Supplemental Information

Impacts of Coal Ash on Methylmercury Production and the Methylating Microbial Community in Anaerobic Sediment Slurries

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Methods

Mercury Speciation Calculations. Mercury equilibrium speciation calculations with Visual MINTEQ.[1] The Hg speciation calculations utilized stability constants shown in Table S1, which were selected based on the analysis described in Hsu-Kim et al. (2013).[2] Ionic strength was set to 0 M. Visual MINTEQ data inputs (SI Tables S4-S6) were the microcosm porewater concentrations of: H^+, Fe(II), AVS, Cl, Hg, and thiols (estimated from DOC measurements). All of the microcosms were amended with 10 mM pyruvate (30 mmol L^{-1} of organic carbon), which does not act as a strong ligand for Hg. In Experiment #3, we observed that the DOC in the porewater of the slurries was initially 33 mM right after construction of the microcosms. Thus, we estimated that approximately 10% of the measured DOC value for other microcosms was attributed to dissolved organic matter with thiol functional groups that bind Hg^{2+}. This assumption results in approximately 1.5 – 3.8 mM (18 - 46 mg L^{-1}) of DOC with the capacity to strongly bind Hg. We then assumed that the DOC had 5 nmol mg^{-1} of thiols that strongly bind Hg, based on previous studies with dissolved organic matter isolates.[3]

Table S1. Stability constants used in Hg Speciation Calculations.

Reaction	log K (I=0, T=25 ˚C)	Reference
$H_2S \Leftrightarrow HS^- + H^+$	-7.02	4
$HS^- \Leftrightarrow S^{2-} + H^+$	-17.4	4
$\beta\text{-}HgS_{(s)} + H^+ \Leftrightarrow Hg^{2+} + HS^-$	log K_{s0} = -38.7 ± 2	2
$Hg^{2+} + HS^- \Leftrightarrow HgSH^+$	30.2	2
$Hg^{2+} + 2HS^- \Leftrightarrow Hg(SH)_2^0$	37.7	2
$Hg^{2+} + 2HS^- \Leftrightarrow HgSH_2^-$	31.5	2
$Hg^{2+} + 2HS^- \Leftrightarrow HgS_2^{2-} + 2H^+$	23.2	4
$Hg^{2+} + RS_2^{2-} \Leftrightarrow Hg(RS_2)$	28.7	2
$RS_2^{2-} + H^+ \Leftrightarrow RS_2H^-$	8.4	2
$RS_2H^- + H^+ \Leftrightarrow RS_2H_2$	8.4	2
$Hg^{2+} + H_2O \Leftrightarrow HgOH^+ + H^+$	-3.4	4
$Hg^{2+} + 2H_2O \Leftrightarrow Hg(OH)_2^0 + 2H^+$	-6.2	4
$Hg^{2+} + 3H_2O \Leftrightarrow Hg(OH)_3^- + 3H^+$	-21.1	4
$Hg^{2+} + Cl^- \Leftrightarrow HgCl^+$	7.3	4
$Hg^{2+} + 2Cl^- \Leftrightarrow Hg(Cl)_2^0$	14	4
$Hg^{2+} + 3Cl^- \Leftrightarrow Hg(Cl)_3^-$	15	4
$Hg^{2+} + Cl^- + H_2O \Leftrightarrow HgOHCl^0 + H^+$	4.2	4
$Fe^{2+} + HS^- \Leftrightarrow FeS_{(s),\ mackinawite} + H^+$	3.6	4
$Fe^{2+} + HS^- \Leftrightarrow Fe(HS)^+$	5.62	5
$Fe^{2+} + 2HS^- \Leftrightarrow Fe(HS)_{2(aq)}$	8.95	4
$Fe^{2+} + 3HS^- \Leftrightarrow Fe(HS)_3^-$	10.99	4
$Fe^{2+} + H_2O \Leftrightarrow FeOH^+ + H^+$	-9.40	4
$Fe^{2+} + 2H_2O \Leftrightarrow Fe(OH)_{2(aq)} + 2H^+$	-20.49	4
$Fe^{2+} + 3H_2O \Leftrightarrow Fe(OH)_3^- + 3H^+$	-30.99	4
$Fe^{2+} + Cl^- \Leftrightarrow FeCl^+$	-0.20	4

Table S2. Experiment 1: Microcosm porewater concentrations used as input parameters in the Visual MINTEQ calculations.

Experiment 1: Sediment-Only						
Timepoint (Hours)	pH	Fe(II) (mM)	HS^- (mM)	Cl^- (mM)	Thiols (mM)	$Hg(II)_T$ (mM)
2	6.48	0.118	0.009	0.085	1.62E-04	2.17E-07
24	6.55	0.003	0.008	0.090	1.70E-04	1.48E-07
48	6.50	0.003	0.011	0.092	1.66E-04	1.52E-07
96	6.39	0.003	0.010	0.091	1.63E-04	1.01E-07
168	6.84	0.001	0.003	0.095	1.36E-04	1.84E-07
Experiment 1: Sediment-Only + Ash						
Timepoint (Hours)	pH	Fe(II) (mM)	HS^- (mM)	Cl^- (mM)	Thiols (mM)	$Hg(II)_T$ (mM)
2	6.93	1.52E-01	0.005	0.092	1.62E-04	1.77E-07
24	6.91	4.86E-02	0.008	0.096	1.62E-04	1.14E-07
48	6.97	6.08E-02	0.009	0.093	1.69E-04	1.19E-07
96	6.92	1.39E-02	0.007	0.092	1.32E-04	6.93E-08
168	7.13	6.13E-02	0.002	0.095	1.64E-04	1.13E-07

Table S3. Experiment 2: Microcosm porewater concentrations used as input parameters in the Visual MINTEQ calculations.

Experiment 2: Sediment Only						
Timepoint (Hours)	pH	Fe(II) (mM)	HS^- (mM)	Cl^- (mM)	Thiols (mM)	$Hg(II)_T$ (mM)
2	6.33	0.306	2.37E-03	0.025	1.99E-04	1.29E-07
12	6.17	0.315	4.32E-03	0.022	1.93E-04	6.00E-08
24	6.33	0.306	7.44E-03	0.011	2.25E-04	9.50E-08
48	6.40	0.312	8.10E-03	0.012	2.22E-04	7.76E-08
120	6.42	0.317	4.88E-03	0.011	1.64E-04	7.27E-08
Experiment 2: Sediment Only + Ash						
Timepoint (Hours)	pH	Fe(II) (mM)	HS^- (mM)	Cl^- (mM)	Thiols (mM)	$Hg(II)_T$ (mM)
2	6.91	0.305	3.12E-05	0.017	1.67E-04	4.45E-08
12	6.68	0.303	3.12E-05	0.016	1.83E-04	3.69E-08
24	6.91	0.266	2.05E-03	0.014	1.79E-04	6.02E-08
48	6.96	0.260	5.95E-03	0.013	1.81E-04	4.28E-08
120	7.06	0.238	3.72E-03	0.011	1.40E-04	4.21E-08

Table S4. Experiment 3: Microcosm porewater concentrations used as input parameters in the Visual MINTEQ calculations.

