Supporting Information

Isolation and microbial reduction of natural Fe(III)-bearing phyllosilicates from subsurface sediments

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Table S1. Total Fe recovered from bulk FRC Area 2 sediment by different chemical extraction procedures (see Materials and Methods). Values represent the mean \pm range of duplicate determinations.

Extraction method	Fe extracted (µmol g ⁻¹)						
AAO	10.2 ± 0.2						
AAO+Fe(II)	327 ± 3.4						
CDB-80C	284 ± 3.5						
CDB-RT	261 ± 3.9						

Table S2. HF-phenanthroline extractable Fe content of FRC Area 2 sediment before and after extraction with AAO+Fe(II), CDB-80C, or CDB-RT. Values represent mean \pm range of duplicate samples.

Material	Fe(II) (µmol g ⁻¹)	Total Fe (μ mol g ⁻¹)	Fe(III) $(\mu mol g^{-1})^b$	% Fe(III) reduced ^c		
Non-extracted sediment	14.1 ± 0.6	429 ± 19.4^{a}	$415\pm20.0^{\rm a}$			
AAO+Fe(II) extracted sediment	18.7 ± 0.9	110 ± 3.4	91.7 ± 13.8	4.7 ± 0.2		
CDB-80C extracted sediment	27.0 ± 2.3	136 ± 1.7	109 ± 0.6	10.5 ± 1.7		
CDB-RT extracted sediment	19.8 ± 2.3	146 ± 10.0	126 ± 12.3	4.3 ± 2.0		

^a Measured but not relevant to calculation of Fe(III) phyllosilicate reduction during wet-chemical extraction.

^b Determined from the difference between total Fe and Fe(II).

^c Determined from the difference between the Fe(II) content of extracted vs. the mean Fe(II) content of non-extracted sediment, divided by the Fe(III) content of the non-extracted material plus the difference between the Fe(II) content of extracted vs. the mean Fe(II) content of non-extracted sediment (e.g. for CDB-80C extracted sediment, % Fe(III) reduced = $(27.0 - 14.1)/[109 + (27.0 - 14.1)] \times 100 = 10.5$)

Table S3. HF-phenanthroline extractable Fe content of FRC Area 2 sediment (< 45 μ m size fraction) that was stripped of Fe(III) oxides by CDB-80C, washed and reoxidized by H₂O₂, and then reextracted with AAO+Fe(II) or CDB-80C. Values represent the mean \pm range of duplicate (non-reextracted) or the mean \pm SD of quadruplicate (reextracted) samples.

Material	Fe(II) (µmol g ⁻¹)	Total Fe (µmol g ⁻¹)	Fe(III) $(\mu mol g^{-1})^a$	% Fe(III) reduced ^b	
Stripped/reoxidized sediment,	14.4 ± 0.25	138 ± 4.7	124 ± 4.9		
no reextraction					
Stripped/reoxidized sediment,	19.8 ± 1.9	137 ± 3.6	117 ± 4.6	5.7 ± 3.7	
AAO+Fe(II) reextraction					
Stripped/reoxidized sediment,	23.3 ± 1.9	131 ± 3.4	107 ± 2.4	13.5 ± 1.9	
CDB-80C reextraction					

^a Determined from the difference between total Fe and Fe(II).

^b Determined from the difference between the Fe(III) content of reextracted vs. non-reextracted sediment, divided by the Fe(III) content of the non-reextracted material.

