAG
Site A Aug, RNA	CCGGCGAA	AAAACGCT	GAGTTTGATCNTGGCTCAG
Site A Aug, DNA	CAGTCATT	AAAACGTG	GAGTTTGATCNTGGCTCAG
Site A Sep, RNA	GAACATTA	AAAACACC	GAGTTTGATCNTGGCTCAG
Site A Sep, DNA	GAACACCA	AAAACACA	GAGTTTGATCNTGGCTCAG
Site C Jul, RNA	CATATGGT	AAAACGCC	GAGTTTGATCNTGGCTCAG
Site C Jul, DNA	TGGCGAGA	AAAACGTC	GAGTTTGATCNTGGCTCAG
Site C Aug, RNA	TCTCTTCT, CTAGCGCA, CTAGCTGA	AAAACGCG, AAAACAAG, AAAACAAT	GAGTTTGATCNTGGCTCAG
Site C Aug, DNA	TTACCAAT, CTAATGGA, CTAGCAA	AAAACGGA, AAAACAAA, AAAACAAC	GAGTTTGATCNTGGCTCAG

Table S2. Homology search results for 18S rRNA gene sequences amplified from DNA extracts of algal samples collected in August 2013.

Algal sample (color)	Primer	Length of detected sequence (bp)	Results of homology search (GenBank acc. no.)	Homology (%)
Site O Aug (Green)	Euk20F/ Euk1179R	1131	<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	99.9
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	99.9
			<i>Tribonema</i> sp. JB9 (KF791558)	99.8
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	99.8
	P45/P47	572	<i>Tribonema</i> sp. JB9 (KF791558)	100
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	100
			<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	100
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	100
Site A Aug (Green)	Euk20F/ Euk1179R	1133	<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	100
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	100
			<i>Tribonema</i> sp. JB9 (KF791558)	99.9
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	99.9
	P45/P47	388	<i>Tribonema</i> sp. JB9 (KF791558)	100
			<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	100
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	100
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	100
Site B Aug (Green)	Euk20F/ /Euk1179R	1132	<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	100
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	100
			<i>Tribonema</i> sp. JB9 (KF791558)	99.9
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	99.9
	P45/P47	446	<i>Tribonema</i> sp. JB9 (KF791558)	99.5
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	99.5
			<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	99.5
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	99.5
Site C Aug (Brown)	Euk20F/ Euk1179R	1133	<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	100
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	100
			<i>Tribonema</i> sp. JB9 (KF791558)	99.9
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	99.9
	P45/P47	461	<i>Tribonema</i> sp. JB9 (KF791558)	100
			<i>Tribonema viride</i> strain SAG 23.94 (AM490825)	100
			<i>Tribonema ulotrichoides</i> strain SAG 21.94 (AM490817)	100
			<i>Tribonema minus</i> strain SAG 880-3 (AM490824)	100

Table S3. Percentages of OTUs detected by 16S rRNA gene-targeted amplicon pyrosequencing for RNA and DNA extracts from the algal samples.

Algal sample (color)	Nucleic acid	Chloroplast (% Total)	Bacteria (% Total)	Gallionellaceae (% Bacteria)	OTU-1 * ¹ (% Bacteria)	OTU-2 * ² (% Bacteria)
Site O Aug (Green)	RNA	68.6	31.4	96.5	81.0	15.5
	DNA	87.7	12.3	96.4	75.7	20.7
Site O Sep (Green)	RNA	21.8	78.2	89.4	76.1	13.3
	DNA	82.0	18.0	91.3	79.3	12.0
Site A Jul (Green)	RNA	85.0	15.0	91.8	34.5	57.3
	DNA	99.2	0.8	79.5	24.1	55.4
Site A Aug (Green)	RNA	63.7	36.3	92.5	39.7	52.8
	DNA	93.6	6.4	88.0	39.7	48.4
Site A Sep (Brown)	RNA	32.7	67.3	82.9	49.9	33.1
	DNA	93.6	6.4	63.0	36.4	26.6
Site C Jul (Brown)	RNA	60.4	39.6	80.8	28.7	52.2
	DNA	98.0	2.0	81.0	19.0	62.0
Site C Aug (Brown)		14.1	85.9	71.2	20.6	50.6
	RNA	17.8	82.2	68.5	26.7	41.8
		21.6	78.4	71.5	26.6	44.9
		87.0	13.0	65.0	15.9	49.1
	DNA	88.8	11.2	54.6	21.0	33.7
		86.6	13.4	68.4	24.2	44.2