Experiment 3: Sediment Only						
Timepoint (Hours)	pH	Fe(II) (mM)	HS^- (mM)	Cl^- (mM)	Thiols (mM)	$Hg(II)_T$ (mM)
2	7.26	0.187	4.08E-03	0.161	1.32E-04	1.29E-07
12	7.11	0.164	5.66E-03	0.167	8.65E-05	2.87E-07
24	7.11	0.176	4.85E-03	0.158	1.28E-04	1.92E-07
48	7.15	0.165	5.50E-03	0.205	1.33E-04	1.73E-07
120	7.15	0.230	1.61E-03	0.238	1.36E-04	3.65E-07
Experiment 3: Sediment Only + Ash						
Timepoint (Hours)	pH	Fe(II) (mM)	HS^- (mM)	Cl^- (mM)	Thiols (mM)	$Hg(II)_T$ (mM)
2	7.62	0.122	8.58E-04	0.278	1.32E-04	3.02E-08
12	7.31	0.018	3.74E-03	0.189	8.13E-05	2.02E-07
24	7.43	0.017	5.48E-03	0.156	1.24E-04	1.01E-07
48	7.48	0.048	9.48E-03	0.184	1.19E-04	1.79E-08
120	7.62	0.038	5.50E-03	0.189	1.29E-04	3.33E-07

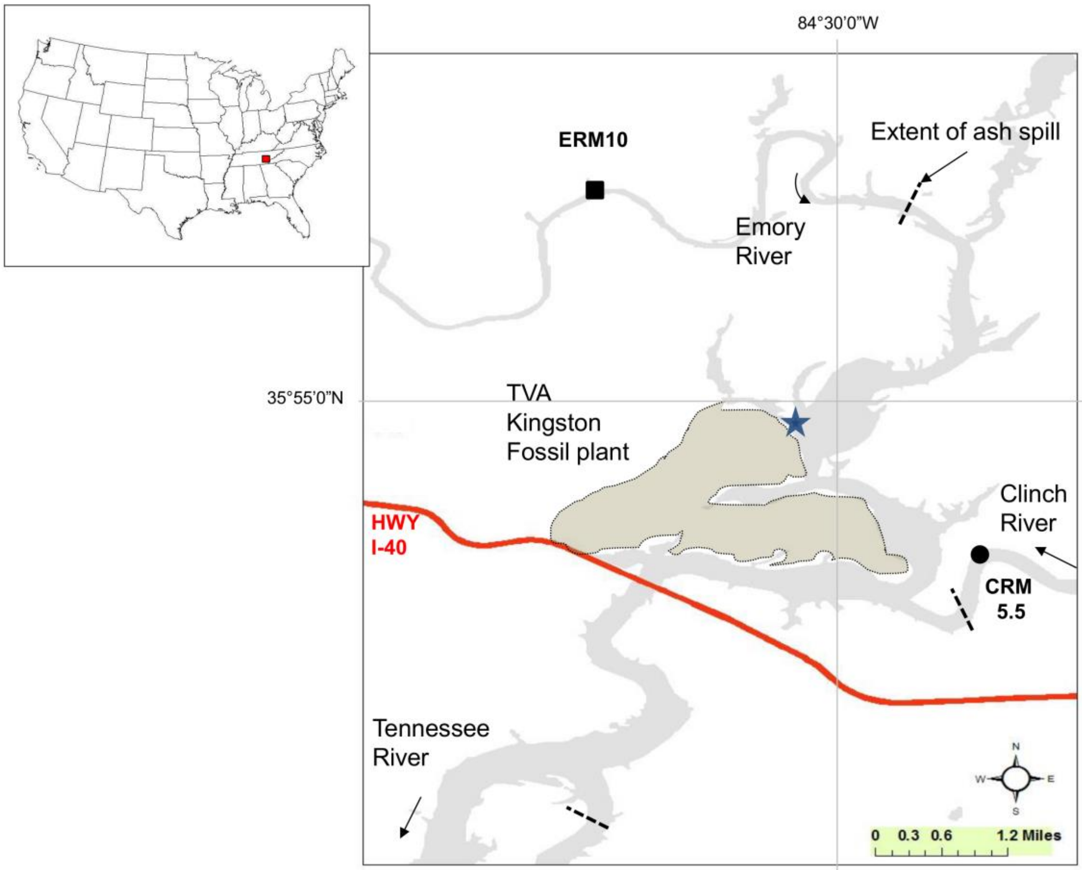


Figure S1. Locations of the sampling sites from which sediment and water samples were collected for the microcosm experiments. Emory River Mile Marker 10 (ERM 10) and Clinch River Mile Marker 5.5 (CRM 5.5) are located upstream of the coal ash spill. The star represents the location of the ash release from the impoundment. The dashed lines indicate the extent of the ash spill.

