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

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Ⓢ *Supporting Information*

ABSTRACT: In January 2014, approximately 37 800 L of crude 4-methylcyclohexanemethanol (crude MCHM) spilled into the Elk River, West Virginia. To understand the long-term fate of 4-MCHM, we conducted experiments under environmentally relevant conditions to assess the potential for the 2 primary compounds in crude MCHM (1) to undergo biodegradation and (2) for sediments to serve as a long-term source of 4-MCHM. We developed a solid phase microextraction (SPME) method to quantify the *cis-* and *trans-*isomers of 4-MCHM. Autoclaved Elk River sediment slurries sorbed 17.5% of *cis-*4-MCHM and 31% of *trans-*4-MCHM from water during the 2-week experiment. Sterilized, impacted, spill-site sediment released minor amounts of *cis-* and up to 35 μg/L of *trans-*4-MCHM into water, indicating 4-MCHM was present in sediment collected 10 months post spill. In anoxic microcosms, 300 μg/L *cis-* and 150 μg/L *trans-*4-MCHM degraded to nondetectable levels in 8−13 days in both impacted and background sediments. Under aerobic conditions, 4-MCHM isomers degraded to nondetectable levels within 4 days. Microbial communities at impacted sites differed in composition compared to background samples, but communities from both sites shifted in response to crude MCHM amendments. Our results indicate that 4-MCHM is readily biodegradable under environmentally relevant conditions.
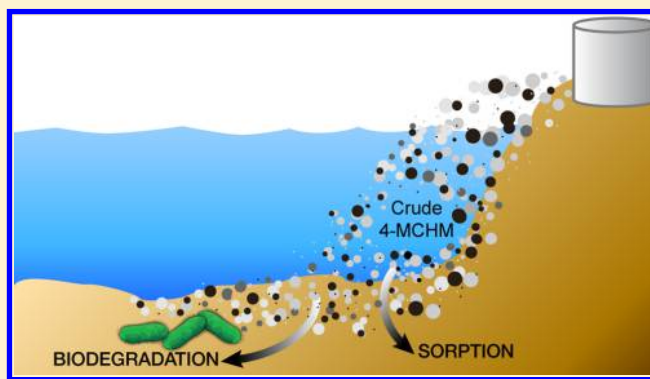
■ INTRODUCTION

In January 2014, an industrial solvent mixture spilled from a storage tank into the Elk River, West Virginia, contaminating 15% of the State's public drinking water supply.[1,2] The spilled chemical mixture, used in coal processing, was composed of crude MCHM and propylene glycol phenyl ethers (PPH).[3] Crude MCHM was composed primarily of *cis-* and *trans-*4-methylcyclohexanemethanol (4-MCHM) and minor components including other cyclohexanes.[3] The contaminants were transported downgradient to the West Virginia American Water's Kanawha Valley Treatment Plant (KVTP), a drinking water plant that served over 300 000 residents, resulting in the loss of drinking water for up to 10 days for the entire community.[4,5] The precise composition of the spilled material was unknown, complicating the response and limiting the guidance health officials could issue to protect the population.[6] In the days following the spill, approximately 14 mg/L 4-MCHM was measured in the Elk River raw water intake of the KVTP drinking water plant.[5] At the time of the spill, no human health data existed for crude MCHM exposures.[7] Residents exposed to impacted tap water reported a list of health effects including respiratory, neurological, and digestive problems, as well as skin irritations, with over half of the households surveyed reporting a person in their home became ill from

exposure.[1,8] In those studies, adverse health effects occurred at concentrations far less than the Centers for Disease Control's (CDC) 4-MCHM screening level of 1000 μg/L.

At the time of the spill, little was known about the physical, chemical and biological properties of 4-MCHM (both *cis-* and *trans-*isomers) that control its fate in the environment and the ultimate impacts of human or animal exposures. 4-MCHM is a saturated alicyclic primary alcohol with a methyl (CH_3) and hydroxymethyl (CH_2OH) group on the cyclohexane ring, which results in *cis-* and *trans-*isomers depending on the spatial positions of these groups. Isomers can have different physical, chemical, and biological activities and ultimately different toxicities.

Following the spill, a rapid scientific response was undertaken to understand the fate of crude MCHM components, including the *cis-* and *trans-*4-MCHM isomers, in water treatment infrastructure and under specific environmental conditions.[7] Studies included investigations of the DNA damage potential of 4-MCHM at subtoxic levels;[9] results of

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these studies raised concerns about the potential carcinogenic and reproductive toxicity of 4-MCHM and its metabolites. Sain et al.[10] found that the *trans*-4-MCHM isomer was more volatile than the *cis* isomer and a higher inhalation exposure to *trans*-than to *cis*-4-MCHM isomers was found for residents during showering. Gallagher et al.[11] found that *cis*- and *trans*-4-MCHM isomers had different human odor detection limits and responses. Additional studies have focused on the solubility of and sorption potential of 4-MCHM in aqueous solution and to activated carbon[12] as well as in water distribution piping[1] and during the wastewater treatment plant processes.[13] Weidhaas et al.[13] demonstrated sorption onto Elk River sediments varied by isomer, with *trans*-4-MCHM preferentially sorbing onto sediment in laboratory studies.

Foreman et al.[14] identified low levels of both the *cis*- and *trans*-isomers of 4-MCHM at distances greater than 600 km downstream from the spill. Whereas many organic contaminants are readily biodegraded under oxic or anoxic conditions by native microbial populations, little is known about the potential for microbial degradation of crude MCHM and the long-term fate of the chemical components in the environment. One of the microbial degradation studies, conducted by Weidhaas et al.[15] showed biological degradation, defined as loss of the parent compound, of both *cis*- and *trans*-4-MCHM isomers (from crude MCHM) and PPH in Elk River sediments in microcosms under oxic conditions; degradation rates were lower at higher MCHM concentrations and when PPH was also present. Weidhaas et al.[15] observed that *trans*-4-MCHM biodegraded faster than *cis*-4-MCHM isomer, in contrast to the results of Yuan et al.[16] who showed faster biodegradation of *cis*-4-MCHM. Differences between the two study results were attributed to the different sediments and sludges used in the microcosms as well as the different redox conditions employed during the experiments.[15] These studies demonstrate the importance of understanding the isomer-specific behavior of *cis*- and *trans*-4-MCHM and the need for observations of the fate of these compounds under a variety of environmental conditions likely to be present at a spill site and afterward.

The potential for persistence of components of crude MCHM at low concentrations in the environment necessitates understanding how they biodegrade at these concentrations. In this study, we developed a quantitative method, using headspace solid phase microextraction followed by gas chromatography and mass spectrometry (HS-SPME-GC-MS) that allowed us to identify *cis*- and *trans*-isomers of 4-MCHM in crude MCHM at low concentrations. Our work builds on the existing scientific knowledge by assessing the isomer-specific fate of low concentrations of 4-MCHM via sorption and anaerobic biodegradation in Elk River sediments previously exposed to spilled crude MCHM and unexposed background sediments.

