Influence of seasonal and geochemical changes on

iron geomicrobiology of an iron-carbonate mineral water spring

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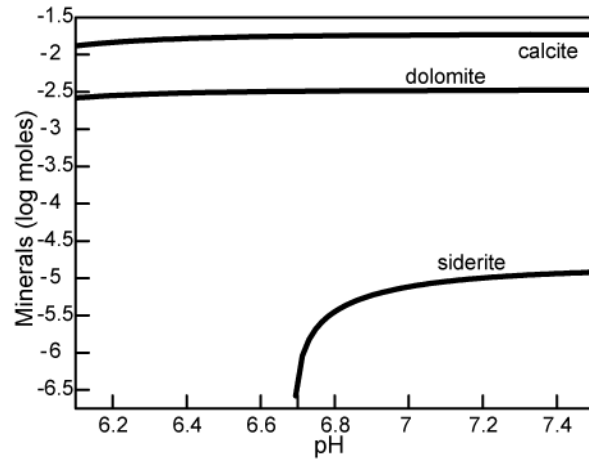
Supplemental Material

FIG S1. Thermodynamic modeling of carbonate mineral precipitation using the React-module of Geochemist's Workbench. The pH-change along the flow channel was used as variable on the x-axis. The y-axis shows the calculated amount of minerals precipitated under the given conditions. Temperature was kept constant at 5°C since temperature changes did not influence the thermodynamic modeling significantly. The ion concentrations measured at the spring were used in the matrix for the calculation.
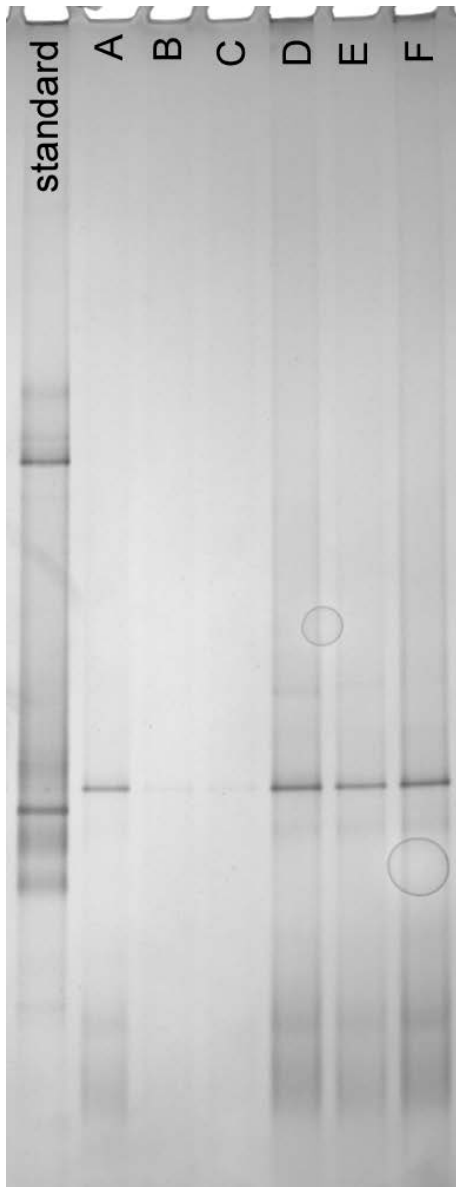
FIG S2. Representative DGGE-gel of the spring 2009 sampling campaign with cyanobacterial primers (TABLE S3). Lane A: flow channel, 0 m from spring outlet, lane B: flow channel, 1.2 m from spring outlet, lane C: flow channel, 2.2 