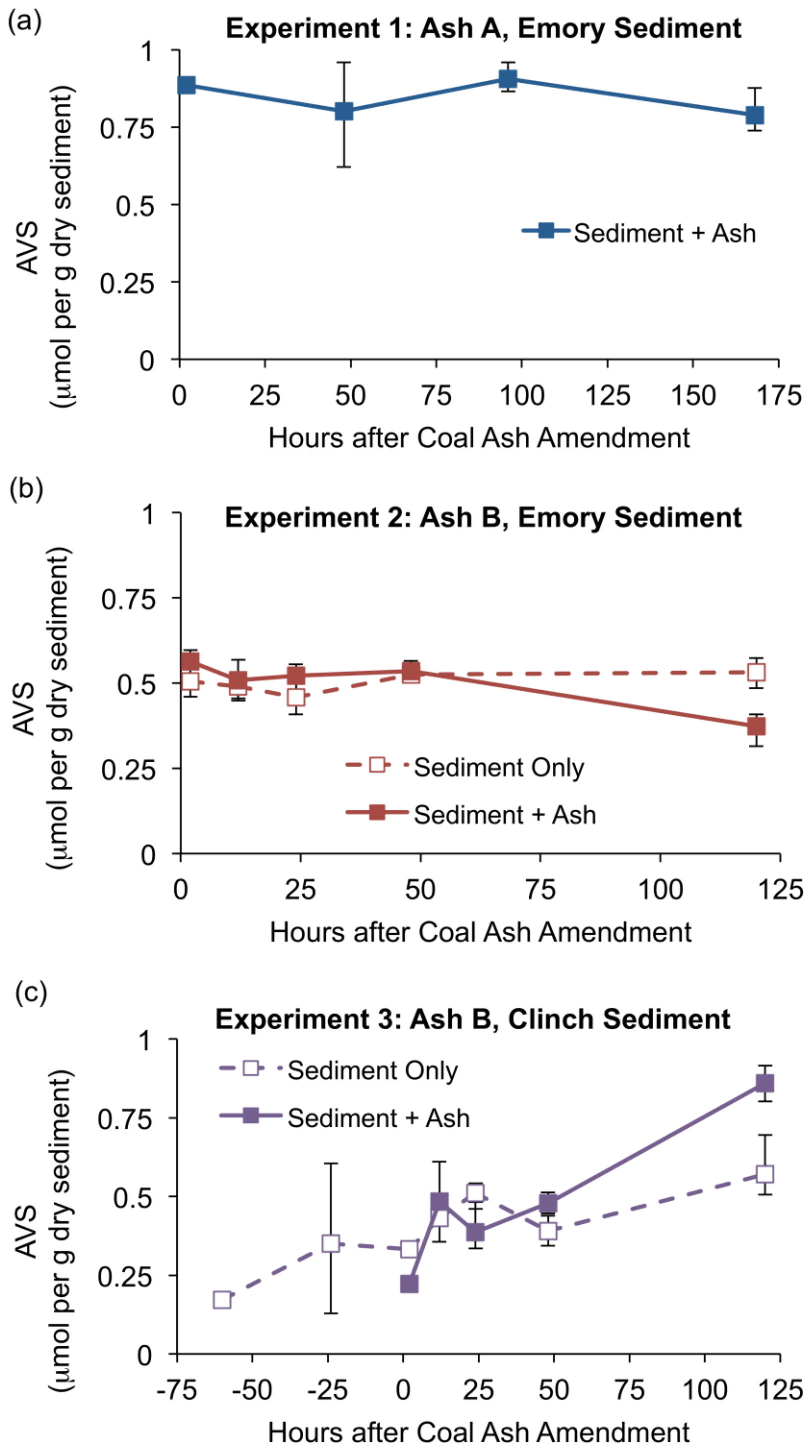


Figure S2. Acid volatile sulfide (AVS) contents in aliquots of whole slurries from the sediment-ash microcosms. (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B; (c) Experiment 3: Clinch Sediment/Ash B. Data points represent replicate microcosms (n=2-3). Error bars represent the range of the samples. AVS data is not available for the Sediment Only treatment from Experiment 1.

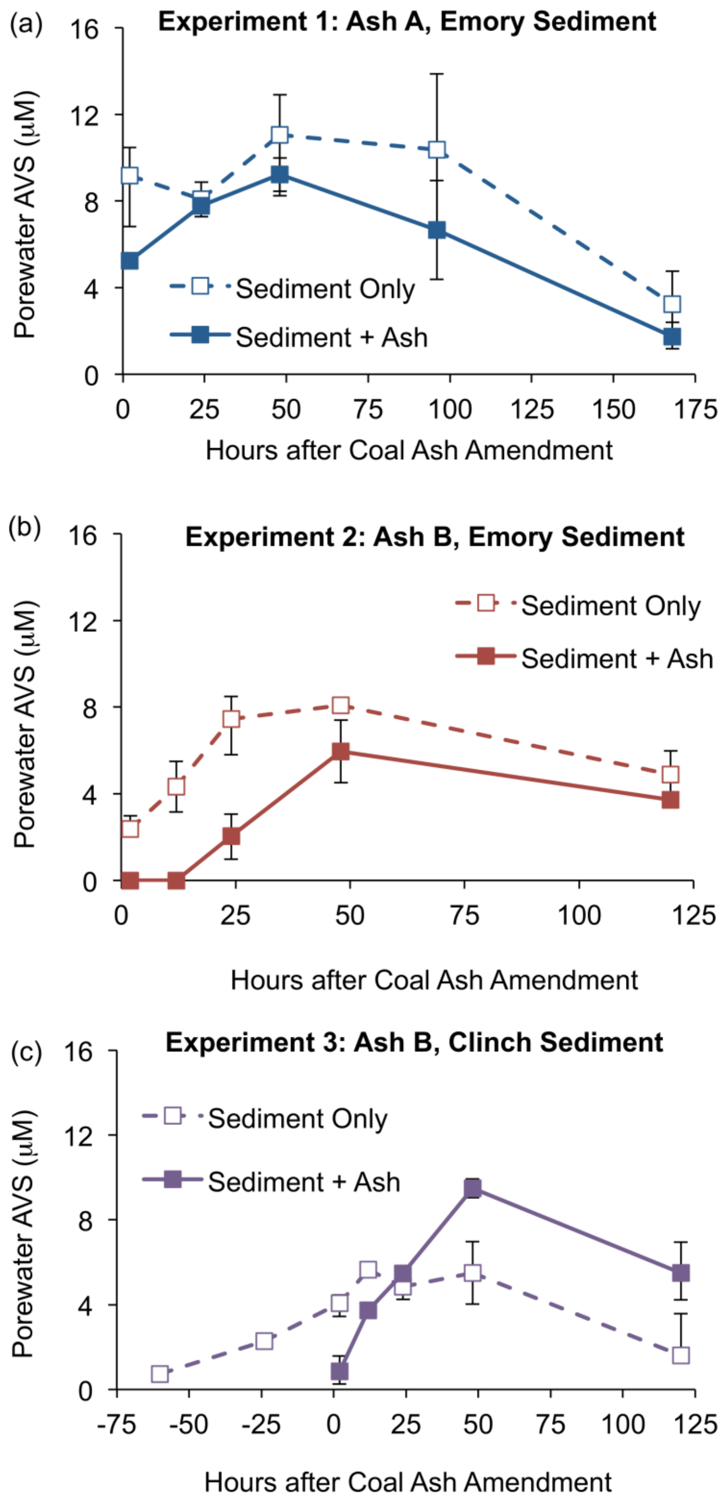


Figure S3. Dissolved acid volatile sulfide concentrations in the porewater of sediment-ash microcosms. (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B; (c) Experiment 3: Clinch Sediment/Ash B. Data points represent the mean of replicate microcosms (n=2-3). Error bars represent the range of replicate samples.