*¹ OTU-1: a major OTU which has high homology with *Gallionella capsiferiformans* ES-2 (CP002159, 96.5% identity) and *Sideroxydans lithotrophicus* ES-1 (CP001965, 96.4% identity)

*² OTU-2: a major OUT which has high homology with *S. lithotrophicus* ES-1 (94.3% identity) and *G. capsiferiformans* ES-2 (97.4% identity)

Plot data for Figure 2.

pH

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		5.90	5.94	5.92
Site A	6.06	6.41	6.04	6.17
Site B	6.29	6.47	5.97	6.24
Site C	6.39	6.50	6.37	6.42

Temperature (°C)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		16.4	15.0	15.7
Site A	16.9	14.9	14.3	15.4
Site B	17.3	15.3	14.4	15.7
Site C	17.5	15.4	14.4	15.8

Conductivity (ms cm⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		4.82	4.92	4.87
Site A	4.84	4.85	4.82	4.84
Site B	4.88	4.79	4.80	4.82
Site C	4.85	4.80	4.77	4.81

Eh (mV)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		187	179	183
Site A	170	143	148	154
Site B	179	141	144	155
Site C	175	133	133	147

Oxygen (mg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		2.00	1.28	1.64
Site A	3.00	5.00	3.69	3.90
Site B	5.50	6.70	5.00	5.73
Site C	6.20	6.90	6.55	6.55

DOC (mg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		4.354	3.154	3.754
Site A		4.053	3.556	3.805
Site B	4.554	3.228	3.696	6.24
Site C	3.680	3.317	3.382	6.42

Sulfate (mM)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		30.70	34.14	32.36
Site A	33.15	27.92	36.02	32.36
Site B	34.03	34.24	32.27	33.51
Site C	36.76	36.51	35.04	36.10

Fe(II) (mM)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		3.014	3.134	3.074
Site A	3.307	2.515	2.602	2.808
Site B	3.298	2.304	2.387	2.663
Site C	3.039	2.289	2.114	2.481