Sample Phase ¹		δ,2	$\Delta \text{ or } \epsilon_o^3$	H^4	$\sigma_{\Delta} (mm/s)^5$	comp.(%) ⁶	χ ^{2 (7)}	<cs>⁸</cs>	$<\Delta>$ or $<\varepsilon>^9$	<h>¹⁰</h>	sd $<\Delta>$ or 11	Phase, % ¹²
	mm/s	mm/s	Tesla	or σ_{H} (Tesla)			mm/s	mm/s	Tesla	mm/s	(stdev)	
Pristine	Clay Fe(III)	0.47	0.68	na 13	0.4	100	1.36	0.48	0.69	na	0.37	13.1 (1.2)
Fe(III	Clay Fe(II)	1.25	2.89	na	0.24	100		1.25	2.89	na	0.24	3.17(0.5)
	Fe(III)-oxyhydro(a)	0.48	-0.13	49.6	0.89	47.9		0.48	-0.13	49.02	2.15	63.4(1.1)
				48.49	2.75	52.1						
	Fe(III)-oxyhydro(b)	0.53*	0*	0	0	100		0.53	0	39.69	29.98	20(16.4)
Pristine, bioreduced	Clay Fe(III)	0.45	0.66	na	0.42	100	1.49	0.45	0.68	na	0.38	10.4(0.8)
	Clay Fe(II)	1.26	2.82	na	0.36	100		1.26	2.82	na	0.36	9.6(0.5)
	Fe(III)-oxyhydro(a)	0.48*	-0.13*	49.43	0.98	55.2		0.48	-0.13	49	2.12	57.7(1)
				48.48	2.9	44.8						
	Fe(III)-oxyhydro(b)	0.53*	0*	-0.13*	50	100		0.53	0	39.67	29.97	22.3(10.5)
Prisitine, CDB-80C extracted	Clay Fe(III)	0.47	0.66	na	0.43	100	0.7	0.47	0.68	па	0.39	25.4(1.4)
	Clay Fe(II)	1.25	2.95	na	0.32	100		1.25	2.95	па	0.32	13.9(0.9)
	Fe(III)-oxyhydro(a)	0.48*	-0.13*	49.21	0.9	46.7		0.48	-0.13	50.55	2.45	11.9(1.4)
				51.7	2.74	53						
Fe(III)-oxyhydro(b	Fe(III)-oxyhydro(b)	0.53*	0*	0*	48	100		0.53	0	38.13	28.8	48.7(18.1)
Prisitine, CDB-80C extracted,	Clay Fe(III)	0.47	0.69	na	0.44	100	0.7	0.47	0.71	na	0.4	29.1(1.7)
H ₂ O ₂ reoxidized	Clay Fe(II)	1.27	2.89	na	0.31	100		1.27	2.89	na	0.31	9.5(1)
	Fe(III)-oxyhydro(a)	0.48*	-0.13*	50.18	2.74*	86		0.48	-0.13	50.1	2.56	18.2(1.6)
				49.57	0.1*	14						
Fe(III)	Fe(III)-oxyhydro(b)	0.53*	0*	0*	50	100		0.53	0	39.76	30	43(22.7)
Prisitine, CDB-80C extracted	Clay Fe(III)	0.46	0.67	na	0.41	100	0.7	0.46	0.69	na	0.38	26.5(1.1)
H_2O_2 reoxidized, bioreduced	Clay Fe(II)	1.24	2.88	na	0.33	100	017	1.25	2.88	na	0.33	14.3(0.7)
		0.49*	-0.12*	10.60	2 7/*	70 0		0.49	0.12	10.60	2.42	21.1(1.1)
		0.40	0.15	49.69	0.1*	21.2		0.40	-0.15	45.05	2.45	21.1(1.1)
	Fe(III)-oxyhydro(b)	0.53*	0*	0*	46	100		0.53	0	36.75	27.8	38.1(13.6)
1 2	2											
[*] Spectral component; [*] cente	r shift; ³ quadrupole spl	itting or qu	adrupole shi	ft; [≁] hyper	fine magneticf fi	eld; ^o std dev of	the comp	ponet; ^o the	weight of the G	aussian comp	onent;	
⁷ reduced chi square; ⁸ average	e center shift; ⁹ average	quadrupo	e or average	e quadrupo	ole shift; ¹⁰ avera	ge magnetic hy	perfine fie	eld ^{, 11} stand	ard deviation; ¹²	spectral perc	ent; ¹³ not applicable	
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Modeling was carried out usin	g voight-based fitting r	nethod of I	kancourt and	a Ping (199	1) WITH RECOIL	Software; * In	ese paran	heters are i	rozen during mo	odeling:	n la.	
No coupling was allowed betw			the A+/A	is in all ele	inetal doublet an	1. A1/A2 and	2/02 are	. the (twice	at 2 and 2	u 01 0.097 mr	11/5,	
No coupling was allowed betw	veen CS, QS of 8 and av	rerage BNT;	ule A+/A- al	eas of dol	ubiet are fixed at	1, A1/A3 and A	AZ/A3 area	as ae fixed	ai 2 dilu 3			
Rancourt D.G, and Ping, Y.Y. (1	1991) "Voigt-based me	thods for a	rbitrary-sha	pe static h	perfine paramet	ter distribution	s in Mosst	aur spectr	oscopy": Nuclea	ar Instrument	s	
and Methods in Physics Resea	rch . B58 . 85-97.											

