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APPENDIX

Formation of green rust and elemental sulfur in an analogue for oxygenated ferro-euxinic transition zones of Precambrian oceans

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METHODS

Sample collection

Water samples for Fe(II) and Fe(III) quantification were fixed in 1 M HCl on site to prevent oxidation by air-O₂. Water samples for sulfide quantification were fixed in 2% (w/v) zinc acetate to prevent oxidation by air-O₂. Water for bicarbonate quantification by titration against 1 M HCl was collected without a headspace in acid-washed Schott bottles that were closed with air-tight butyl stoppers. For elemental sulfur and polysulfide analysis, red and white flocs were collected in acid-washed bottles that were filled to the brim with Arvadi Spring water and closed with oxygen-tight butyl stoppers. Red and white floc samples for synchrotron-based X-ray absorption spectroscopy and Mössbauer spectroscopy were collected anoxically. The sampling bottles were flushed with N₂, while red and white flocs sedimented to the bottom of the sampling bottles and most of the liquid supernatant was removed. Samples were transported on ice and in the dark to the geomicrobiology laboratory at the University of Tuebingen (Germany) where they were immediately frozen at -80°C upon return. The samples were later freeze-dried under anoxic conditions and thereafter stored under dark and anoxic conditions.

Wet-chemical water analyses

Fe(II) and total Fe (Fe(tot)) were quantified with the spectrophotometric ferrozine assay after Stookey (1970). For Fe(tot) quantification, samples were incubated in 10% (w/v) hydroxylamine hydrochloride (HAHCl) for 30 minutes to reduce all Fe(III) to Fe(II). After addition of ferrozine solution and an incubation for 5 minutes, Fe(II) concentrations were quantified by measuring the absorption spectrophotometrically with a microplate reader (FlashScan 550, Analytic Jena) at

562 nm in HAHCl-reduced and non-reduced samples. Fe(III) concentrations were calculated by subtracting Fe(II) in non-reduced samples from Fe(tot) in HAHCl-reduced samples.

Hydrogen sulfide was quantified with the methylene blue method after Cline (1969). Thereby, 50 μ l of sample were added to 50 μ l of N,N-dimethyl-p-phenyldiamine sulfate and 50 μ l of $\text{NH}_4\text{Fe(III)(SO}_4)_2 \times 12 \text{ H}_2\text{O}$ prior to measuring the absorbance at 664 nm with a microplate reader (FlashScan 550, Analytic Jena).

Synchrotron based X-ray absorption spectroscopy (XAS) at Fe and S K-edge

Fe and S K-edge spectra were collected at the SUL-X beamline at ANKA, Karlsruhe Institute of Technology, Germany. The ring current and energy were set at 90-150 mA and 2.5 GeV, respectively. Spectra were collected at room temperature and under vacuum, using a 7 element Si (Li) fluorescence detector (SGX sensortech (MA) Ltd). Si ([1 1 1], $\Phi=0$) double-crystal monochromators and a Si-coated mirror were used for high-energy cutoff, i.e. harmonic suppression. The hutch slits were set at 0.5 \times 0.5 mm for bulk measurements. The spatial elemental distribution and speciation were investigated in white flocs samples using a combination of μ -XRF and μ -XANES on the Fe and S K-edges. The hutch slits were set at 100 \times 100 μ m for this analysis. Three spectra were recorded per sample, and spectra of elemental Fe and Scots tape were simultaneously recorded to allow for internal calibration. Fe samples were prepared anoxically in form of pressed pellets mixed with cellulose (Aldrich) sandwiched between Kapton tape and later mounted on an aluminum sample stage. The S samples were prepared anoxically as a thin layer of dry sample on Kapton tape and transported in a VacuShut that only opens when vacuum is established and thus reduces the exposure of ambient air to the sample. Furthermore, the sample compartment was flushed with N_2 gas prior to applying vacuum.

