Supplemental Material

Identification of Anthraquinone-Degrading Bacteria in Soil Contaminated with Polycyclic Aromatic Hydrocarbons

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Chemical Characterization of [U-^{13}C]Anthraquinone

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra are shown in Figs. S1 and S2, respectively. The total ion chromatogram (TIC) and mass spectrum are shown in Fig. S3.

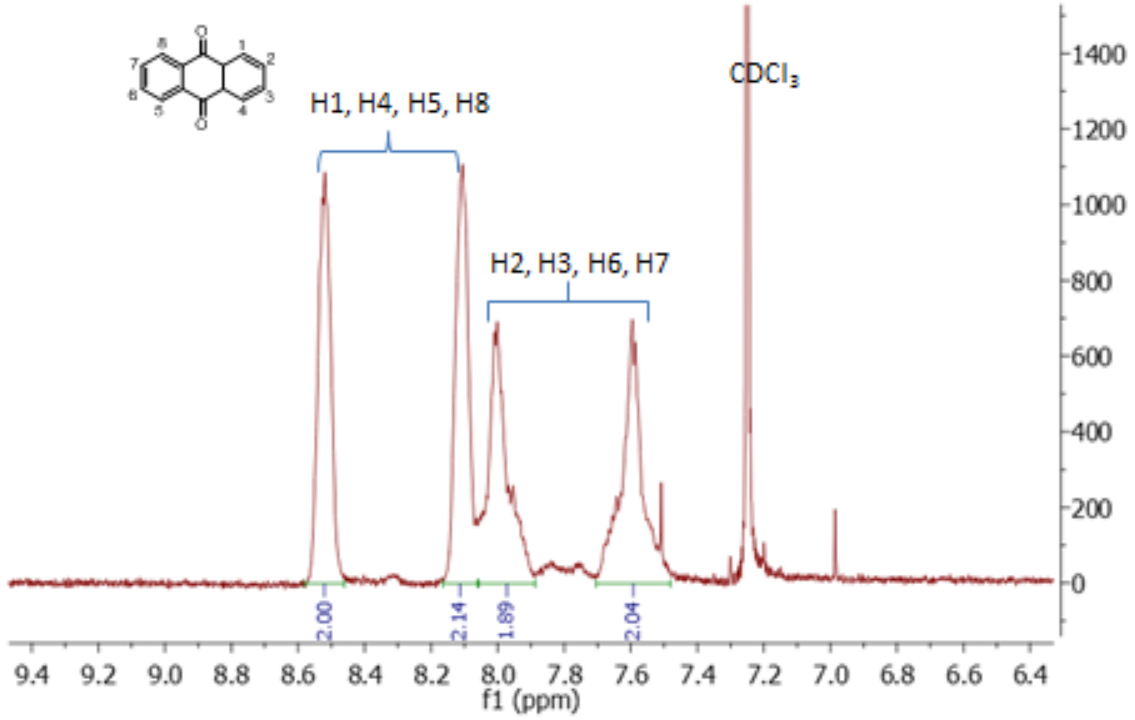

FIG. S1. ^{1}H NMR ($CDCl_3$, 400 MHz) of [U-^{13}C]anthracene-9,10-dione. Assignment based on natural abundance anthracene-9,10-dione. (Data were obtained from the National Institute of Advanced Industrial Science and Technology (Japan))

