Supporting Information for
Recovery of Phenanthrene-Degrading Bacteria After Simulated *In Situ* Persulfate Oxidation in Contaminated Soil

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Number of pages: 11
Number of Figures: 6
Number of Tables: 3

Prepared for *Environmental Science and Technology*

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Column soil characterization

Physical properties and PAH characterization of the column soil are summarized in Tables S1 to S3. The porosity and particle density of the 30/40 silica sand support layer are 0.35 and 2.67 g/cm^3, respectively *(S6)*.

Table S1. Properties of the Column Soil

Property	Unit	Value (mean ± std. dev.)	No. of Replicates	Method (reference)
Sand content	%	82.9	1	S1
Silt content	%	13.8	1	S1
Clay content	%	3.3	1	S1
Moisture content	%	7.50 ± 0.01	6	S2
Soil pH		7.56 ± 0.11	3	S3
Inorganic carbon	%	7.0 ± 1.6	6	S4
Organic matter (375°C)	%	8.3 ± 1.2	6	S4
Black carbon (550°C)	%	0.88 ± 0.26	3	S4
Soil particle density	g/cm^3	2.62 ± 0.04	3	S5
Bulk density	g/cm^3	1.65 ± 0.03	3	

Table S2. Select Properties of the Column System

Property	Unit	Value
Dry mass of column soil	g	13,000
Porosity	%	37.0
Pore volume	ml	2,810
Volume of persulfate solution injected	ml	17,400
Mass of persulfate injected	g	348

Table S3. PAH Concentrations in the Column Soil (mean ± std. dev.; n=33)

Analyte	Abbreviation	Concentration (mg/kg)
Naphthalene	NAP	9.5 ± 4.2
Acenaphthene	ACE	11.9 ± 2.7
Fluorene	FLU	9.5 ± 2.0
Phenanthrene	PHN	129 ± 31
Anthracene	ANT	10.5 ± 2.2
Fluoranthene	FLA	25.2 ± 5.0
Pyrene	PYR	40.9 ± 7.8
Benz[*a*]anthracene	BaA	13.8 ± 3.0
Chrysene	CHR	14.0 ± 3.6
Benzo[*b*]fluoranthene	BbF	6.9 ± 1.7
Benzo[*k*]fluoranthene	BkF	4.2 ± 1.1
Benzo[*a*]pyrene	BaP	13.5 ± 4.2
Dibenz[*a,h*]anthracene	DBA	1.0 ± 0.5
Benzo[*g,h,i*]perylene	BgP	5.3 ± 1.6
TOTAL		295 ± 65