XAS data fitting

All spectra were energy calibrated to 7111.09 eV (Fe) or 2481.4 eV (S) and averaged using SixPack (Webb, 2005). The spectra were carefully inspected for beam damage, energy shift and subjected to further XAS analysis. The Fe spectra were subjected to linear combination fitting (LCF) using Athena (Ravel & Newville, 2005) and shell-by-shell fitting in Viper (Klementev, 2001). The LCF was conducted using 2L-ferrihydrite, goethite, siderite, pyrite, lepidocrocite, hematite, green rust (CO_3^{2-} , Cl^- , SO_4^{2-}), FeS, Fe(III)-NOM complexes, magnetite, and mackinawite as Fe reference compounds. LCF was conducted on the XANES (-20 to 30 eV) and EXAFS ($k=3-10 \text{ \AA}^{-1}$ using k^3 -weighting) regions. The sum was not forced to equal 1 and non-negative boundary conditions were applied. For the shell-by-shell fitting, the background was subtracted and the spline-fitted spectra were k^3 -weighted and analyzed using back-scattering parameters obtained via ab initio calculations (FEFF7, Zabinsky et al., 1995). Goethite was used as input structure for these calculations (Szytula et al., 1968). The number of free variables was restricted by correlating coordination numbers and fixing the Debye-Waller factor (σ^2) to literature values. The amplitude reduction factor was set to 0.75. The S K-edge data fitting was conducted using peak fitting in Athena (Ravel & Newville, 2005). Arctan step functions were used, as well as Gaussian peak functions for fitting the normalized spectra in the range -20 to 30 eV relative to the S K-edge.

Mössbauer spectroscopy

The Mössbauer spectrometer was calibrated with a 7 μm thick α - ^{57}Fe foil at room temperature, which was also used to determine the half width at half maximum (fixed to 0.128 mm/s during fitting). Fitting was carried out using Recoil (University of Ottawa) with the Voigt based fitting routine (VBF) (Rancourt et al., 1991). The purpose of these ^{57}Fe Mössbauer measurements was

to determine Fe(II)/Fe(III) ratios and mineral compositions as a complementary analysis to the synchrotron-based Fe K-edge X-ray absorption spectroscopy analysis.

Elemental sulfur analysis

125 μL anoxic ultrapure water (MilliQ) and 125 μL of 2% (w/v) zinc acetate were added to 500 μL of unfiltered sample. The samples were stored dark and at 4°C for 8 days. To extract elemental sulfur, 6 mL of methanol were added to the refrigerated samples, which were placed on a rolling shaker for 3 h with occasional shaking. The samples were then centrifuged for 5 minutes at 4000 rpm. Liquid samples were subsequently analyzed for elemental sulfur by HPLC on an Ultrasphere ODS column, operated with an isocratic mixture of 98% methanol and 2% H₂O run with a flow rate of 0.8 mL/min and detection at 265 nm.

Polysulfide analysis

Total and aqueous polysulfides were determined according to Rizkov et al. (2004) and Kamyshny et al. (2006). For total polysulfide analysis, 267 μL unfiltered sample ($n=3$) and 8 μL methyl trifluoromethanesulfonate were added simultaneously to 1067 μL anoxic methanol and then incubated at 4°C for ca. 36 h in the dark. Afterwards, the derivatized samples were filtered (0.45 μm) in a glovebox (100% N₂ atmosphere) and stored anoxically in the dark at 4°C until analysis. For aqueous polysulfide analysis, 200 μL of filtered sample ($n=3$) and 6 μL methyl trifluoromethanesulfonate were added to 800 μL anoxic methanol and stored anoxically in the dark and at 4°C until analysis. Polysulfides were quantified via HPLC (Merck Hitachi, L-2130 pump, L-2200 autosampler, L-2420 UV-vis detector) using a C18 column (Waters-Spherisorb, ODS2) after Kamyshny et al. (2006).

TABLES

TABLE A1. QUALITY OF FIT FROM LCF OF FE K-EDGE XANES AND EXAFS.

XANES			EXAFS		
Sample	R-factor	Chi-square	Sample	R-factor	Chi-square
B1	0.000488	0.0488	B1	0.0951	46.0
B2	0.000174	0.0001	B2	0.0463	0.0287
B3	0.000461	0.0144	B3	0.0670	30.3
HP1	0.000080	0.0054			
HP2	0.000068	0.0046			
HP3	0.000633	0.0568			
HP4	0.000143	0.0100			
HP5	0.000145	0.0090			
HP6	0.000113	0.0079			
HP7	0.000102	0.0068			

Table A2. Fe K-EDGE EXAFS SHELL-BY-SHELL FIT RESULTS OF RED FLOC SAMPLES.

