Supplemental Information for
Desorption and Bioavailability of PAHs in Contaminated Soil Subjected to Long-Term In Situ Biostimulation

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Soil preparation and column design

Contaminated soil was collected from a former MGP site in Salisbury, NC, USA. Soil was collected at a depth of 4 ft below ground surface in the vicinity of the former tar well during active remediation activities by the site owner. The collected soil was sieved through a 10-mm wire screen, mixed with sterile 40/50 grade silica sand (Unimin Corporation, Le Sueur, MN) at a 50:50 ratio (dry weight), and stored at 4°C prior to column packing. Addition of the silica sand was necessary to maintain low-pressure flow during long-term column operation; preliminary column studies with the source material yielded very high inlet pressures (>100 psi). In subsequent discussion, the final packing material is referred to as "column soil".

Two 110-cm long, 10.2-cm diameter stainless-steel columns (Figure S1), each containing a 100-cm zone of column soil, were operated for ~780 d at 20°C, receiving a continuous supply of simulated groundwater in a downward flow direction. The columns were operated at a flowrate of 2.1 L/d for ~630 d that was subsequently reduced to 1.4 L/d to stabilize rising inlet pressures near the end of the experiment. One of the columns was subjected to biostimulation by amending simulated groundwater with pure oxygen and inorganic nutrients; the second column served as a control, receiving air-saturated, unamended groundwater. Simulated groundwater contained 1.83 g $CaCl_2 \cdot H_2O$, 1.01 g $MgSO_4 \cdot 7H_2O$, 2.19 g $NaHCO_3$, 1 ml of an 88 mg/L KCl solution, and 1 ml of 1 N H_2SO_4 in 20 L of sterile-filtered reagent water, based on historical ion concentrations of groundwater in the region of the MGP site [S1]. For the biostimulated column, the simulated groundwater was supplemented with 1 ml of a nutrient stock solution, containing 59.4 g/L of NH_4NO_3 and 29.2 g/L of K_2HPO_4, yielding final nitrogen and phosphorus concentrations of 1.0 mg/L and 0.3 mg/L, respectively. Initially, both columns were run under control conditions for eight months to allow for bed consolidation and stabilization of inlet pressures before biostimulation was initiated in one of the columns.

Each column was equipped with three ports (Ports A, B, and C) positioned 30, 55, and 80 cm below the top of each column, respectively, for periodic soil sample collection, along with nine additional smaller ports for monitoring porewater dissolved oxygen (DO) concentrations (Figure S1). As part of a larger study, soil samples were collected from the surface soil and Ports A, B, and C of the control and biostimulated columns immediately after the 8-month equilibration phase (t = 0) and 31, 93, 184, 380, and 534 d after biostimulation commenced. For this study, desorption experiments, soil extraction, and quantification of PAHs were conducted on the original (untreated) column soil and treated soil from Port A of the control and biostimulated columns on day 534. Port A was selected because the DO concentration was saturated at this depth (30 cm) in both columns at the time of sampling. Additional soil was collected from Port A of both columns at day 593 for density separation into two soil fractions: carbon-rich particles and the bulk mineral fraction, defined as "low-density" and "high-density" material.