Column design

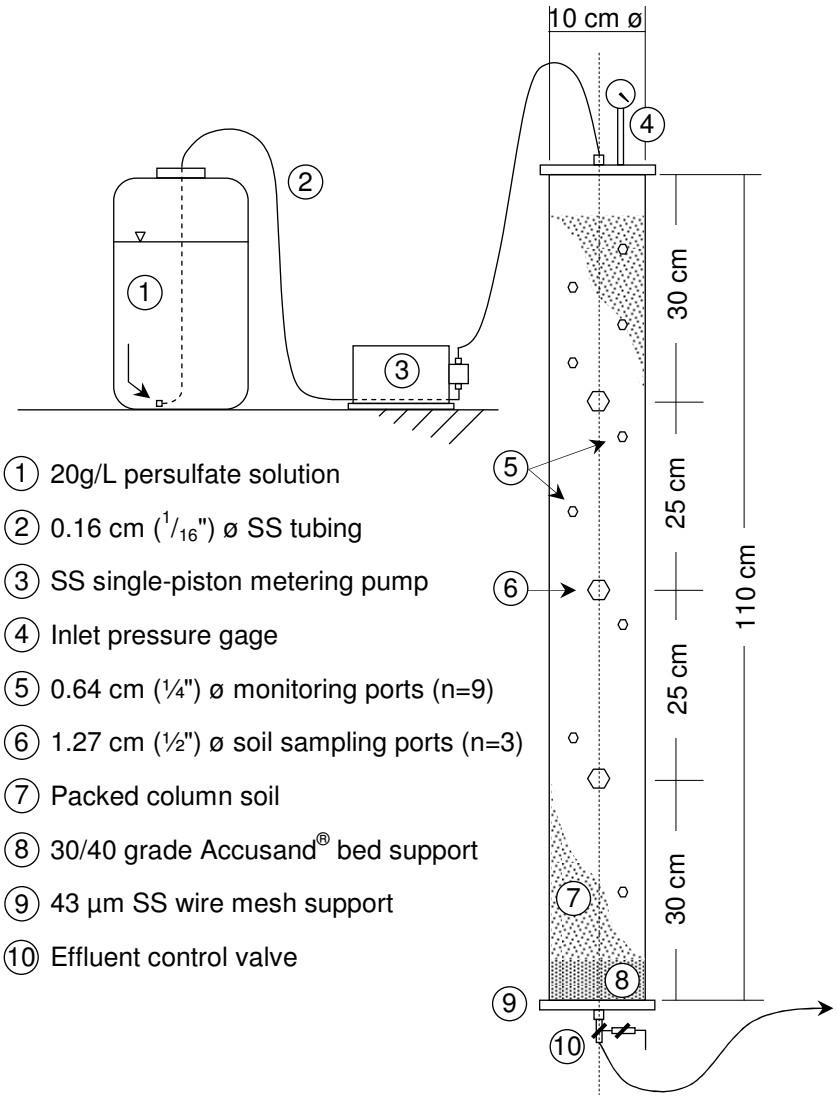

Figure S1. Schematic of the column system. SS, stainless steel.

Procedures for chemical and molecular analyses

PAH Analysis. Aliquots of centrifuged column soil (5 g wet wt. each) were transferred to triplicate 30-mL glass centrifuge vials for extraction. To each vial was added sodium sulfate (6-7 g), 5-mm glass beads to improve mixing, and 10 mL each of dichloromethane and acetone. An internal standard, 0.2 mL of 100 mg/L anthracene-d_{10} in acetonitrile, was also included to evaluate the recovery efficiency. All vials were sealed with screw-top caps with Teflon-lined septa, vigorously shaken for 24 h, and centrifuged for 15 min at 3,500 rpm. The supernatant from each vial was filtered through a 0.2 μm pore-size nylon filter (Millipore, Burlington, MA) and transferred to a 50-mL volumetric flask. The vials were replenished with dichloromethane and acetone (10 mL each), returned to the shaker for 24 h, and centrifuged as described above. The second-day extracts were filtered and combined with the initial extract in the volumetric flask. The combined extracts were brought up to volume, transferred to amber serum vials, and stored in the dark at 4°C prior to HPLC analysis. The HPLC system included a Waters (Milford, MA) 600E system controller, a Waters 717 Plus autosampler, and a Perkin Elmer (Beaconsfield, UK) LS40 fluorescence detector. Samples were injected through a 3-μm particle-size Supelcosil™ LC-PAH column (Sigma-Aldrich, St. Louis, MO) using a gradient mobile phase of filtered acetonitrile and reagent water. Initial conditions consisted of 60% acetonitrile and 40% filtered water at a flowrate of 1 mL/min. The proportion of acetonitrile was increased linearly to 100% during the first 10 min of each sample run, followed by a flowrate increase to 2 mL/min at 12.5 min. Analyte standards were prepared from an EPA 610 Polynuclear Aromatic Hydrocarbons Mixture stock (Sigma-Aldrich, St. Louis, MO) and used to create a four-point calibration curve for sample quantification. Of the 16 EPA-regulated PAHs, acenaphthylene and indeno[1,2,3-*cd*]pyrene were not detected using this method. Moisture content was measured in triplicate for each sample to normalize PAH concentrations on a dry mass basis. Recovery of anthracene-d_{10} was ≥90%.