Sample	Path	Coordination number	σ^{2*} (\AA^2)	Bond distance (\AA)	ΔE_0^\dagger	F_i^\S
1	Fe-O	5.8	0.0158	2.02	0.20	6.75
	Fe-Fe	1.6	0.0100 [#]	3.05		
	Fe-Fe	0.9	0.0100 [#]	3.37		
2	Fe-O	6.1	0.0144	2.00	-0.85	6.47
	Fe-Fe	1.8	0.0100 [#]	3.03		
	Fe-Fe	1.0	0.0100 [#]	3.42		
3	Fe-O	5.6	0.015	2.02	0.36	6.55
	Fe-Fe	1.3	0.0100 [#]	3.06		
	Fe-Fe	0.7	0.0100 [#]	3.41		

The amplitude reduction factor was set to 0.75.

*Debye-Waller factor

[†] E_0 was assumed to be identical for all shells.

[§] F_i is defined as $(\sum(k^3\chi_{exp}-k^3\chi_{fit})^2/\sum(k^3\chi_{exp})^2)\times 100$, where χ_{exp} and χ_{fit} represent experimental and fitted data points, respectively.

[#]Fixed to 0.0100 according to Maillot et al. (2011).

Table A3. MÖSSBAUER HYPERFINE PARAMETERS IN RED FLOCS MEASURED AT 77 K.

Fe species	δ^* [mm/s]	ΔE_Q^\dagger [mm/s]	ϵ^\S [mm/s]	$B_{hf}^\#$ [T]	R.A. [%]	\pm	Fe(II)/Fe(III)	\pm
Fe(II)	1.27	2.75			55.6	5.8	1.51	0.34
FeS?	-0.02	0.64			4.5	3.2		
Paramagnetic Fe(III)	0.55	0.39			8.3	3.2		
Goethite	0.43		-0.11	48.4	8.3	1.6		
Poorly ordered Fe(III)	0.94		-0.67	32.0	23.3	7.1		

*chemical shift with respect to Fe(0)
 \dagger quadrupole splitting
 \S quadrupole shift $\#$ hyperfine magnetic field
R.A. = relative abundance

TABLE A4. QUALITY PARAMETERS FOR S K-EDGE PEAK FIT USING ATHENA.

		B1	HP1	HP2	HP3	HP4	HP5	HP6	HP7
Arctangent	Step	0.998	1.056	1.057	1.019	1.048	1.028	1.085	1.052
	e0	2476.83	2473.99	2474.53	2475.95	2472.31	2477.28	2477.6	2473.01
	Width	0.981	2.368	2.257	2.17	2.498	3.029	2.551	2.662
Gaussian 1	Height	0.049	3.701	3.536	2.775	2.765	1.795	4.056	3.38
	Center (eV)	2468.56	2471.38	2471.44	2471.61	2471.13	2471.48	2471.63	2471.18
	Sigma	0.402	1.814	1.574	1.525	1.563	0.952	1.824	1.877
	Area	0.049	3.701	3.536	2.775	2.765	1.795	4.056	3.38
Gaussian 2	Height	1.379	0.216	0.463	0.424	0.195	0.462	0.281	0.085
	Center (eV)	2471.4	2473.99	2473.95	2474.33	2473.68	2473.56	2474.27	2473.8
	Sigma	1.078	0.781	0.861	0.978	0.72	0.966	0.806	0.664
	Area	1.379	0.216	0.463	0.424	0.195	0.462	0.281	0.085
Gaussian 3	Height	1.357	0.353	0.394	1.373	0.415	2.316	1.941	0.593
	Center (eV)	2481.48	2477.89	2477.96	2481.42	2478.91	2481.4	2476.95	2478.71
	Sigma	0.834	1.373	1.378	0.714	1.908	0.768	1.957	2.553
	Area	1.357	0.353	0.394	1.373	0.415	2.316	1.941	0.593
Gaussian 4	Height	0.111	0.206	0.208	-	-	-	0.471	0.025
	Center (eV)	2484.84	2481.24	2481.22	-	-	-	2481.38	2481.54
	Sigma	0.653	0.947	0.877	-	-	-	0.937	0.463
	Area	0.111	0.206	0.208	-	-	-	0.471	0.025
R factor		0.000385	0.000208	0.000287	0.002684	0.000293	0.008652	0.000312	0.000216
Chi-squared		0.0422	0.0325	0.0439	0.4107	0.0458	1.31399	0.0472	0.0338