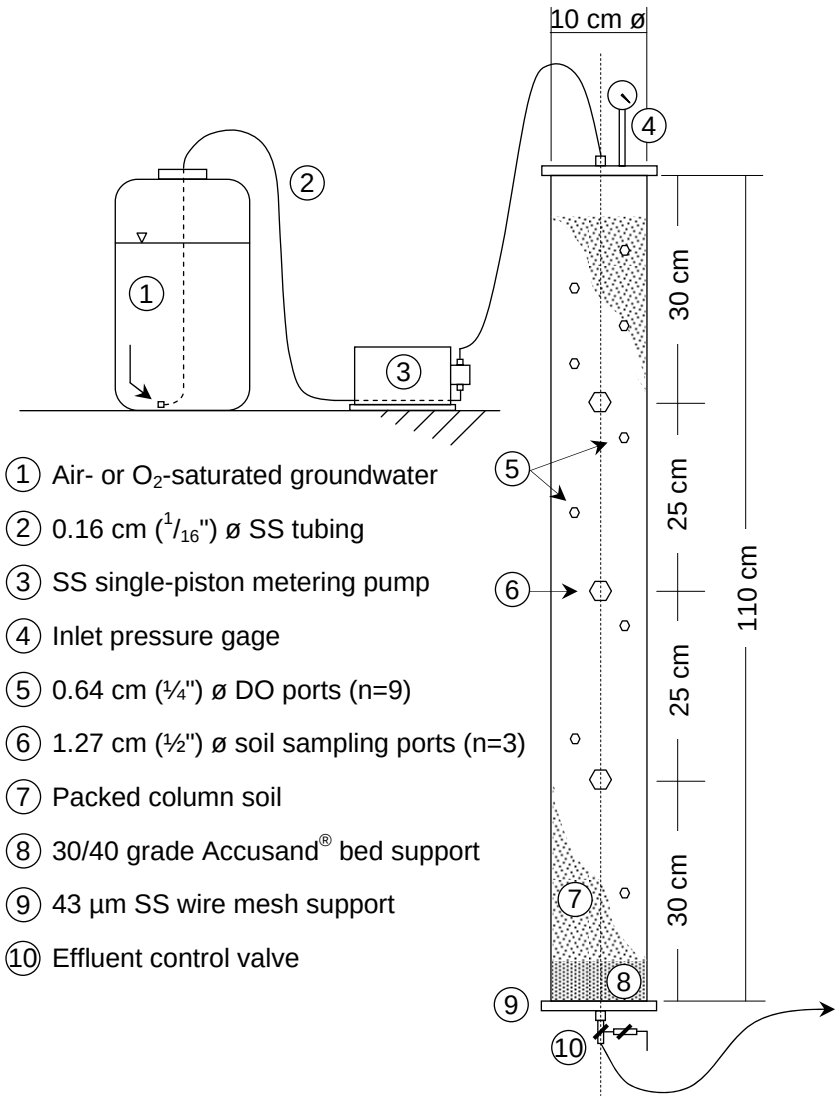

Figure S1. Schematic of the column system. SS, stainless steel.

Procedure for PAH quanitification in soil

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%.

PAH concentrations in high- and low-density material

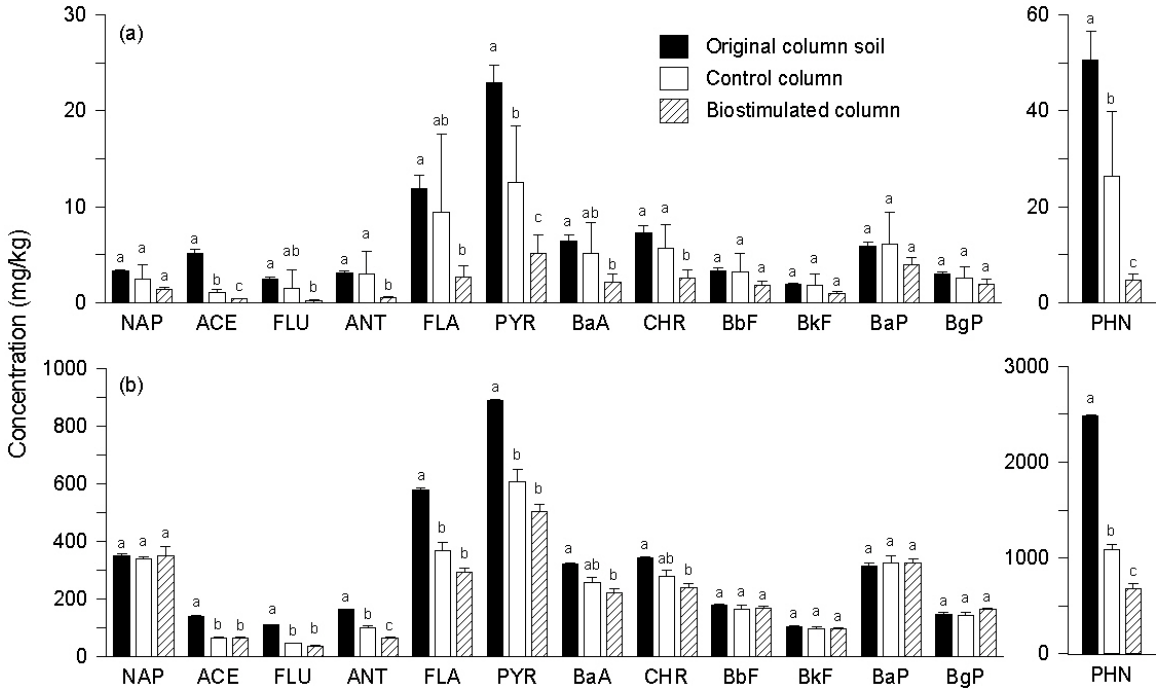

Figure S2. PAH concentrations in the high-density (a) and low-density (b) materials of the original column soil and soil from Port A of the control and biostimulated columns at day 593. Note that phenanthrene concentrations are plotted separately in each panel. Error bars represent one standard deviation in panel (a) and the range in panel (b). The letters above the error bars represent the results of significance analyses using the Tukey-Kramer HSD test. For each analyte, conditions sharing a common letter are not significantly different ($p > 0.05$). Abbreviations: NAP – naphthalene, ACE – acenaphthene, FLU – fluorene, PHN – phenanthrene, ANT – anthracene, FLA – fluoranthene, PYR – pyrene, BaA – benz[*a*]anthracene, CHR – chrysene, BbF – benzo[*b*]fluoranthene, BkF – benzo[*k*]fluoranthene, BaP – benzo[*a*]pyrene, BgP – benzo[*g,h,i*]perylene.

