Supporting Information

Degradation of Crude 4-MCHM (4-methylcyclohexanemethanol) in Sediments from Elk River, West Virginia

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Supplemental Methods

Site description and sampling.

Sediment for microbial community analysis was collected the impacted and background sites (Fig. 1 and Table S1) into sterile Whirl–Pak® bags (Nasco, Fort Atkinson, Wisconsin USA) and frozen immediately on dry ice in the field, and then at -80ºC in the lab. For microcosm experiments, 3-4 kg of sediment from each site were collected into 450 mL glass jars (5 jars per site) and stored on water ice in the field. River water (10 L) was collected into glass bottles at the background, upstream site in the approximate center of the river using a peristaltic pump (Geopump™ Peristaltic Pump Series II, Geotech Environmental Equipment, Inc., Denver, Colorado USA). Bottles were rinsed 3 times with river water prior to sample collection and water was stored on water ice in the field. Sediment and water samples were stored at 4ºC in the lab and used for microcosm construction within 2 weeks of collection.

Sediment porewaters were collected in the lab (3 days after field collection) for anion determination by shaking sediment-filled bottles and then allowing the solids to settle for 5 min. Overlying water was then filtered using a 0.22 µm Supor® Filters (Pall Corporation, Port Washington, New York USA) and stored at 4ºC.

Microcosm design, construction, and sampling.

In all microcosms, glass or Teflon™ lab supplies, e.g., bottles and syringes, were used to prevent contamination from phthalates. Prior to constructing sorption and biodegradation microcosms, the multiple jars of sediments were homogenized in a sterile, glass container for each site. Leaves, twigs and clams were removed from sediments during homogenization. Crude MCHM was obtained from Eastman Chemical Company (Kingsport, Tennessee, USA) and was used for all experiments. Fluids from the tanks at the Freedom Industries spill site were not obtained.

Sorption experiments.

Abiotic sorption microcosms were constructed by adding 10 g of homogenized, background sediments into 18 preweighed, amber glass 40 mL VOA vials (precleaned, poly cap with Teflon™/silicone septum, Scientific Specialties Service, Inc., Hanover, Maryland USA). The loaded vials were autoclaved twice at 121ºC for 30 min and then weighed again. To the sediment samples, 15 mL of sterile (autoclaved) Burdick & Jackson™ water (Honeywell Burdick & Jackson™, Mexico City, Mexico) was added. A solution of crude MCHM was prepared by adding 10 µL of crude MCHM (Eastman Chemical Company, Kingsport, Tennessee, USA) to 10 mL of Burdick & Jackson™ water. Then, 15 µL of the crude MCHM solution was added to each of the sediment-water filled VOA vials. The amended VOA vials were incubated at room temperature in the dark while shaking at approximately 175 rpm. Loss of 4-MCHM in the water phase was measured in triplicate vials sacrificed at days 0, 1, 2, 5, 13 and 25. Day 0 samples were collected after allowing the vials to shake for 1 hour prior to sampling. For each test day, a 2 mL aliquot of water was transferred to a corresponding solid phase microextraction (SPME) vial containing 0.5 g NaCl; the SPME vials were then stored at -20ºC until analysis. 4-MCHM isomer concentrations were analyzed by solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) as described below.

Biodegradation experiments.

To evaluate the potential for microbial degradation of 4-MCHM, a microcosm experiment was constructed to monitor microbial activity and population dynamics under anaerobic conditions in impacted and background sediments amended with 1 mg/L crude MCHM or unamended (Table 1). Control treatments included autoclaved microcosms (killed) that were amended with crude MCHM or unamended. Triplicate microcosms were constructed for each treatment (24 total bottles) by adding 100 g of homogenized sediments into a sterile 500 mL Schott bottle (Schott AG, Mainz, Germany). Bottles were sealed with Schott DURAN® GL45 red caps lined with a PTFE faced silicone liners (Schott AG, Mainz, Germany), and modified to include gas and liquid sampling ports (Fig. S1). The ports were inserted into the bottle lids with Female luer bulkheads (Cole-Parmer, Vernon Hills, Illinois, USA, Item # 45508-30) with septa (World Precision Instruments, Sarasota, Florida, USA, Item # 14034-40) attached to sample gases, and PTFE tubing (Cole-Parmer, Vernon Hills, Illinois, USA, Item # EW-06605-27) to access the microcosm liquid phase. The liquid sampling port was closed with a Stopcock with Luer Connections (Cole-Parmer, Vernon Hills, Illinois, USA, Item # EW-30600-00).

Bottles were flushed with sterile N_2 for 15 min (100 mL/min) prior to transferring to a Coy anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, Michigan, USA). In the anaerobic chamber, 200 mL of 0.22 μm-filtered, anoxic Elk River water from the background site were added to each bottle. Bottles were then flushed again with N_2 for 15 min. Some bottles experienced leakage around the sampling ports; to prevent further leakage, the ports on all bottles were sealed with epoxy. Killed control microcosms were sterilized by autoclaving once for 30 min at 121ºC; following autoclaving, killed controls were reflushed for a third time with N_2 for 15 min. The same solution of crude MCHM used for the sorption experiments was utilized to amend the biodegradation microcosms to an initial concentration of 1 mg/L crude MCHM in the whole microcosm. Microcosms were vortex mixed briefly and then shaken on rocker shaker for 1 hour prior to the day 0 sampling.

Liquid samples were collected from microcosms over time for analysis of 4-MCHM, terminal electron acceptor (e.g., Fe(II), nitrate, and sulfate), and non-volatile dissolved organic carbon (NVDOC) concentrations. Baked, glass syringes and disposable 23G syringe needles (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) were used for all sampling and only overlying water was sampled. To sample, first sterile N_2 was injected into the gas sampling port septa. Then ~0.5 mL of liquid was removed from the liquid sampling port and discarded to flush the Teflon tubing. Two ml of liquid were removed and immediately placed into a Restek SPME vial (Restek Corporation, Bellefonte, Pennsylvania, USA) with 0.5 g NaCl and 0.1 mg $HgCl_2$ added to inhibit biotic activity. For NVDOC and terminal electron acceptors, ~4.5 mL of liquid was sampled and filtered using 0.22 μm Supor® filters. Samples for NVDOC were filtered into baked amber-glass VOA vials with Teflon septa, preserved with hydrochloric acid (HCl) to pH 2, and then stored at 4ºC until analysis. Dissolved Fe(II) was measured immediately by transferring 0.25 mL of sampled water into the ferrozine assay, as described below. Samples for nitrate and sulfate determination were stored at -20ºC until analysis. To verify that the microcosms were anoxic headspace oxygen was measured 7 days after construction using gas chromatography (GC), as described below. At the end of the experiment (day 25), the live microcosms were deconstructed and 30 g of sediment was frozen at -80ºC for microbial community characterization and 20 mL of aqueous sample was used immediately for analysis of

formaldehyde. The autoclaved killed controls were stored at 4ºC for evaluation of sterilization success.

Sterilization evaluation.

After the experiments ended, effective sterilization of sediments in the biodegradation and sorption microcosms was evaluated for a subset of microcosms by adding an electron donor to the systems to stimulate residual microbial activity. Acetate (~5 mM final concentration in the whole microcosm) was added to the killed + crude MCHM biodegradation microcosms 9 days after the completion of the experiment. Duplicate samples collected on day 13 and 25 of the sorption experiments were also monitored for residual activity, with one vial left unamended whereas the second vial was amended with ~5 mM acetate. Concentrations of acetate and headspace CO_2 were measured over time, as described below. The presence of active microorganisms is indicated by the loss of acetate in parallel with the production of CO_2 due to active conversion of the substrate for growth. Samples for acetate measurement were 0.22 µm filtered with Supor® filters, then an 1 ml aliquot was acidified with 10 µL of 0.2 N HCl.

Analytical methods

MCHM determination.

MCHM was quantified in water samples using headspace SPME-GC-MS by headspace analysis and data reduction using Selective Ion Monitoring mode. A 2 or 4 mL sample aliquot was transferred to a Restek SPME vial (Restek Corporation, Bellefonte, Pennsylvania, USA) with 0.5 or 1 g, respectively, of baked NaCl and the vial was crimped and capped. For the biotic and abiotic experiments, samples were analyzed within a few days. For the sorption experiments, 0.1 mg $HgCl_2$ was also added to the SPME vial, and the samples were analyzed at the end of the experiment.

The headspace of each SPME vial was sampled using a HTA HT280T auto analyzer (HTA S.R.L., Italy). The headspace was exposed to a 100-µm polydimethylsiloxane-coated fiber (Supelco, Inc., Bellefonte, Pennsylvania, USA) for 15 min at 50°C with constant agitation of the vial contents by vibration. The fiber was then inserted into the heated inlet (250°C) of an Agilent 6890A gas chromatograph (GC; Agilent Technologies, Santa Clara, California USA) interfaced with an Agilent 5973 mass spectrometer (MS) and desorbed for 10 min. The GC (splitless mode) was equipped with a 30-m DB-5ms column (0.25 mm ID and 0.25 µm thick coating) and programmed as follows: held at 50° C for 2 min; 5°/min to 100°C; 15°/min to 290° and held for 3 min. The MS source was operated in the electron impact mode with an ionization energy of 70 eV at source temperature of 250°C, with data collected in full scan mode. A molecular ion of 55 was used to quantify the *cis*- and *trans*-4-MCHM isomers. A standard of *cis*- and *trans*-4-MCHM from TCI America (Portland, Oregon USA) was used to quantify the data. HS-SPME-GC-MS of the TCI America standard resulted in 2 peaks. Using the National Institute of Standards and Technology (NIST) mass spectrometry database, the peaks were identified as the 2 isomers, *cis*- and *trans*-4-MCHM. The standard was composed of 32.4% *trans*-4-MCHM and 67.6% *cis*-4-MCHM, which was verified by Foreman et al.[1] The standard was first diluted in methanol and then ultrapure water to a concentration of 52 µg/mL of total 4-MCHM, then diluted in ultrapure water to a range of 88-1060 µg/L *cis*- and 42-500 µg/L *trans*-4-MCHM. The detection limit was 10 µg/L for *cis*-4-MCHM and 23 µg/L for *trans*-4-MCHM.

Sediment characterization.

Aliquots of homogenized sediment were collected at the time of microcosm set up for percent (%) water and % organic carbon (C) and nitrogen (N) for both impacted and background sites. Easily visible leaf & twig parts (>3 mm) were removed when sediment was prepared for organic C analysis. A total of 5 subsamples were collected for each sediment type. Approximately 5 g of wet sediment was placed in a pre-weighed Al dish, dried overnight at 50ºC, and then reweighed to determine the % water content. For % C and N, approximately 8 mg of dried sediment was weighed into Ag cups and exposed for 24 hours to concentrated HCl acid fumes. The samples were re-dried in an oven, inserted into Sn cups then sealed. Percent organic C and N were analyzed using a ThermoScientific Flash 2000 Elemental Analyzer.

Microcosm geochemistry.