FIGURES

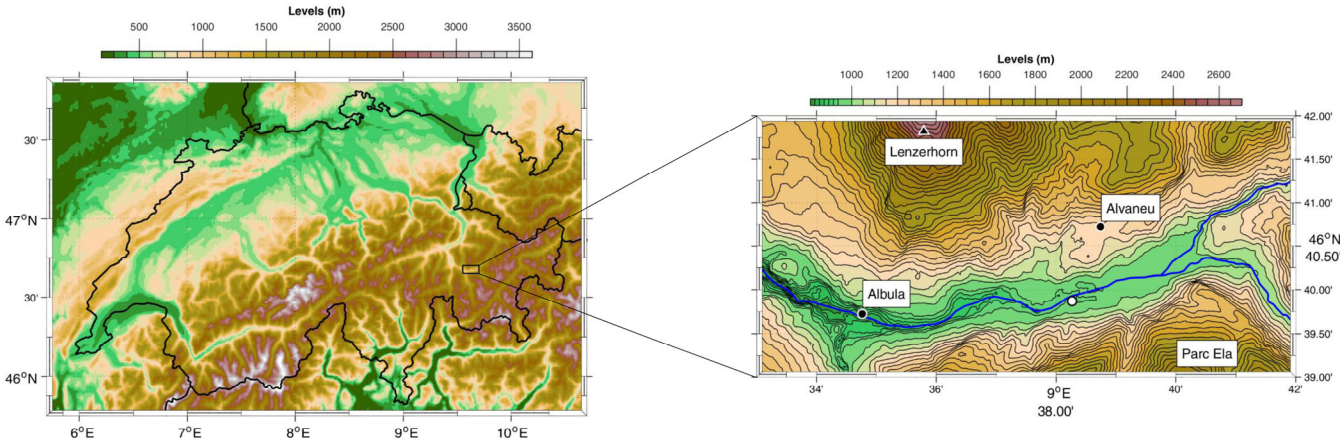


Figure A1. Arvadi Spring topographic location map. The rectangular box shows the Albula valley, and the white circle shows the Arvadi Spring location.

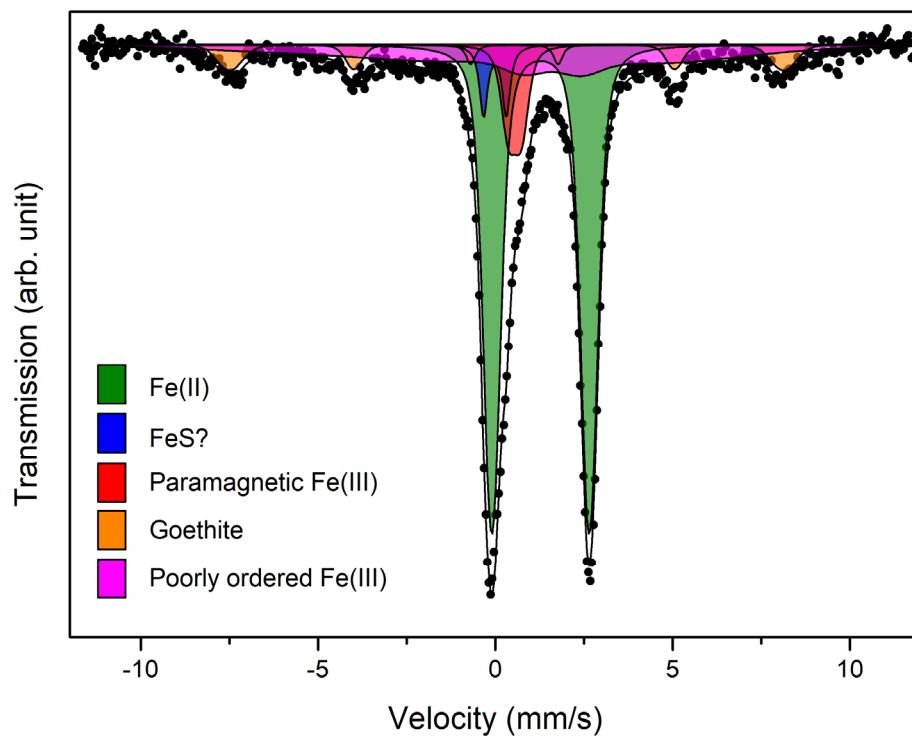


Figure A2. ^{57}Fe Mössbauer spectra collected at 77 K for red flocs. The Fe(III) crystalline phase likely corresponds to ordered Fe(III) such as goethite. The Fe(II) phase is best fitted with parameters that show close similarity to green rust. The Fe(III) paramagnetic phase likely corresponds to poorly crystalline phases such as ferrihydrite or lepidocrocite. A minor FeS phase appears to be present.