■ MATERIALS AND METHODS

Site Description and Sampling. The spill occurred at a Freedom Industries facility ∼0.3 km upriver from Charleston, West Virginia, USA on the Elk River. Detailed information on the spill can be obtained from the U.S. Chemical Safety and Hazard Investigation Board Report.[5] Background, unimpacted (upstream background site) and impacted (spill zone) sediments (Figure 1) were collected from the Elk River 11 months after the spill on November 18, 2014 by using a petite PONAR sampler. The background site is ∼700 m upriver from the spill location and was not exposed to the MCHM spill.
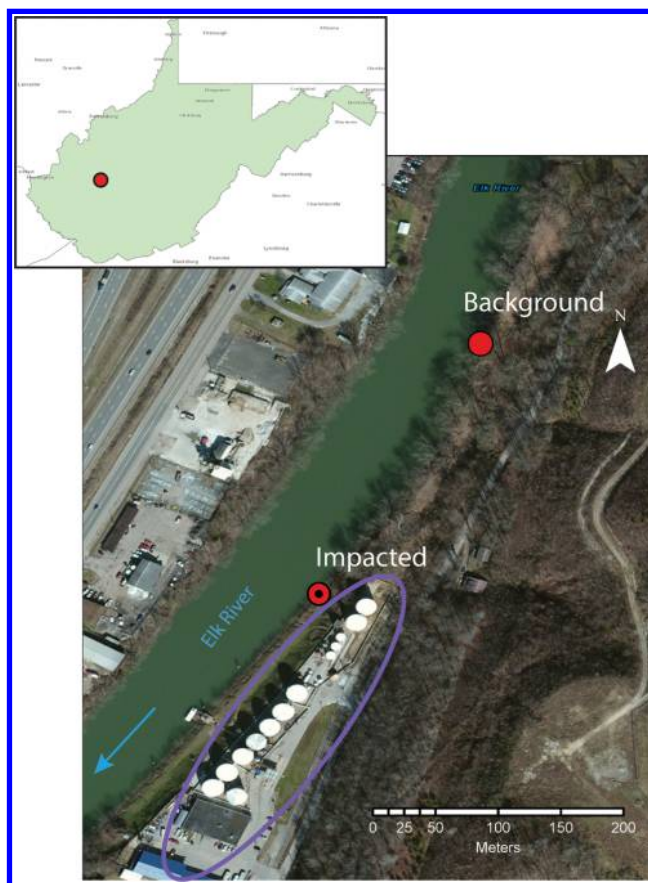
Figure 1. Location of sampling sites along the Elk River in Charleston, WV. The river flows from northeast to southwest, as indicated by the blue arrow; the Freedom Industries site is indicated by the purple ellipse. Specific locations for sampling sites can be found in Table S1 and Cozzarelli et al. (2017).[21] Source: Esri. DigitalGlobe, GeoEy, i-cubed, Earthstar Geographies, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.

Background sediment and river water were collected close to the riverbank, as described in the Supporting Information (SI) Methods. Impacted sediment was collected directly downhill from the Freedom Industries facility at the riverbank, behind a boom in place for remediation purposes. Sediment and porewater chemistry were determined as described in the SI Methods.

Microcosm Design, Construction, and Sampling. Two experiments were carried out to investigate abiotic and biotic degradation of components of crude MCHM via (1) sorption and (2) biodegradation, as summarized in Table 1. Biodegradation in this study was aimed at monitoring loss of *cis*- and *trans*-4-MCHM isomers. Full details on microcosm design, construction, and sampling can be found in the SI Methods. For all experiments, crude MCHM (without PPH) obtained from Eastman Chemical Company (Kingsport, TN) was used. Tank liquid or spilled fluids were not obtained for this study.

Abiotic sorption microcosms were constructed by adding 10 g of homogenized, background sediment into 18 amber glass 40 mL VOA vials (precleaned, poly cap with Teflon/silicone septum, Scientific Specialties Service, Inc., Hanover, MD). The loaded vials were autoclaved twice at 121 °C for 30 min, and then 15 mL of sterile (autoclaved) ultrapure water (Honeywell Burdick & Jackson, Mexico City, Mexico) was added. The

Table 1. Treatment Conditions for Crude MCHM Abiotic and Biotic Degradation Microcosms[a]

Experiment	Treatment Name	Sediment	Media	Amendment
Abiotic Sorption	Sorption	Background (autoclaved)	Sterile ultrapure water	1 mg/L crude MCHM
Biotic	+MCHM	Background Impacted	River water	1 mg/L crude MCHM
	+MCHM, killed	Background Impacted	River water	1 mg/L crude MCHM, autoclaved
	Unamended	Background Impacted	River water	Unamended
	Unamended, killed	Background Impacted	River water	Unamended, autoclaved

[a]Triplicate microcosms were prepared for each treatment. Autoclaved treatments represent killed controls.

sediment-water filled VOA vials were amended with 15 μL of a crude MCHM solution to an initial concentration of 1 mg/L in the water layer. The amended VOA vials were incubated (oxic conditions) at room temperature in the dark while shaking at approximately 175 rpm. Loss of 4-MCHM in the water phase was measured in triplicate sacrificial samples collected at days 0, 1, 2, 5, 13, and 25 using HS-SPME-GC-MS.

Microbial degradation of 4-MCHM was evaluated in microcosms constructed to monitor microbial activity and population dynamics under anaerobic (intended) and aerobic (unintended) conditions in impacted and background sediments amended with 1 mg/L crude MCHM or unamended (Table 1). Control treatments included autoclaved (once for 30 min at 121 °C) microcosms (killed) that were amended with MCHM or unamended. Triplicate microcosms were constructed for each treatment (24 total bottles) and each bottle contained 100 g of homogenized sediment and 200 mL of 0.22 μm-filtered, anoxic Elk River water from the background site in 500 mL Schott bottles (Schott AG, Mainz, Germany). River water was made anoxic by flushing with sterile N_2. Bottles were sealed with Schott DURAN GL45 red caps lined with a PTFE faced silicone liner (Schott AG, Mainz, Germany), flushed to form a N_2 headspace, and modified to include gas and liquid sampling ports (SI Figure S1), as described in the SI Methods. Some bottles experienced leakage around the sampling ports; to prevent further leakage, ports on all bottles were sealed with epoxy.

Liquid samples were collected from microcosms over time to measure concentrations of cis- and trans-4-MCHM isomers, indicators of terminal electron accepting processes (e.g., Fe(II), nitrate, and sulfate), and nonvolatile dissolved organic carbon (NVDOC), as described in the SI Methods. To verify that the microcosms were anoxic, the concentration of oxygen in the headspace was measured after 7 days using gas chromatography (GC) as described in the SI Methods. At the end of the experiment (day 25), the live microcosms were deconstructed and 30 g of sediment was frozen at −80 °C for microbial community characterization and 20 mL of aqueous sample was used immediately for analysis of formaldehyde. The autoclave-killed controls were stored at 4 °C for evaluation of sterilization success for a subset of the microcosms as described in the SI Methods.