Desorption data for whole soil samples and high and low-density material

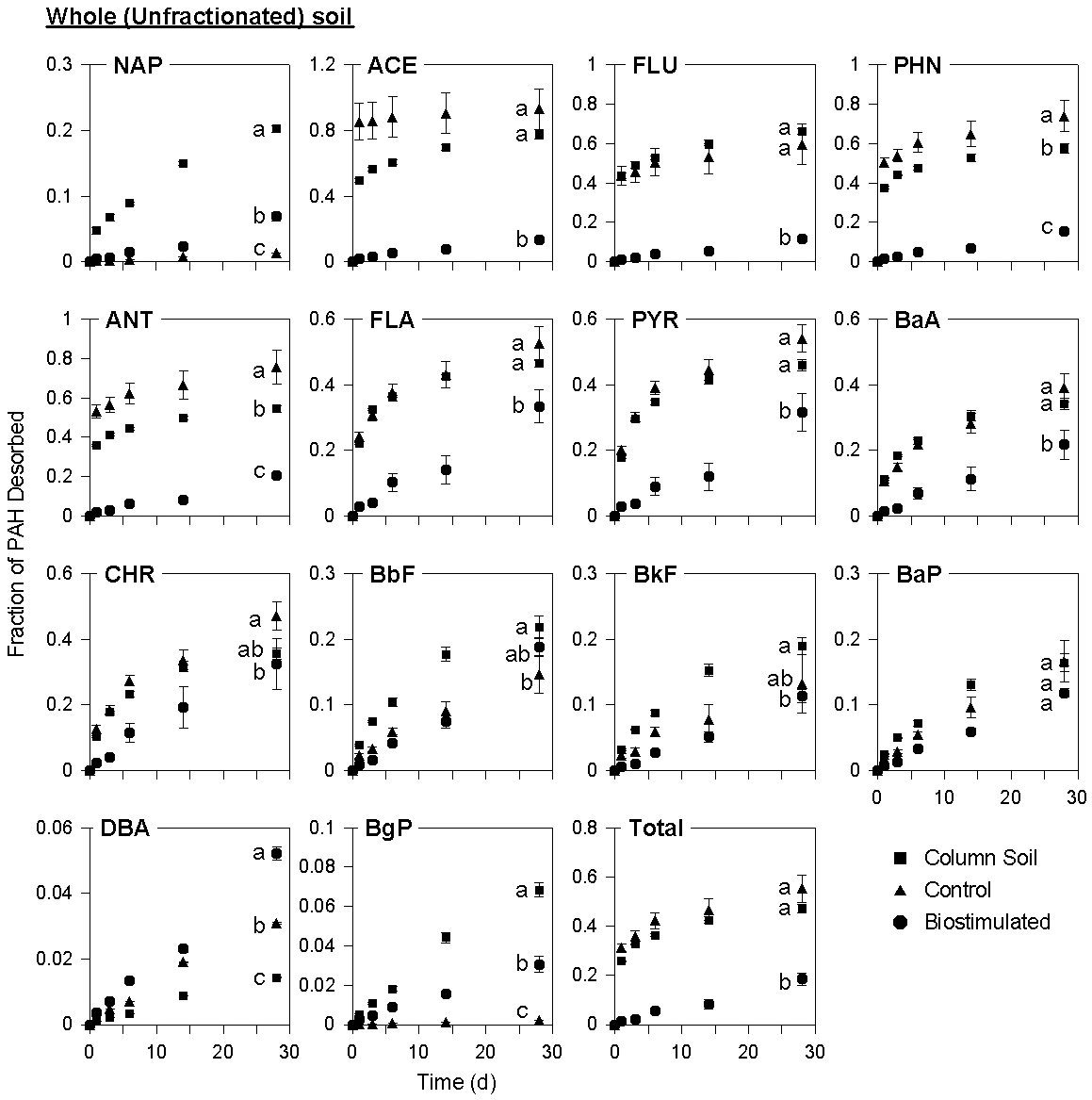

Figure S3. Desorption of PAHs vs. time for the original column soil and samples collected from Port A of the control and biostimulated columns at day 534. Symbols are the mean values of duplicate analyses from triplicate vessels. Error bars represent the standard deviation and are within the size of the symbol if not visible. The letters adjacent to the 28-d time points represent the results of significance analyses using the Tukey-Kramer HSD test. For each analyte, conditions sharing a common letter are not significantly different. Refer to Figure S2 for definitions of acronyms for the PAHs.