Mineralization Assay. Approximately 5 g (wet wt.) of centrifuged column soil was transferred to an acid-washed 30-mL glass vial and slurried with 20 mL of sterile simulated groundwater as a source of inoculum for mineralization incubations. Assays with each sample were performed in triplicate. Each replicate consisted of a 40-mL amber EPA vial containing 1 mL of soil slurry, 4 mL of sterile simulated groundwater, a sterile glass tube containing filter paper saturated with 60 μL of 2 N KOH, and 1 μL of the radiolabeled substrate, corresponding to 20,000 disintegrations per min (dpm) for ^{14}C-acetate and 30,000 dpm for ^{14}C-phenanthrene. Triplicate killed control incubations were prepared similarly but were amended with 40 μL of phosphoric acid to reduce the pH below 2. Each incubation vial was sealed with an aluminum foil-lined septum and screw-top cap and shaken continuously (60 rpm) at room temperature for 15 d. At designated time points, the filter paper CO_2 trap was removed and replaced, combined with Ultima Gold™ XR scintillation cocktail, and analyzed on a Packard Tri-Carb Liquid Scintillation Analyzer (Meriden, CT; Model 1900 TR). After 15 d, all vials were acidified with phosphoric acid and incubated for an additional 48 h to collect any residual $^{14}CO_2$. For all incubations, there was negligible recovery of residual $^{14}CO_2$ following acidification. To quantify unmineralized radiolabeled compound in the incubations, 2 mL of ethyl acetate was added to each slurry, shaken overnight, and the activity in the extract quantified by scintillation counting. Total recovery of the radiolabeled compounds as $^{14}CO_2$ plus extractable ^{14}C ranged from 82% to 132% in the live incubations.

16S rRNA Clone Libraries. To identify dominant bacterial groups before and after persulfate injection, clone libraries of 16S rRNA genes were constructed from surface soil DNA extracts from the pre-oxidation sample and 100 d post-oxidation. PCR amplification was performed using primers 8f *(S7)* and 1492r *(S8)* as described elsewhere *(S9)*. PCR products were cloned into an Invitrogen TA cloning kit (Carlsbad, CA) per the manufacturer's instructions and resulting inserts were partially sequenced with primer 8f by Functional Biosciences, Inc. (Madison, WI). The resulting partial 16S rRNA sequences were trimmed using Sequencher 4.7 (Gene Codes; Ann Arbor, MI) and close relatives identified by BLASTN searches of GenBank *(S10)*. Using ClustalX *(S11)*, sequences were aligned and a neighbour-joining phylogenetic tree was constructed (bootstrapped 1,000 times without considering gaps). Chimeras were resolved using the Bellerophon server *(S12)* and confirmed with BLAST analyses. Partial 16S rRNA gene sequences recovered from this work were submitted to GenBank with accession numbers HM622160-HM622262.

qPCR primer sets

Table S4. qPCR primers used in this study

Target Group	Primer Name	Primer Sequence (5´→3´)	Reference
Bacteria	341F	CCT ACG GGA GGC AGC AG	S13
	517R	ATT ACC GCG GCT GCT GG	S13
Pyrene Group 1	Eub399F	ACT CCT ACG GGA GGC AGC	S14
	PG1R	TAG CAG GCC GTA TTA AGA C	S14
Pyrene Group 2	PG2F	GCA CAG GGT AGC TTG CTA TC	S14
	PG2.1R	CGC AGG CTC ATC GTT CC	This study

Effluent persulfate breakthrough

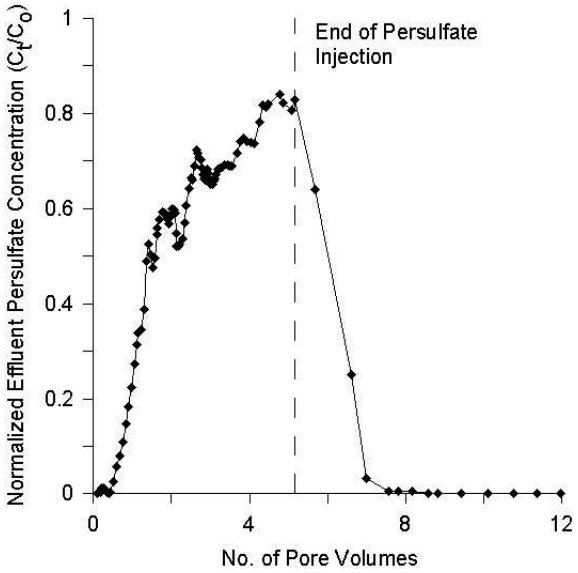

Figure S2. Breakthrough curve of effluent persulfate.