Nitrate and sulfate concentrations in microcosms samples were determined by ion chromatography (Dionex ICS 1000 IC with electrochemical detector and AS14 column). NVDOC concentrations in water were analyzed by high-temperature combustion using a TOC-Vcsn Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan).
Dissolved Fe(II) concentrations were determined using a ferrozine assay modified from Stookey (1970)[2] for use on a microplate reader.[3] Briefly, 250 µL of sample were added to 20 µL 0.01 N HCl, 130 µL of ferrozine reagent, and 100 µL of acetate buffer, then vortex mixed. The ferrozine reagent contained 12.7 µM ferrozine in 1 mM HCl; the acetate buffer contained 34.95 g sodium acetate and 15 mL glacial acetic acid in 100 mL of ultrapure water. Absorbance (in triplicate) was measured at 562 nm using a MRXe Revelation 96 Well Microplate Reader (Thermo Labsystems, Chantilly, Virginia, USA) and converted to iron concentrations based on a standard curve of known Fe(II) concentrations.

Samples for headspace oxygen and carbon dioxide were collected from microcosm bottles from the gassing sampling port using a "sampling valve" and pressure lock, and gas tight syringes (Valco Instruments Co. Inc., Houston, Texas, USA) with non-coring needles. The "sampling valve" was composed of a Hamilton HV Plug Valve (Hamilton Company, Reno, Nevada, USA), sealed with Thermogreen™ LB-2 5mm septa (Supelco, Bellefonte, Pennsylvania, USA), and Kel-F® female and male luer fittings (Hamilton No. 35031 and No. 35030, Hamilton Company, Reno, Nevada, USA). A sterile syringe needle was attached to the male end, then inserted into the flamed port of a microcosm bottle. Using the pressure lock syringe 0.1 mL of gas was removed and then injected into a HP6890 gas chromatograph (Hewlett Packard HP 5890 Series GC, Global Medical Instrumentation Inc., Ramsey, Minnesota USA). Gases were separated on a Haysepn 80-100 mesh column with a 3m 1/8 inch Nafion Dryer and analyzed with a thermal conductivity detector. The GC operated with nitrogen as the carrier gas (20 mL min^{-1} total flow), temperatures of 40ºC, 155ºC, and 180ºC for the oven, injector, and detector, respectively, and an injector flow rate of (20 $mLmin^{-1}$ total flow). GC signals were analyzed using VP Class 7.3 software (Shimadzu, Columbia, Maryland, USA). Instrument responses were standardized using mixed oxygen and carbon dioxide standards ranging in concentration from 0.5 to 20% O_2 and CO_2 (BuyCalGas, Cross Instrumentation, Conyers, Georgia, USA).

Acetate was measured using high performance liquid chromatography. Twenty-five µL of acidified sample was run on an Agilent 1220 Infinity liquid chromatograph (Agilent Technologies, Santa Clara, California, USA) with a UV detector and an Acclaim™ Organic Acid

column (5 µM particle size, 4 × 150 mm; Thermo Fisher Scientific, Waltham, Massachusetts, USA). The mobile phase was 100 mM Na_2SO_4 acidified to pH 3.0 with methanesulfonic acid and set to isocratic flow at 0.6 mL min^{-1} for 5 minutes then 1 mL min^{-1} for 55 minutes. The column oven was set to 30ºC and peaks were detected at 254 nm. Peak areas were converted to concentrations based on a standard curve of a custom organic acid certified reference material (Inorganic Ventures, Christiansburg, Virginia, USA).

Significant loss of *cis*- and *trans*-4-MCHM in sorption microcosms was calculated using t-tests in Prism 6 (GraphPad Software, Inc., La Jolla, California USA). Rates of degradation were calculated using linear regression in Prism 6. Percent loss of 4-MCHM was calculated by the difference in concentrations from the beginning to the end of the experiment multiplied by 100.

Microbial community characterization.

DNA extraction and sequencing.

Samples for microbial community characterization were collected from homogenized background and impacted sediments on day 0 and from each of the "live" microcosms on day 25 of the incubation. Duplicate extractions were done for the following anoxic microcosms: background MCHM amended bottle 2, impacted MCHM amended bottle 5, background unamended bottle 14, and impacted unamended bottle 17. Sediment samples (0.24 to 0.35 g) were extracted using the PowerSoil® DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, California, USA) according to the manufacturer's instructions and DNA was quantified via Qubit® dsDNA High Sensitivity assay (Invitrogen, Carlsbad, California, USA). DNA samples (n = 20) were sent to the Michigan State University Genomics Core Facility for sequencing on an Illumina MiSeq (San Diego, California, USA), where the 16S rRNA gene V4 region from both Bacteria and Archaea was amplified with barcoded and Illumina-compatible primers 515F and 806R [4] and the amplicons normalized and pooled for 2 × 250 base pair sequencing using a standard MiSeq flow cell and a 500 cycle reagent cartridge (both v2).

Bioinformatics

Paired end reads from the samples were merged using the fastq_mergepairs script from usearch[5] and converted to fasta files for import into mothur v.1.36.1.[6] The total number of merged reads was 2,392,239 with library sizes ranging from 75,151 to 172,167 sequences. Sequences were clustered into OTUs at a cutoff distance of 0.03 and classified following the guidelines of Kozich et al. (2013).[7] In brief, de-replicated sequences were aligned to Silva v119 SSU reference database,[8] chimera-checked using uchime,[9] classified against Greengenes 13_8[10] via the naïve Bayesian classifier,[11] and clustered into OTUs using the cluster.split command with binning of sequences at the Order level.[12] Post-processing, the data set consisted of 1,896,644 sequences with library sizes ranging from 58,591 to 134,300. Sequences are available under BioProject PRJNA389713 in the NCBI Short Read Archive (https://www.ncbi.nlm.nih.gov/sra).

Following bioinformatics processing, mothur data files were imported into R version 3.2.2[13] using phyloseq version 1.12.2.[14] We used phyloseq to bin OTUs by genus designations and calculate alpha diversity measures (number of genera, Simpson Evenness Index, and Chao1 Richness Estimator) on libraries (without singleton OTUs) subsampled to the smallest size (n_{seq} = 58,587). To determine if background and impacted sediment microbial communities differed in

composition, we subjected a Bray-Curtis dissimilarity matrix constructed from the subsampled libraries to permutational MANOVA (PERMANOVA) with site as a factor (background versus impacted), time (T0 or Day 25) as a block, and 999 permutations, and visualized the dissimilarity matrix using nonmetric multidimensional scaling (NMDS). These analyses were performed using the adonis and metamds functions, respectively, in vegan version 2.3-3.[15] We used the corr.axes function in mothur[6] to calculate Spearman correlations between genera and each NMDS axis to determine if any genera were significantly correlated with the NMDS dimensions. The relative abundances of genera that were highly and significantly correlated (Spearman correlations between 0.7 to 1 or -0.7 to -1 and $P < 0.05$) with NMDS axis 1 were plotted as a heatmap with the color scale log base 10 transformed. The R package ggplot2[16] was used to generate NMDS and heatmap plots.