Microbial Community Characterization. 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. Sediment samples (0.24−0.35 g) were extracted using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA), and then sent to the Michigan State University Genomics Core Facility for Illumina 16S iTag sequencing (San Diego, CA), as described in the SI. Sequences were processed using usearch[17] and mothur v.1.36.1[18] as described in the SI Methods. Sequences are available under BioProject PRJNA389713 in the NCBI Short Read Archive. Diversity measures and statistical analyses were performed in R[19] as described in the SI Methods.

RESULTS AND DISCUSSION

Sediment and Porewater Characteristics. Porewater chemistry near the spill site and at the upstream location had similar concentrations of inorganic anions (SI Table S1). The water samples had low concentrations of anions (each less than 10 mg/L); sulfate was the dominant anion. Nitrate concentrations were less than 1 mg/L. Sediments collected from the background and impacted sites had <0.1% N and <2% organic carbon. The impacted site had 2−3 times higher concentrations of both of these elements than the background site (SI Table S1). It is unknown whether the higher %C in the impacted sediments represents residual crude MCHM or is due to natural variability.

Abiotic Sorption Experiments. Sorption experiments were conducted over 25 days with slurries of Elk River sediments collected from an unimpacted area of the river upstream from the spill (Figure 1) in ultrapure water. Significant loss of both cis- and trans-4-MCHM was observed within 2 days ($p < 0.05$, Figure 2, SI Table S2). However, this loss was only statistically significant in the beginning of the experiment (over the first 0−5 days, SI Table S2). We

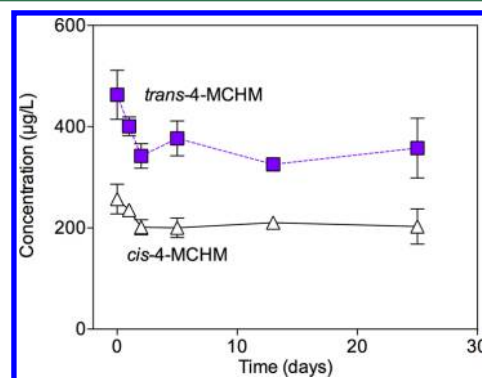**Figure 2.** Sorption experiments for cis- and trans-4-MCHM over 25 days of incubation. White and purple symbols are for cis- and trans-4-MCHM in the water layer, respectively. Results are averages ± standard deviations for triplicate incubations sacrificed at each time point.

hypothesize that the insignificant loss later in the experiment was due to variability among the triplicate sacrificial samples or desorption from the sediments. Average losses between the beginning and end of the experiment were 17.5% for *cis*-4-MCHM and 31% for *trans*-4-MCHM, with most of the loss occurring within the first 3 days. These results indicate that despite the low organic content of the upstream, background sediments (0.66% C, SI Table S1), there is the potential for these compounds to be retained in Elk River sediments. Dietrich et al.[12] also conducted partitioning experiments and found less sorption of the *cis*- than *trans*-4-MCHM isomer onto activated carbon. Although those experiments were conducted at 100−200 mg/L concentrations, our observations are in agreement with Dietrich et al.[12] that sorption behavior is isomer specific. However, it should be noted that our sorption experiments were performed with sediments in ultrapure water and behavior of 4-MCHM will differ under the in situ conditions of the Elk River.

Biodegradation Experiments. Loss of both *cis*- and *trans*-4-MCHM was observed under oxic and anoxic conditions in the crude MCHM amended microcosms constructed with background or impacted Elk River sediment (Figure 3). Gas chromatographic headspace analyses confirmed that two of the microcosms contained oxygen, presumably due to failure to create a gastight seal around the cap sampling ports; those microcosms are plotted as "oxic" in comparison to the "anoxic" microcosms (Figure 3 and SI Figure S1, Table S3). Degradation of both *cis*- and *trans*-4-MCHM in either background or impacted sediment occurred faster under oxic conditions (200−250 μg L^{-1} day^{-1}) compared with anoxic conditions (33−70 μg L^{-1} day^{-1}), after the initial 24 h period (Figure 3A, B). By day 4, both isomers presumably completely degraded (below detection level) in the oxygenated bottles. These observations support those of Weidhaas et al.[15] indicating that aerobic metabolism results in rapid degradation of 4-MCHM. By day 13, both isomers were completely degraded in the anoxic treatments. Formaldehyde was below detection level in all end-point samples (data not shown). Although the first samples (day 0) were collected after the microcosms were allowed to shake for 1 h to allow for initial sediment sorption, a portion of rapid losses in the first 24 h for all treatments likely reflect continued sorption onto the Elk River sediments, consistent with the observations from the sorption experiments that indicated sorption occurrence over the first 3 days of those experiments.

Sediment source (impacted or background) played a role in the degradation of both isomers under anoxic conditions. Impacted sediment microcosms had faster complete loss of both *cis*- and *trans*-4-MCHM compared to background sediments; complete loss occurred within 8 days (Figure 3A, B). Complete loss in the background sediment microcosms occurred within 13 days, with anoxic impacted microcosms degrading 4-MCHM faster than anoxic background microcosms with loss rates of 56.4 vs 34.0 μg L^{-1} day^{-1}, respectively. Sulfate and Fe(II) concentrations measured over time (Figure 3 C, D) indicated that iron and sulfate reduction were the dominant anaerobic processes. Sulfate was completely reduced in the anoxic microcosms, whereas, in the oxic microcosms, reduced sulfur was subsequently oxidized (Figure 3C). Sulfur oxidation was observed in both live, oxic and killed anoxic bottles. Abiotic and biotic mechanisms could contribute to oxidation but further research is needed to assess the specific pathways involved. Iron reduction was evident in all anoxic
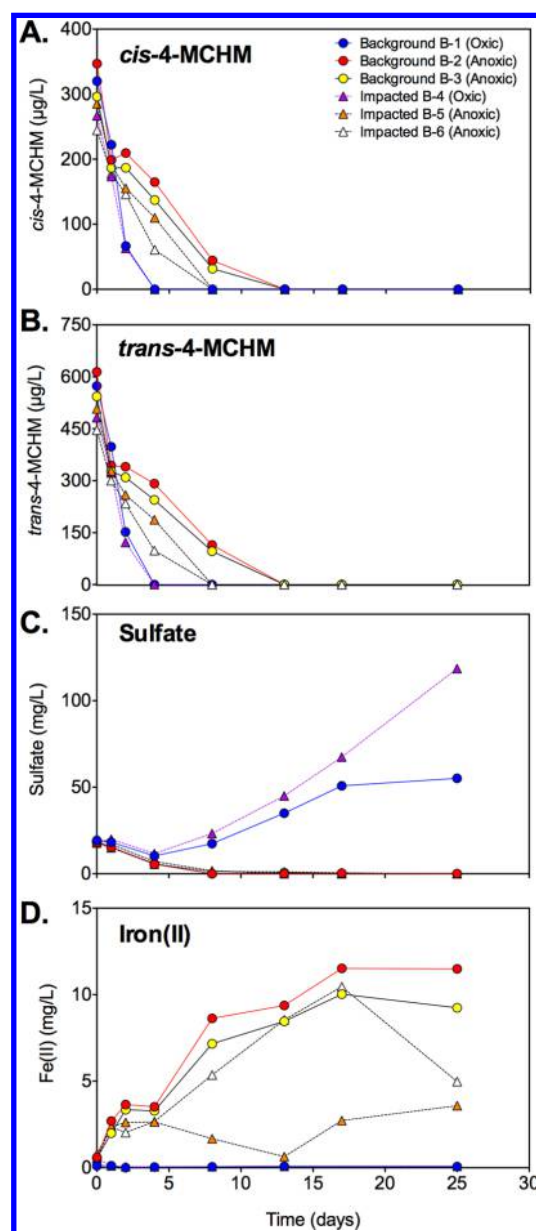
Figure 3. Concentrations in water layer of (A) *cis*-4-MCHM, (B) *trans*-4-MCHM, (C) sulfate, and (D) iron(II) over time in crude MCHM-amended microcosms with background (circles) and impacted (triangles) sediments. Results for individual microcosms are presented as some bottles were oxic and others anoxic.