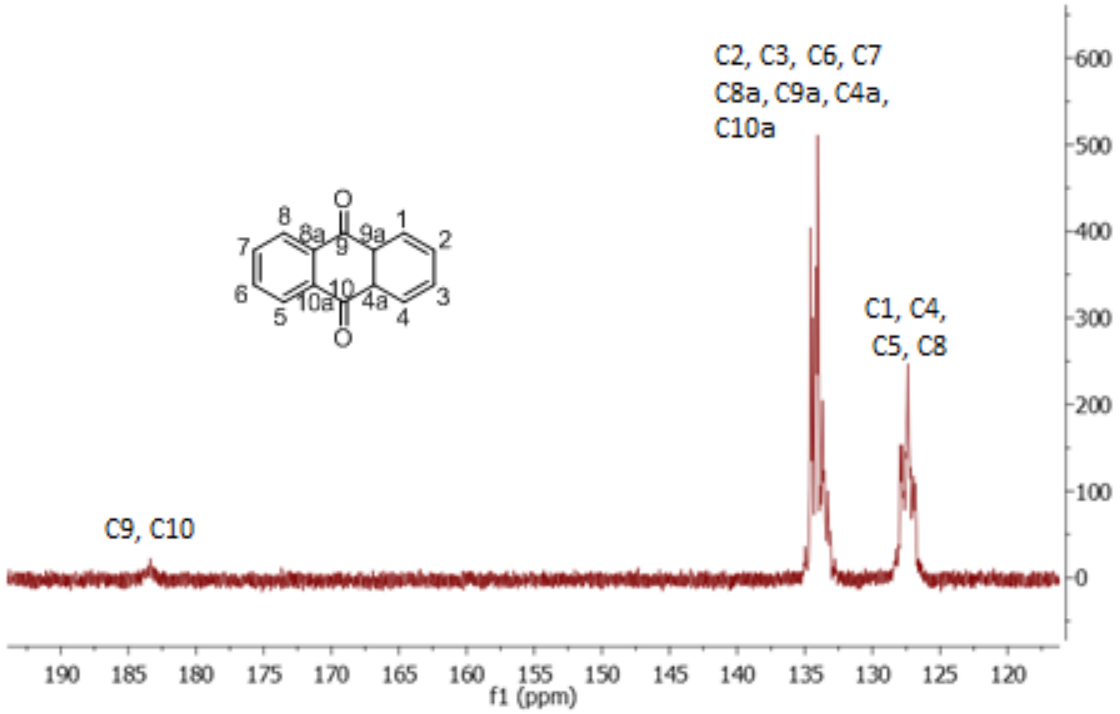

FIG. S2. ^{13}C NMR ($CDCl_3$, 100 MHz) of [U-^{13}C]anthracene-9,10-dione. Assignment based on natural abundance anthracene-9,10-dione (1).