Sequence processing scripts

```
# merged reads using fastq_mergepairs script
# for each file, ran the following command:
usearch8.0.1517_i86linux64 -fastq_mergepairs R1_001.fastq -reverse
R2_001.fastq -fastqout merged.fastq

# converted fastq to fasta files using prinseq-lite (v 0.20.4; Schmieder R
and Edwards R: Quality control and preprocessing of metagenomic datasets.
Bioinformatics 2011, 27:863-864. [PMID: 21278185])
# for each file, ran the following command:
perl prinseq-lite.pl -fastq file.fastq -out_format 1 -out_good
filename_merged

# processed sequences into OTUs using mothur mothur v.1.36.1 (Schloss, P.D.,
et al., Introducing mothur: Open-source, platform-independent, community-
supported software for describing and comparing microbial communities. Appl
Environ Microbiol, 2009. 75(23):7537-41.)
# made a group file, merged fasta files, screened, dereplicated, and counted
sequences
make.group(fasta=MCHM_13_B_T25_merged.fasta-MCHM_15_B_T25_merged.fasta-
MCHM_17_I_T25_R2_merged.fasta-MCHM_2_B_T25_R1_merged.fasta-
MCHM_4_I_T25_merged.fasta-MCHM_6_I_T25_merged.fasta-
MCHM_I_T0_R1_merged.fasta-MCHM_14_B_T25_R1_merged.fasta-
MCHM_16_I_T25_merged.fasta-MCHM_18_I_T25_merged.fasta-
MCHM_2_B_T25_R2_merged.fasta-MCHM_5_I_T25_R1_merged.fasta-
MCHM_B_T0_R1_merged.fasta-MHCH_I_T0_R2_merged.fasta-
MCHM_14_B_T25_R2_merged.fasta-MCHM_17_I_T25_R1_merged.fasta-
MCHM_1_B_T25_merged.fasta-MCHM_3_B_T25_merged.fasta-
MCHM_5_I_T25_R2_merged.fasta-MCHM_B_T0_R2_merged.fasta, groups=MCHM_13_B_T25-
MCHM_15_B_T25-MCHM_17_I_T25_R2-MCHM_2_B_T25_R1-MCHM_4_I_T25-MCHM_6_I_T25-
MCHM_I_T0_R1-MCHM_14_B_T25_R1-MCHM_16_I_T25-MCHM_18_I_T25-MCHM_2_B_T25_R2-
MCHM_5_I_T25_R1-MCHM_B_T0_R1-MHCH_I_T0_R2-MCHM_14_B_T25_R2-MCHM_17_I_T25_R1-
MCHM_1_B_T25-MCHM_3_B_T25-MCHM_5_I_T25_R2-MCHM_B_T0_R2)
merge.files(input=MCHM_13_B_T25_merged.fasta-MCHM_15_B_T25_merged.fasta-
MCHM_17_I_T25_R2_merged.fasta-MCHM_2_B_T25_R1_merged.fasta-
MCHM_4_I_T25_merged.fasta-MCHM_6_I_T25_merged.fasta-
MCHM_I_T0_R1_merged.fasta-MCHM_14_B_T25_R1_merged.fasta-
MCHM_16_I_T25_merged.fasta-MCHM_18_I_T25_merged.fasta-
MCHM_2_B_T25_R2_merged.fasta-MCHM_5_I_T25_R1_merged.fasta-
MCHM_B_T0_R1_merged.fasta-MHCH_I_T0_R2_merged.fasta-
MCHM_14_B_T25_R2_merged.fasta-MCHM_17_I_T25_R1_merged.fasta-
MCHM_1_B_T25_merged.fasta-MCHM_3_B_T25_merged.fasta-
MCHM_5_I_T25_R2_merged.fasta-MCHM_B_T0_R2_merged.fasta, output=MCHM.fasta)
screen.seqs(fasta=MCHM.fasta, group=MCHM.groups, maxambig=0, maxlength=300,
processors=60)
unique.seqs(fasta=current)
count.seqs(name=MCHM.good.names, group=MCHM.good.groups, processors=60)

# Aligned sequences to Silva v119, removed sequences that failed to align,
and summarized sequences
# Customized Silva v119 to v4 region
pcr.seqs(fasta=silva.nr_v119.align, taxonomy=silva.nr_v119.tax,
oligos=primer.oligos, pdiffs=3, processors=60)
summary.seqs(fasta=silva.nr_v119_U515F806R.pcr.align, processors=60) #
renamed in shell
```

```
screen.seqs(fasta=silva.nr_v119_U515F806R.pcr.align,
taxonomy=silva.nr_v119_U515806.pcr.tax, maxambig=0, maxlength=300,
processors=60)
summary.seqs(fasta=silva.nr_v119_U515F806R.pcr.good.align, processors=60)
align.seqs(fasta=MCHM.good.unique.fasta,
reference=../silva.nr_v119_U515F806R.pcr.good.align, flip=T, processors=60)
remove.seqs(accnos=MCHM.good.unique.flip.accnos,
fasta=MCHM.good.unique.align, alignreport=MCHM.good.unique.align.report)
remove.seqs(accnos=MCHM.good.unique.flip.accnos, group=MCHM.good.groups)
remove.seqs(accnos=MCHM.good.unique.flip.accnos, name=MCHM.good.names)
remove.seqs(accnos=MCHM.good.unique.flip.accnos, count=MCHM.good.count_table)
summary.seqs(fasta=MCHM.good.unique.pick.align,
count=MCHM.good.pick.count_table, processors=60)

# Pre-processed sequences prior to OTU clustering
screen.seqs(fasta=MCHM.good.unique.pick.align,
count=MCHM.good.pick.count_table, summary=MCHM.good.unique.pick.summary,
start=13862, end=23444, maxhomop=8, minlength=240, maxlength=260,
processors=60)
summary.seqs(fasta=MCHM.good.unique.pick.good.align,
count=MCHM.good.pick.good.count_table, processors=60)
filter.seqs(fasta=MCHM.good.unique.pick.good.align, vertical=T, trump=.,
processors=60)
unique.seqs(fasta=MCHM.good.unique.pick.good.filter.fasta,
count=MCHM.good.pick.good.count_table)
summary.seqs(fasta=current, count=current, processors=60)
pre.cluster(fasta=current, count=current, diffs=2, processors=60)
summary.seqs(fasta=current, count=current)

# Checked sequences for chimeras and removed chimeric reads
chimera.uchime(fasta=MCHM.good.unique.pick.good.filter.unique.precluster.fast
a, count=MCHM.good.unique.pick.good.filter.unique.precluster.count_table,
dereplicate=t, processors=2)
remove.seqs(fasta=MCHM.good.unique.pick.good.filter.unique.precluster.fasta,
accnos=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.accn
os)
summary.seqs(fasta=current,
count=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.pick.
count_table, processors=60)

# Classified sequences against GreenGenes version 13_8 and removed unknowns
and non-bacterial/archaeal sequences
classify.seqs(fasta=MCHM.good.unique.pick.good.filter.unique.precluster.pick.
fasta,
count=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.pick.
count_table, reference=../gg_13_8_99.fasta, taxonomy=../gg_13_8_99.gg.tax,
cutoff=80, processors=60)
remove.lineage(fasta=MCHM.good.unique.pick.good.filter.unique.precluster.pick
.fasta,
count=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.pick.
count_table,
taxonomy=MCHM.good.unique.pick.good.filter.unique.precluster.pick.gg.wang.tax
onomy,  taxon=unknown-Mitochondria-Chloroplast-Eukaryota)
summary.seqs(fasta=current, count=current, processors=60)

# Clustered sequences into OTUs and classified OTUs
```

```
cluster.split(fasta=MCHM.good.unique.pick.good.filter.unique.precluster.pick.
pick.fasta,
count=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.pick.
pick.count_table,
taxonomy=MCHM.good.unique.pick.good.filter.unique.precluster.pick.gg.wang.pic
k.taxonomy, splitmethod=classify, taxlevel=4, cutoff=0.15, large=T,
processors=60)
make.shared(list=MCHM.good.unique.pick.good.filter.unique.precluster.pick.pic
k.an.unique_list.list,
count=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.pick.
pick.count_table, label=0.03)
classify.otu(list=MCHM.good.unique.pick.good.filter.unique.precluster.pick.pi
ck.an.unique_list.list,
count=MCHM.good.unique.pick.good.filter.unique.precluster.denovo.uchime.pick.
pick.count_table,
taxonomy=MCHM.good.unique.pick.good.filter.unique.precluster.pick.gg.wang.pic
k.taxonomy, label=0.03)

# imported output files (MCHM.0.03.shared and MCHM.0.03.cons.taxonomy) into R
for summary and statistical analysis
```

R analysis scripts

```
# libraries needed to import mothur files, process OTU tables, perform
# statistical analyses, and make graphs
library(phyloseq)
library(genefilter)
library(vegan)
library(ggplot2)
library(biomformat)
library(reshape2)
library(scales)
# imported mothur shared, taxonomy, and metadata file
MCHM <- import_mothur(mothur_shared_file = "MCHM.0.03.shared",
mothur_constaxonomy_file = "MCHM.0.03.cons.taxonomy")
samples <- read.csv("MCHM_sample_data.csv")
class(samples$Rep)
samples$Rep <- as.factor(samples$Rep)
class(samples$Rep)
rownames(samples) <- samples[,1]
samples <- sample_data(samples)
colnames(tax_table(MCHM))[1] <-"Domain"
colnames(tax_table(MCHM))[2] <-"Phylum"
colnames(tax_table(MCHM))[3] <-"Class"
colnames(tax_table(MCHM))[4] <-"Order"
colnames(tax_table(MCHM))[5] <-"Family"
colnames(tax_table(MCHM))[6] <-"Genus"
colnames(tax_table(MCHM))[7] <-"Species"
rank_names(MCHM)
MCHM <- merge_phyloseq(MCHM, samples)
sample_data(MCHM)
sum(taxa_sums(MCHM)) # 1,896,644
min(sample_sums(MCHM)) # 58,591
max(sample_sums(MCHM)) # 134,300
# converted OTU counts to relative abundance
MCHM_RA <- transform_sample_counts(MCHM, function(OTU)(OTU/sum(OTU))*100)
# collapsed samples by taxonomy into counts and relative abundances
Domain <- tax_glom(MCHM, taxrank=rank_names(MCHM)[1])
Domain_RA <- transform_sample_counts(Domain, function(OTU)(OTU/sum(OTU))*100)
Phyla <- tax_glom(MCHM, taxrank=rank_names(MCHM)[2])
Phyla_RA <- transform_sample_counts(Phyla, function(OTU)(OTU/sum(OTU))*100)
Class <- tax_glom(MCHM, taxrank=rank_names(MCHM)[3])
Class_RA <- transform_sample_counts(Class, function(OTU)(OTU/sum(OTU))*100)
Order <- tax_glom(MCHM, taxrank=rank_names(MCHM)[4])
Order_RA <- transform_sample_counts(Order, function(OTU)(OTU/sum(OTU))*100)
Family <- tax_glom(MCHM, taxrank=rank_names(MCHM)[5])
Family_RA <- transform_sample_counts(Family, function(OTU)(OTU/sum(OTU))*100)
Genus <- tax_glom(MCHM, taxrank=rank_names(MCHM)[6])
Genus_RA <- transform_sample_counts(Genus, function(OTU)(OTU/sum(OTU))*100)
# analyzed sequences using OTUs collapsed into Genera
# diversity analysis on samples: removed singleton and subsampled
ns <- prune_taxa(taxa_sums(Genus)>1, Genus)
ns_subsamp <- rarefy_even_depth(ns, sample.size=min(sample_sums(ns)),
rngseed=1358, replace=FALSE, trimOTUs=TRUE)
sample_sums(ns_subsamp) # 58,587
ns_richness <- estimate_richness(ns_subsamp)
write.csv(ns_richness, "Genus_ns_richness.csv")
min(ns_richness$Observed) # 702
```

```
max(ns_richness$Observed) # 789
mean(ns_richness$Observed) # 757
sd(ns_richness$Observed) # 21
# NMDS analysis on all sequences
tns_subsamp <- as.data.frame(t(otu_table(ns_subsamp)))
ns_subsamp_NMDS <- metaMDS(tns_subsamp, distance="bray", k=3)
ns_subsamp_NMDS # stress is 0.086
stressplot(ns_subsamp_NMDS)
ordiplot(ns_subsamp_NMDS, type="p", display="sites")
orditorp(ns_subsamp_NMDS, display="sites", pos=4, air=0.1)
# plot of NMDS plot with ggplot2
ns_NMDS_data <- as.data.frame(ns_subsamp_NMDS$points)
ns_NMDS_data <- cbind(ns_NMDS_data, sample_data(ns_subsamp)$Location,
sample_data(ns_subsamp)$Time, sample_data(ns_subsamp)$Bottle,
sample_data(ns_subsamp)$Amend)
fix(ns_NMDS_data) # changed headers
ns_NMDS_ggplot <- ggplot(ns_NMDS_data, aes(y=NMDS2, x=NMDS1,
shape=factor(Time))) +
  theme_bw(base_size=12) + theme(panel.grid=element_blank())
# bottle numbers only plot
ns_NMDS_ggplot + geom_point(size=2, aes(color=factor(Location))) +
  scale_shape_discrete(labels=c("Day 25","Initial")) +
  scale_x_continuous(limits=c(-0.1,0.11)) +
  scale_color_manual(values=c("blue","red"),
guide=guide_legend(override.aes=aes(shape=15))) +
  theme(legend.position="bottom", legend.text=element_text(size=12),
legend.title=element_blank()) +
  geom_text(aes(label=ns_NMDS_data$Bottle), hjust=-.25, vjust=0.5, size=2.5)
+
  annotate("text", label="Stress = 0.086", x=0.1, y=0.1, size=3)
ggsave("NMDS_Genus_btl_num_plot.eps", width=6.5, height=5)
# bottle numbers and amendment, oxic/anoxic
ns_NMDS_ggplot + geom_point(size=2, aes(color=factor(Location))) +
  scale_shape_discrete(labels=c("Day 25","Initial")) +
  scale_color_manual(values=c("blue","red"),
guide=guide_legend(override.aes=aes(shape=15))) +
  scale_x_continuous(limits=c(-0.1,0.11)) +
  theme(legend.position="bottom", legend.text=element_text(size=12),
legend.title=element_blank()) +
  geom_text(aes(label=ns_NMDS_data$Amend), hjust=1.2, vjust=0.45, size=2.5) +
  annotate("text", label="Stress = 0.086", x=0.1, y=0.1, size=3)
ggsave("NMDS_Genus_amend_plot.eps", width=6.5, height=5)
# no labels
ns_NMDS_ggplot + geom_point(size=2, aes(color=factor(Location))) +
  scale_shape_discrete(labels=c("Day 25","Initial")) +
  scale_color_manual(values=c("blue","red"),
guide=guide_legend(override.aes=aes(shape=15))) +
  scale_x_continuous(limits=c(-0.1,0.11)) +
  theme(legend.position="bottom", legend.text=element_text(size=12),
legend.title=element_blank()) +
  annotate("text", label="Stress = 0.086", x=0.1, y=0.1, size=3)
ggsave("NMDS_Genus_nolabels_plot.eps", width=6.5, height=5)
# plot of common NMDS plot with ggplot2
# tested for homogeneity of variances using location as group
ns_ss_hv_BI <- betadisper(tns_subsamp_BC, group=samples$Location,
type="centroid")
ns_ss_hv_BI
```

```
ns_ss_hv_BI_pt <- permutest(ns_ss_hv_BI, pairwise=TRUE, permutations=999)
ns_ss_hv_BI_pt # not significant
# permanova on BC distance matrices
tns_subsamp_BC <- vegdist(tns_subsamp, distance="bray")
# compared background and impacted with time as blocking variable
ns_ss_pAOV_BI <- adonis(tns_subsamp_BC ~ sample_data(ns_subsamp)$Location,
strata=sample_data(ns_subsamp)$Time, permuations=999)
ns_ss_pAOV_BI # df(1,18) = 7.73; P<0.001; R2 0.300
ns_subsamp_biom <- make_biom(otu_table(ns_subsamp))
write_biom(ns_subsamp_biom, "Genus_ns_subsamp.biom")
write.table(ns_subsamp_NMDS$points, "Genus_ns_subsamp_NMDS.axes", sep="\t")
# opened in excel and modified header to Group, axis1, axis2
### in mothur, ran the following commands
### make.shared(biom=Genus_ns_subsamp.biom)
### corr.axes(shared=Genus_ns_subsamp.shared, axes=ns_subsamp_NMDS.axes,
method=spearman)
# read in correlation results and subset to include only OTUs with
correlations
# more than 1 >=0.5 or -1 < -0.5
ns_OTU_NMDS_corr <- read.table("Genus_ns_subsamp.spearman.corr.axes",
header=TRUE, row.names="OTU", sep="\t")
ns_OTU_NMDS_sig_cor <-
ns_OTU_NMDS_corr[which(ns_OTU_NMDS_corr$axis1>=0.5|ns_OTU_NMDS_corr$axis1<=-
0.5),]
# converted subsampled libraries to relative abundances
ns_subsamp_RA <- transform_sample_counts(ns_subsamp,
function(OTU)(OTU/sum(OTU))*100)
# retreived OTUs from ns_subsamp that are significantly correlated
ns_OTU_NMDS_corr_sig_list <- rownames(ns_OTU_NMDS_sig_cor)
ns_ss_RA_OTU_NMDS_corr_sig <-  prune_taxa(ns_OTU_NMDS_corr_sig_list,
ns_subsamp_RA)
min(sample_sums(ns_ss_RA_OTU_NMDS_corr_sig)) # 53.1%
max(sample_sums(ns_ss_RA_OTU_NMDS_corr_sig)) # 57.1%
# exported csv files of RA and taxonomy tables and significant correlations
write.csv(otu_table(ns_ss_RA_OTU_NMDS_corr_sig),
"Genus_ns_ss_RA_OTU_NMDS_corr_sig.csv")
write.csv(tax_table(ns_ss_RA_OTU_NMDS_corr_sig),
"Genus_ns_ss_RA_OTU_NMDS_corr_sig_tax.csv")
write.csv(ns_OTU_NMDS_sig_cor, "Genus_ns_OTU_NMDS_sig_cor.csv")
### combined tables in excel and added column to sum OTU abundances across
samples
### and calculated an effect size change by looking at the overall % change
in OTU
### abundances b/w background and impacted samples
ns_ss_RA_OTU_NMDS_corr_sig_comb <-
read.table("Genus_ns_combined_OTU_NMDS_sig_cor.txt", sep="\t", header=TRUE,
row.names="OTU")
# removed OTUs with summed abundances less than 2 across samples
OTU_NMDS_corr_sig <-
ns_ss_RA_OTU_NMDS_corr_sig_comb[which(ns_ss_RA_OTU_NMDS_corr_sig_comb$OTU_sum
>2),]
# removed OTUs with abundance changes less than 30%
OTU_NMDS_corr_sig <-
OTU_NMDS_corr_sig[which(OTU_NMDS_corr_sig$Effect_Size>=30|OTU_NMDS_corr_sig$E
ffect_Size<=-30),]
# removed OTUs with correlations b/w -0.69 and 0.69
```

```
OTU_NMDS_corr_sig <-
OTU_NMDS_corr_sig[which(OTU_NMDS_corr_sig$axis1>=0.7|OTU_NMDS_corr_sig$axis1<
=-0.7),]
# modified table in excel to make a combined taxonomy column with Spearman
Correlation
ns_top_OTUs <-
as.matrix(read.table("Genus_ns_combined_OTU_NMDS_sig_cor_most_abund_OTUs_for_
plot.txt", header=TRUE, row.names="Taxonomy", sep="\t"))
ns_top_OTUs_melt <- melt(ns_top_OTUs)
head(ns_top_OTUs_melt)
OTUhmlabels_ns <- row.names(ns_top_OTUs)
ns_top_OTUs_hm <- ggplot(data=ns_top_OTUs_melt, aes(x=Var2, y=Var1)) +
theme_bw() + theme(plot.margin=grid::unit(c(0,0,0,0), "mm"))
ns_top_OTUs_hm + geom_tile(aes(fill=value)) +
  scale_fill_gradient(name="Abundance", low="#FFCC33",
na.value="lightyellow", high="#CC0000", trans=log_trans(10)) +
  theme(axis.text.x=element_text(vjust=0.5, angle=90, hjust=1),
axis.title=element_blank()) +
  scale_y_discrete(expand=c(0,0), limits=OTUhmlabels_ns) +
  scale_x_discrete(expand=c(0,0), labels=c("Bck_R1","Bck_R2","Bck_U-
1","Bck_U-2_R1","Bck_U-2_R2","Bck_U-3","Bck_M-13","Bck_M-14_R1","Bck_M-
14_R2","Bck_M-15","Imp_R1","Imp_R2","Imp_U-4","Imp_U-5_R1","Imp_U-
5_R2","Imp_U-6","Imp_M-16","Imp_M-17_R1","Imp_M-17_R2","Imp_M-18"))
ggsave("Genus_ns_top_OTUs_NMDS_cor.eps", width=11, height=7)
```