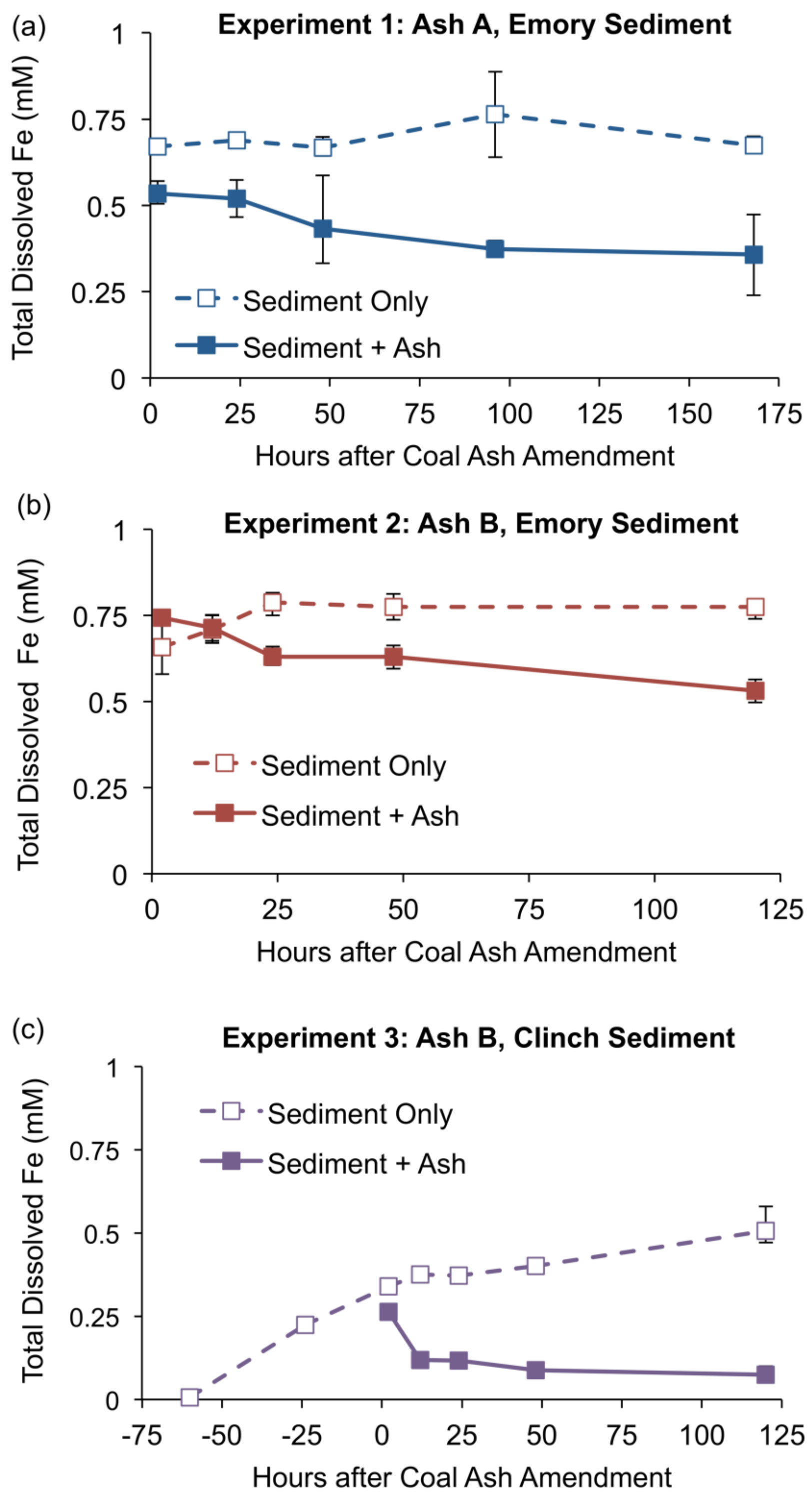


Figure S4. Dissolved iron concentrations in the porewater of sediment-ash microcosms. (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B; (c) Experiment 3: Clinch Sediment/Ash B. Each data point represents replicate microcosms (n=2-3). Error bars represent the range of replicate samples.

