Effects of extraction conditions on the redox properties of soil organic matter (SOM) and its ability to stimulate microbial iron(III) mineral reduction by electron shuttling

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SUPPORTING INFORMATION

Number of tables in supporting information: 5

Number of figures in supporting information: 4

Total number of pages of supporting information: 10 (including cover page)

Table S1. Two-way ANOVA statistical analysis of the measured specific UV absorbance values at 254 nm (SUVA$_{254}$) for all NOM extracts. The two-way ANOVA analysis was conducted using the SPSS software to test whether the oxic extraction conditions, the extraction method, or both of them have significant impact on the SUVA$_{254}$ values of the extracts. $P<0.05$ indicates the rejection of the null hypotheses, meaning that the corresponding condition has significant influence on the SUVA$_{254}$.

[a]Dependent Variable: SUVA$_{254}$

Conditions	Sum of Squares	[b]Degrees of freedom	[c]Mean Square	[d]F	P
Oxic extraction conditions (oxic *vs.* anoxic)	0.002	1	0.002	11.181 F (1.20) =4.35	<0.05
Extraction method (water *vs.* chemical)	0.125	4	0.031	166.808 F (4.20) =2.87	<0.05
Oxic extraction conditions and extraction method	0.077	4	0.019	103.209 F (4.20) =2.87	<0.05
Error (within group variances)	0.004	20	0.000 (0.00018)		

[a]Three null hypotheses of the two-way ANOVA analysis:
Hypothesis$_{n1}$: the oxic conditions do not have significant impact on the measured SUVA$_{254}$ values of the extracts
Hypothesis$_{n2}$: the extraction method does not have significant impact on the measured SUVA$_{254}$ values of the extracts
Hypothesis$_{n3}$: the oxic conditions and the extraction method together do not have any significant impact on the measured SUVA$_{254}$ values of the extracts

[b]Degree of Freedom was calculated based on the number of variables under each condition. For example, under oxic extraction conditions, there are two variables, i.e., oxic conditions or anoxic conditions, so the degree of freedom is 2-1=1. For the extraction method, there are five variables, i.e., water-extracted SOM, water-extracted FA, chemically-extracted FA, water-extracted HA, chemically extracted HA, therefore the degree of freedom is 5-1 =4. The degree of freedom of both conditions (the third row: oxic conditions and extraction method) was calculated by multiplying the degree of freedom of oxic extraction conditions and the degree of freedom of extraction method, therefore 1×4 =4.

[c]Mean Square =Sum of Square/Degree of Freedom

[d]F ratio =variance of the group means (Mean Square)/mean of the within group variances (Mean Square Error). The calculated F ratio should be compared to the critical F ratio based on the degree of freedom (as shown in the brackets), and in all cases above, the F ratio was higher than the critical F ratio, indicating the null hypotheses should be rejected.

Table S2. ^{13}C-NMR analysis of SOM, FA and HA. All extracts were isolated from a forest soil (Schönbuch forest, Baden-Wuerttemberg, Germany) under oxic or anoxic conditions. ^{13}C-NMR was conducted from freeze-dried extracts. The relative intensities of the signals were obtained by integration of the specific chemical shift ranges by an integration routine with MESTRE NOVA. Data for water-extracted FA cannot be presented due to the lack of an adequate amount of sample for analysis.

Percentage distribution of ^{13}C within indicated ppm regions (%)

Sample/ppm range	**0-45**	**45-60**	**60-90**	**90-110**	**110-140**	**140-160**	**160-185**	**185-225**	**Aromaticity[a] (%)**
	Alkyl C	*N*-Alkyl C	*O*-Alkyl C	*O*-Alkyl & *C*/*H*-Aryl C	*C*/*H*-Aryl C	O-Aryl C	Carbonyl C	Aldehyde & Ketone	
Water-extracted SOM, oxic	20	10	30	10	12	6	7	4	20
Water-extracted SOM, anoxic	35	10	14	6	13	6	15	1	23
Chem.-extracted FA, oxic	21	9	32	9	9	4	11	3	15
Chem.-extracted FA, anoxic	22	10	19	8	14	8	12	6	27
Water-extracted HA, oxic	31	11	19	7	13	7	7	2	23
Water-extracted HA, anoxic	23	9	17	8	15	9	10	8	30
Chem.-extracted HA, oxic	24	11	22	8	15	8	9	3	20
Chem.-extracted HA, anoxic	27	11	21	8	14	7	8	3	24