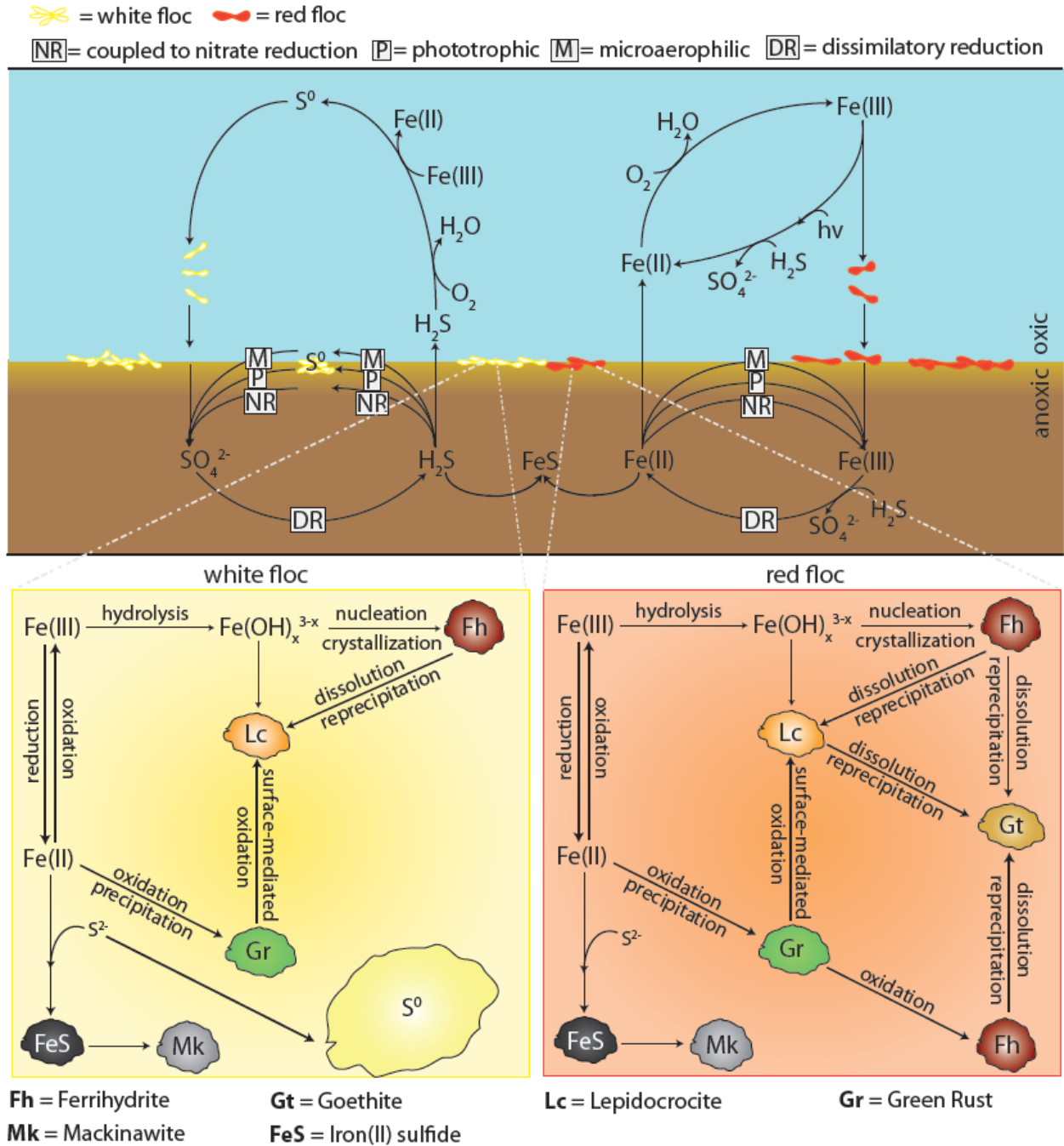


Figure A3. Fe-S-redox processes in the Arvadi Spring water and sediment (top) as well as an overview about the Fe-mineral transformation processes in white and red floc precipitates that settle on the Arvadi Spring sediment (bottom left and right, respectively). FeS accounts for amorphous FeS and nanoparticulate mackinawite.

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