References

1. Foreman, W. T.; Rose, D. L.; Chambers, D. B.; Crain, A. S.; Murtagh, L. K.; Thakellapalli, H.; Wang, K. K., Determination of (4-methylcyclohexyl)methanol isomers by heated purge-and-trap GC/MS in water samples from the 2014 Elk River, West Virginia, chemical spill. *Chemosphere* **2015,** *131*, 217-224, http://dx.doi.org/10.1016/j.chemosphere.2014.11.006.
2. Stookey, L. L., Ferrozine---a new spectrophotometric reagent for iron. *Anal. Chem.* **1970,** *42*, (7), 779-781, http://dx.doi.org/10.1021/ac60289a016.
3. Fleming, E. J.; Cetinić, I.; Chan, C. S.; Whitney King, D.; Emerson, D., Ecological succession among iron-oxidizing bacteria. *ISME J.* **2014,** *8*, (4), 804-815, http://dx.doi.org/10.1038/ismej.2013.197.
4. Caporaso, J. G.; Lauber, C. L.; Walters, W. A.; Berg-Lyons, D.; Lozupone, C. A.; Turnbaugh, P. J.; Fierer, N.; Knight, R., Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Science* **2011,** *108*, (Supplement 1), 4516-4522, http://dx.doi.org/10.1073/pnas.1000080107.
5. Edgar, R. C.; Flyvbjerg, H., Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **2015,** *31*, (21), 3476-3482, http://dx.doi.org/10.1093/bioinformatics/btv401.
6. Schloss, P. D.; Westcott, S.; Ryabin, T.; Hall, J.; Hartmann, M.; Hollister, E.; Lesniewski, R.; Oakley, B.; Parks, D.; Robinson, C.; Sahl, J.; Stres, B.; Thallinger, G.; Van Horn, D.; Weber, C., Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009,** *75*, (23), 7537-41, http://dx.doi.org/10.1128/AEM.01541-09.
7. Kozich, J. J.; Westcott, S. L.; Baxter, N. T.; Highlander, S. K.; Schloss, P. D., Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* **2013,** *79*, (17), 5112-20, http://dx.doi.org/10.1128/AEM.01043-13.
8. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O., The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **2013,** *41*, (D1), D590-D596, http://dx.doi.org/10.1093/nar/gks1219.
9. Edgar, R. C.; Haas, B. J.; Clemente, J. C.; Quince, C.; Knight, R., UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011,** *27*, (16), 2194-2200, http://dx.doi.org/10.1093/bioinformatics/btr381.
10. DeSantis, T. Z.; Hugenholtz, P.; Larsen, N.; Rojas, M.; Brodie, E. L.; Keller, K.; Huber, T.; Dalevi, D.; Hu, P.; Andersen, G. L., Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **2006,** *72*, (7), 5069-5072, http://dx.doi.org/10.1128/aem.03006-05.
11. Wang, Q.; Garrity, G. M.; Tiedje, J. M.; Cole, J. R., Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007,** *73*, (16), 5261-5267, http://dx.doi.org/10.1128/aem.00062-07.
12. Schloss, P. D.; Westcott, S. L., Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. *Appl. Environ. Microbiol.* **2011,** *77*, (10), 3219-3226, http://dx.doi.org/10.1128/AEM.02810-10.
13. R Core Team *R: A language and environment for statistical computing*, R Foundation for Statistical Computing: Vienna, Austria, 2015.

14. McMurdie, P. J.; Holmes, S., phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **2013,** *8*, (4), e61217, http://dx.doi.org/10.1371/journal.pone.0061217.
15. Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H.H., Wagner, H. *vegan: Community Ecology Package. R package version 2.2-1*, 2014.
16. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis* Springer-Verlag: New York, 2009.
17. Cozzarelli, I. M.; Akob, D. M.; Baedecker, M. J.; Spencer, T.; Jaeschke, J.; Dunlap, D. S.; Mumford, A.; Poret-Peterson, A. T., MCHM Degradation Studies Data Release. *U. S. Geological Survey Data Release* **2017**, http://dx.doi.org/10.5066/F73R0R41.