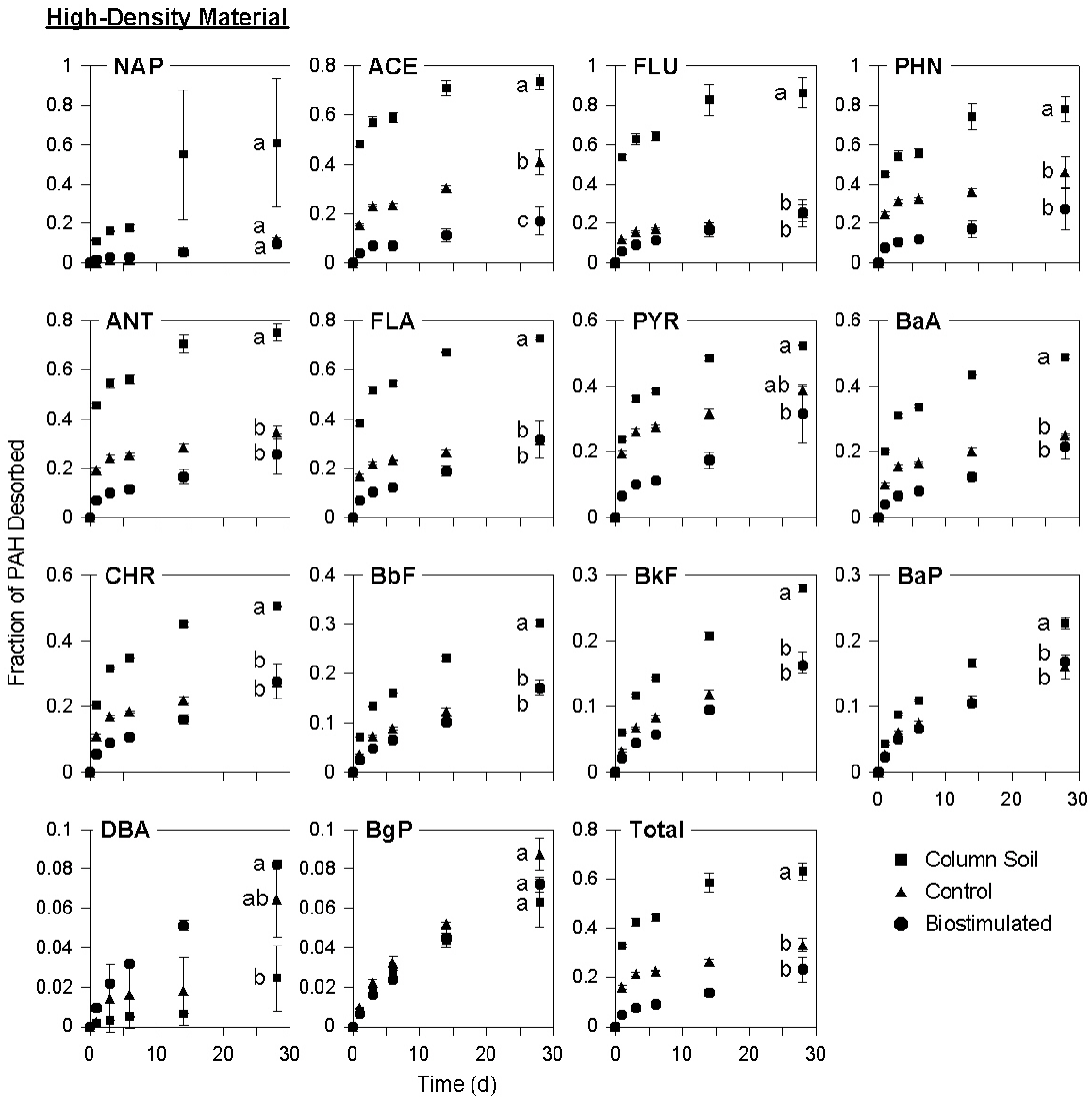

Figure S4. Desorption of PAHs vs. time for the high-density material separated from the original column soil and soil collected from Port A of the control and biostimulated columns at day 593. Notes as in Figure S3.