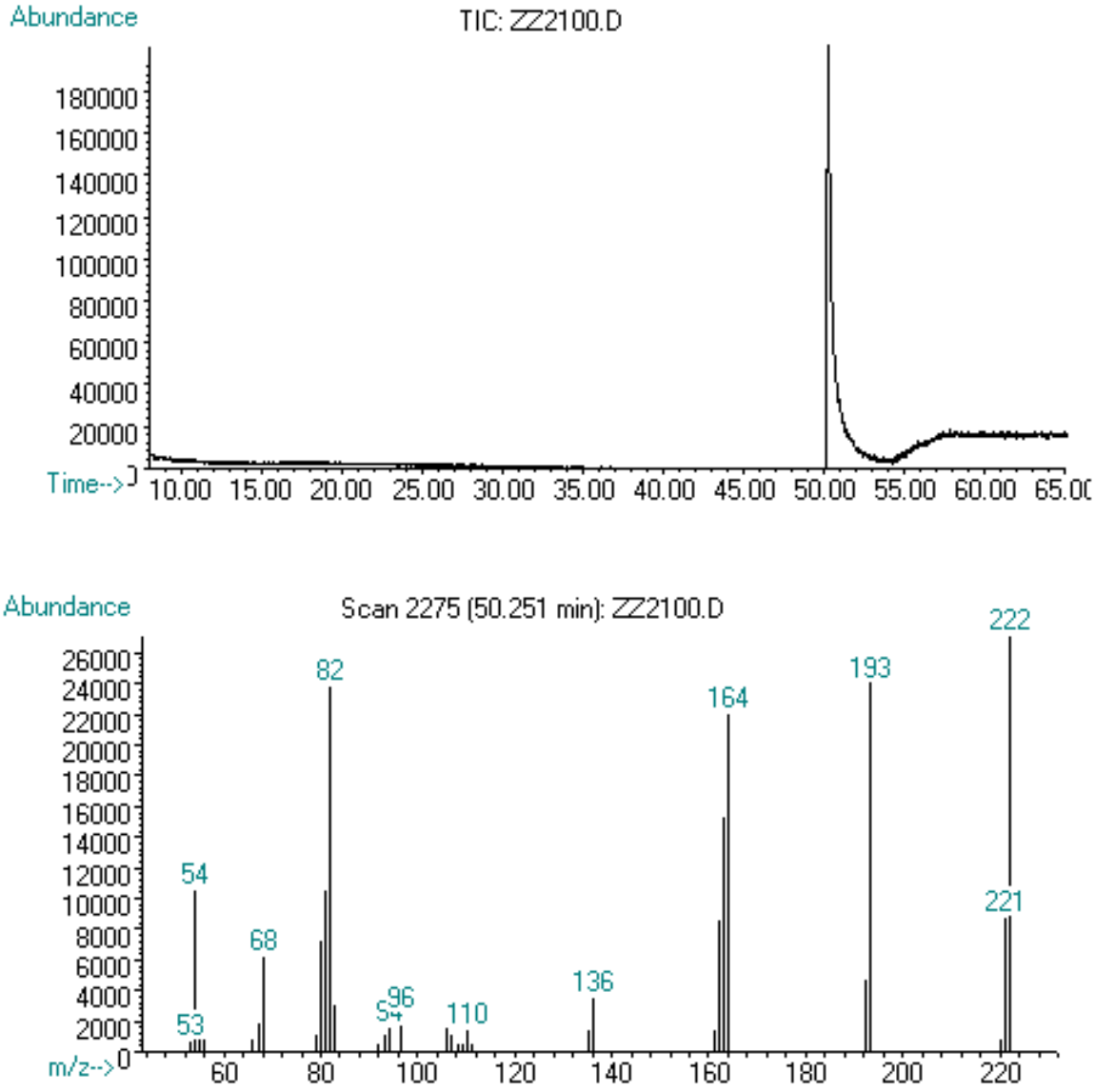

FIG. S3. Total ion gas chromatogram (top) and electron-impact mass spectrum (bottom) of [U-^{13}C]anthracene-9,10-dione.

Substrate Removal in SIP Incubations

Incubations of feed or treated soil samples with unlabeled anthraquinone or anthracene were conducted in parallel with the SIP incubations that contained ^{13}C-labeled substrate. Fig. S4 illustrates removal of most of the spiked substrate over the 20-day incubation period and no change in concentration in acid-inhibited controls.