Dissolved oxygen profiles

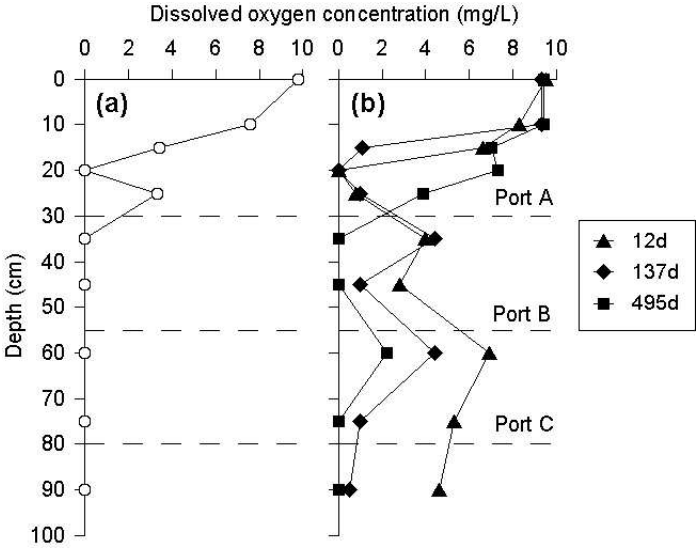

Figure S3. Dissolved oxygen profiles before persulfate injection (a) and 12, 137, and 495 d after injection (b). Oxygen concentrations at the top of the column (depth = 0) were measured in the feed reservoir for the simulated groundwater; note that the soil bed surface prior to persulfate injection was at a depth of approximately 5 cm. Data for post-oxidation time points other than those shown are omitted for clarity. The absence of oxygen at a depth of 20 cm shown in (a) and (b) was attributed to a blockage of the screened cannula due to fines accumulation.