[a] Aromaticity (%)= [Aromatic C peak area (110-160 ppm)] × 100/[Total peak area (0-160 ppm)]

Table S3. Total phosphate-leachable Fe, Fe(II) and Fe(III) in the isolated SOM, FA and HA samples. All samples were prepared by dissolving the freeze-dried SOM, FA, and HA extracts (powder) in 50 mM of phosphate buffer (pH 7) at a concentration of 100 mg C L^{-1}. After overnight agitation at 300 rpm at room temperature, samples were filtered through 0.22 μm syringe filter (mixed cellulose ester (MCE), Millipore, Germany). Iron concentrations were quantified in the phosphate-buffer extracts with the spectrophotometric ferrozine assay in a microtiterplate assay. Errors represent the standard deviation calculated from 3 parallels. Missing data is due to concentration below detection.

	Fe total (μmol L^{-1})	**Fe(II) (μmol L^{-1})**	**Fe(III) (μmol L^{-1})**	**[1]Contribution of Fe(II) to electron accepting capacity (EDC) (μmol e$^-$ mmol C^{-1})/%**	**[2]Contribution of Fe(III) to electron accepting capacity (EAC) (μmol e$^-$ mmol C^{-1})/%**	**[3]Total contribution of Fe to electron exchange capacity (EEC) (μmol e$^-$ mmol C^{-1})/%**
SOM (water-extracted, oxic)	32.8±1.8	17.4±1.6	15.4±1.3	2.1±0.19/11.7-14.0	1.9±0.16/10.9-12.9	4.0±0.21/11.8-13.0
SOM (water-extracted, anoxic)	123.1±0.8	79.2±0.8	43.9±0.9	9.5±0.10/33.4-33.7	5.3±0.11/32.3-33.7	14.8±0.10/33.0-33.4
FA (water-extracted, oxic)	18.8±1.3	7.2±0.4	11.6±0.7	0.9±0.05/7.7-8.6	1.4±0.08/59.6-66.8	2.3±0.16/16.1-18.5
FA (water-extracted, anoxic)	22.6±1.0	10.0±1.0	12.6±1.6	1.2±0.12/5.5-6.8	1.5±0.19/27.5-35.4	2.7±0.12/10.6-11.6
FA (chem.-extracted, oxic)	–	–	–	–	–	–
FA (chem.-extracted, anoxic)	–	–	–	–	–	–
HA (water-extracted, oxic)	15.9±2.1	7.2±0.3	8.7±0.5	0.9±0.04/1.6-1.7	1.0±0.06/14.1-15.9	1.9±0.25/2.0-2.6
HA (water-extracted, anoxic)	60.7±2.6	27.8±0.4	32.9±0.6	3.3±0.05/38.5-39.7	3.9±0.07/13.6-14.1	7.3±0.31/46.3-50.4
HA (chem.-extracted, oxic)	–	–	–	–	–	–
HA (chem.-extracted, anoxic)	–	–	–	–	–	–

[1]Calculation of contribution of Fe(II) to the EDC:

Take water-extracted SOM, oxic as an example, Fe(II) concentration = 17.4 μmol L^{-1}

The volume of SOM solution used for EDC analysis is 200 μL

Mole quantity of Fe(II) = 17.4 μmol L^{-1} * 200 μL = 0.00348 μmol Fe(II)

1 mol Fe(II) can donate 1 mol e$^-$, so 0.00348 μmol Fe(II) can donate 0.00348 μmol e$^-$

Mole quantity of C = 100 mg C L^{-1} 10^{-3} * 200 μL /12 g mol^{-1} = 0.00167 mmol C

Therefore, the contribution of Fe(II) to the EDC = 0.00348 μmol e$^-$/0.00167 mmol C = 2.1 μmol e$^-$ mmol C^{-1}

[2]Calculation of contribution of Fe(III) to the EAC:

Take water-extracted SOM, oxic as an example, Fe(III) concentration = 15.4 μmol L^{-1}

The volume of SOM solution used for EAC analysis is 200 μL

Mole quantity of Fe = 15.4 μmol L^{-1} * 200 μL = 0.00308 μmol Fe

1 mol Fe(III) can accept 1 mol e$^-$, so 0.00308 μmol Fe(III) can accept 0.00308 μmol e$^-$