treatments, but absent in the oxic microcosms (Figure 3D, SI Table S3). 4-MCHM contains eight carbons per molecule and the addition of 1 mg/L could account for the majority of the observed Fe(III) reduction. Although there was enough 4-MCHM to support the observed production of Fe(II) in the microcosms, degradation of the NVDOC present in the microcosms (SI Table S3) could also have resulted in production of Fe(II). Therefore, further research is needed to establish whether 4-MCHM biodegradation is directly coupled to Fe(III) or sulfate reduction.

Although the biologically active microcosms showed substantially more loss for both *cis*- and *trans*-4-MCHM over time (Figure 3) compared to the controls, there was some loss in the autoclaved controls (supposedly "killed" microcosms) (SI Figure S2). After the experiment was completed, these

microcosm bottles were evaluated for residual microbial activity. Acetate consumption and headspace CO_2 during the sterilization evaluation study revealed that three of the bottles, B-7, B-8, and B-10, were biologically active (SI Table S4). These observations suggest that either some microorganisms within the sediment survived autoclaving treatment and were able to recover by the end of the experiment, or that contamination occurred at some point during sampling.

The unamended, "killed" treatments with impacted sediments (Table 1) provided an opportunity to observe the potential for desorption of cis- and trans-4-MCHM from spill-zone sediments (Figure 4) into the water layer. All three
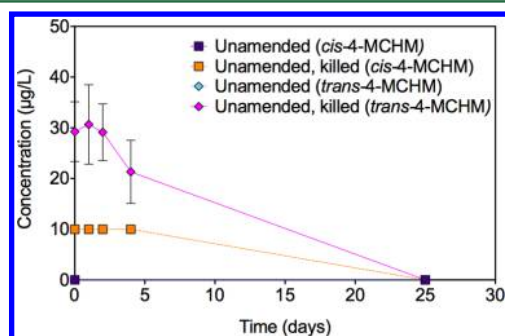
Figure 4. Concentrations of cis- and trans-4-MCHM in the water layer of unamended (no crude MCHM added) microcosms constructed with impacted sediments. Microcosms sterilized by autoclaving are indicated by "killed." Results are averages ± standard deviations for triplicate microcosms. Values for trans-4-MCHM on day 4 were above detection limit of 23 μg/L but below the lowest standard. cis-4-MCHM was detected on days 0−4 but values were below the detection limit of 10 μg/L.

autoclaved impacted-sediment microcosms contained measurable concentrations of trans-4-MCHM (23−38 μg/L) and detectable concentrations of cis-4-MCHM (<10 μg/L) (SI Table S3). The unamended microcosms that were not autoclaved had no measurable concentrations of either isomer. Both isomers of MCHM persisted in the microcosms with killed sediment for at least 4 days (Figure 4). We hypothesize that these losses were caused by resorption onto the sediment; there was no evidence of sulfate loss or Fe(II) production over time in these microcosms, however aerobic degradation cannot be ruled out. Nevertheless, the release of 4-MCHM from autoclaved impacted sediments collected 10 months after the spill demonstrates that 4-MCHM persisted in soils at the impacted site. Further research is needed to evaluate the conditions that control desorption of 4-MCHM from riverbed sediments and the potential for impacted sediments to serve as a long-term source of 4-MCHM to the river or shallow groundwater.

Microbial communities in homogenized background and impacted nonautoclaved sediments used to prepare the microcosms (day 0) and from each of the "live" microcosms on day 25 of the incubation were characterized using 16S iTag sequencing (SI Table S5). Diversity in the + MCHM and unamended microcosms and site sediments were similar across samples (SI Table S5). The number of genera per library (subsampled to 58 587 sequences) ranged from 702 to 789 with an average of 757 genera (±21). No samples were dominated by particular operational taxonomic units (OTUs), as all libraries had Simpson Evenness index values >0.98. The samples were also similar in composition at high taxonomic

ranks. Bacteria dominated the microbial communities with very few Archaea detected, generally <2% of total sequence reads (SI Figure S3A). The low detection of Archaea is not surprising, as the universal primers we used are known to overlook >90% of archaeal diversity in gut microbiomes.[20] At the phylum level, communities were generally similar to approximately 15 phyla present at greater than 1% abundance across all samples (SI Figure S3B). Members of the Proteobacteria dominated the communities, accounting for at least 41% of OTUs (average of 44.4 ± 1.7%). The second most abundant phylum was Bacteroidetes (average of 9.49% ± 0.98), followed Acidobacteria (6.66 ± 1.20), Chloroflexi (5.28 ± 0.89), Verrucomicrobia (4.57 ± 0.58), Actinobacteria (3.21 ± 0.41), and Nitrospirae (3.66 ± 0.55), Planctomycetes (2.6 ± 0.38) (SI Figure S3B). OTUs not classifiable at the phylum level constituted 7.80 ± 0.81 of the communities and may represent novel diversity. These results are consistent with the work of Weidhaas et al.,[15] which saw dominance of Proteobacteria-related OTUs in clone libraries from microcosms degrading high concentrations of 4-MCHM and PPH. In the Weidhaas et al.[15] study, sediments were collected ∼700 m downstream of the spill site and were exposed to the spill.