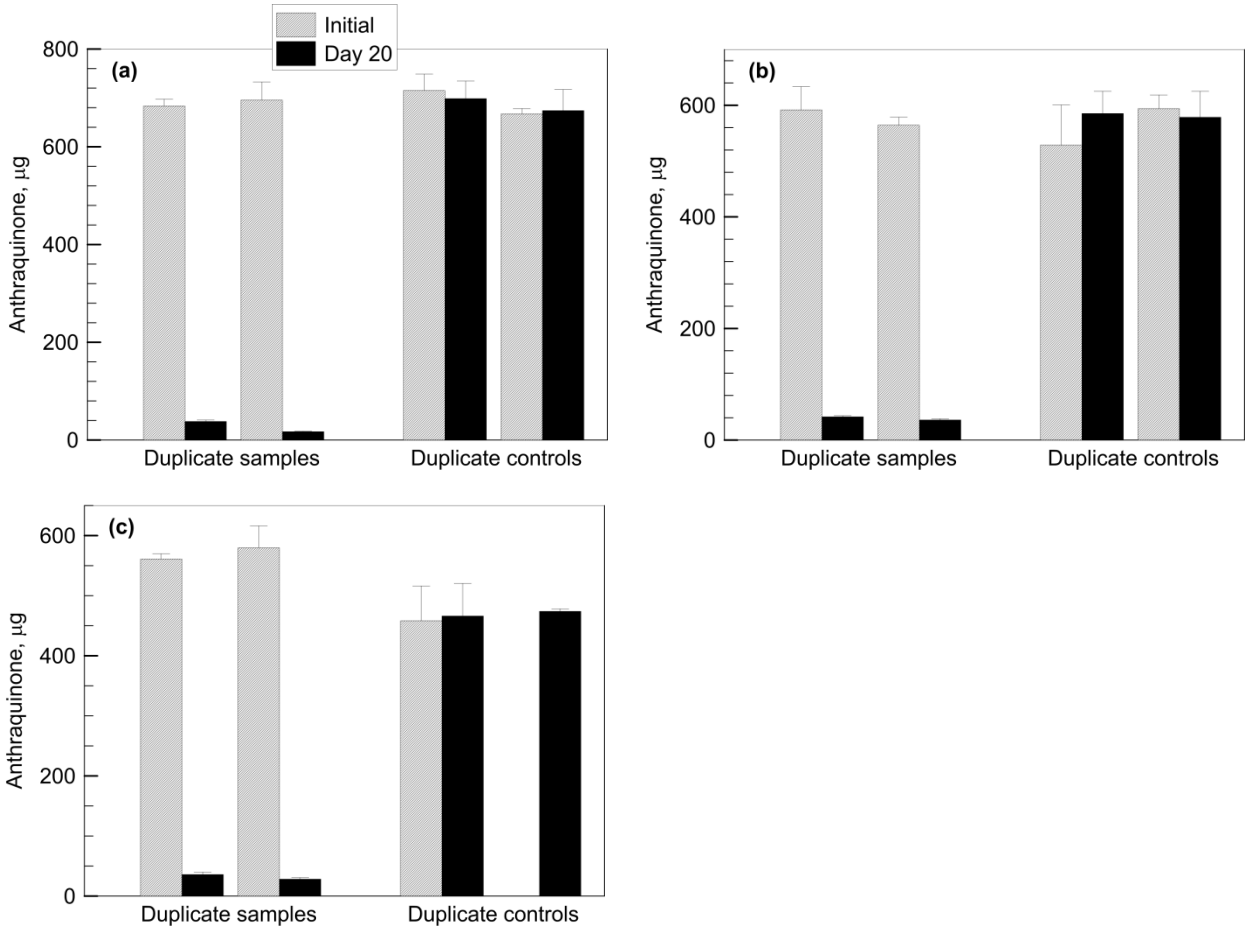

FIG. S4. Initial and final (Day 20) amounts of added substrates in incubations with **(a)** anthraquinone in feed soil, **(b)** anthraquinone in bioreactor-treated soil, and **(c)** anthracene in feed soil, in comparison to acid-inhibited controls. For panel (c), the initial concentration of anthracene was not measured in one of the acid-inhibited controls, so only the final amount is shown. Incubations contained 1 g soil dry weight.

The initial concentration of each substrate shown in Fig. S4 would have included any residual amount of the substrate in the soil before being spiked with unlabeled substrate. Not accounting for removal of either anthracene or anthraquinone during the two-day pre-incubation of each soil sample before spiking the unlabeled substrate, the native concentrations would have been negligible compared to the spiked amount (see Supplementary Table S1 in Hu et el., 2014) (2). In the feed soil, the anthracene and anthraquinone concentrations are approximately 5 and 18 µg/g, respectively. In the bioreactor-treated soil, the anthraquinone concentration is approximately 5 µg/g.

Identification of Heavy Fractions in Ultracentrifuge Tubes from SIP Incubations

Shown below are DGGE images and DNA concentrations for every fraction in each incubation from the SIP experiments. For each combination of substrate (anthraquinone or anthracene) and inoculum (feed soil or bioreactor-treated soil), duplicate incubations were conducted with unlabeled substrate and duplicate incubations were conducted with ^{13}C-labeled substrate. The DGGE image and DNA concentration graph for a given duplicate are paired vertically in each Figure. The fractions selected as containing "heavy" DNA for generating clone libraries are identified for each of the incubations conducted with a ^{13}C-labeled substrate.