Mole quantity of C = 100 mg C L^{-1} 10^{-3} * 200 μL /12 g mol^{-1} = 0.00167 mmol C

Therefore, the contribution of Fe(III) to the EAC = 0.00308 μmol e$^-$/0.00167 mmol C =1.9 μmol e$^-$ mmol C^{-1}

[3]Calculation of contribution of Fe to the EEC:

Take water-extracted SOM, oxic as an example, Fe total concentration = 32.8 μmol L^{-1}

The volume of SOM solution used for EEC analysis is 200 μL

Mole quantity of Fe total = 32.8 μmol L^{-1} * 200 μL = 0.00656 μmol Fe(II)

1 mol Fe(II) can exchange (either donate or accept) 1 mol e$^-$, so 0.00656 μmol Fe(II) can donate 0.00656 μmol e$^-$

Mole quantity of C = 100 mg C L^{-1} 10^{-3} * 200 μL /12 g mol^{-1} = 0.00167 mmol C

Therefore, the contribution of Fe(II) to the EDC = 0.00656 μmol e$^-$/0.00167 mmol C = 4.0 μmol e$^-$ mmol C^{-1}

Table S4. Summary table for the two-way ANOVA of the measured electron exchange capacity (EEC) of all extracts. The two-way ANOVA analysis was conducted using the SPSS software to test whether the oxic extraction conditions or the extraction method, or both of them have significant impact on the EEC values of the extracts. $P<0.05$ indicates the rejection of the null hypotheses, meaning that the corresponding condition has significant influence on the EEC.

[a]Dependent Variable: Electron exchange capacity (EEC)

Condition	Sum of Squares	[b]Degrees of freedom	[c]Mean Square	[d]F	P
Oxic extraction conditions (oxic *vs*. anoxic)	18073	1	18073.6	657.912 F (1.20) =4.35	<0.05
Extraction method (water *vs*. chemical)	21448	4	5362.1	195 F (4.20) =2.87 .191	<0.05
Oxic extraction conditions and extraction method	9722	4	2430.8	88.477 F (4.20) =2.87	<0.05
Error (within group variances)	150	20	7.5		

[a]Three null hypotheses of the two-way ANOVA analysis:
Hypothesis$_{n1}$: the oxic extraction conditions do not have significant impact on the measured EEC values of the extracts
Hypothesis$_{n2}$: the extraction method does not have significant impact on the measured EEC values of the extracts
Hypothesis$_{n3}$: the oxic extraction conditions and the extraction method together do not have any significant impact on the measured EEC values of the extracts

[b]Degree of Freedom was calculated based on the number of variables under each condition. For example, under oxic extraction conditions, there are two variables, i.e., oxic conditions and anoxic conditions, so the degree of freedom is 2-1=1. For the extraction method, there are five variables, i.e., water-extracted SOM, water-extracted FA, chemically-extracted FA, water-extracted HA, chemically extracted HA, therefore the degree of freedom is 5-1 =4. The degree of freedom of both conditions (the third row: oxic extraction conditions and extraction method) was calculated by multiplying the degree of freedom of oxic extraction conditions and the degree of freedom of extraction method, therefore 1×4 =4.

[c]Mean Square =Sum of Square/Degree of Freedom

[d]F ratio =variance of the group means (Mean Square)/mean of the within group variances (Mean Square Error). The calculated F ratio should be compared to the critical F ratio based on the degree of freedom (as shown in the brackets), and in all cases above, the F ratio was higher than the critical F ratio, indicating the null hypotheses should be rejected.

Table S5. Metal content analyzed by Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (4100, Agilent Inc., Santa Clara, CA, USA) in the extracted SOM, HA and FA samples. Prior to the MP-AES analysis, 0.5 g sample was digested with 10 mL 2% HNO_3 in microwave oven at 190 °C (800 w) for 10 min, after cooling down to room temperature, centrifuged for 10 min at 14000 rpm and the supernatant was used for the analysis. The data for FA (chemically-extracted, oxic) and HA (water-extracted, oxic) are missing due to the lack of enough samples. The unit of all metal concentrations is mg kg^{-1}, blank means the concentration of the corresponding metal is too low to be detected.