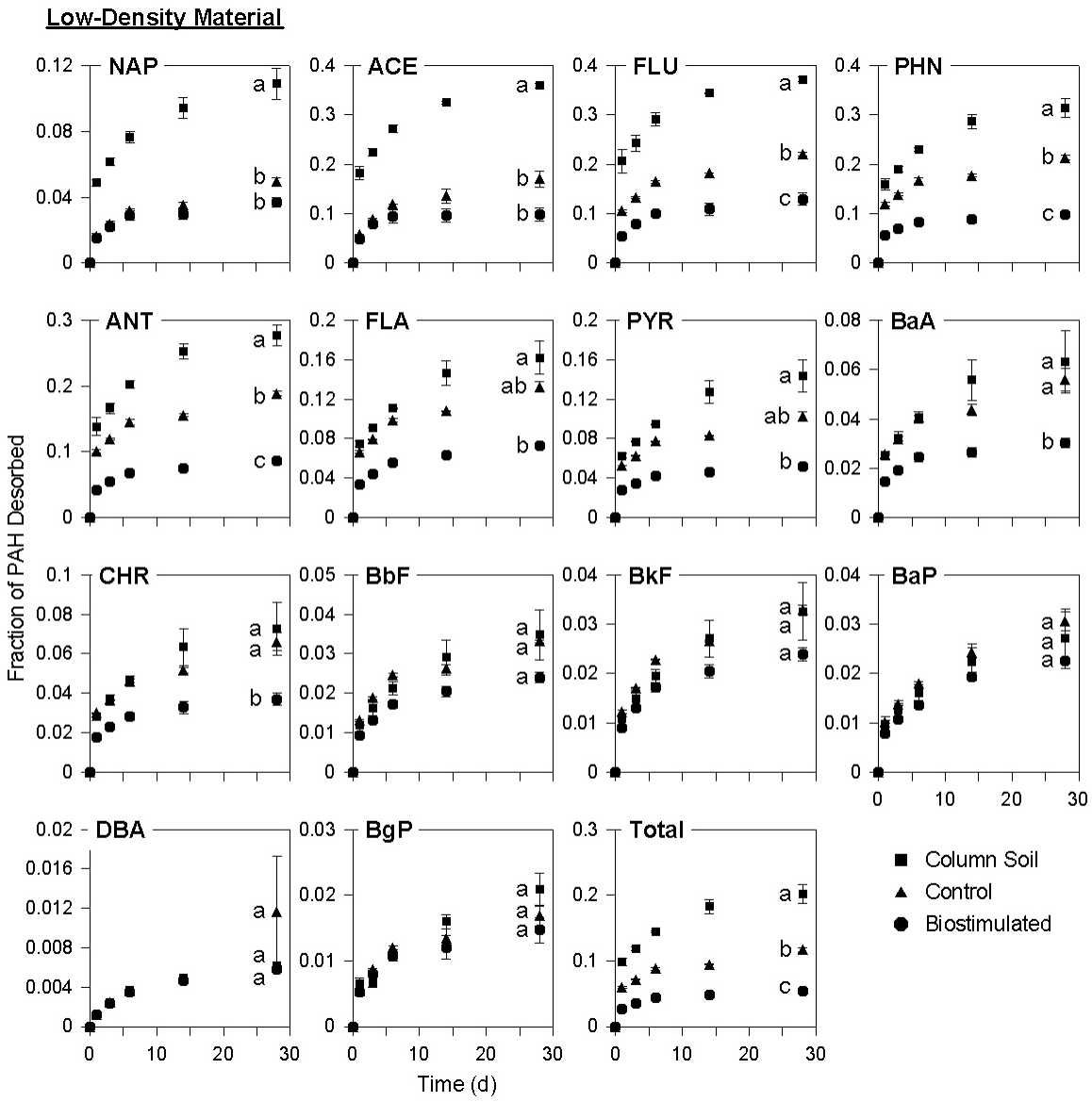

Figure S5. Desorption of PAHs vs. time for the low-density material separated from the column soil and soil collected from Port A of the control and biostimulated columns at day 593. Notes as in Figure S3.

Table S1. Fitted parameter values for the original column soil and soil collected from Port A of the biostimulated and control columns at day 534. [a]