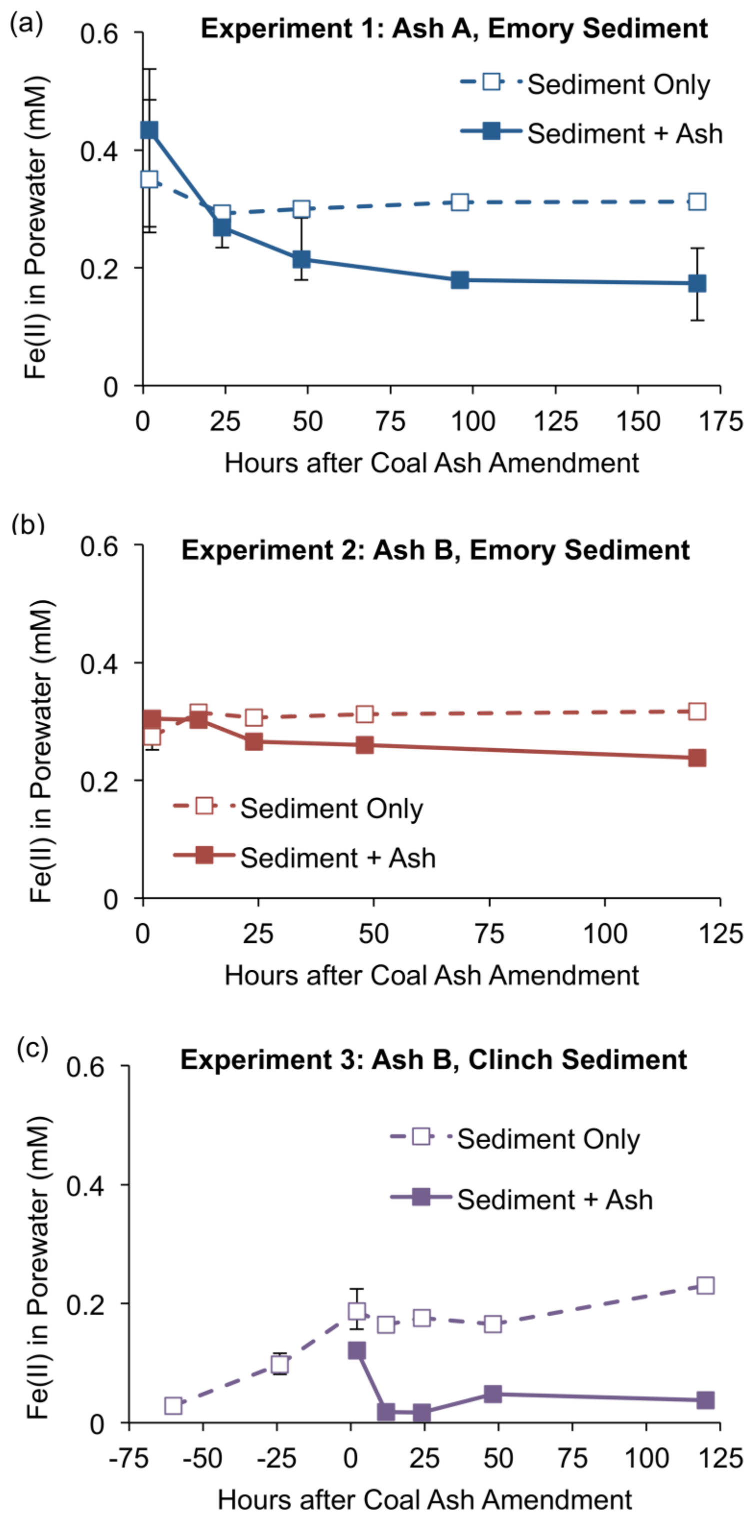


Figure S5. Ferrous iron (Fe(II)) concentrations in the porewater of sediment-ash microcosms; (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B (c) Clinch Sediment/Ash B. Data points represent the mean of replicate microcosms (n=2-3). Error bars represent the range of replicate samples.

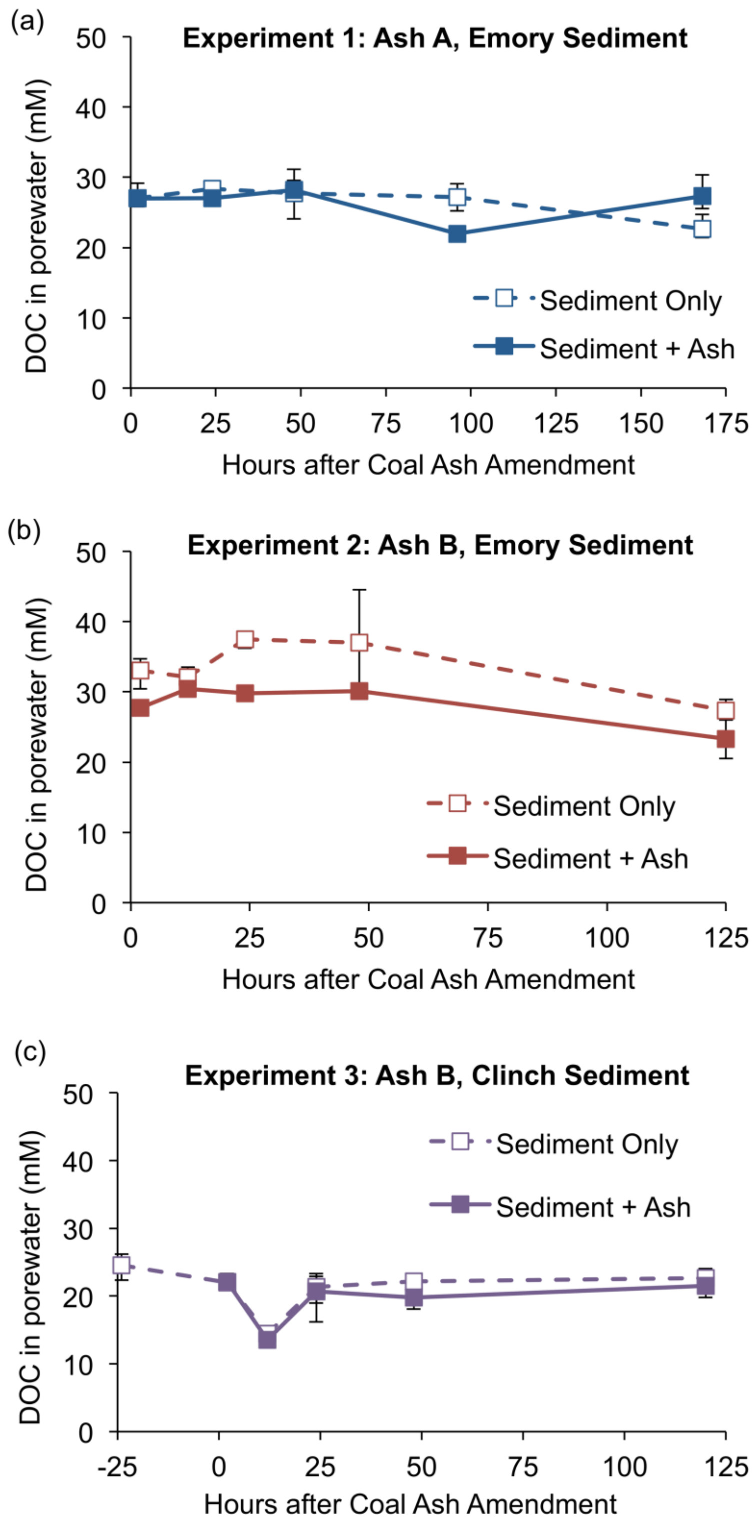


Figure S6. Dissolved organic carbon (DOC) concentrations in the porewater of sediment-ash microcosms (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B; (c) Experiment 3: Clinch Sediment/Ash B. Data points represent replicate microcosms (n=2-3). Error bars represent the range of replicate samples.