	B	Zn	Ca	Mg	Cu	Ag	Ba	Mn	Pb	Al	Ga
SOM (water-extracted, oxic)	1.65	0.04	10.09	3.03	0.10	0.02	0.07	3.82	0.10	0.99	0.33
SOM (water-extracted, anoxic)	0.11	0.13	-	8.97	0.11	0.01	0.68	10.64	0.14	12.64	0.01
FA (water-extracted, oxic)	0.34	0.01	14.02	0.20	0.08	0.01	0.03	0.45	0.07	0.26	0.03
FA (water-extracted, anoxic)	0.06	0.01	0.84	-	0.05	0.01	0.01	0.31	0.03	0.82	0.02
FA (chemically-extracted, anoxic)	0.09	0.11	-	11.13	0.19	0.02	1.05	3.40	0.12	14.87	0.01
HA (water-extracted, anoxic)	0.03	0.03	6.41	2.78	0.01	0.01	0.26	2.07	0.10	19.64	0.02
HA (chemically-extracted, oxic)	0.08	0.04	0.03	7.91	3.92	0.09	0.02	0.32	1.6	0.15	64.12
HA (chemically-extracted, anoxic)	0.02	0.02	0.01	6.46	2.94	0.05	0.03	0.09	0.15	0.09	7.14