Supplementary Figures

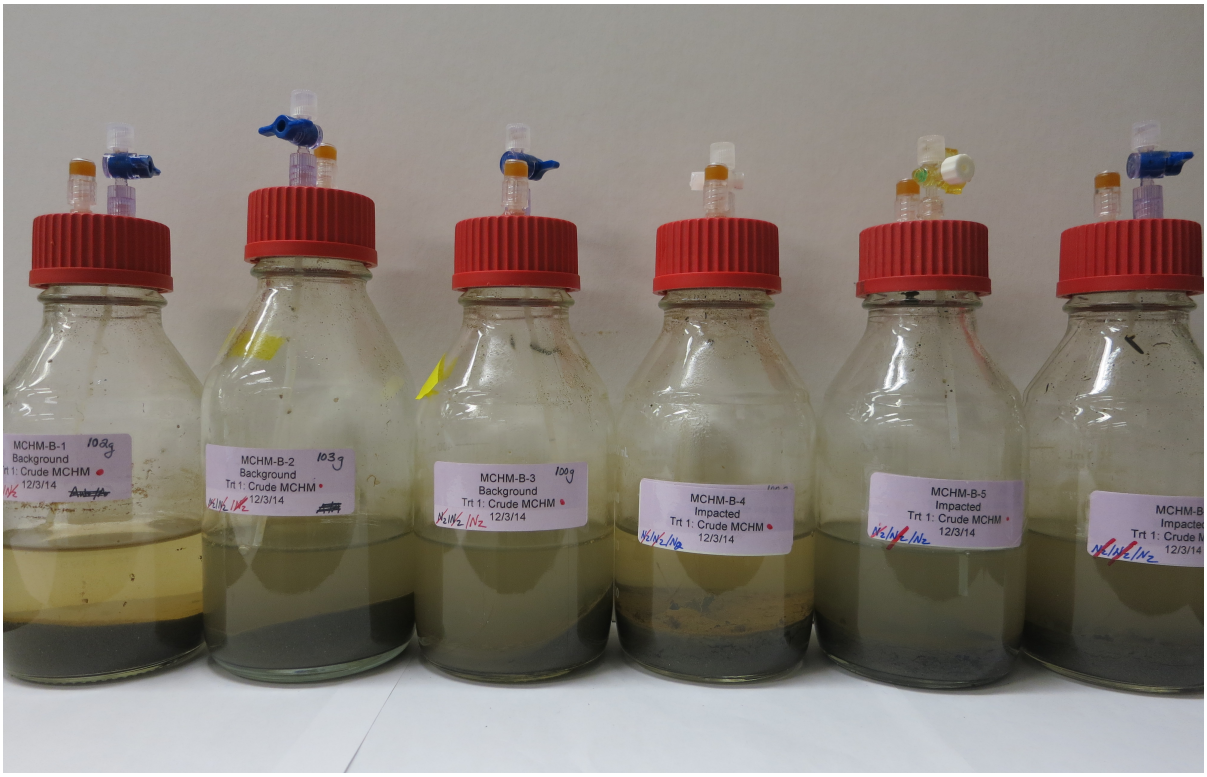

Figure S1. Photograph of biodegradation microcosms on day 25 amended with 1 mg/L crude MCHM. Left to right: Background sediment bottles B-1, B-2, and B-3; Impacted sediment bottles B-4, B-5, and B-6. Note the darker color in B-2, B-3, B-5, and B-6 which corresponds to the presence of Fe(II) from microbial iron reduction. Bottles B-1 and B-4 have a lighter color consistent with the lack of Fe(II) production observed due to air leakage (Table S3).

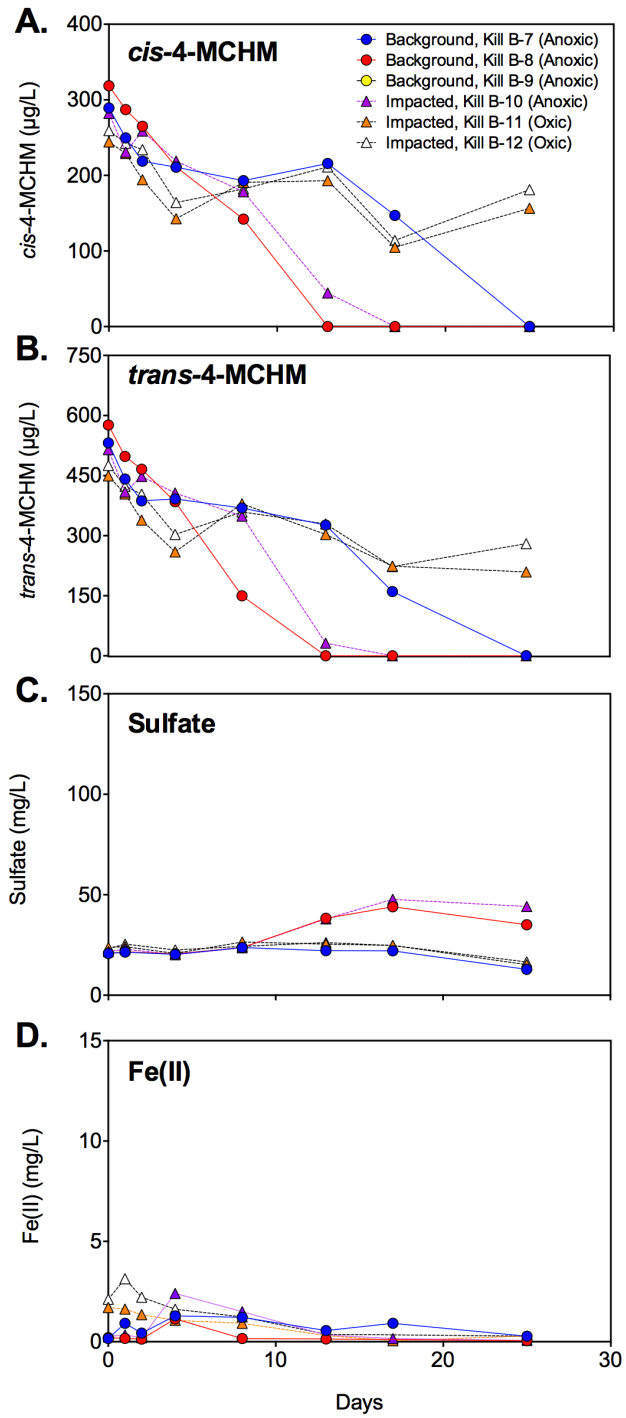

Figure S2. Concentrations of (A) *cis*-4-MCHM, (B) *trans*-4-MCHM, (C) sulfate, and (D) Fe(II) over time in crude MCHM-amended, killed microcosms with background (circles) and impacted (triangles) sediments. Results for individual microcosms are presented as some bottles were oxic and others anoxic. The background treatment had duplicate bottles (B-7 and B-8), whereas impacted was incubated in triplicate.

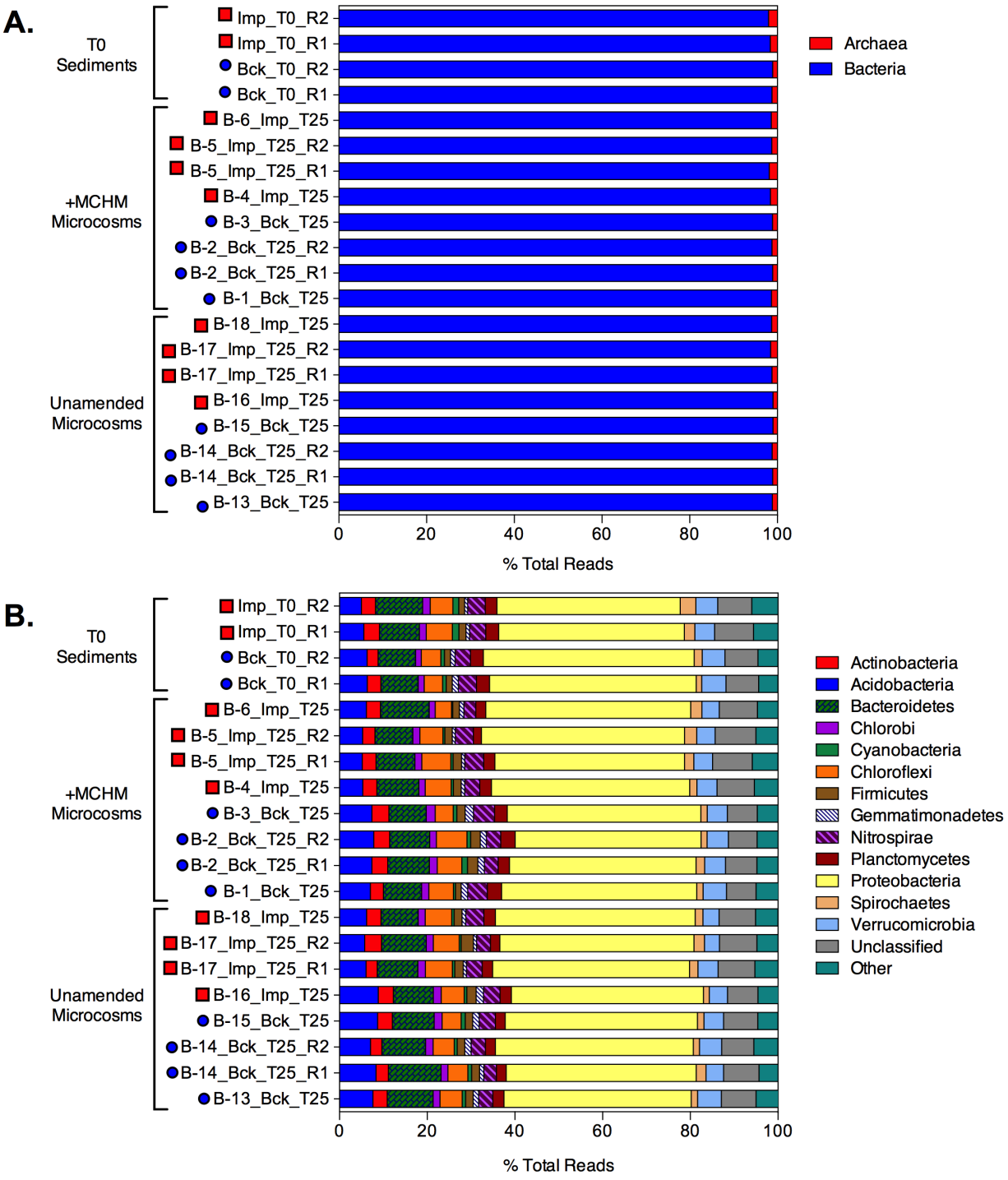

Figure S3. Phylogenetic affiliation of 16S rRNA gene sequences from non-autoclaved sediments and biodegradation microcosms at the (A) Domain and (B) Phylum level. Microcosm samples collected on day 25 (T25) are named by bottle number (B-#) and sediments sources are abbreviated "Imp" (red squares) and "Bck" (blue circles) for impacted and background sites, respectively. R1 or R2 indicates extraction replicates. Sequences in phyla that represented <1% of total reads in all samples were combined into the group "Other" and included OTUs affiliated

with the Caldiserica, Caldithrix, Caldithrix, Tenericutes, Thermi, Poribacteria, Lentisphaerae, Chlamydiae, Crenarchaeota, Elusimicrobia, Euryarchaeota, Fibrobacteres, Fusobacteria, Parvarchaeota, Armatimonadetes, and candidate phyla. Candidate phyla included the AC1, AD3, AncK6, BHI80-139, BRC1, FBP, FCPU426, GAL15, GN02, GN04, GOUTA4, H-178, Hyd24-12, KSB3, LCP-89, LD1, MAT-CR-M4-B07, MVS-104, NC10, NKB19, OC31, OD1, OP1, OP11, OP3, OP8, OP9, PAUC34f, SBR1093, SC4, SR1, TA06, TM6, TM7, TPD-58, VHS-B3-43, WPS-2, WS1, WS2, WS3, WS4, WS5, WWE1, and ZB3 phyla.