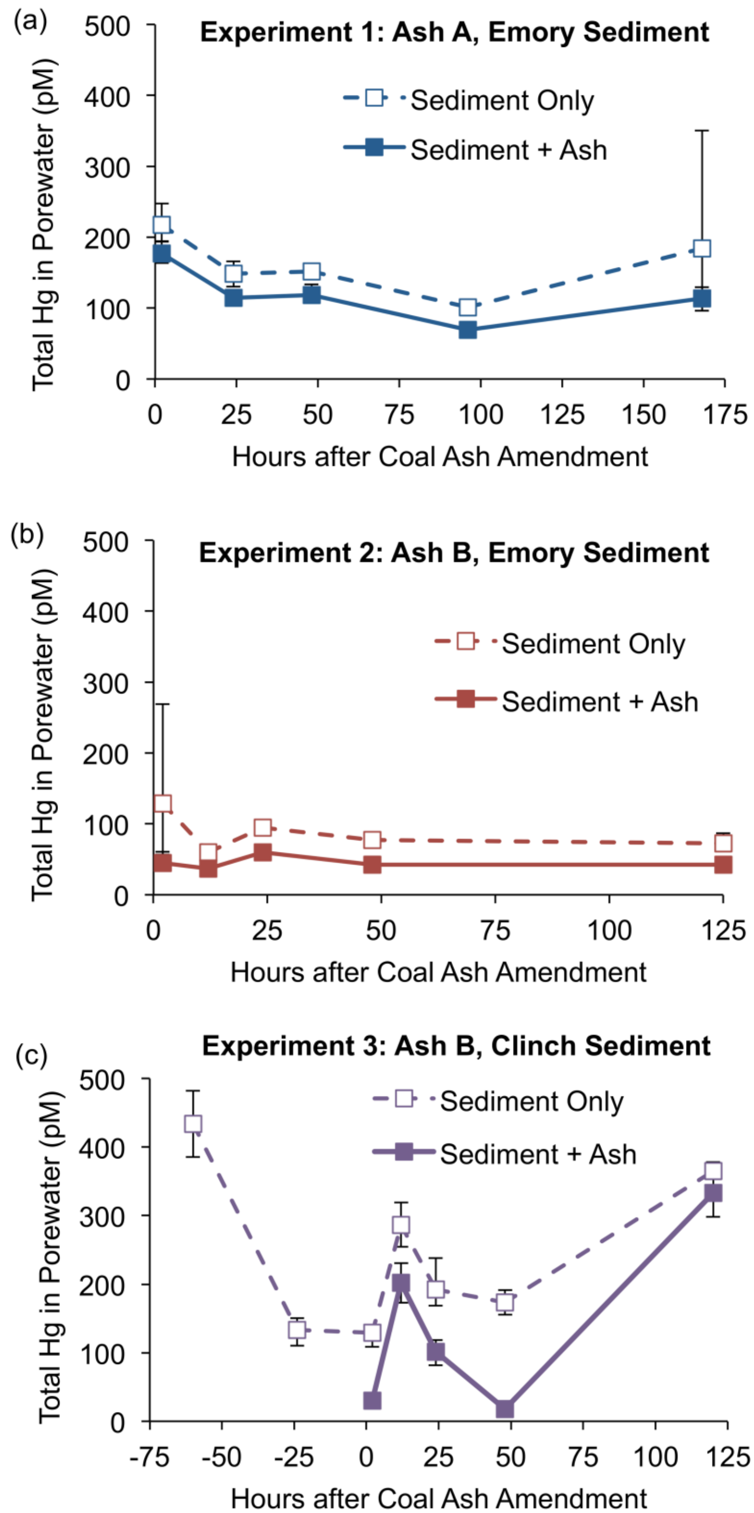


Figure S7. Total Hg concentrations in the porewater of sediment-ash microcosms; (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B; (c) Clinch

Sediment/Ash B. Data points represent the mean of replicate microcosms (n=2-3). Error bars represent the range of replicate samples.

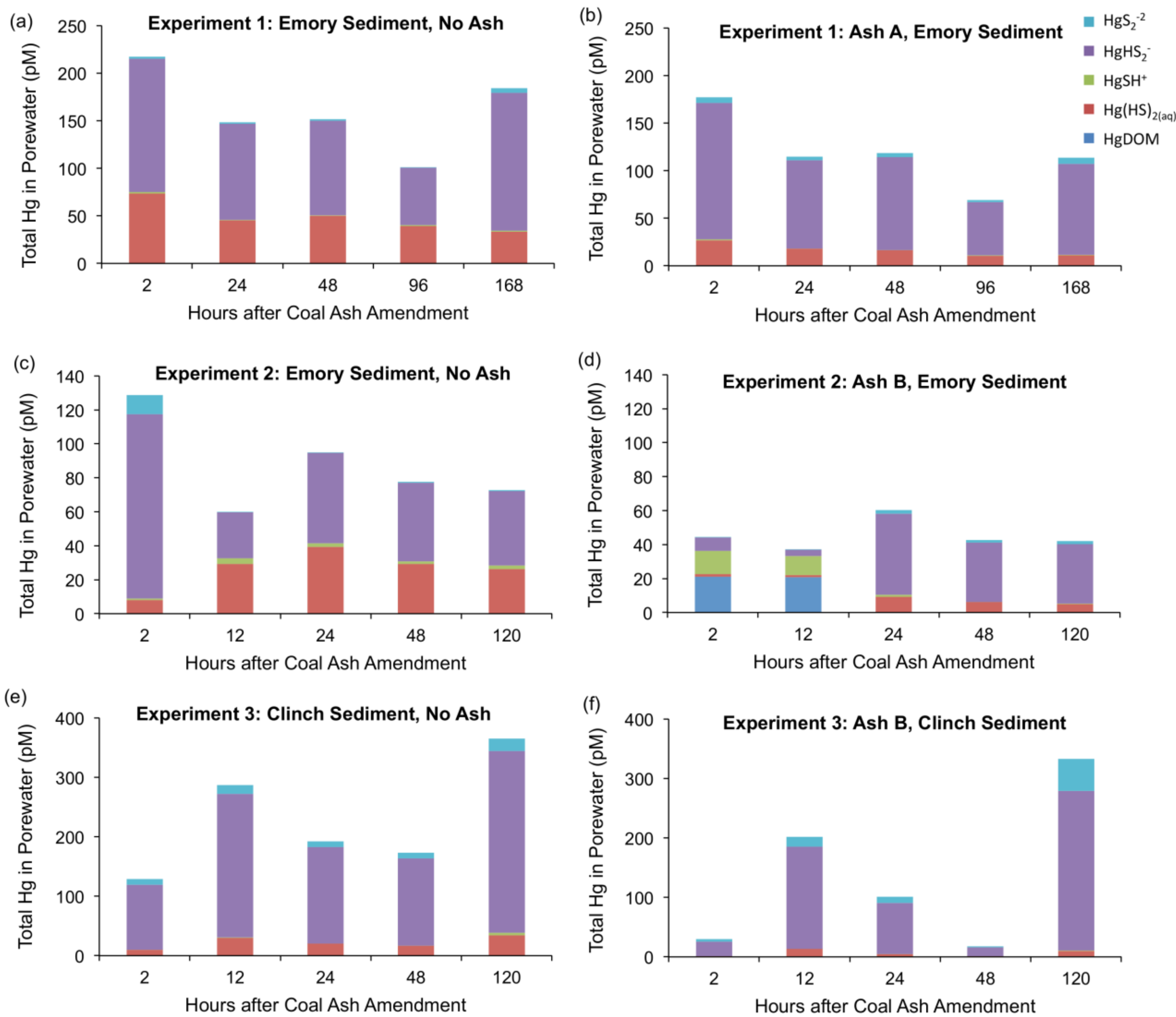


Figure S8. Calculated speciation of porewater Hg in sediment-ash microcosms (a) Experiment 1: Emory Sediment-only microcosms; (b) Experiment 1: Emory Sediment/Ash A microcosms (c) Experiment 2: Emory Sediment-only microcosms; (d) Experiment 2: Emory Sediment/Ash B microcosms; (e) Experiment 3: Clinch Sediment-only microcosms; (f) Clinch Sediment/Ash B microcosms.