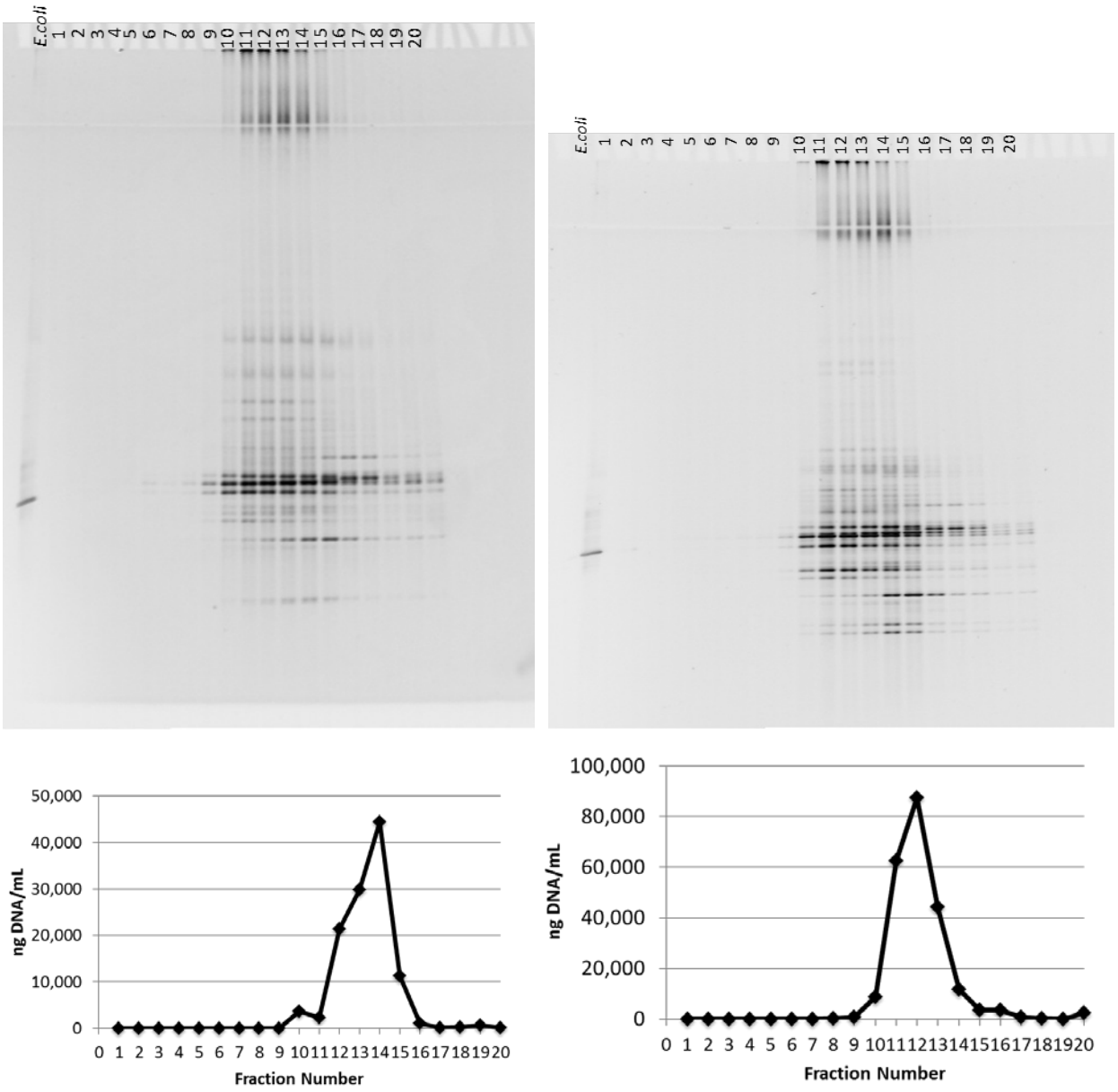

FIG. S5. DGGE reverse image and DNA concentration in each fraction from duplicate incubations of **unlabeled anthraquinone in feed soil**.