2. Supplementary Figures

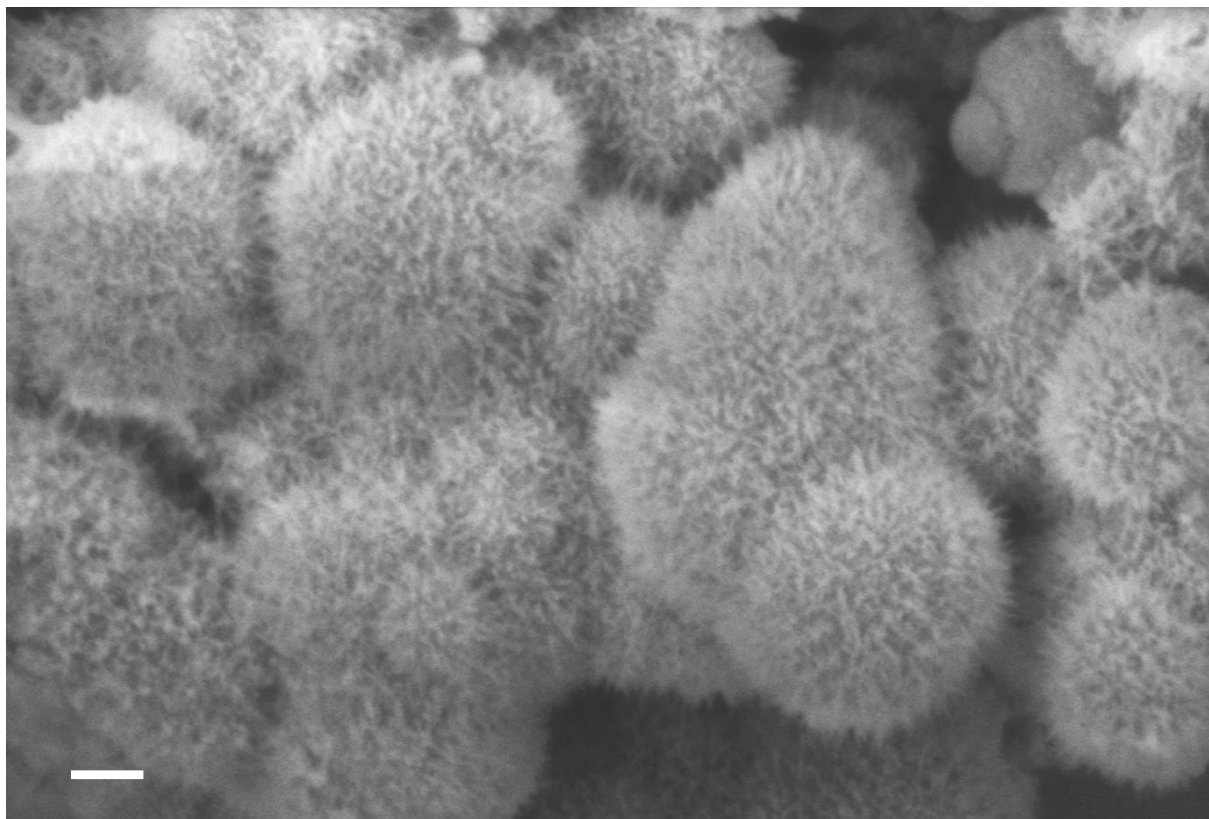


Figure S1. High resolution secondary electron image of Fe-oxides around the algae. Characteristic morphologies of schwertmannite (nano-needles) and ferrihydrite (upper left part) were represented. Scale bar indicates 200 nm.