Despite phylum level similarities, there were differences in community composition between background and impacted samples based on NMDS and PERMANOVA using a Bray−Curtis dissimilarity matrix of subsampled libraries at the genus-level. Communities from background and impacted sediment and microcosm samples separated along NMDS1, although one impacted microcosm sample (UnAm B-16, unamended treatment, bottle B-16) clustered with background samples (Figure 5). The overall composition of background and impacted samples was significantly different ($F_{1,18}$ = 7.27; $P < 0.001$) based on a PERMANOVA with "time" (T0 and T25) as a block. The difference in genera based on shared OTUs between the sites corresponds with the variable rates of 4-MCHM biodegradation between the background and impacted micro-
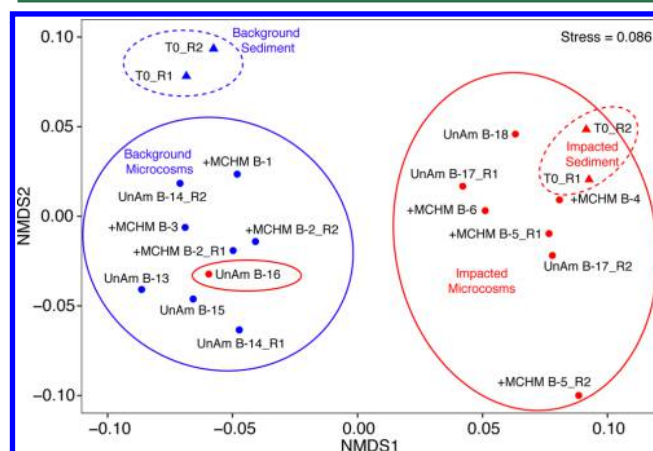
Figure 5. Nonmetric multidimensional scaling (NMDS) plot of Elk River sediment samples and microcosm microbial communities. Samples from the background and impacted sites are indicated by blue and red symbols, respectively. Circle and triangle symbols indicate sediment and microcosm samples, respectively. Amended microcosms are denoted by "+MCHM" and those unamended by "UnAm", followed by the bottle number. R1 or R2 indicates extraction replicates. Dashed circles highlight the microbial communities from collected sediment samples whereas the solid circles indicate microbial communities in samples from the microcosm experiments.

cosms (Figure 3). Impacted sediments degraded 4-MCHM faster than those collected from the background site suggesting that in situ microbial communities had undergone selection due to the spill. Differences between impacted and background sediments in the abundance of genera highly correlated with NMDS1 (SI Figure S4) could reflect a shift to MCHM tolerant microbes. 4-MCHM and PPH were toxic to Elk River microbial cultures that were exposed to these compounds in laboratory studies.[15] Therefore, we would expect that communities in impacted sediments would differ from background due to their exposure to the crude MCHM/PPH spill, although it is not known which components of the chemical spill cause the observed changes.

Implications. Our laboratory microcosm studies, using crude MCHM as the source material, demonstrated substantial sorption and biodegradation of cis- and trans-4-MCHM in Elk River sediment. Biodegradation was faster in sediments previously exposed to crude MCHM, suggesting microbial communities in impacted sediments appear to be primed for 4-MCHM degradation, possibly due to selective pressure from the 2014 spill. Indeed, profiles of microbial communities in impacted and background sites were different, underscoring the potential importance of using in situ communities for bioremediation. The in situ microbial community apparently acclimated to the presence of the 4-MCHM in less than 1 year following the spill. Understanding how the microbial community composition and diversity changes in sediments after exposure provides insight into how these communities respond to chemical spills.

Examination of impacted sediments collected 10 months post spill revealed measurable concentrations of sorbed 4-MCHM. The observation of sediment-sorbed 4-MCHM occurred despite the potential for rapid aerobic and anaerobic degradation, shown here and in other studies.[15,16] These observations highlight the potential of sediment to serve as a long-term source of 4-MCHM to the river water or shallow groundwater. In the event of future desorption of 4-MCHM from impacted sediments, our results indicate biodegradation or readsorption should occur in a matter of days. Degradation, however, does not necessarily reduce toxicity. Lan et al.[9] found that metabolites of 4-MCHM (likely aldehydes and carboxylic acids) were more toxic, particularly to human cells, than 4-MCHM. They concluded that 4-MCHM could be related to carcinogenesis and reproductive toxicity due to the DNA damage effects they observed on human cells, raising concerns for the impacts of chronic exposures, even at low concentrations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b03142.

Data are summarized in Tables S1–S6. All data and meta data are available in Cozzarelli et al.[21] (PDF)

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Whelton, A. J.; McMillan, L.; Connell, M.; Kelley, K. M.; Gill, J. P.; White, K. D.; Gupta, R.; Dey, R.; Novy, C. Residential tap water contamination following the Freedom Industries chemical spill: perceptions, water quality, and health impacts. *Environ. Sci. Technol.* 2015, 49 (2), 813–23.

(2) Rosen, J. S.; Whelton, A. J.; McGuire, M. J.; Clancy, J. L.; Bartrand, T.; Eaton, A.; Patterson, J.; Dourson, M.; Nance, P.; Adams, C. The crude MCHM chemical spill in Charleston, W. Va. *J. Am. Water Works Assoc.* 2017, 106 (9), 65–74.

(3) National Toxicology Program *NTP Research Program on Chemicals Spilled into the Elk River in West Virginia—Final Update*; U.S. Department of Health and Human Services: Durham, NC, 2016; pp 1–7, https://ntp.niehs.nih.gov/go/792827.

(4) WVDHSEM WV American Water Emergency. http://www.dhsem.wv.gov/West%20Virginia%20Public%20Water%20Supply%20Study%20Commission/Pages/WV-American-Water-Emergency.aspx.

(5) U.S. Chemical Safety and Hazard Investigation Board *Investigation Report: Chemical Spill Contaminates Public Water Supply in Charleston, West Virginia, Report No. 2014−01-I-WV*; U.S. Chemical Safety Board: Washington, DC, 2017; pp 1–145, http://www.csb.gov/assets/1/7/FINAL_Freedom_Industries_Investigation_Report_(5-11-2017)_(2).pdf.

(6) Whelton, A.; McMillan, L.; Novy, C. L.-R.; White, K.; Huang, X. Case study: the crude MCHM chemical spill investigation and recovery in West Virginia USA. *Environmental Science: Water Research & Technology* 2017, 3 (2), 312–332.

(7) Cooper, W. J. Responding to Crisis: The West Virginia Chemical Spill. *Environ. Sci. Technol.* 2014, 48 (6), 3095–3095.

(8) Thomasson, E. D.; Scharman, E.; Fechter-Leggett, E.; Bixler, D.; Ibrahim, S.; Duncan, M. A.; Hsu, J.; Scott, M.; Wilson, S.; Haddy, L.; Pizon, A.; Burrer, S. L.; Wolkin, A.; Lewis, L. Acute health effects after the Elk River chemical spill, West Virginia, January 2014. *Public Health Rep.* 2017, 132 (2), 196–202.

