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High-pH and anoxic conditions during soil organic matter extraction increases its electron-exchange capacity and ability to stimulate microbial Fe(III) reduction by electron shuttling

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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-extractable OM, FA isolated from water-extractable OM, FA isolated from soil, HA isolated from water-extractable OM, HA isolated from soil, 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 OM, 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 FA isolated from water-extractable OM 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-extractable OM, oxic	20	10	30	10	12	6	7	4	20
Water-extractable OM, anoxic	35	10	14	6	13	6	15	1	23
FA (Isolated from soil, oxic)	21	9	32	9	9	4	11	3	15
FA (Isolated from soil, anoxic)	22	10	19	8	14	8	12	6	27
HA (Isolated from water-extractable OM, oxic)	31	11	19	7	13	7	7	2	23
HA, (Isolated from water-extractable OM, anoxic)	23	9	17	8	15	9	10	8	30
HA (Isolated from soil, oxic)	24	11	22	8	15	8	9	3	20
HA (Isolated from soil, 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 OM, FA and HA samples. All samples were prepared by dissolving the freeze-dried OM, 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})/%**
Water-extractable OM, 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
Water-extractable OM, 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 (Isolated from water-extractable OM, 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 Isolated from water-extractable OM, 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 (Isolated from soil, oxic)	–	–	–	–	–	–
FA (Isolated from soil, anoxic)	–	–	–	–	–	–
HA (Isolated from water-extractable OM, 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 Isolated from water-extractable OM, 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 (Isolated from soil, oxic)	–	–	–	–	–	–
HA (Isolated from soil, anoxic)	–	–	–	–	–	–

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

Take water-extractable OM, oxic as an example, Fe(II) concentration = 17.4 μmol L^{-1}
The volume of OM 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-extractable OM, oxic as an example, Fe(III) concentration = 15.4 μmol L^{-1}
The volume of OM 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-extractable OM, oxic as an example, Fe total concentration = 32.8 μmol L^{-1}
The volume of OM 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-extractable OM, FA isolated from water-extractable OM, FA isolated from soil, HA isolated from water-extractable OM, HA isolated from soil, 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 water-extractable OM, 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 (Isolated from soil, oxic) and HA (Isolated from water-extractable OM, 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
Water-extractable OM, oxic	1.65	0.04	10.09	3.03	0.10	0.02	0.07	3.82	0.10	0.99	0.33
Water-extractable OM, anoxic	0.11	0.13	-	8.97	0.11	0.01	0.68	10.64	0.14	12.64	0.01
FA (Isolated from water-extractable OM, oxic)	0.34	0.01	14.02	0.20	0.08	0.01	0.03	0.45	0.07	0.26	0.03
FA (Isolated from water-extractable OM, anoxic)	0.06	0.01	0.84	-	0.05	0.01	0.01	0.31	0.03	0.82	0.02
FA (Isolated from soil, anoxic)	0.09	0.11	-	11.13	0.19	0.02	1.05	3.40	0.12	14.87	0.01
HA (Isolated from water-extractable OM, anoxic)	0.03	0.03	6.41	2.78	0.01	0.01	0.26	2.07	0.10	19.64	0.02
HA (Isolated from soil, oxic)	0.08	0.04	0.03	7.91	3.92	0.09	0.02	0.32	1.6	0.15	64.12
HA (Isolated from soil, anoxic)	0.02	0.02	0.01	6.46	2.94	0.05	0.03	0.09	0.15	0.09	7.14

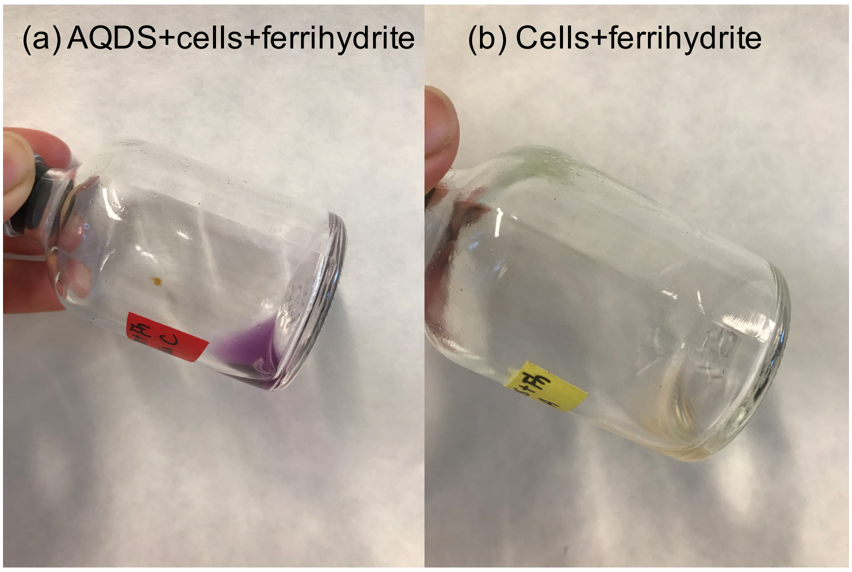

Figure S1. Addition of ferrozine to incubation bottles at the end of the experiment shows sorption or precipitation of Fe(II) at the glass walls of experiments containing AQDS, *Shewanella oneidensis* MR-1 and ferrihydrite (a) but not when containing *Shewanella oneidensis* MR-1 and ferrihydrite without AQDS (b). Before adding the ferrozine solution, original solutions in the bottles were discarded completely in the anoxic glovebox.