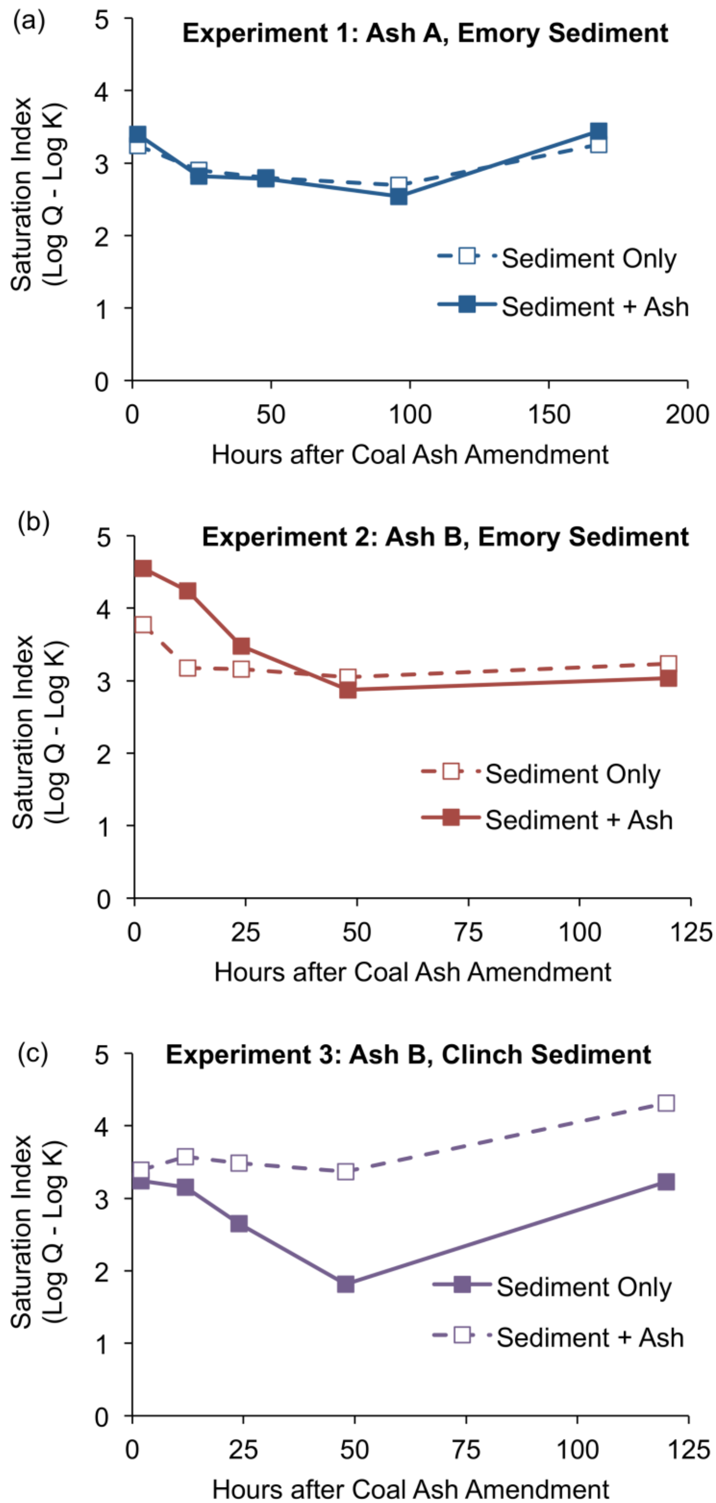
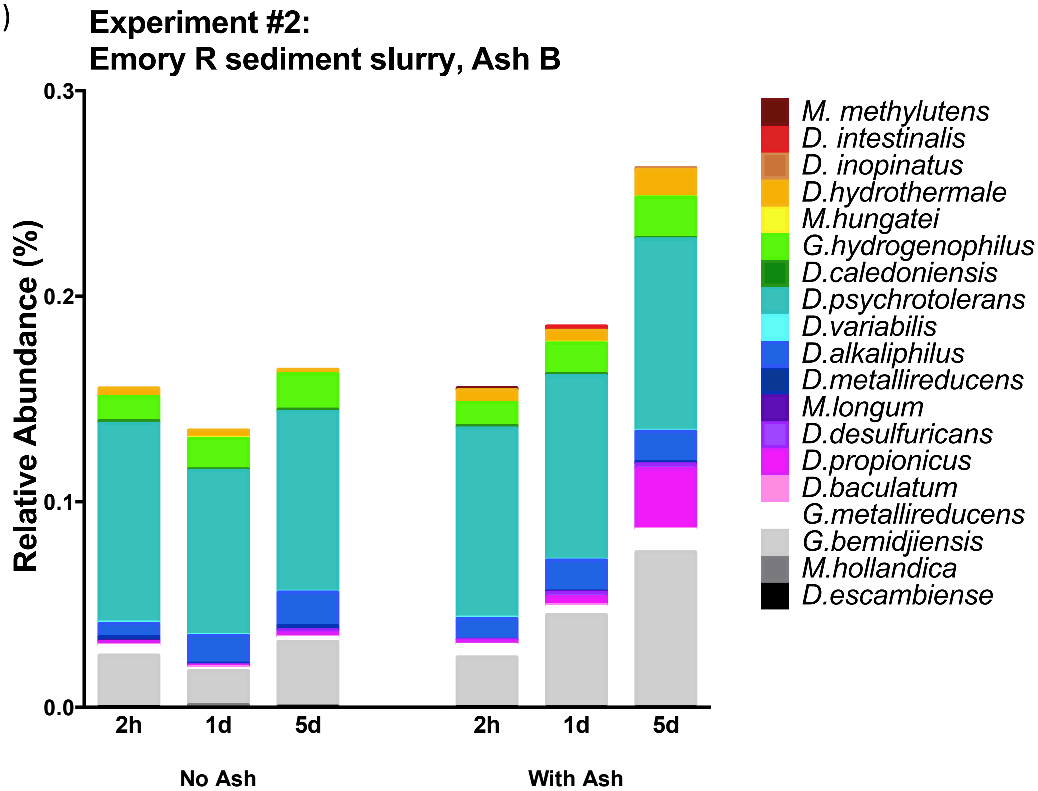


Figure S9. Saturation indices for metacinnabar in sediment-ash microcosm porewater (a) Experiment 1: Emory Sediment/Ash A microcosms; (b) Experiment 2: Emory Sediment/Ash B microcosms; (c) Experiment 3: Clinch Sediment/Ash B microcosms. Positive saturation index values indicate oversaturation with respect to $HgS_{(s)}$.