m from spring outlet, lane D: microbial mat, 0 m from spring outlet, lane E: microbial mat, 1.2 m from spring outlet, lane F: microbial mat, 2.2 m from spring outlet.
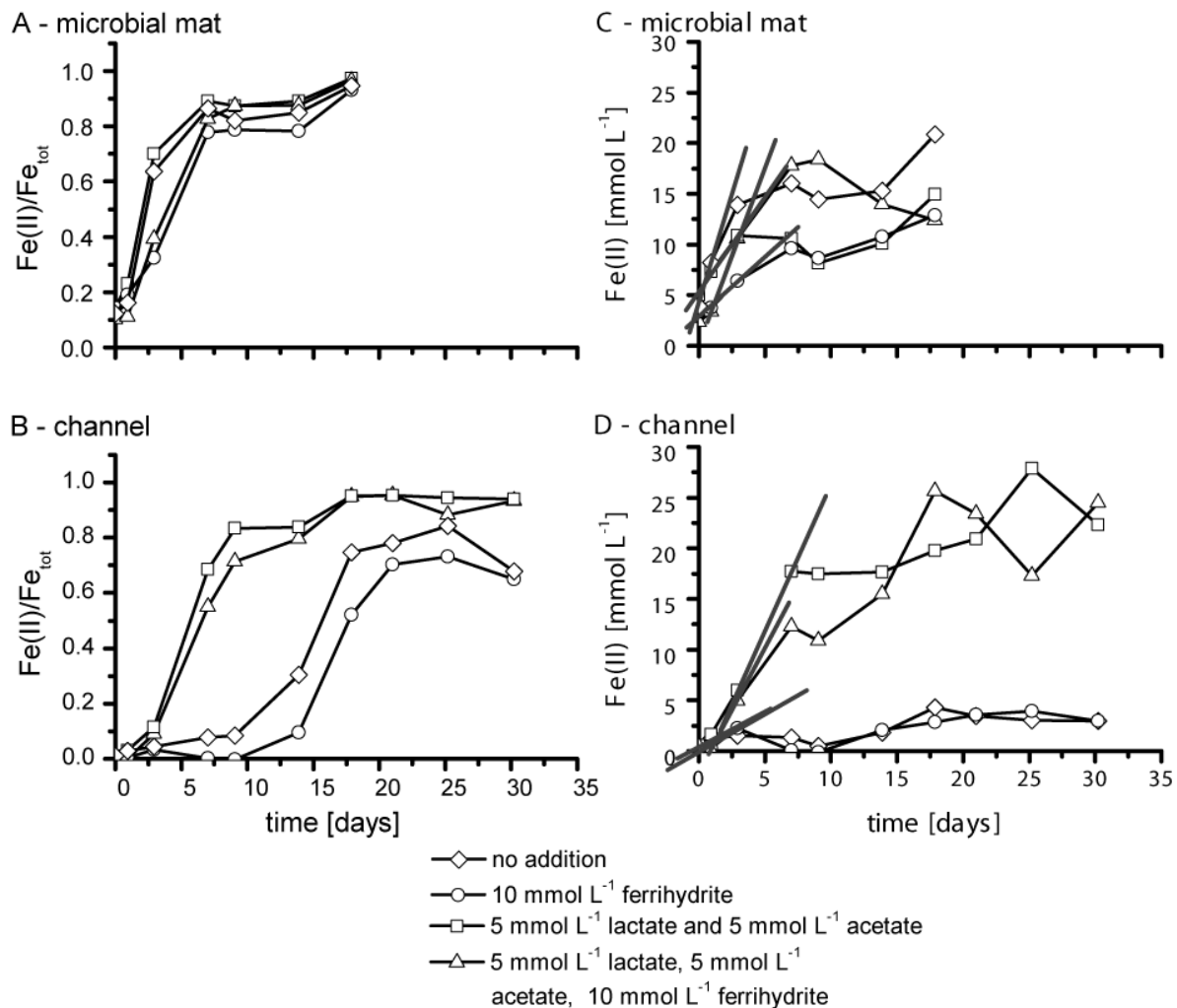
FIG S3. Reduction of Fe(III) in 1 g of wet sample collected from the Fuschna spring. A and C: sample collected from the side of the stream. B: sample collected from the middle of the channel. Samples were collected in summer 2010. A and B show $Fe(II)/Fe_{tot}$ while C and D show $Fe(II)_{tot}$. This figure corresponds to Fig. 5 in the main manuscript but in addition it shows the regression lines used for the determination of the Fe(III) reduction rates for Fig. C and D.

TABLE S1. Calculation of expected Fe(II) concentrations considering chemical oxidation of Fe(II) in the flow channel in comparison to measured values. For the calculation the initial measured Fe^{2+} concentration, the O_2 concentrations and pH at the different sampling points and the flow rate were used.