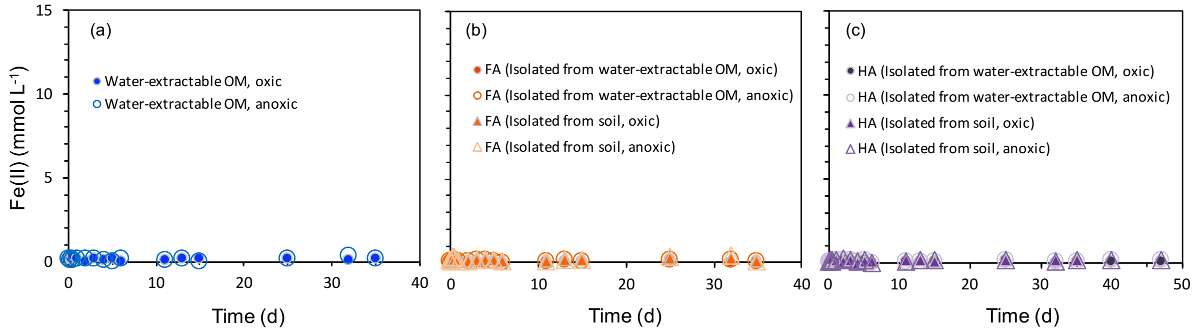

Figure S2. Abiotic reduction of ferrihydrite (15 mmol L^{-1}) in the presence of 50 mg C L^{-1} water-extractable OM (a), FA (b) and HA (c) and 15 mmol L^{-1} lactate, presented as total Fe(II) formed over time. All setups were incubated in air-tight 100 mL glass serum bottles flushed with N_2 at 30 °C in the dark.

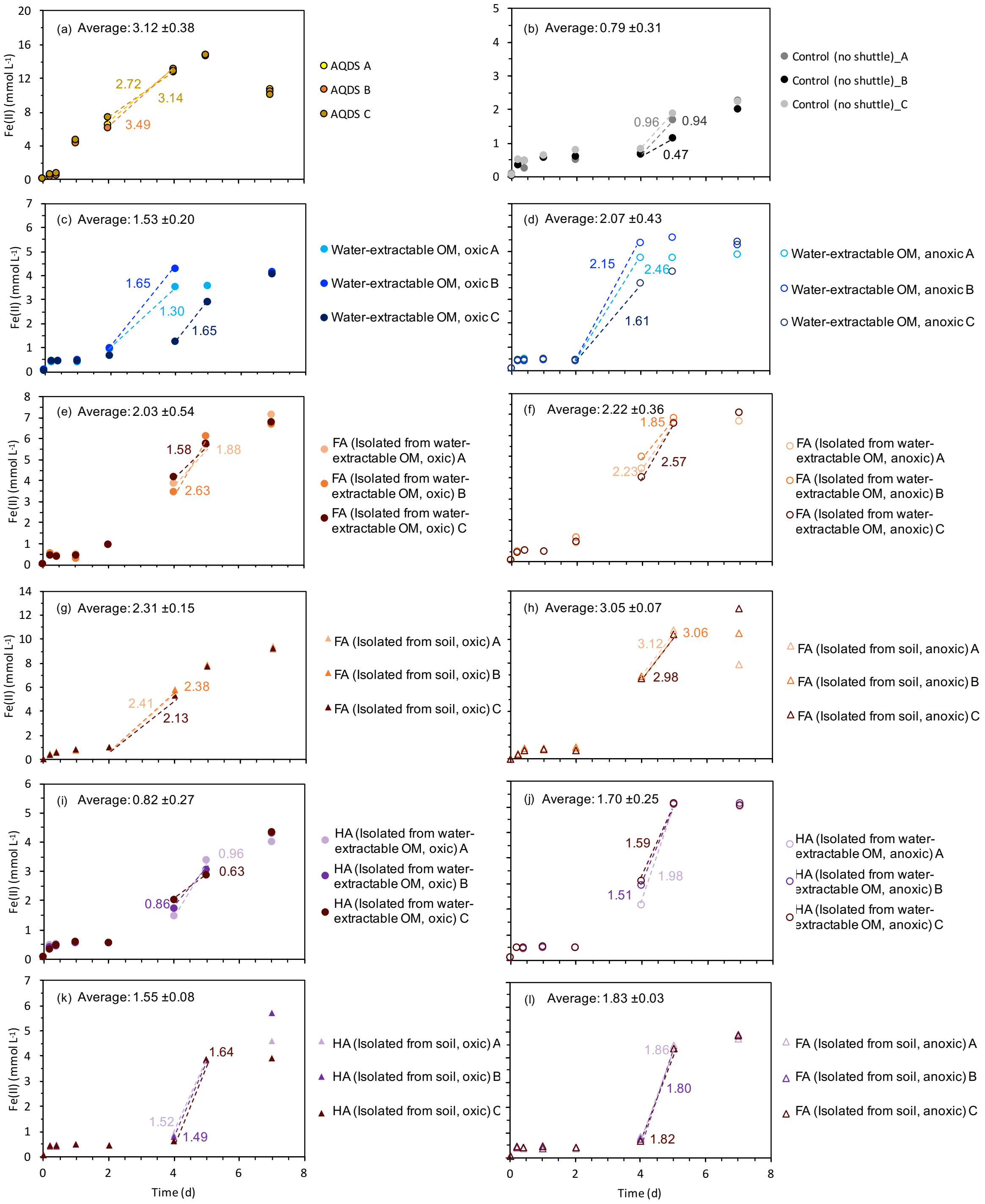

Figure S3. Calculation of the fastest initial microbial Fe(III) reduction rates in setups amended with AQDS (a), *Shewanella oneidensis* MR-1 only (control, b), water-extractable OM (c-d), FA (e-h), HA (i-l). The reduction rate was calculated separately for each of the triplicates. The average of the 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).