	Fast-desorbing fraction, f			Fast-desorbing rate constant, k_1 (10^{-3}/hr)			Slow-desorbing rate constant, k_2 (10^{-3}/hr)		
Analyte[b]	Column Soil	Control	Biostim.	Column Soil	Control	Biostim.	Column Soil	Control	Biostim.
NAP	0.06 ± 0.01	0 [c]	0 [c]	48.7 ± 28.9	0 [c]	0 [c]	0.26 ± 0.03	0.02 ± 0.001[c]	0.10 ± 0.01[c]
ACE	0.53 ± 0.02	0.85 ± 0.12	0 [c]	98.1 ± 21.5	229 ± 1941	0 [c]	1.15 ± 0.16	1.20 ± 3.10	0.23 ± 0.03[c]
FLU	0.48 ± 0.02	0.45 ± 0.07	0 [c]	94.7 ± 17.7	125 ± 181	0 [c]	0.68 ± 0.09	0.47 ± 0.37	0.18 ± 0.01[c]
PHN	0.43 ± 0.02	0.53 ± 0.06	0 [c]	79.0 ± 14.6	114 ± 91.5	0 [c]	0.46 ± 0.09	0.91 ± 0.40	0.25 ± 0.27[c]
ANT	0.41 ± 0.01	0.55 ± 0.06	0 [c]	85.1 ± 14.2	122 ± 115	0 [c]	0.41 ± 0.06	0.91 ± 0.45	0.33 ± 0.03[c]
FLA	0.34 ± 0.01	0.30 ± 0.04	0 [c]	41.8 ± 5.6	59.2 ± 26.5	0 [c]	0.34 ± 0.06	0.58 ± 0.15	0.57 ± 0.07[c]
PYR	0.32 ± 0.02	0.32 ± 0.04	0 [c]	30.3 ± 5.4	34.2 ± 11.3	0 [c]	0.36 ± 0.08	0.59 ± 0.15	0.52 ± 0.07[c]
BaA	0.21 ± 0.03	0.15 ± 0.03	0 [c]	24.5 ± 8.0	40.0 ± 27.0	0 [c]	0.28 ± 0.08	0.51 ± 0.10	0.37 ± 0.04[c]
CHR	0.23 ± 0.04	0.18 ± 0.04	0 [c]	17.9 ± 5.7	33.2 ± 20.2	0 [c]	0.27 ± 0.10	0.64 ± 0.13	0.61 ± 0.09[c]
BbF	0.14 ± 0.06	0.03 ± 0.02	0 [c]	7.9 ± 4.5	23.2 ± 41.7	0 [c]	0.14 ± 0.12	0.19 ± 0.05	0.29 ± 0.02[c]
BkF	0.13 ± 0.06	0.03 ± 0.03	0 [c]	7.2 ± 4.1	25.3 ± 72.9	0 [c]	0.11 ± 0.11	0.16 ± 0.08	0.17 ± 0.01[c]
BaP	0.13 ± 0.09	0.02 ± 0.02	0 [c]	5.6 ± 4.3	64.3 ± 255	0 [c]	0.07 ± 0.15	0.25 ± 0.04	0.19 ± 0.01[c]
BgP	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0.11 ± 0.007[c]	0.003 ± 0.0002[c]	0.05 ± 0.003[c]
Total	0.33 ± 0.02	0.35 ± 0.04	0 [c]	60.2 ± 11.5	82.1 ± 42.7	0 [c]	0.39 ± 0.07	0.56 ± 0.17	0.30 ± 0.03[c]
3-ring	0.44 ± 0.02	0.53 ± 0.06	0 [c]	81.2 ± 14.5	115 ± 95.5	0 [c]	0.50 ± 0.09	0.89 ± 0.40	0.25 ± 0.02[c]
4-ring	0.30 ± 0.02	0.28 ± 0.04	0 [c]	32.5 ± 6.3	41.8 ± 16.8	0 [c]	0.34 ± 0.07	0.59 ± 0.14	0.52 ± 0.06[c]
5,6-ring	0.11 ± 0.07	0.02 ± 0.02	0 [c]	6.3 ± 4.3	26.4 ± 65.7	0 [c]	0.09 ± 0.11	0.16 ± 0.04	0.16 ± 0.01[c]

[a] Values are presented as the best-fit value ± 95% confidence interval. r^2 values were greater than 0.90 for all model fits, with the exception of BkF (control; r = 0.85).

[b] Abbreviations are defined in the caption to Figure S2.

[c] Regression did not converge on unique values for f, k_1, and k_2, so regression was performed assuming a single desorption rate (*i.e.*, assuming $k_1 = 0$).

Table S2. Fitted parameter values for the high-density material in the original column soil and soil collected from Port A of the biostimulated and control columns at day 593. [a]