	0 m	0.7 m	1.2 m	1.5 m	2.2 m
Fe(II) [µmol/L] in water calculated	15	4	1.5	0.6	0.2
Fe(II) [µmol/L] in water measured	15	13	13	15	13

TABLE S2. Modeling parameters for Mössbauer spectra collected at 77K from samples of August 2009 at three different locations from the Fuschna spring outlet. For the sample collected at 1.2 m an additional spectrum was collected at 5K.

	outlet	1.2 m downstream	2.3 m downstream	ferrihydrite reference[**]
δ_0 (77K)[*]	0.47	0.48	0.47	0.46
$\Delta\varepsilon$ (77K)[*]	0.99	0.94	0.90	0.70
σ_Δ (77K)[*]	0.73	0.57	0.47	NA
δ_0 (5K)[*]	ND	0.49	ND	0.48
ε (5K)[*]	ND	-0.05	ND	-0.02
H (5K)[*]	ND	44.3	ND	45.3 and 49.3

[*]δ_0 – center shift, $\Delta\varepsilon$ – quadrupole splitting, σ_Δ – sigma, H – hyperfine field, 2 separate sextet were used to fit the data in the reference, ND – not determined, NA – not applicable.

[**]ferrihydrite reference prepared according to Raven et al., 1998.

TABLE S3. Primers used for DGGE, clone library construction, and quantitative PCR. PCRs with *Archaea* primers yielded no products in this study.

Primer	Target group	Sequence (5'->3')	Application	Reference
Cya359F-GC	most Cyanobacteria	CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACG GGGGGGGGGAATYTTCCGCAATGGG	DGGE	(9)
CYA785R	most Cyanobacteria	GACTACWGGGGTATCTAATCC	DGGE	(4)
GC-341-F (GM5-F-GC)	most Bacteria	CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGC CCGCCTACGGGAGGCAGCAG	DGGE	Modified after (7)
907-R	most Bacteria	CCGTCAATTCCTTTRAGTTT	DGGE	(6)
GC-ARCH344f	most Archaea	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACG GGGGACGGGGYGCAGCAGGCGCGA	DGGE	(3)
ARCH519r	most Archaea	GWATTACCGCGGCKGCTG	DGGE	(1)
GC-ARCH357f	most Archaea	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACG GGGGCCCTACGGGGCGCAGCAG	DGGE	(12)
ARCH693r	most Archaea	GGATTACARGATTTC	DGGE	(12)
GM3-8F	most Bacteria	AGAGTTTGATCMTGGCTCAG	PCR; clone library	(7)
Uni1392R	most Bacteria	ACGGGCGGTGTGTRC	PCR; clone library	(10)
341F	most Bacteria	CCTACGGGAGGCAGCAG	qPCR	(7)
797R	most Bacteria	GGACTACCAGGGTATCTAATCCTGTT	qPCR	(8)
GAL214F	*mainly Gallionella* spp.	CCTCTCGCTTTCGGAGTGGCCG	qPCR	(5)
GAL384R	*mainly Gallionella* spp.	GGTATGGCTGGATCAGGC	qPCR	(2)

TABLE S4. Geochemical parameters of the spring, samples collected in August 2009. Concentrations for sodium, nitrate, nitrite and bromide were below the detection limit of ion chromatography.

Distance from outlet [m]	°C	O_2 [μM]	pH	Fe(II) [μM]	Fe(III) [μM]	P_{total} [μM]
0 ± 0.05	10.8 ± 0.1	15 ± 1	6.3 ± 0.05	15.9 ± 0.2	3.8 ± 0.2	0.09 ± 0.01
1.2 ± 0.05	12.5 ± 0.1	140 ± 7	6.5 ± 0.05	12.7 ± 0.9	6.6 ± 0.9	0.24 ± 0.02
2.2 ± 0.05	14.5 ± 0.01	217 ± 11	6.7 ± 0.05	12.6 ±0.1	7.2 ± 0.1	0.21 ± 0.02

Distance from outlet [m]	K^+ [mM]	Mg^{2+} [mM]	Ca^{2+} [mM]	F^- [μM]	Cl^- [mM]	SO_4^{2-} [mM]
0 ± 0.05	*0.3* ± 0.03	*2.2* ± 0.01	*17.4* ± 0.6	32 ± 1	0.2 ± 0.003	0.5 ± 0.03
1.2 ± 0.05	0.3 ± 0.01	2.2 ± 0.2	16.0 ± 0.7	28 ± 1	0.2 ± 0.01	0.8 ± 0.07
2.2 ± 0.05	0.3 ± 0.01	2.2 ± 0.2	13.4 ± 0.8	19 ± 0.1	0.2 ± 0.01	0.8 ± 0.06

Distance from outlet [m]	CO_3^{2-} [mM]	alkalinity [mM]	delivery [L/min]
0 ± 0.05	44 ± 1	51 ± 2	1.1 ± 0.05
1.2 ± 0.05	28 ± 1	51 ± 2	
2.2 ± 0.05	20 ± 0.1	48 ± 2	

TABLE S5. Phylogenetic affiliations and frequencies of bacterial 16S rRNA gene clones from the Fuschna spring. Samples were collected 1.2 m of the spring outlet in August 2009 from the middle of the flow channel and the flanking microbial mat, respectively. Each row represents a different operational taxonomic unit with a similarity cut-off of 97%. Taxonomic names in quotation marks are not yet validly published.