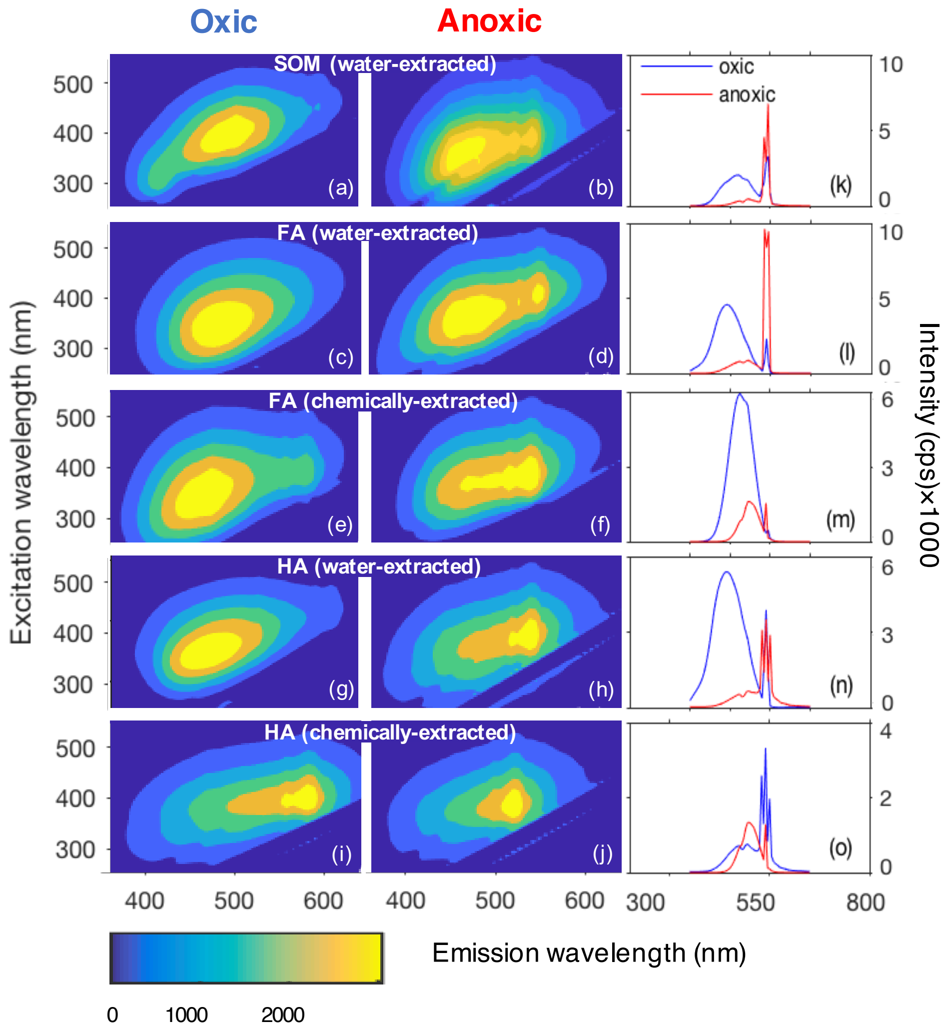

Figure S1. Fluorescence excitation-emission matrices (EEM) of SOM (a,b), FA (c-f), HA (g-j) and emission spectra at a fixed 440 nm excitation wavelength for SOM (k), FA (l,m) and HA (n,o). Freeze-dried SOM/FA/HA powders were dissolved in Milli-Q water (pH 7) at a concentration of 100 mg C L^{-1} and the solutions were agitated for 12 h at 300 rpm at room temperature, samples were then filtered through 0.45 µm syringe filters (mixed cellulose ester (MCE), Millipore, Germany). For fluorescence analysis, samples were prepared by stepwise dilution of extract solutions with Milli-Q water (pH 7) until absorbance values of 0.300 at 254 nm wavelength were reached. A wavelength step size of 10 nm was used for the collection of EEM spectra. The excitation and emission wavelength ranges were from 200-600 and 300-700 nm, respectively. A shift of the emission peak to higher wavelengths indicates a higher aromaticity of the OM compounds.

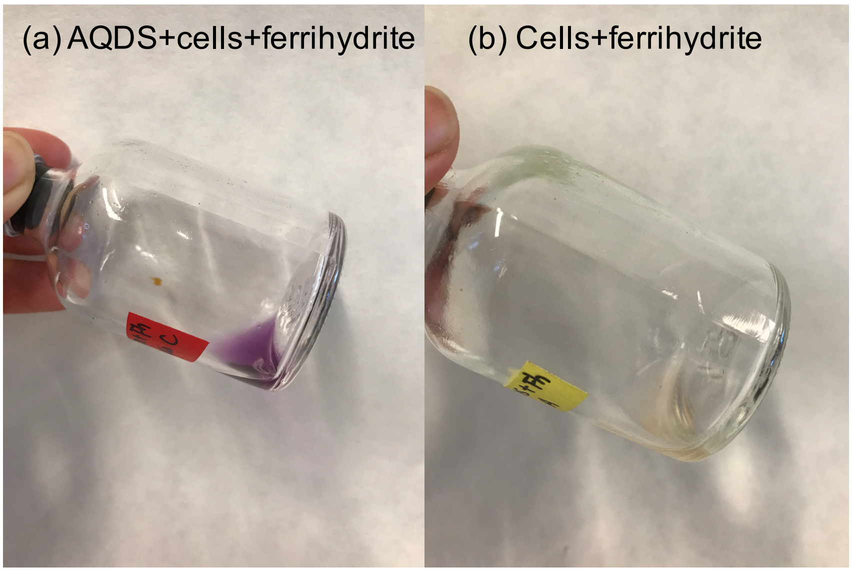

Figure S2. Addition of ferrozine to incubation bottles at the end of the experiment shows sorption or precipitation of Fe(II) at the glass walls of experiments containing AQDS, *Shewanella oneidensis* MR-1 and ferrihydrite (a) but not when containing *Shewanella oneidensis* MR-1 and ferrihydrite without AQDS (b). Before adding the ferrozine solution, original solutions in the bottles were discarded completely in the anoxic glovebox.

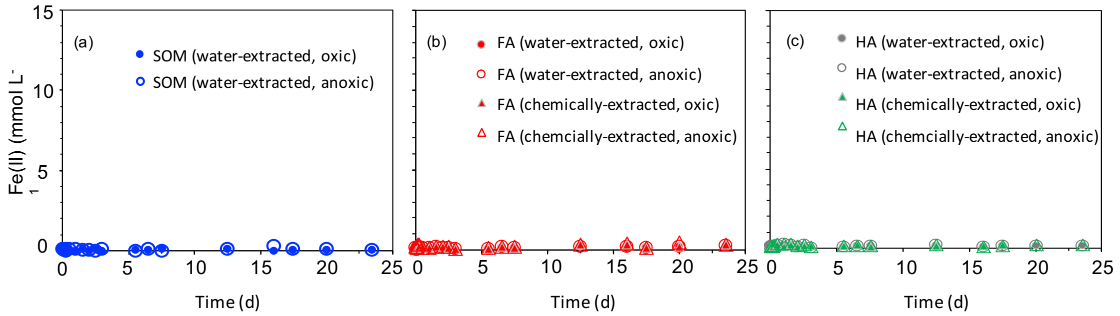

Figure S3. Abiotic reduction of ferrihydrite (15 mmol L^{-1}) in the presence of 50 mg C L^{-1} SOM (A), FA (B) and HA (C) and 15 mmol L^{-1} lactate, presented as total Fe(II) formed over time. All setups were incubated in air-tight 100 mL glass serum bottles flushed with N_2 at 30°C in the dark.