	Fast-desorbing fraction, f			Fast-desorbing rate constant, k_1 (10^{-3}/hr)			Slow-desorbing rate constant, k_2 (10^{-3}/hr)		
Analyte[b]	Column Soil	Control	Biostim.	Column Soil	Control	Biostim.	Column Soil	Control	Biostim.
NAP	ND [c]	0 [d]	0.01 ± 0.004	ND [c]	0 [d]	125 ± 403	ND [c]	0.19 ± 0.02[d]	0.13 ± 0.01
ACE	0.55 ± 0.05	0.20 ± 0.03	0.05 ± 0.03	82.8 ± 38.9	59.2 ± 29.5	53.0 ± 104	0.92 ± 0.41	0.45 ± 0.09	0.20 ± 0.07
FLU	0.56 ± 0.13	0.15 ± 0.02	0.07 ± 0.04	105 ± 132	68.1 ± 37.6	46.9 ± 76.6	2.10 ± 1.34	0.20 ± 0.06	0.32 ± 0.10
PHN	0.49 ± 0.10	0.28 ± 0.03	0.08 ± 0.05	91.5 ± 94.6	80.8 ± 47.8	88.7 ± 295	1.50 ± 0.79	0.40 ± 0.14	0.35 ± 0.14
ANT	0.51 ± 0.06	0.23 ± 0.01	0.07 ± 0.04	84.9 ± 48.1	73.1 ± 19.7	75.5 ± 174	1.15 ± 0.44	0.24 ± 0.05	0.32 ± 0.11
FLA	0.49 ± 0.04	0.21 ± 0.01	0.07 ± 0.03	58.2 ± 16.1	64.6 ± 9.7	90.7 ± 254	1.02 ± 0.26	0.21 ± 0.03	0.45 ± 0.10
PYR	0.36 ± 0.03	0.24 ± 0.01	0.07 ± 0.01	42.4 ± 12.0	63.1 ± 10.0	72.6 ± 50.1	0.48 ± 0.14	0.31 ± 0.04	0.34 ± 0.04
BaA	0.30 ± 0.03	0.15 ± 0.01	0.04 ± 0.02	42.2 ± 11.4	44.1 ± 7.5	71.7 ± 124	0.49 ± 0.10	0.19 ± 0.02	0.29 ± 0.05
CHR	0.31 ± 0.03	0.16 ± 0.01	0.06 ± 0.02	41.0 ± 11.8	43.9 ± 7.0	70.2 ± 134	0.53 ± 0.12	0.21 ± 0.02	0.39 ± 0.07
BbF	0.13 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	27.9 ± 8.19	26.2 ± 11.2	35.7 ± 16.8	0.34 ± 0.04	0.18 ± 0.03	0.23 ± 0.01
BkF	0.11 ± 0.01	0.06 ± 0.01	0.03 ± 0.005	27.2 ± 8.44	25.8 ± 11.8	38.5 ± 19.5	0.32 ± 0.04	0.17 ± 0.03	0.22 ± 0.01
BaP	0.08 ± 0.02	0.05 ± 0.01	0.04 ± 0.005	24.7 ± 11.4	25.5 ± 15.5	29.9 ± 9.9	0.26 ± 0.04	0.18 ± 0.03	0.22 ± 0.01
BgP	0.02 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	11.6 ± 21.2	22.6 ± 18.9	11.9 ± 9.3	0.06 ± 0.05	0.11 ± 0.01	0.09 ± 0.01
Total	0.40 ± 0.06	0.20 ± 0.01	0.05 ± 0.02	65.9 ± 39.0	65.8 ± 18.6	73.7 ± 154	0.81 ± 0.32	0.27 ± 0.05	0.31 ± 0.07
3-ring	0.50 ± 0.10	0.27 ± 0.03	0.08 ± 0.04	90.4 ± 85.5	79.1 ± 43.8	83.1 ± 249	1.44 ± 0.75	0.37 ± 0.12	0.34 ± 0.13
4-ring	0.38 ± 0.03	0.20 ± 0.01	0.06 ± 0.03	46.6 ± 12.8	58.0 ± 8.0	101 ± 360	0.59 ± 0.15	0.24 ± 0.02	0.41 ± 0.09
5,6-ring	0.08 ± 0.01	0.05 ± 0.01	0.03 ± 0.004	25.3 ± 10.8	25.9 ± 14.0	30.4 ± 10.2	0.22 ± 0.03	0.16 ± 0.02	0.19 ± 0.01

[a] Values are presented as the best-fit value ± 95% confidence interval. r^2 values were greater than 0.90 for all model fits, with the exception of ACE, PHN, and ANT (biostimulated; r = 0.86, 0.83, and 0.88, respectively).

[b] Abbreviations are defined in the caption to Figure S2.

[c] Values for NAP in the original column soil were not determined due to poor reproducibility between replicates at the 28-d time point.

[d] Regression did not converge on unique values for f, k_1, and k_2, so regression was performed assuming a single desorption rate (*i.e.*, assuming $k_1 = 0$).