(a)


Experiment #2:
Emory R sediment slurry, Ash B
Relative Abundance (%)
0.3
0.2
0.1
0.0
2h
1d
5d
2h
1d
5d
No Ash
With Ash
M. methylutens
D. intestinalis
D. inopinatus
D.hydrothermale
M.hungatei
G.hydrogenophilus
D.caledoniensis
D.psychrotolerans
D.variabilis
D.alkaliphilus
D.metallireducens
M.longum
D.desulfuricans
D.propionicus
D.baculatum
G.metallireducens
G.bemidjiensis
M.hollandica
D.escambiense

(b)

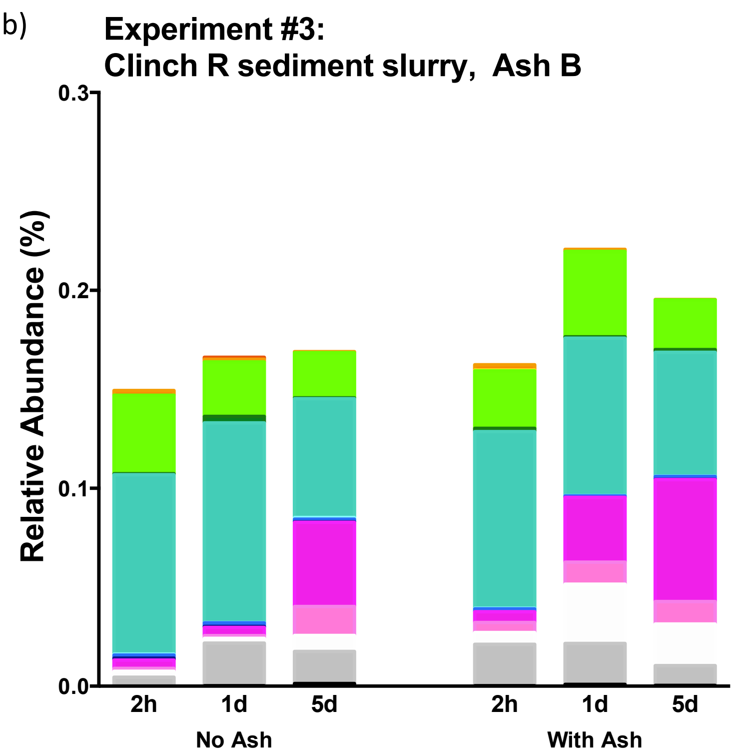

Experiment #3:
Clinch R sediment slurry, Ash B
Relative Abundance (%)
0.3
0.2
0.1
0.0
2h
1d
5d
2h
1d
5d
No Ash
With Ash

Figure S10. Fraction of the total relative abundance of methylating species in sediment-ash microcosms from (a) Experiment #2 and; (b) Experiment #3.

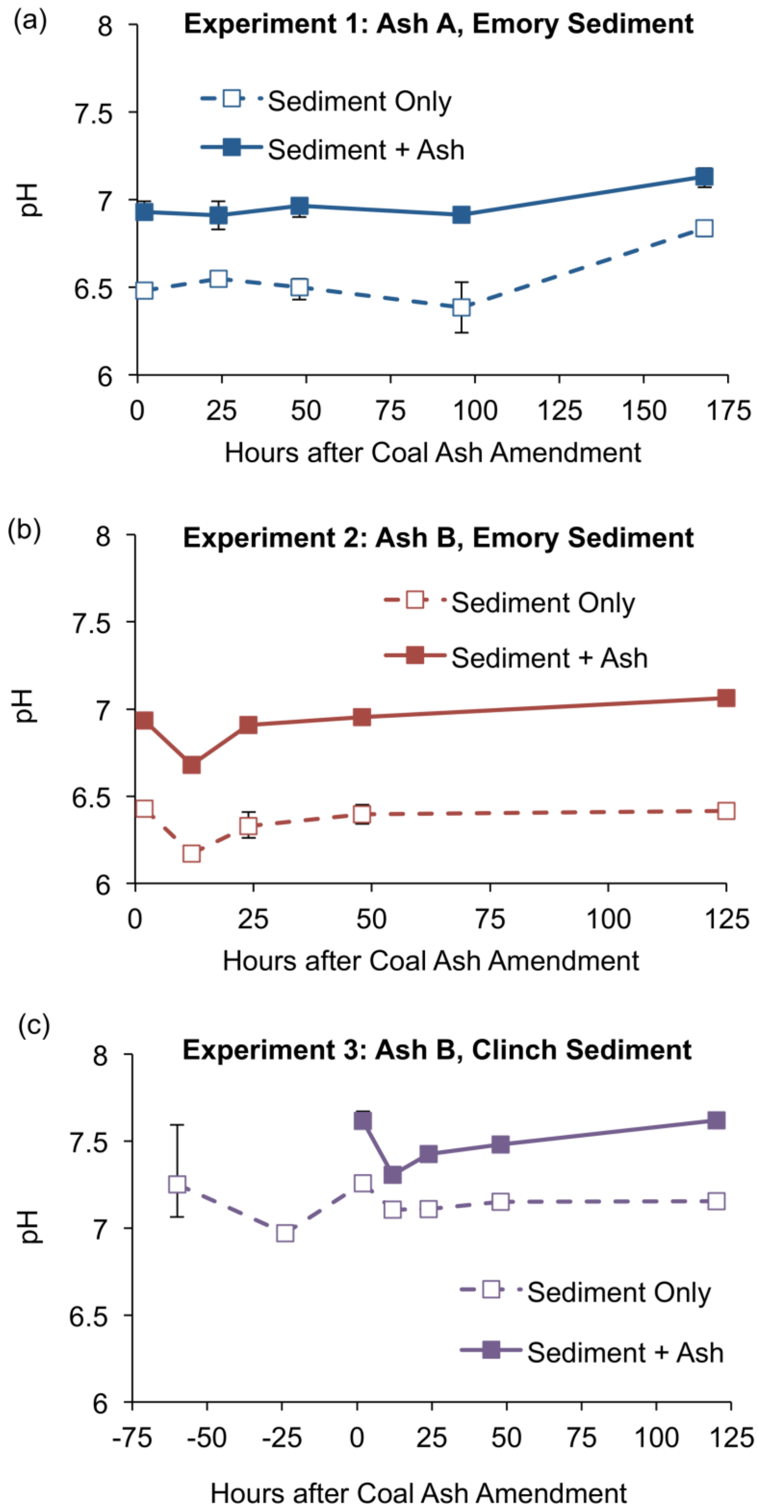


Figure S11. pH values in the sediment-ash microcosms. (a) Experiment 1: Emory Sediment/Ash A; (b) Experiment 2: Emory Sediment/Ash B; (c) Experiment 3: Clinch Sediment/Ash B. Data points represent replicate microcosms (n=2-3). Error bars represent the range of replicate samples.

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