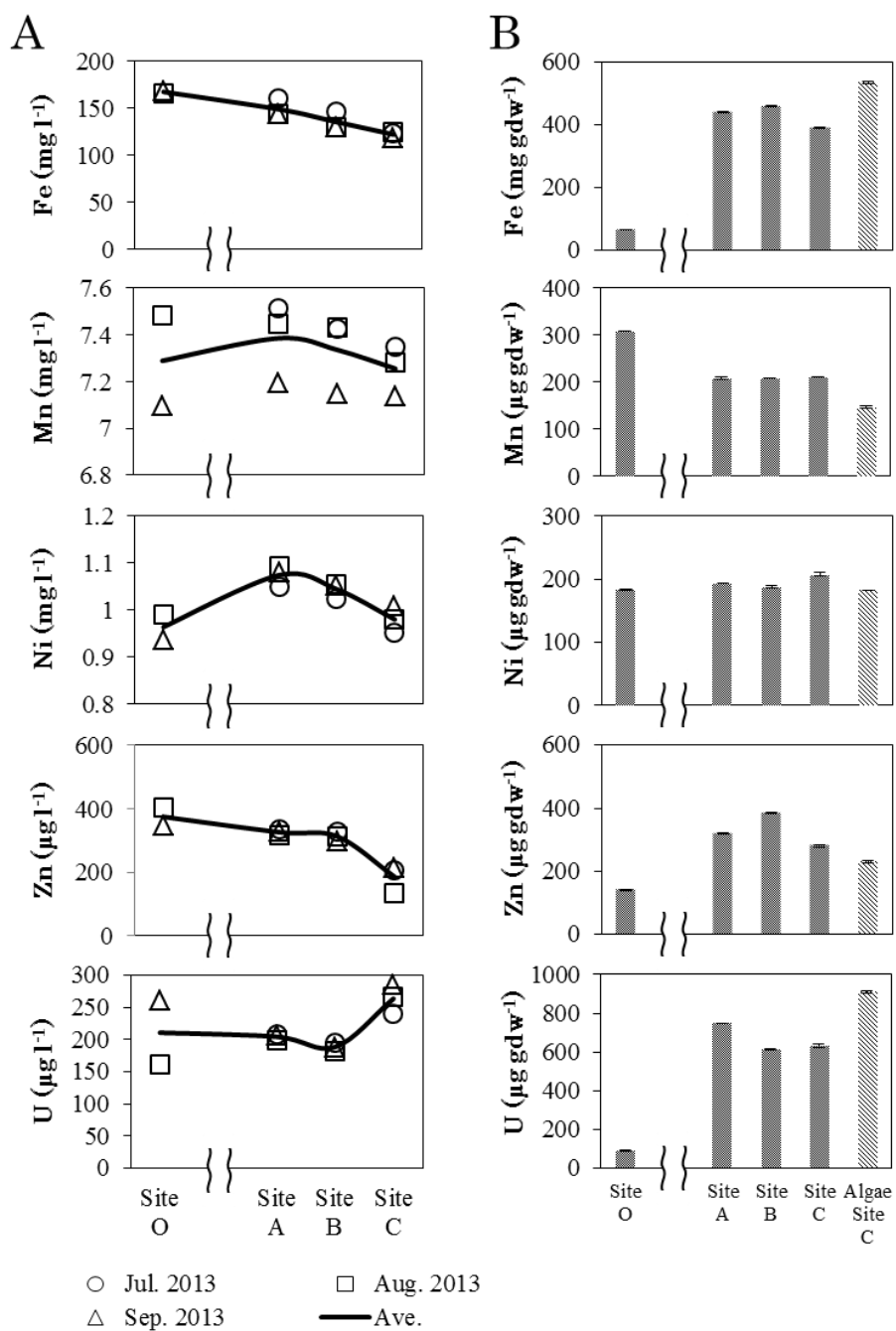


Figure S2. ICP-MS/OES measurement for metals dissolved in water (A) and in sediments or in brown algae collected at site C in the outflow water stream (B) at each sampling site in July, August, and September 2013. B: n=3, error bars indicate standard deviations.

Plot data for Figure S2.

A

Fe (mg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		166.45	168.80	167.63
Site A	160.63	143.40	143.53	149.19
Site B	146.27	130.43	130.45	135.72
Site C	123.33	125.33	118.03	122.23

Mn (mg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		7.485	7.094	7.290
Site A	7.516	7.446	7.196	7.386
Site B	7.425	7.434	7.150	7.336
Site C	7.347	7.282	7.138	7.256

Ni (mg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		0.991	0.938	0.965
Site A	1.050	1.095	1.080	1.075
Site B	1.025	1.056	1.053	1.045
Site C	0.953	0.980	1.009	0.981

Zn (µg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		402.2	347.0	374.6
Site A	336.0	315.0	328.2	326.4
Site B	329.0	311.0	299.0	313.0
Site C	207.0	133.0	215.0	185.0

U (µg l⁻¹)

	Jul 2013	Aug 2013	Sep 2013	Average
Site O		161.0	260.6	210.8
Site A	208.3	199.0	206.2	204.5
Site B	196.0	182.0	188.0	188.7
Site C	239.4	267.0	284.5	263.6

B

Fe (mg gdw⁻¹)

	Average (n=3)	SD
Sediment site O	66.33	0.17
Sediment site A	441.77	3.76
Sediment site B	461.63	1.00
Sediment site C	391.81	1.17
Algae site C	535.88	2.29

Mn (µg gdw⁻¹)

	Average (n=3)	SD
Sediment site O	307.2	0.0
Sediment site A	208.0	2.0
Sediment site B	208.8	0.5
Sediment site C	211.4	0.0
Algae site C	147.0	2.0

Ni (µg gdw⁻¹)

	Average (n=3)	SD
Sediment site O	183.0	1.0
Sediment site A	192.7	0.3
Sediment site B	187.0	2.0
Sediment site C	207.0	3.0
Algae site C	182.7	0.0

Zn (µg gdw⁻¹)

	Average (n=3)	SD
Sediment site O	141.0	2.0
Sediment site A	320.0	1.0
Sediment site B	385.1	0.7
Sediment site C	281.0	4.0
Algae site C	229.0	4.0

U (µg gdw⁻¹)

	Average (n=3)	SD
Sediment site O	92	1.0
Sediment site A	746	0.1
Sediment site B	614	4.0
Sediment site C	631	8.0
Algae site C	909	6.0