Table S3. Fitted parameter values for the low-density material in the original column soil and soil collected from Port A of the biostimulated and control columns at day 593. [a]

	Fast-desorbing fraction, f			Fast-desorbing rate constant, k_1 (10^{-3}/hr)			Slow-desorbing rate constant, k_2 (10^{-3}/hr)		
Analyte[b]	Column Soil	Control	Biostim.	Column Soil	Control	Biostim.	Column Soil	Control	Biostim.
NAP	0.06 ± 0.01	0.02 ± 0.004	0.02 ± 0.01	50.8 ± 31.9	41.4 ± 22.5	34.2 ± 22.1	0.08 ± 0.03	0.04 ± 0.01	0.02 ± 0.01
ACE	0.23 ± 0.03	0.10 ± 0.02	0.09 ± 0.02	56.5 ± 24.2	28.3 ± 17.5	28.4 ± 18.5	0.29 ± 0.09	0.12 ± 0.06	0.01 ± 0.05
FLU	0.25 ± 0.03	0.14 ± 0.01	0.09 ± 0.02	64.6 ± 35.6	54.2 ± 16.3	36.4 ± 21.3	0.29 ± 0.12	0.16 ± 0.03	0.07 ± 0.04
PHN	0.20 ± 0.03	0.14 ± 0.01	0.07 ± 0.01	61.2 ± 37.3	71.5 ± 25.6	56.4 ± 27.6	0.26 ± 0.10	0.13 ± 0.03	0.04 ± 0.02
ANT	0.17 ± 0.03	0.12 ± 0.01	0.06 ± 0.01	59.7 ± 37.3	67.2 ± 23.4	46.9 ± 20.3	0.22 ± 0.09	0.12 ± 0.03	0.05 ± 0.02
FLA	0.09 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	56.8 ± 46.8	63.8 ± 24.5	43.6 ± 15.4	0.13 ± 0.06	0.09 ± 0.02	0.04 ± 0.01
PYR	0.08 ± 0.02	0.06 ± 0.01	0.04 ± 0.005	55.6 ± 49.4	66.5 ± 26.9	51.0 ± 23.3	0.12 ± 0.05	0.06 ± 0.02	0.02 ± 0.01
BaA	0.03 ± 0.01	0.03 ± 0.005	0.02 ± 0.003	43.9 ± 56.3	57.1 ± 31.2	42.2 ± 21.6	0.05 ± 0.03	0.04 ± 0.01	0.01 ± 0.01
CHR	0.04 ± 0.01	0.04 ± 0.004	0.02 ± 0.004	43.8 ± 54.2	61.4 ± 30.4	45.2 ± 26.6	0.06 ± 0.04	0.05 ± 0.01	0.02 ± 0.01
BbF	0.02 ± 0.01	0.02 ± 0.002	0.02 ± 0.003	34.6 ± 45.2	36.4 ± 11.8	31.6 ± 15.2	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
BkF	0.02 ± 0.01	0.02 ± 0.002	0.02 ± 0.003	35.4 ± 46.1	35.6 ± 12.3	29.3 ± 14.7	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
BaP	0.01 ± 0.01	0.01 ± 0.003	0.01 ± 0.002	43.3 ± 69.5	41.6 ± 24.2	36.3 ± 22.8	0.02 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
BgP	0.01 ± 0.00	0.01 ± 0.002	0.01 ± 0.003	79.1 ± 151	25.3 ± 15	27.6 ± 22.6	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Total	0.12 ± 0.02	0.07 ± 0.006	0.04 ± 0.01	58.4 ± 37.5	64.6 ± 23.1	46.8 ± 22.2	0.15 ± 0.06	0.07 ± 0.02	0.03 ± 0.01
3-ring	0.20 ± 0.03	0.14 ± 0.01	0.07 ± 0.01	61.0 ± 36.0	68.5 ± 24.1	51.2 ± 25.4	0.26 ± 0.10	0.13 ± 0.03	0.04 ± 0.03
4-ring	0.07 ± 0.02	0.06 ± 0.006	0.03 ± 0.004	54.0 ± 49.4	64.0 ± 26.2	46.8 ± 20.3	0.10 ± 0.05	0.06 ± 0.02	0.03 ± 0.01
5,6-ring	0.01 ± 0.01	0.01 ± 0.002	0.01 ± 0.002	40.3 ± 57.2	36.4 ± 15.2	31.5 ± 17.1	0.02 ± 0.01	0.02 ± 0.005	0.01 ± 0.01

[a] Values are presented as the best-fit value ± 95% confidence interval. r^2 values were greater than 0.90 for all model fits, with the exception of BaP (column soil; r = 0.86).

[b] Abbreviations are defined in the caption to Figure S2.

Literature Cited

S1. Groves, M.R. Preliminary Report on Groundwater Resources in Rowan County, North Carolina. North Carolina Department of Natural and Economic Resources, 1976.