Phylogenetic Affiliation			Sampling Location		Representative Clones	Closest Cultured Relative in	Sequence
			Flow channel	Mat	from this Study	SILVA 108 NR Database	Similarity [%]
Actinobacteria	*Actinomycetales*	*Microbacteriaceae*	0	1	Fuschna-P4-H05	*Salinibacterium amurskyense* (AF539697)	96
Bacteroidetes	*Bacteroidales*	*Porphyromonadaceae*	0	2	Fuschna-P5-H01	*Paludibacter propionicigenes* (AB078842)	97
		Porphyromonadaceae	0	4	Fuschna-P5-E06	*Paludibacter propionicigenes* (AB078842)	95
	Flavobacteriales	*Cryomorphaceae*	2	0	Fuschna-P4-G09	*Owenweeksia hongkongensis* (AB125062)	88
		Flavobacteriaceae	0	1	Fuschna-P3-G09	*Actibacter sediminis* (EF670651)	91
	Sphingobacteriales	*Chitinophagaceae*	0	3	Fuschna-P4-D06	*Ferruginibacter lapsinanis* (FJ177532)	94
		Chitinophagaceae	2	0	Fuschna-P5-C08	*Sediminibacterium salmoneum* (EF407879)	97
		Sphingobacteriaceae	0	1	Fuschna-P4-H01	*Solitalea canadensis* (AB078046)	91
		Sphingobacteriaceae	0	2	Fuschna-P5-A04	*Pedobacter terricola* (EF446147)	93
		NS11-12 marine group	0	1	Fuschna-P3-C07	*Solitalea koreensis* (EU787448)	86
Cyanobacteria	*Oscillatoriales*		1	6	Fuschna-P5-F06	"*Tychonema bourrellyi*" (AB045897)	99
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	0	1	Fuschna-P4-C05	"*Leptothrix ginsengisoli*" (FM886840)	99
		Comamonadaceae	4	22	Fuschna-P5-D04, P4-F04, P5-E01, P3-B07	*Albidiferax ferrireducens* (AF435948))	97-98
		Comamonadaceae	0	2	Fuschna-P5-G03	*Albidiferax ferrireducens* (AF435948)	98
		Comamonadaceae	0	1	Fuschna-P4-A03	*Albidiferax ferrireducens* (AF435948)	98

	Hydrogenophilales	*Hydrogenophilaceae*	0	1	Fuschna-P4-H03	*Thiobacillus aquaesulis* (U58019)	92
	Nitrosomonadales	*Gallionellaceae*	69	2	Fuschna-P4-E07, P3-A09	*Gallionella ferruginea* (L07897)	97
	H2SRC138 cluster		1	0	Fuschna-P4-B09	n/a	
Deltaproteobacteria	*Bdellovibrionales*	*Bacteriovoraceae*	6	0	Fuschna-P5-E09	*Bacteriovorax stolpii* (AJ288899)	96
Gammaproteobacteria	*Alteromonadales*	*Shewanellaceae*	0	6	Fuschna-P5-C01	*Shewanella putrefaciens* (X81623)	99
	Aeromonadales	*Aeromonadaceae*	0	31	Fuschna-P5-C02, P3-H07, P4-B04, P5-B06, P5-D02, P4-B02, P4-B06, P3-D09	*Aeromonas punctata* subsp. *caviae* (X74674)	99
	Enterobacteriales	*Enterobacteriaceae*	0	6	Fuschna-P5-H02	*Buttiauxella noackiae* (AJ293689)	99
			0	1	Fuschna-P3-D08	*Plesiomonas shigelloides* (X60418)	93
	Legionellales	*Legionellaceae*	0	1	Fuschna-P5-F02	*Legionella taurinensis* (DQ667196)	96
Verrucomicrobia	*Verrucomicrobiales*	*Verrucomicrobiaceae*	0	1	Fuschna-P5-F05	*Prosthecobacter fusiformis* (U60015)	88

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