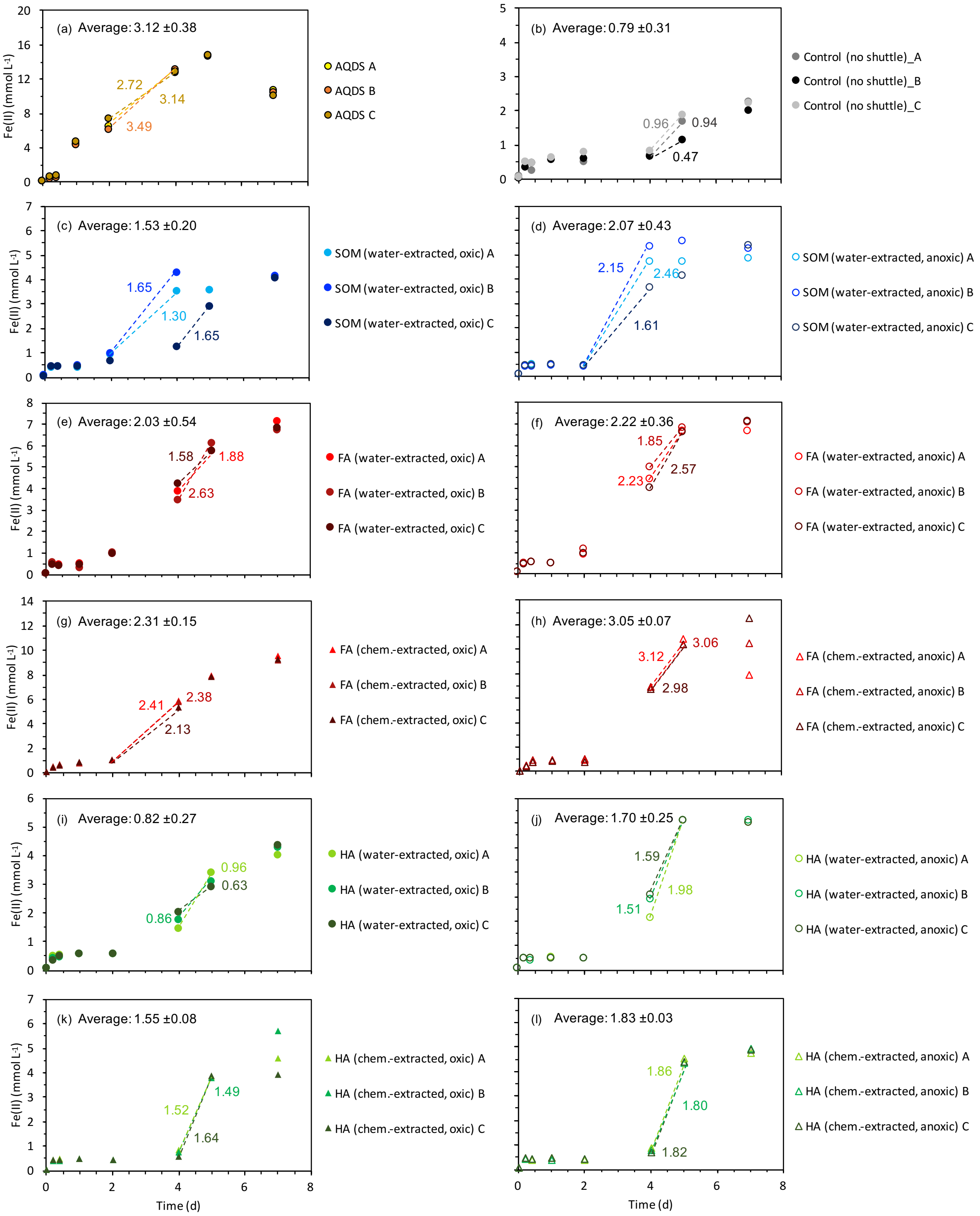

Figure S4. Calculation of the fastest initial microbial Fe(III) reduction rates in setups amended with SOM (a-b), FA (c-f), HA (g-j). The reduction rate was calculated separately for each of the triplicates. The average of the three rates was calculated and used for the correlation of reduction rate with either EEC or EAC. The standard deviation for the average of the three rates was calculated and is indicated in each panel (e.g. AQDS, 3.12±0.38).