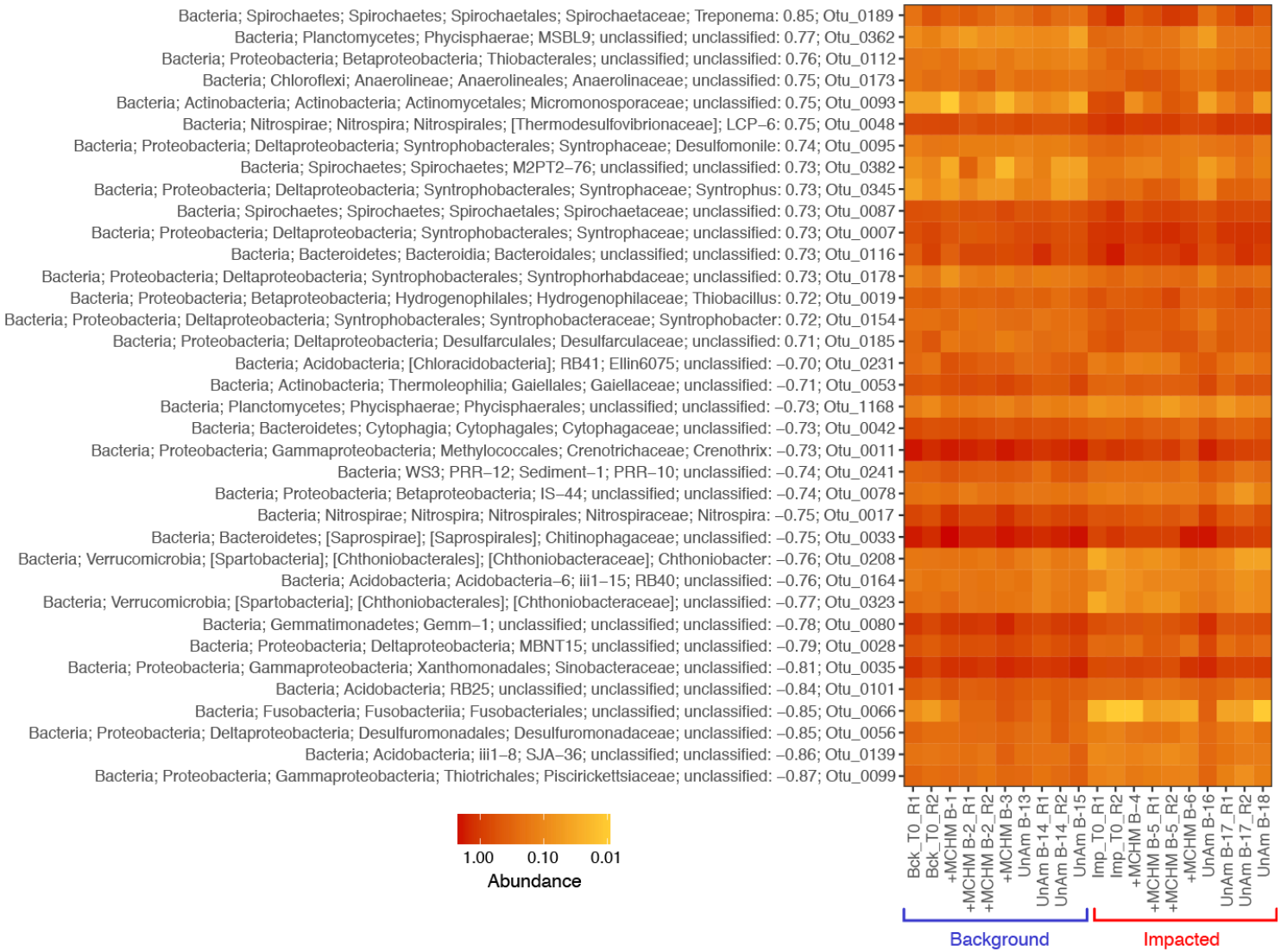

Figure S4. Heat map of genera significantly ($P < 0.05$) and highly (Spearman's $r > 0.7$ or < -0.7) correlated with NMDS1. Row labels are taxonomic designations followed by Spearman correlations, and a representative OTU number. The color scale is log10 transformed.

Supplementary Tables

Table S1. Characteristics of Sediments used for crude MCHM sorption and biodegradation studies. Averages and standard deviations (SD) of 5 replicate samples. Leaf & twig parts >3 mm (easily visible) were removed when sediment prepared for organic C analysis.

Site	Latitude	Longitude	Sediment						Porewater				
			%N	%N SD	% Org. C	% Org. C SD	%Water Content	%Water Content SD	Cl (mg/L)	Br (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)
Impacted	38.37012	-81.60605	0.10	0.01	1.96	0.20	61.02	0.56	6.6	<0.014	0.3	<0.016	8.4
Background	38.37192	-81.60489	0.05	0.00	0.66	0.08	29.27	1.86	6.7	<0.014	0.8	<0.016	9.5

Table S2: Results of crude MCHM sorption experiments. A) Concentrations of cis- and trans-4-MCHM over time in sorption experiments. Concentrations were measured using HS-SPME-GC-MS. B) Results of t-tests for sorption experiments.

A.

Day	*cis*-4-MCHM (µg/L)			*trans*-4-MCHM (µg/L)		
	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
0	257	228	286	465	414	511
1	236	225	245	392	388	422
2	184	206	214	317	345	365
5	192	187	222	364	351	416
13	218	214	199	332	333	311
25	171	240	198	301	419	353

B.

***cis*-4-MCHM**		
	p-value	**significant**
day 0 vs. day 2	0.043	yes
day 0 vs. day 5	0.049	yes
day 0 vs. day 25	0.109	no

***trans*-4-MCHM**		
	p-value	**significant**
day 0 vs. day 2	0.018	yes
day 0 vs. day 5	0.066	no
day 0 vs. day 25	0.075	no

Table S3: Chemistry over time in crude MCHM biodegradation experiments. NC= no sample collected; ND=not detected. In Blue = above detection limit; below lowest standard; BDL= below detection limit of 10 ug/L for cis-4-MCHM and 23 µg/L for trans-4-MCHM.

A. *cis*-4-MCHM concentrations

				***cis*-4-MCHM µg/L**							
			Date	12/4/14	12/5/14	12/6/14	12/8/14	12/12/14	12/17/14	12/21/14	12/30/14
Treatment	**Site**	**Bottle Name**	**Day**	**0**	**1**	**2**	**4**	**8**	**13**	**17**	**25**
+MCHM	Background	MCHM-B-1		320	222	67	BDL	BDL	BDL	BDL	BDL
+MCHM	Background	MCHM-B-2		347	199	210	165	44	BDL	BDL	BDL
+MCHM	Background	MCHM-B-3		296	187	187	137	32	BDL	BDL	BDL
+MCHM	Impacted	MCHM-B-4		267	174	63	BDL	BDL	BDL	BDL	BDL
+MCHM	Impacted	MCHM-B-5		285	191	155	110	BDL	BDL	BDL	BDL
+MCHM	Impacted	MCHM-B-6		245	178	146	61	BDL	BDL	BDL	BDL
+MCHM, killed	Background	MCHM-B-7		289	250	219	211	193	216	147	BDL
+MCHM, killed	Background	MCHM-B-8		319	287	265	211	142	BDL	BDL	BDL
+MCHM, killed	Impacted	MCHM-B-10		282	231	259	219	179	44.6	BDL	BDL
+MCHM, killed	Impacted	MCHM-B-11		244	229	194	143	191	193	105	157
+MCHM, killed	Impacted	MCHM-B-12		259	243	234	164	182	211	114	181
Unamended	Background	MCHM-B-13		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Background	MCHM-B-14		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Background	MCHM-B-15		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Impacted	MCHM-B-16		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Impacted	MCHM-B-17		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Impacted	MCHM-B-18		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Background	MCHM-B-19		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Background	MCHM-B-20		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Background	MCHM-B-21		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Impacted	MCHM-B-22		<10	<10	<10	<10	NC	NC	NC	BDL
Unamended, killed	Impacted	MCHM-B-23		<10	<10	<10	<10	NC	NC	NC	BDL
Unamended, killed	Impacted	MCHM-B-24		<10	<10	<10	<10	NC	NC	NC	BDL

B. *trans*-4-MCHM concentrations

Treatment	Site	Bottle Name	Date	*trans*-4-MCHM µg/L							
				12/4/14	12/5/14	12/6/2014	12/8/14	12/12/14	12/17/14	12/21/14	12/30/14
			Day	**0**	**1**	**2**	**4**	**8**	**13**	**17**	**25**
+MCHM	Background	MCHM-B-1		574	398	152	BDL	BDL	BDL	BDL	BDL
+MCHM	Background	MCHM-B-2		614	344	340	292	114	BDL	BDL	BDL
+MCHM	Background	MCHM-B-3		543	327	310	245	96.5	BDL	BDL	BDL
+MCHM	Impacted	MCHM-B-4		482	322	122	BDL	BDL	BDL	BDL	BDL
+MCHM	Impacted	MCHM-B-5		508	328	259	188	BDL	BDL	BDL	BDL
+MCHM	Impacted	MCHM-B-6		446	300	234	98	BDL	BDL	BDL	BDL
+MCHM, killed	Background	MCHM-B-7		532	441	387	392	369	326	160	BDL
+MCHM, killed	Background	MCHM-B-8		576	498	466	385	150	BDL	BDL	BDL
+MCHM, killed	Impacted	MCHM-B-10		515	409	448	407	349	31.9	BDL	BDL
+MCHM, killed	Impacted	MCHM-B-11		449	404	339	259	380	303	224	209
+MCHM, killed	Impacted	MCHM-B-12		476	423	403	303	359	329	223	280
Unamended	Background	MCHM-B-13		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Background	MCHM-B-14		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Background	MCHM-B-15		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Impacted	MCHM-B-16		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Impacted	MCHM-B-17		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended	Impacted	MCHM-B-18		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Background	MCHM-B-19		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Background	MCHM-B-20		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Background	MCHM-B-21		BDL	NC	NC	NC	NC	NC	NC	BDL
Unamended, killed	Impacted	MCHM-B-22		34.6	38.4	35.4	27.8	NC	NC	NC	BDL
Unamended, killed	Impacted	MCHM-B-23		22.9	22.8	24.7	<23	NC	NC	NC	BDL
Unamended, killed	Impacted	MCHM-B-24		30.1	30.8	27.3	<23	NC	NC	NC	BDL