(9) Lan, J.; Hu, M.; Gao, C.; Alshawabkeh, A.; Gu, A. Z. Toxicity Assessment of 4-Methyl-1-cyclohexanemethanol and Its Metabolites in Response to a Recent Chemical Spill in West Virginia, USA. *Environ. Sci. Technol.* 2015, 49 (10), 6284–6293.

(10) Sain, A. E.; Dietrich, A. M.; Smiley, E.; Gallagher, D. L. Assessing human exposure and odor detection during showering with crude 4-(methylcyclohexyl)methanol (MCHM) contaminated drinking water. *Sci. Total Environ.* 2015, 538, 298–305.

(11) Gallagher, D. L.; Phetxumphou, K.; Smiley, E.; Dietrich, A. M. Tale of two isomers: complexities of human odor perception for cis-

and trans-4-methylcyclohexane methanol from the chemical spill in West Virginia. *Environ. Sci. Technol.* **2015**, *49* (3), 1319−27.

(12) Dietrich, A. M.; Thomas, A.; Zhao, Y.; Smiley, E.; Shanaiah, N.; Ahart, M.; Charbonnet, K. A.; DeYonker, N. J.; Alexander, W. A.; Gallagher, D. L. Partitioning, aqueous solubility, and dipole moment data for *cis*- and *trans*-(4-Methylcyclohexyl)methanol, principal contaminants of the West Virginia chemical spill. *Environ. Sci. Technol. Lett.* **2015**, *2* (4), 123−127.

(13) Weidhaas, J.; Lin, L.-S.; Buzby, K. A case study for orphaned chemicals: 4-methylcyclohexanemethanol (MCHM) and propylene glycol phenyl ether (PPH) in riverine sediment and water treatment processes. *Sci. Total Environ.* **2017**, *574*, 1396−1404.

(14) 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.

(15) Weidhaas, J.; Lin, L.-S.; Buzby, K.; Li, X. Biodegradation of MCHM and PPH in river microcosms and activated sludge. *J. Environ. Eng.* **2016**, *142* (11), 1−9.

(16) Yuan, L.; Zhi, W.; Liu, Y.; Smiley, E.; Gallagher, D.; Chen, X.; Dietrich, A.; Zhang, H. Aerobic and anaerobic microbial degradation of crude (4-methylcyclohexyl)methanol in river sediments. *Sci. Total Environ.* **2016**, *547*, 78−86.

(17) Edgar, R. C.; Flyvbjerg, H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **2015**, *31* (21), 3476−3482.

(18) 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.

(19) R Core Team *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2015.

(20) Raymann, K.; Moeller, A. H.; Goodman, A. L.; Ochman, H. Unexplored archaeal diversity in the great ape gut microbiome. *mSphere* **2017**, *2* (1), e00026−17.

(21) 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.