Phylogenetic tree of all sequences recovered in the clone libraries

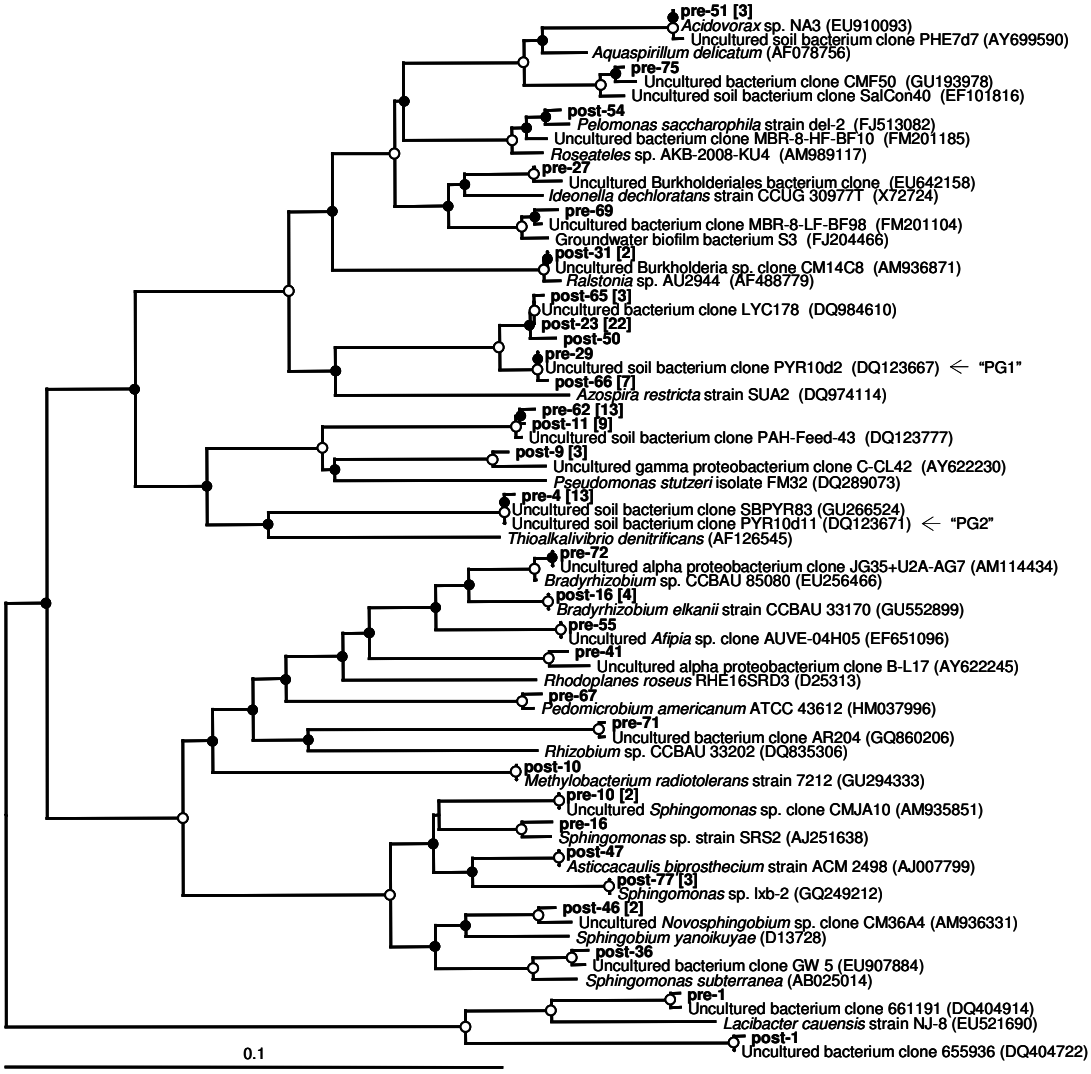

Figure S4. Neighbor-joining phylogenetic tree of 16S rRNA gene sequences recovered pre- and post-oxidation (100 d) from the column surface soil and selected close relatives. Clones from this study (in bold) follow the naming scheme of "pre" (pre-oxidation) and "post" plus an assigned number for identification purposes. Square brackets (in bold) include the number of sequences within an OTU represented by the clonal sequence shown. GenBank accession numbers are shown in parentheses for the selected reference sequences. Bootstrap values are indicated on nodes with an open (○) and closed (●) circle representing ≥95 and ≥50% bootstrap support, respectively. *Thermus aquaticus* YT-1 (L09663) was used as an outgroup (not shown). Sequences associated with groups PG1 and PG2 are indicated with an arrow.