C. Fe(II) concentrations

Treatment	Site	Bottle Name	Date	Fe(II) mg/L							
				12/4/14	12/5/14	12/6/14	12/8/14	12/12/14	12/17/14	12/21/14	12/29/14
			Day	0	1	2	4	8	13	17	25
+MCHM	Background	MCHM-B-1		0.11	0.11	0.00	0.04	0.05	0.08	BDL	0.07
+MCHM	Background	MCHM-B-2		0.59	2.65	3.58	3.46	8.47	9.21	11.31	11.28
+MCHM	Background	MCHM-B-3		0.55	1.96	3.29	3.23	7.03	8.30	9.85	9.07
+MCHM	Impacted	MCHM-B-4		0.54	0.11	0.05	0.05	0.06	0.08	BDL	0.09
+MCHM	Impacted	MCHM-B-5		0.78	2.33	2.58	2.60	1.65	0.63	2.68	3.53
+MCHM	Impacted	MCHM-B-6		0.55	2.31	1.99	2.63	5.26	8.40	10.28	4.89
+MCHM, killed	Background	MCHM-B-7		0.15	0.90	0.42	1.26	1.20	0.55	0.91	0.26
+MCHM, killed	Background	MCHM-B-8		0.19	0.17	0.13	1.12	0.16	0.14	BDL	0.05
+MCHM, killed	Impacted	MCHM-B-10		0.27	0.34	0.20	2.37	1.47	0.33	0.15	0.07
+MCHM, killed	Impacted	MCHM-B-11		1.68	1.60	1.33	1.05	0.91	0.30	0.05	0.28
+MCHM, killed	Impacted	MCHM-B-12		2.08	3.08	2.18	1.59	1.22	0.36	BDL	0.28
Unamended	Background	MCHM-B-13		0.86	1.97	1.43	2.27	NC	NC	2.54	0.89
Unamended	Background	MCHM-B-14		0.83	0.42	0.07	0.47	NC	NC	0.03	0.02
Unamended	Background	MCHM-B-15		1.07	2.40	3.16	3.42	NC	NC	1.70	0.14
Unamended	Impacted	MCHM-B-16		1.06	2.09	1.54	1.04	NC	NC	BDL	0.03
Unamended	Impacted	MCHM-B-17		1.30	2.66	2.61	2.68	NC	NC	0.27	3.96
Unamended	Impacted	MCHM-B-18		1.40	2.09	2.09	0.19	NC	NC	0.03	0.02
Unamended, killed	Background	MCHM-B-19		0.21	0.17	0.15	0.87	NC	NC	BDL	0.03
Unamended, killed	Background	MCHM-B-20		0.53	0.12	0.07	0.57	NC	NC	BDL	0.06
Unamended, killed	Background	MCHM-B-21		0.55	0.32	0.86	0.31	NC	NC	BDL	0.16
Unamended, killed	Impacted	MCHM-B-22		2.20	1.03	0.79	1.05	NC	NC	0.22	0.20
Unamended, killed	Impacted	MCHM-B-23		2.27	4.30	1.10	0.70	NC	NC	0.02	0.21
Unamended, killed	Impacted	MCHM-B-24		1.42	0.68	0.31	1.89	NC	NC	BDL	0.07

D. Sulfate concentrations

				Sulfate (mg/L)							
			Date	12/4/14	12/5/14	12/6/4	12/8/14	12/12/14	12/17/14	12/21/14	12/29/14
Treatment	**Site**	**Bottle Name**	**Day**	**0**	**1**	**2**	**4**	**8**	**13**	**17**	**25**
+MCHM	Background	MCHM-B-1		19.24	18.30	NC	10.30	17.41	35.02	50.86	55.20
+MCHM	Background	MCHM-B-2		18.08	15.40	NC	5.47	BDL	BDL	0.31	BDL
+MCHM	Background	MCHM-B-3		18.62	15.51	NC	5.84	BDL	0.64	0.29	0.03
+MCHM	Impacted	MCHM-B-4		18.68	19.95	NC	11.66	23.26	45.00	67.46	118.60
+MCHM	Impacted	MCHM-B-5		17.79	14.98	NC	5.56	1.29	1.10	0.62	0.02
+MCHM	Impacted	MCHM-B-6		20.38	17.08	NC	7.15	1.73	BDL	BDL	BDL
+MCHM, killed	Background	MCHM-B-7		20.76	21.46	NC	20.31	23.73	22.16	22.12	12.90
+MCHM, killed	Background	MCHM-B-8		20.84	21.59	NC	20.62	23.76	38.28	44.04	35.10
+MCHM, killed	Impacted	MCHM-B-10		21.78	22.96	NC	20.16	23.64	38.01	47.75	44.20
+MCHM, killed	Impacted	MCHM-B-11		23.78	24.16	NC	20.83	26.51	25.46	24.82	15.20
+MCHM, killed	Impacted	MCHM-B-12		23.29	25.42	NC	22.53	24.27	26.23	24.72	16.60
Unamended	Background	MCHM-B-13		18.98	16.46	NC	7.76	NC	NC	0.36	BDL
Unamended	Background	MCHM-B-14		19.92	17.18	NC	6.69	NC	NC	29.67	37.90
Unamended	Background	MCHM-B-15		18.28	14.69	NC	6.15	NC	NC	0.63	0.10
Unamended	Impacted	MCHM-B-16		21.20	18.97	NC	9.87	NC	NC	36.83	77.90
Unamended	Impacted	MCHM-B-17		20.40	17.80	NC	7.90	NC	NC	1.05	0.20
Unamended	Impacted	MCHM-B-18		22.15	20.72	NC	10.37	NC	NC	77.24	111.00
Unamended, killed	Background	MCHM-B-19		21.66	21.32	NC	20.51	NC	NC	50.73	39.10
Unamended, killed	Background	MCHM-B-20		20.51	20.76	NC	20.00	NC	NC	50.47	38.20
Unamended, killed	Background	MCHM-B-21		22.95	23.01	NC	24.86	NC	NC	31.59	26.30
Unamended, killed	Impacted	MCHM-B-22		23.75	23.45	NC	22.98	NC	NC	25.08	16.10
Unamended, killed	Impacted	MCHM-B-23		23.24	22.57	NC	22.80	NC	NC	26.24	15.30
Unamended, killed	Impacted	MCHM-B-24		22.51	21.87	NC	21.64	NC	NC	25.56	14.20

E. Nitrate concentrations

				Nitrate (mg/L)							
			Date	12/4/14	12/5/14	12/6/14	12/8/14	12/12/14	12/17/14	12/21/14	12/29/14
Treatment	**Site**	**Bottle Name**	**Day**	**0**	**1**	**2**	**4**	**8**	**13**	**17**	**25**
+MCHM	Background	MCHM-B-1		0.32	BDL	NC	0.25	0.77	1.40	0.87	0.70
+MCHM	Background	MCHM-B-2		0.42	BDL	NC	0.25	BDL	BDL	BDL	BDL
+MCHM	Background	MCHM-B-3		BDL	0.03	NC	0.10	0.10	0.23	0.51	BDL
+MCHM	Impacted	MCHM-B-4		BDL	0.14	NC	0.02	0.04	2.60	0.66	0.30
+MCHM	Impacted	MCHM-B-5		BDL	0.02	NC	BDL	BDL	BDL	BDL	0.06
+MCHM	Impacted	MCHM-B-6		BDL	0.10	NC	BDL	BDL	0.40	0.39	0.04
+MCHM, killed	Background	MCHM-B-7		BDL	BDL	NC	BDL	0.14	0.20	0.30	BDL
+MCHM, killed	Background	MCHM-B-8		0.09	0.22	NC	BDL	BDL	0.35	BDL	0.21
+MCHM, killed	Impacted	MCHM-B-10		0.58	0.20	NC	BDL	BDL	0.34	BDL	0.20
+MCHM, killed	Impacted	MCHM-B-11		0.40	0.20	NC	0.38	0.32	0.61	0.74	0.35
+MCHM, killed	Impacted	MCHM-B-12		0.22	0.23	NC	0.38	0.26	0.35	0.58	0.49
Unamended	Background	MCHM-B-13		0.20	0.20	NC	0.05	NC	NC	BDL	BDL
Unamended	Background	MCHM-B-14		BDL	BDL	NC	BDL	NC	NC	0.61	0.24
Unamended	Background	MCHM-B-15		BDL	BDL	NC	BDL	NC	NC	0.55	0.11
Unamended	Impacted	MCHM-B-16		BDL	0.03	NC	BDL	NC	NC	0.57	0.19
Unamended	Impacted	MCHM-B-17		BDL	BDL	NC	0.13	NC	NC	0.28	0.14
Unamended	Impacted	MCHM-B-18		BDL	BDL	NC	0.28	NC	NC	0.61	0.28
Unamended, killed	Background	MCHM-B-19		0.13	BDL	NC	0.16	NC	NC	0.33	BDL
Unamended, killed	Background	MCHM-B-20		BDL	BDL	NC	0.12	NC	NC	0.32	BDL
Unamended, killed	Background	MCHM-B-21		0.28	0.42	NC	0.52	NC	NC	0.39	BDL
Unamended, killed	Impacted	MCHM-B-22		0.37	0.51	NC	0.51	NC	NC	0.31	BDL
Unamended, killed	Impacted	MCHM-B-23		0.64	0.60	NC	0.71	NC	NC	0.85	0.17
Unamended, killed	Impacted	MCHM-B-24		0.37	0.31	NC	BDL	NC	NC	0.27	BDL

F. Oxygen and non-volatile dissolved organic carbon (DOC) concentrations

				Oxygen (%)	NVDOC (mg/L C)		
		Bottle	Date	12/10/14	12/4/14	12/8/14	12/29/14
Treatment	**Site**	**Name**	**Day**	**6**	**0**	**4**	**25**
+MCHM	Background	MCHM-B-1		9.17	10.0	5.5	2.6
+MCHM	Background	MCHM-B-2		BDL	13.0	14.0	11.0
+MCHM	Background	MCHM-B-3		BDL	13.0	12.0	9.4
+MCHM	Impacted	MCHM-B-4		3.44	8.6	5.0	3.1
+MCHM	Impacted	MCHM-B-5		BDL	15.0	37.0	8.9
+MCHM	Impacted	MCHM-B-6		BDL	8.3	7.7	7.6
+MCHM, killed	Background	MCHM-B-7		BDL	48.0	45.0	29.0
+MCHM, killed	Background	MCHM-B-8		BDL	53.0	46.0	17.0
+MCHM, killed	Impacted	MCHM-B-10		BDL	54.0	53.0	25.0
+MCHM, killed	Impacted	MCHM-B-11		BDL	142.0	126.0	113.0
+MCHM, killed	Impacted	MCHM-B-12		7.65	151.0	138.0	126.0
Unamended	Background	MCHM-B-13		5.95	12.0	10.0	4.3
Unamended	Background	MCHM-B-14		NC	10.0	6.8	2.0
Unamended	Background	MCHM-B-15		NC	11.0	11.0	4.3
Unamended	Impacted	MCHM-B-16		NC	8.8	8.1	2.4
Unamended	Impacted	MCHM-B-17		NC	14.0	11.0	8.5
Unamended	Impacted	MCHM-B-18		NC	11.0	7.3	4.9
Unamended, killed	Background	MCHM-B-19		NC	56.0	44.0	17.0
Unamended, killed	Background	MCHM-B-20		NC	48.0	39.0	16.0
Unamended, killed	Background	MCHM-B-21		NC	92.0	77.0	43.0
Unamended, killed	Impacted	MCHM-B-22		NC	134.0	116.0	78.0
Unamended, killed	Impacted	MCHM-B-23		NC	146.0	126.0	122.0
Unamended, killed	Impacted	MCHM-B-24		NC	74.0	63.0	29.0

Table S4: Tests for residual microbial activity in killed microcosms. Select bottles from the biotic and sorption experiments were amended with ~5 mM acetate at the end of the experiment to verify that autoclaving inhibited microbial activity. Acetate concentrations determined using HPLC. Headspace % CO2 determined using gas chromatography. BD: below detection limit; NC: not collected.