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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[TM] 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$axis1<=-
0.5),]
# converted subsampled libraries to relative abundances
ns_subsamp_RA <- transform_sample_counts(ns_subsamp,
function(OTU)(OTU/sum(OTU))*100)
# retrieved OTUs from ns_subsamp that are significantly correlated
ns_OTU_NMDS_corr_sig_list <- rownames(ns_OTU_NMDS_sig_cor)
ns_ss_RA_OTU_NMDS_corr_sig <-  prune_taxa(ns_OTU_NMDS_corr_sig_list,
ns_subsamp_RA)
min(sample_sums(ns_ss_RA_OTU_NMDS_corr_sig)) # 53.1%
max(sample_sums(ns_ss_RA_OTU_NMDS_corr_sig)) # 57.1%
# exported csv files of RA and taxonomy tables and significant correlations
write.csv(otu_table(ns_ss_RA_OTU_NMDS_corr_sig),
"Genus_ns_ss_RA_OTU_NMDS_corr_sig.csv")
write.csv(tax_table(ns_ss_RA_OTU_NMDS_corr_sig),
"Genus_ns_ss_RA_OTU_NMDS_corr_sig_tax.csv")
write.csv(ns_OTU_NMDS_sig_cor, "Genus_ns_OTU_NMDS_sig_cor.csv")
### combined tables in excel and added column to sum OTU abundances across
samples
### and calculated an effect size change by looking at the overall % change
in OTU
### abundances b/w background and impacted samples
ns_ss_RA_OTU_NMDS_corr_sig_comb <-
read.table("Genus_ns_combined_OTU_NMDS_sig_cor.txt", sep="\t", header=TRUE,
row.names="OTU")
# removed OTUs with summed abundances less than 2 across samples
OTU_NMDS_corr_sig <-
ns_ss_RA_OTU_NMDS_corr_sig_comb[which(ns_ss_RA_OTU_NMDS_corr_sig_comb$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
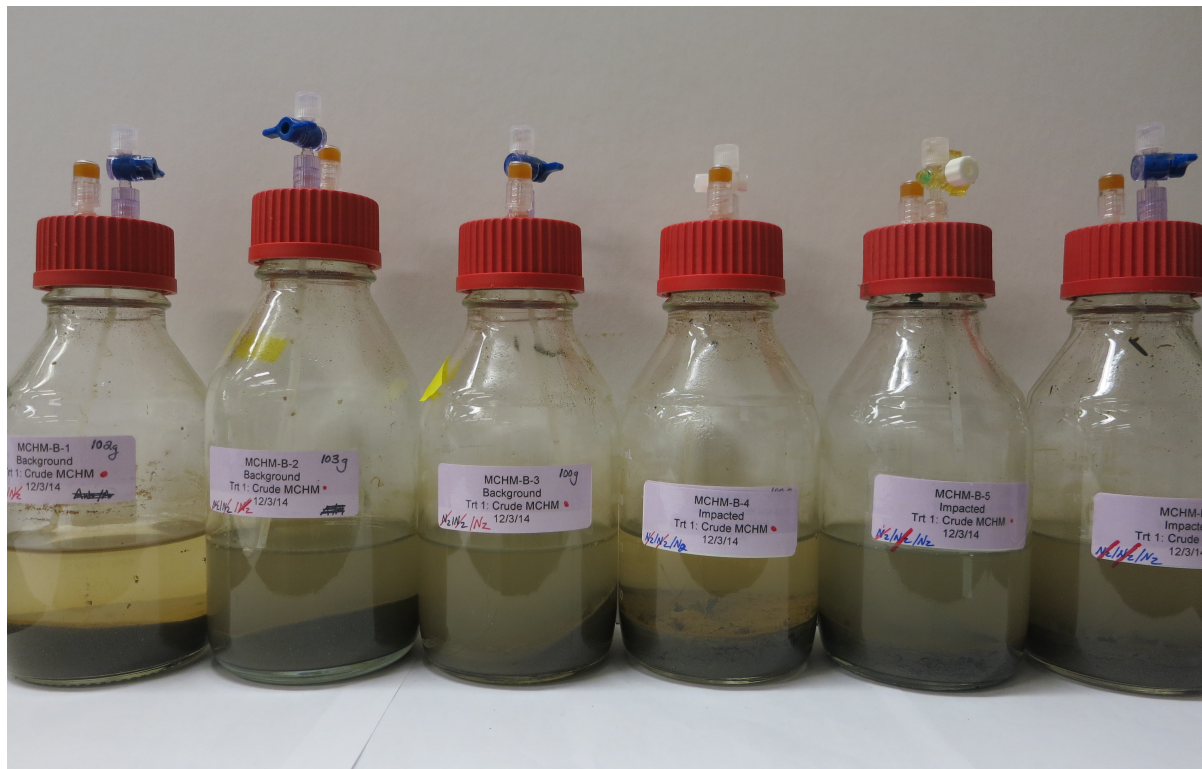
Figure S1. Photograph of biodegradation microcosms on day 25 amended with 1 mg/L crude MCHM. Left to right: Background sediment bottles B-1, B-2, and B-3; Impacted sediment bottles B-4, B-5, and B-6. Note the darker color in B-2, B-3, B-5, and B-6 which corresponds to the presence of Fe(II) from microbial 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).
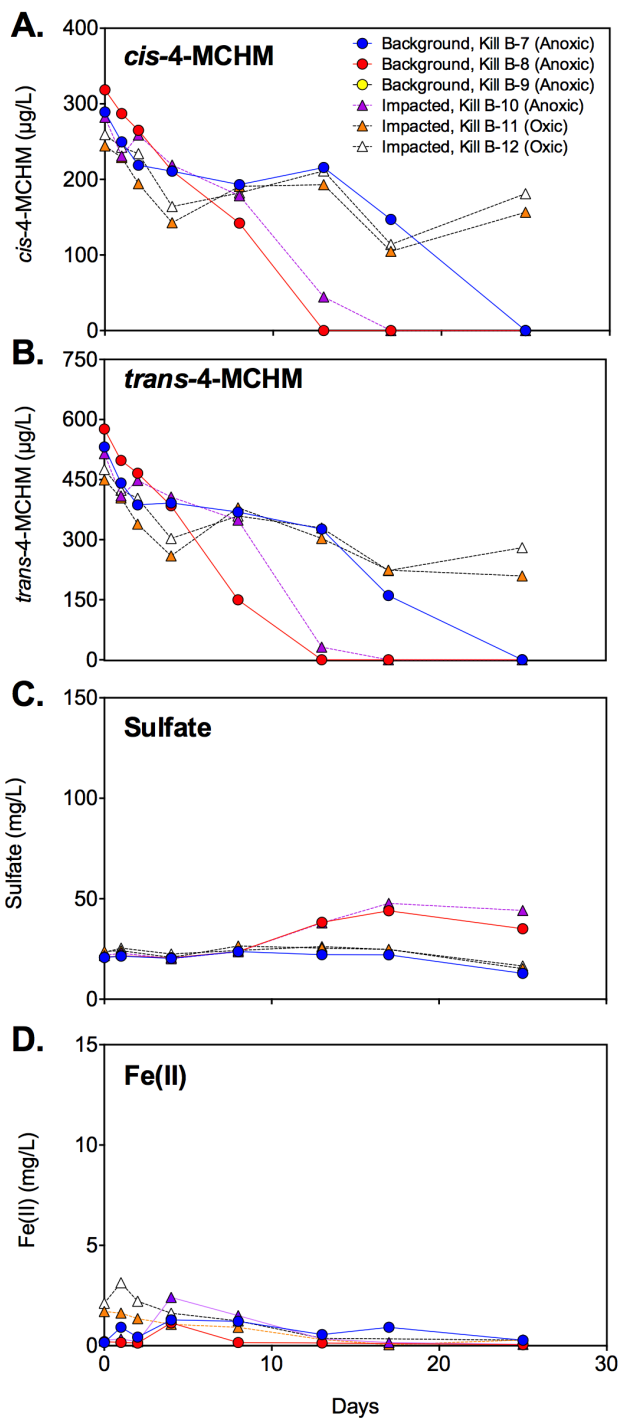