Table S4. Fitting and calculated Mossbauer parameters for 12 K spectra of FRC Area 2 sediments.

Table S5. Calculation of % Fe(III) phyllosilicate reduction from Mossbauer spectroscopic data in Figure 3.

A. Pristine sediment, CDB-80C reduction

Fractional (% total) Fe(III) phyllosilicate content of unreduced sediment = 13.1 ± 1.2 % Fractional (% total) Fe(II) phyllosilicate content of unreduced sediment = 3.2 ± 0.5 % Total fractional phyllosilicate content of unreduced sediment = (13.1 + 3.2) = 16.3 % ^aUncertainty = $[(1.2^2 + 0.5^2)^{1/2}] = 1.3$ % Fe(III) phyllosilicate/total phyllosilicate Fe for unreduced sediment = $(13.1/16.3) \times 100 = 80.5$ ^bUncertainty = $\{[(1.2^2 \times 13.1^2) + (1.3^2 \times 16.3^2)] \times 80.5\}^{1/2} = 9.8$ %

Fractional (% total) Fe(III) phyllosilicate content of CDB-80C extracted sediment = $25.4 \pm 1.4 \%$ Fractional (% total) Fe(II) phyllosilicate content of CDB-80C extracted sediment = $13.9 \pm 0.9 \%$ Total fractional phyllosilicate content of CDB-80C extracted sediment = (25.4 + 13.9) = 39.3 %^aUncertainty = $[(1.4^2 + 0.9^2)^{1/2}] = 1.7 \%$ Fe(III) phyllosilicate/total phyllosilicate Fe for CDB-80C extracted sediment = $(25.4/39.3) \times 100 = 64.6$ ^bUncertainty = $\{[(1.4^2 \times 25.4^2) + (1.7^2 \times 39.3^2)] \times 64.6\}^{1/2} = 4.5 \%$

Change in Fe(III) phyllosilicate/total phyllosilicate Fe during CDB extraction = (80.5 - 64.6) = 15.9 %^aUncertainty = $[(9.8^2 + 80.5^2)^{1/2}] = 10.8 \%$ % Fe(III) phyllosilicate reduction = $(15.9/80.5) \times 100 = 19.7$ ^bUncertainty = $\{[(10.8^2 \times 15.9^2) + (9.8^2 \times 80.5^2)] \times 19.7\}^{1/2} = 13.6 \%$

B. CDB-80C extracted, H₂O₂ reoxidized sediment, microbial reduction

Fractional Fe(III) phyllosilicate content of unreduced sediment = $29.1 \pm 1.7 \%$ Fractional Fe(III) phyllosilicate content of microbially reduced sediment = $26.5 \pm 1.1 \%$ Change in % Fe(III) phyllosilicate content = (29.1 - 26.5) = 2.6 %^aUncertainty = $[(1.7^2 + 1.1^2)^{1/2}] = 2.0 \%$ % Fe(III) phyllosilicate reduction = $2.6/29.1 \times 100 = 8.9 \%$ ^bUncertainty = $\{[(2.0^2 \times 2.6^2) + (1.7^2 \times 29.1^2)] \times 8.9\}^{1/2} = 7.0 \%$