Treatment	Site	Bottle Name	Date	Acetate (mM)				CO2 (%)			
				1/7/15	1/14/15	1/21/15	1/29/15	1/7/15	1/14/15	1/21/15	1/29/15
			Day	0	7	14	22	0	7	14	22
+MCHM, killed	Background	MCHM-B-7		5.11	0.31	0.39	0.20	1.14	2.73	3.97	NC
+MCHM, killed	Background	MCHM-B-8		5.00	NC	0.39	0.21	0.63	2.45	3.59	NC
+MCHM, killed	Impacted	MCHM-B-10		5.39	1.11	0.18	0.21	1.32	2.31	3.36	NC
+MCHM, killed	Impacted	MCHM-B-11		5.39	3.94	4.08	1.69	0.05	0.49	0.91	NC
+MCHM, killed	Impacted	MCHM-B-12		5.39	4.91	5.06	1.87	BD	BD	0.30	NC
Sorption (Killed+MCHM)	Background	SX-A (Day 13)		0.64	0.50	0.44	0.75	NC	NC	NC	NC
Sorption (Killed+MCHM)	Background	SX-B (Day 13)		0.70	0.48	0.44	1.44	NC	NC	NC	NC
Sorption (Killed+MCHM)	Background	SY-B (Day 25)		NC	5.33	4.74	4.22	NC	BD	BD	NC
Sorption (Killed+MCHM)	Background	SY-A (Day 25)		NC	5.01	4.36	0.71	NC	BD	BD	NC

Table S5: Results of 16S rRNA gene amplicon sequencing of biodegradation experiments and in situ sediments. The V4 region of the 16S rRNA gene was targeted using the primers 515F/806R. Raw Illumina reads (as fastq.gz files) can be downloaded from NCBI BioProject PRJNA389713. Samples were analyzed using mothur to generate OTUs at a cutoff of 0.03 and these OTUs were classified using GreenGenes v 13_8. Statistical analyses were performed in mothur and R using packages phyloseq and vegan.

A. Sample information and sequencing summary.

Treatment	Site	Bottle Name	Short Name	Time Point	Library Name	Initial Number of Reads	Number of Reads in Shared OTU Libraries	% of Initial Number of Reads
+MCHM	Background	MCHM-B-1	B-1	Day 25	MCHM-1-B-T25	115657	62872	54.4
+MCHM	Background	MCHM-B-2, rep. 1	B-2, rep. 1	Day 25	MCHM-2-B-T25-R1	99260	53390	53.8
+MCHM	Background	MCHM-B-2, rep. 2	B-2, rep. 2	Day 25	MCHM-2-B-T25-R2	114966	61647	53.6
+MCHM	Background	MCHM-B-3	B-3	Day 25	MCHM-3-B-T25	134300	77553	57.7
+MCHM	Impacted	MCHM-B-4	B-4	Day 25	MCHM-4-I-T25	97463	50551	51.9
+MCHM	Impacted	MCHM-B-5, rep. 1	B-5, rep. 1	Day 25	MCHM-5-I-T25-R1	87905	45620	51.9
+MCHM	Impacted	MCHM-B-5, rep. 2	B-5, rep. 2	Day 25	MCHM-5-I-T25-R2	58591	30075	51.3
+MCHM	Impacted	MCHM-B-6	B-6	Day 25	MCHM-6-I-T25	74648	35882	48.1
Unamended	Background	MCHM-B-13	B-13	Day 25	MCHM-13-B-T25	83049	43665	52.6
Unamended	Background	MCHM-B-14, rep. 1	B-14, rep. 1	Day 25	MCHM-14-B-T25-R1	90861	45875	50.5
Unamended	Background	MCHM-B-14, rep. 2	B-14, rep. 2	Day 25	MCHM-14-B-T25-R2	97358	52210	53.6
Unamended	Background	MCHM-B-15	B-15	Day 25	MCHM-15-B-T25	89714	49433	55.1
Unamended	Impacted	MCHM-B-16	B-16	Day 25	MCHM-16-I-T25	105436	59304	56.2
Unamended	Impacted	MCHM-B-17, rep. 1	B-17, rep. 1	Day 25	MCHM-17-I-T25-R1	80048	42024	52.5
Unamended	Impacted	MCHM-B-17, rep. 2	B-17, rep. 2	Day 25	MCHM-17-I-T25-R2	74657	39538	53.0
Unamended	Impacted	MCHM-B-18	B-18	Day 25	MCHM-18-I-T25	96045	50856	53.0
None	Background	Homogenized Sediment	T0	Day 0	MCHM-B-T0-R1	94094	50287	53.4
None	Background	Homogenized Sediment	T0	Day 0	MCHM-B-T0-R2	102170	54030	52.9
None	Impacted	Homogenized Sediment	T0	Day 0	MCHM-I-T0-R1	107241	54741	51.0
None	Impacted	Homogenized Sediment	T0	Day 0	MHCH-I-T0-R2	93181	51637	55.4

B. Genus-level Diversity Measures on Libraries Subsampled to 58,587 sequences

Library Name	Observed Genera	Chao1	Chao1 Standard Error	Simpson Index
MCHM-1-B-T25	741	799	16	0.985
MCHM-2-B-T25-R1	769	828	16	0.985
MCHM-2-B-T25-R2	776	819	13	0.987
MCHM-3-B-T25	748	798	14	0.985
MCHM-4-I-T25	785	851	17	0.982
MCHM-5-I-T25-R1	763	813	14	0.982
MCHM-5-I-T25-R2	702	725	9	0.980
MCHM-6-I-T25	737	765	10	0.981
MCHM-13-B-T25	761	779	7	0.984
MCHM-14-B-T25-R1	747	787	12	0.982
MCHM-14-B-T25-R2	753	814	16	0.985
MCHM-15-B-T25	753	796	13	0.984
MCHM-16-I-T25	757	784	9	0.985
MCHM-17-I-T25-R1	753	776	8	0.982
MCHM-17-I-T25-R2	745	774	10	0.981
MCHM-18-I-T25	753	804	15	0.982
MCHM-B-T0-R1	780	832	15	0.985
MCHM-B-T0-R2	789	838	14	0.984
MCHM-I-T0-R1	782	846	17	0.982
MHCH-I-T0-R2	748	787	12	0.983

Table S6: Phylogenetic affiliation of 16S rRNA gene sequences from sediments and biodegradation microcosms. The V4 region of the 16S rRNA gene was targeted using the primers 515F/806R. Raw Illumina reads (as fastq.gz files) can be downloaded from NCBI BioProject PRJNA389713. Samples were analyzed using mothur to generate OTUs at a cutoff of 0.03 and these OTUs were classified using GreenGenes v 13_8. All values are presented as percent (%) total reads per sample.

Sample	Bacteria	Archaea	Acidobacteria	Actinobacteria	Bacteroidetes	Chlorobi	Chloroflexi	Cyanobacteria	Firmicutes	Gemmatimonadetes	Nitrospirae	Planctomycetes	Proteobacteria	Spirochaetes	Unclassified	Verrucomicrobia	Other
B-13_Bck_T25	98.83	1.17	7.64	3.25	10.44	1.58	5.06	0.78	1.74	1.24	3.23	2.56	42.70	1.46	7.92	5.40	5.00
B-14_Bck_T25_R1	98.94	1.06	8.34	2.80	12.02	1.57	4.53	0.89	1.79	0.99	2.87	2.20	43.36	2.24	8.08	3.97	4.35
B-14_Bck_T25_R2	98.82	1.18	7.08	2.59	9.94	1.75	4.82	0.66	1.74	1.43	3.27	2.29	45.12	1.42	7.32	5.08	5.51
B-15_Bck_T25	98.99	1.01	8.68	3.39	9.52	1.81	4.35	0.93	1.69	1.49	3.70	2.22	43.84	1.50	7.80	4.49	4.60
B-16_Imp_T25	99.01	0.99	8.83	3.48	9.09	1.80	5.23	0.60	2.12	1.67	3.79	2.59	43.81	1.32	6.94	4.17	4.56
B-17_Imp_T25_R1	98.78	1.22	6.06	2.56	9.25	1.73	6.15	0.55	1.92	0.76	3.57	2.38	44.91	1.91	8.40	4.57	5.28
B-17_Imp_T25_R2	98.46	1.54	5.74	3.86	10.16	1.62	5.91	0.45	2.77	0.76	3.13	2.17	44.25	2.42	8.57	3.39	4.79
B-18_Imp_T25	98.67	1.33	6.20	3.27	8.47	1.60	6.06	0.54	1.81	0.87	4.11	2.60	45.59	1.77	8.31	3.68	5.11
B-1_Bck_T25	98.64	1.36	7.04	2.99	8.70	1.66	5.59	0.46	1.31	1.49	4.45	3.27	44.52	1.44	6.73	5.33	5.01
B-2_Bck_T25_R1	98.91	1.09	7.38	3.63	9.50	1.80	5.60	1.29	2.34	1.48	3.14	2.62	42.52	1.96	7.21	4.74	4.79
B-2_Bck_T25_R2	98.77	1.23	7.82	3.60	9.14	1.54	6.97	0.84	2.21	1.38	3.25	3.31	42.39	1.36	6.55	4.86	4.78
B-3_Bck_T25	98.9	1.1	7.39	3.88	8.51	2.05	4.10	0.82	1.87	1.94	4.77	2.91	44.14	1.47	6.82	4.58	4.74
B-4_Imp_T25	98.4	1.6	5.31	3.23	9.56	1.47	5.92	0.49	1.77	0.82	3.41	2.68	45.20	1.64	8.51	4.61	5.38
B-5_Imp_T25_R1	98.15	1.85	5.20	3.12	8.86	1.58	6.64	0.51	1.93	0.74	4.29	2.57	43.24	2.14	9.07	4.25	5.87
B-5_Imp_T25_R2	98.71	1.29	5.26	2.83	8.62	1.65	5.22	0.44	1.69	0.69	4.14	1.83	46.35	2.76	9.32	4.20	5.02
B-6_Imp_T25	98.62	1.38	6.13	3.24	11.09	1.35	3.72	0.31	1.50	1.03	2.70	2.27	46.76	2.54	8.68	3.98	4.70
Bck_T0_R1	98.76	1.24	6.33	3.11	8.50	1.39	4.19	0.83	1.38	1.39	4.13	2.99	47.11	1.26	7.45	5.53	4.41
Bck_T0_R2	98.93	1.07	6.31	2.49	8.57	1.29	4.47	0.80	1.37	1.06	3.50	2.94	48.10	1.82	7.54	5.18	4.56
Imp_T0_R1	98.37	1.63	5.55	3.58	9.08	1.58	5.96	1.51	1.58	0.83	3.69	2.93	42.37	2.40	8.87	4.51	5.58
Imp_T0_R2	97.99	2.01	4.99	3.21	10.78	1.70	5.18	1.35	1.33	0.72	3.97	2.67	41.81	3.57	7.76	4.99	5.98