Figure S2. Concentrations of (A) *cis*-4-MCHM, (B) *trans*-4-MCHM, (C) sulfate, and (D) Fe(II) over time in crude MCHM-amended, killed microcosms with background (circles) and impacted (triangles) sediments. Results for individual microcosms are presented as some bottles were oxic and others anoxic. The background treatment had duplicate bottles (B-7 and B-8), whereas impacted was incubated in triplicate.
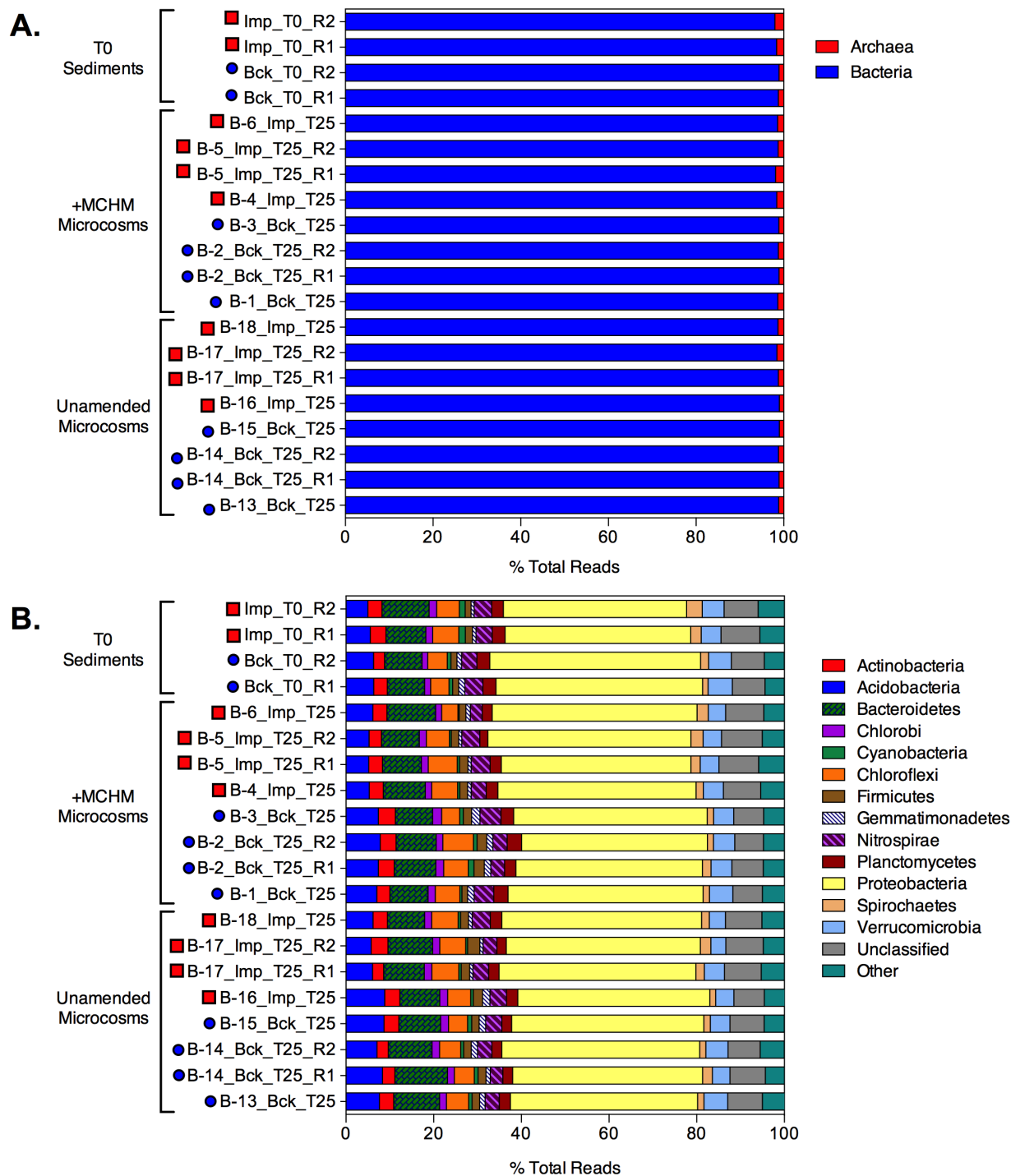
Figure S3. Phylogenetic affiliation of 16S rRNA gene sequences from non-autoclaved sediments and biodegradation microcosms at the (A) Domain and (B) Phylum level. Microcosm samples collected on day 25 (T25) are named by bottle number (B-#) and sediments sources are abbreviated "Imp" (red squares) and "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.
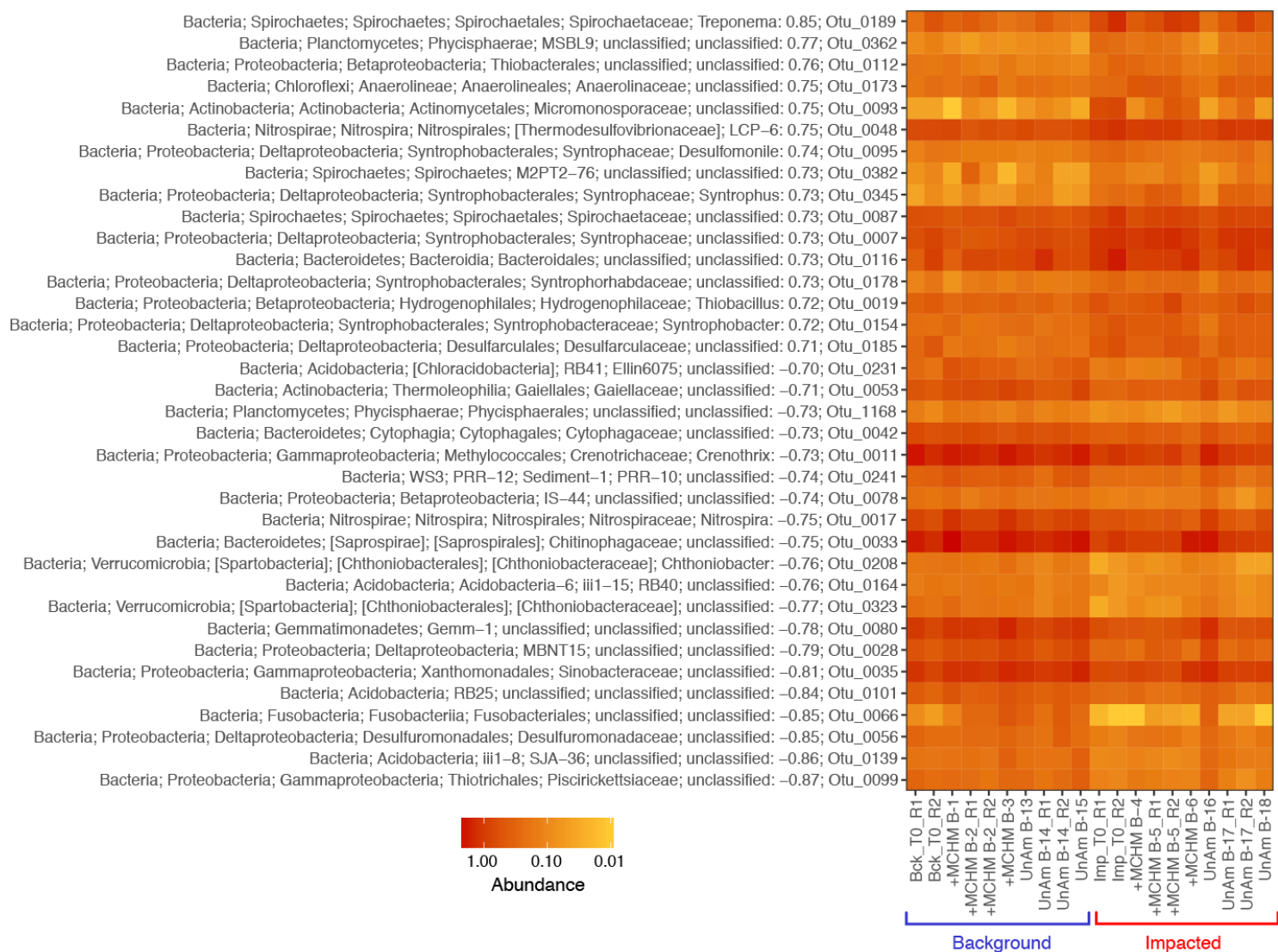
Figure S4. Heat map of genera significantly (P < 0.05) and highly (Spearman's r > 0.7 or <-0.7) correlated with NMDS1. Row labels are taxonomic designations followed by Spearman correlations, and a representative OTU number. The color scale is log10 transformed.

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	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 Day	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
				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

Treatment	Site	Bottle Name	Date Day	Sulfate (mg/L)							
				12/4/14 0	12/5/14 1	12/6/4 2	12/8/14 4	12/12/14 8	12/17/14 13	12/21/14 17	12/29/14 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

Treatment	Site	Bottle Name	Date Day	Nitrate (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
				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

Treatment	Site	Bottle Name	Date Day	Oxygen (%) 12/10/14 6	NVDOC (mg/L C) 12/4/14 0	12/8/14 4	12/29/14 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 % CO_2 determined using gas chromatography. BD: below detection limit; NC: not collected.

Treatment	Site	Bottle Name	Date	Acetate (mM)				CO_2 (%)			
				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