Soil PAH concentrations

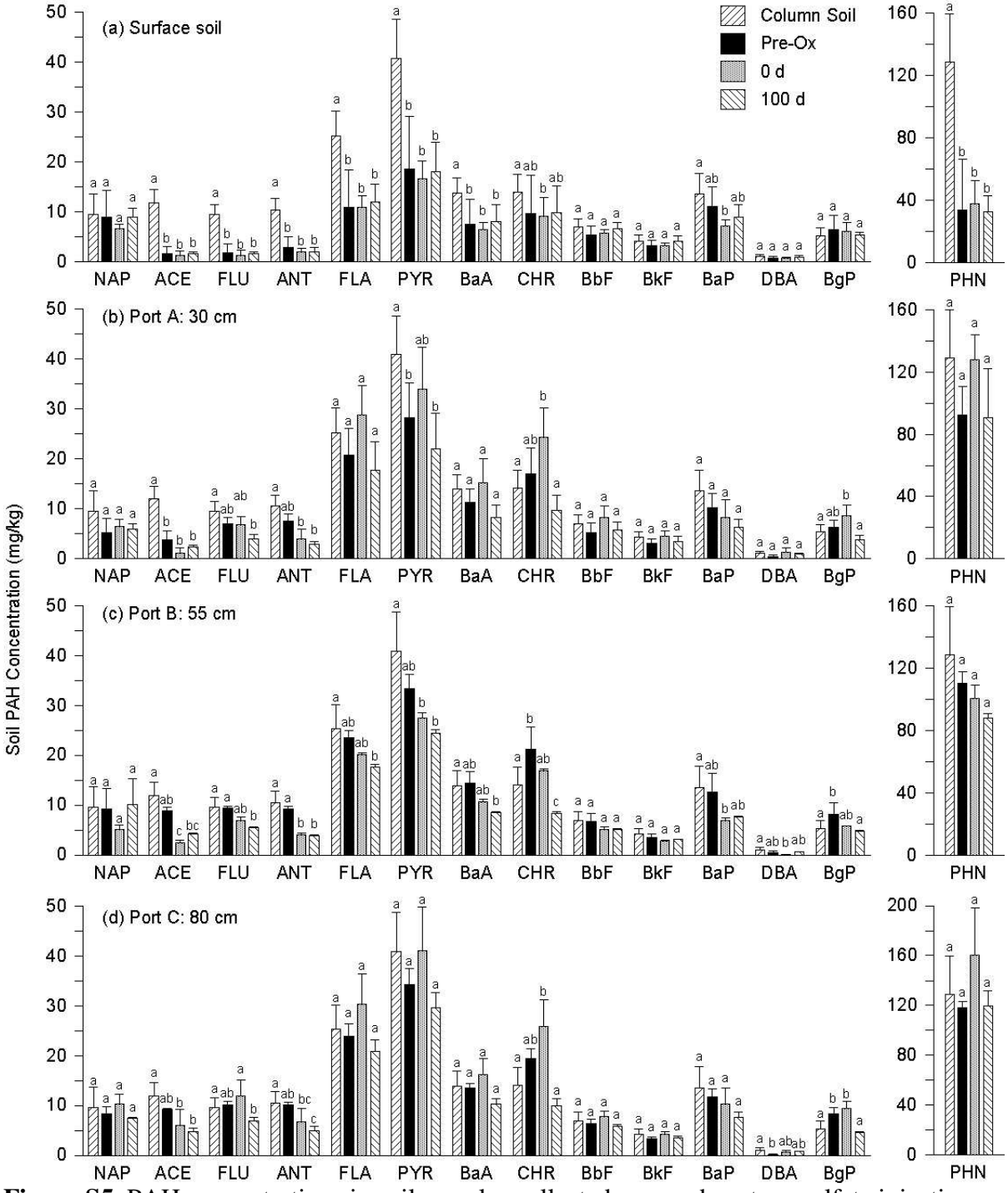

Figure S5. PAH concentrations in soil samples collected pre- and post-persulfate injection from the surface soil (a), Port A (b), Port B (c), and Port C (d). Note that phenanthrene concentrations are plotted separately in each panel. Error bars represent one standard deviation. The letters above the error bars represent the results of significance analyses using the Tukey-Kramer HSD test. For each analyte, a pair of conditions sharing a common letter is not significantly different from each other at a 95% significance level.

Batch persulfate efficacy

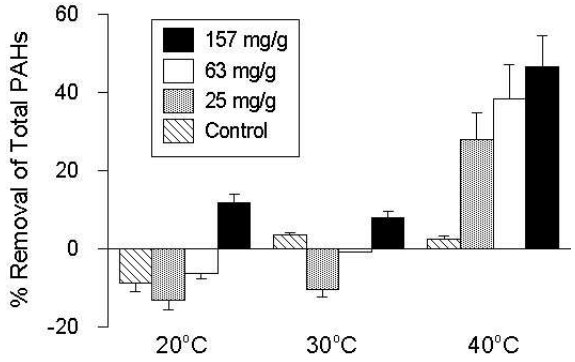

Figure S6. Percent removal of total PAHs in the column soil after persulfate oxidation at various doses, at activation temperatures of 20°C, 30°C, and 40°C. Values represent the means and standard deviation of triplicate 16 d incubations.

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