C. Pristine sediment, microbial reduction

Fractional Fe(III) phyllosilicate content of unreduced sediment = $13.1 \pm 1.2 \%$ Fractional Fe(III) phyllosilicate content of microbially reduced sediment = $10.4 \pm 0.8 \%$ Change in % Fe(III) phyllosilicate content = (13.1 - 10.4) = 2.7 %^aUncertainty = $[(1.2^2 + 0.8^2)^{1/2}] = 1.4 \%$ % Fe(III) phyllosilicate reduction = $2.7/13.1 \times 100 = 20.6$ ^bUncertainty = { $[(1.4^2 \times 2.7^2) + (1.2^2 \times 13.1^2)] \times 20.6$ }^{1/2} = 11.1 %

^a From p. 45 in Bevington and Robinson (1992) (reference 57 in the text), if *x* is the difference between *u* and *v* (i.e. x = u - v), the uncertainty in $x(\sigma_x)$ is calculated as $\sigma_x = [\sigma_u^2 + \sigma_v^2 - 2\sigma_{uv}^2]^{1/2}$, where σ_u and σ_v are the uncertainties in *u* and *v*, and σ_{uv} is uncertainty related to the interaction between *u* and *v*. If *u* and *v* are independent of each other, $\sigma_{uv} = 0$ and the uncertainty in *x* is simply $\sigma_x = [\sigma_u^2 + \sigma_v^2]^{1/2}$.

^b From p. 46 in Bevington and Robinson (1992), if *x* is the ratio of *u* to *v* (i.e. x = u/v), σ_x is calculated as $\sigma_x = \{[(\sigma_u^2/u^2 + \sigma_v^2/v^2 - 2\sigma_{uv}^2/(u \times v)] \times x^2\}^{1/2}$. As above, assuming *u* and *v* are independent of each other, $\sigma_{uv} = 0$, and the uncertainty in *x* is $\sigma_x = [(\sigma_u^2/u^2 + \sigma_v^2/v^2) \times x^2]^{1/2}$.



Figure S1. XRD patterns for the high and low density fractions of FRC Area 2 sediment (clay size fraction) obtained by density gradient centrifugation, compared to the original, pristine sediment. SM is smectite; IL is illite; KA is kaolinite, Qz is quartz; and Gt is goethite. The numbers associated with SM, IL and KA indicate the (hkl) planes in the minerals. The d-spacing values for SM, IL, and KA are the same as those in Figure 1; the d-spacing value for Gt is 4.168 Å.



Figure S2. TEM image of the high density fraction of FRC Area 2 sediment obtained by density gradient centrifugation.



Figure S3. TEM image of the low density fraction of FRC Area 2 sediment obtained by density gradient centrifugation.



Figure S4. XRD patterns for FRC Area 2 sediment collected at different times during AAO+Fe(II) extraction. Abbreviations and d-spacing values for the identified minerals are the same as those as in Figure S1.



Figure S5. 060 reflections of FRC Area 2 sediment after CDB-RT extraction and following H_2O_2 reoxidation, compared to original, unextracted sediment. These XRD analyses were conducted with Rigaku Rapid II X-ray diffraction system (Mo K α radiation). The shaded areas indicate regions where peaks for dioctahedral versus trioctahedral smectites are expected (ref. 39 in the text). Abbreviations as in Figure S1.





Figure S6. SEM images of single particles of quartz and iron oxides aggregate (Qz-FeOX aggregate) (A,C) and XRD analysis for the single particles above (B,D), respectively. Qz = quartz; Hm = hematite; Mgh = maghemite; Gt = goethite. These XRD analyses were conducted with Rigaku Rapid II X-ray diffraction system (Mo K α radiation)