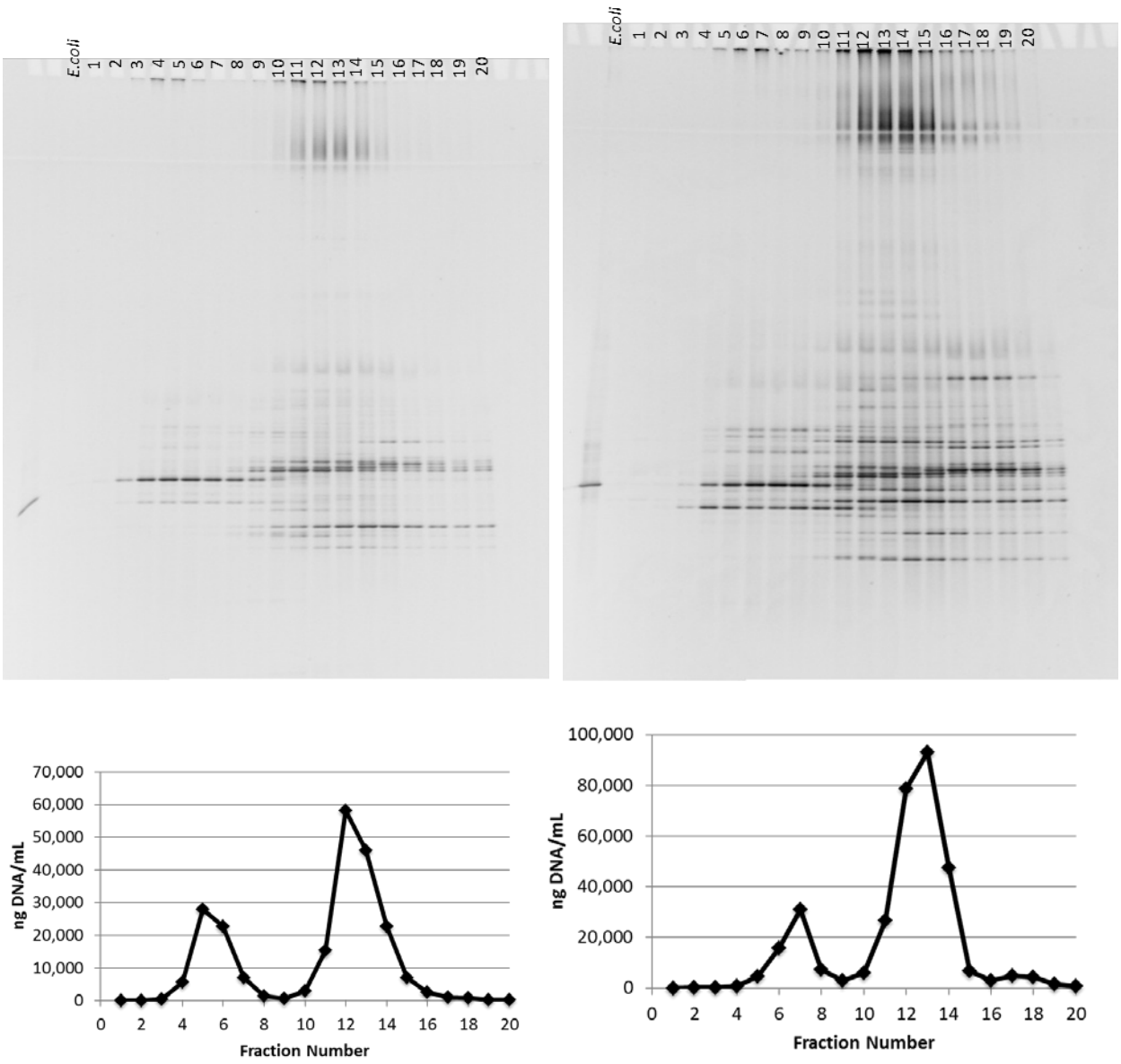

FIG. S6. DGGE reverse image and DNA concentration in each fraction from duplicate incubations of **^{13}C-labeled anthraquinone in feed soil**. From these data, fractions 5, 6, 7, and 8 were selected as the fractions containing ^{13}C-enriched ("heavy") DNA in each of the incubations.

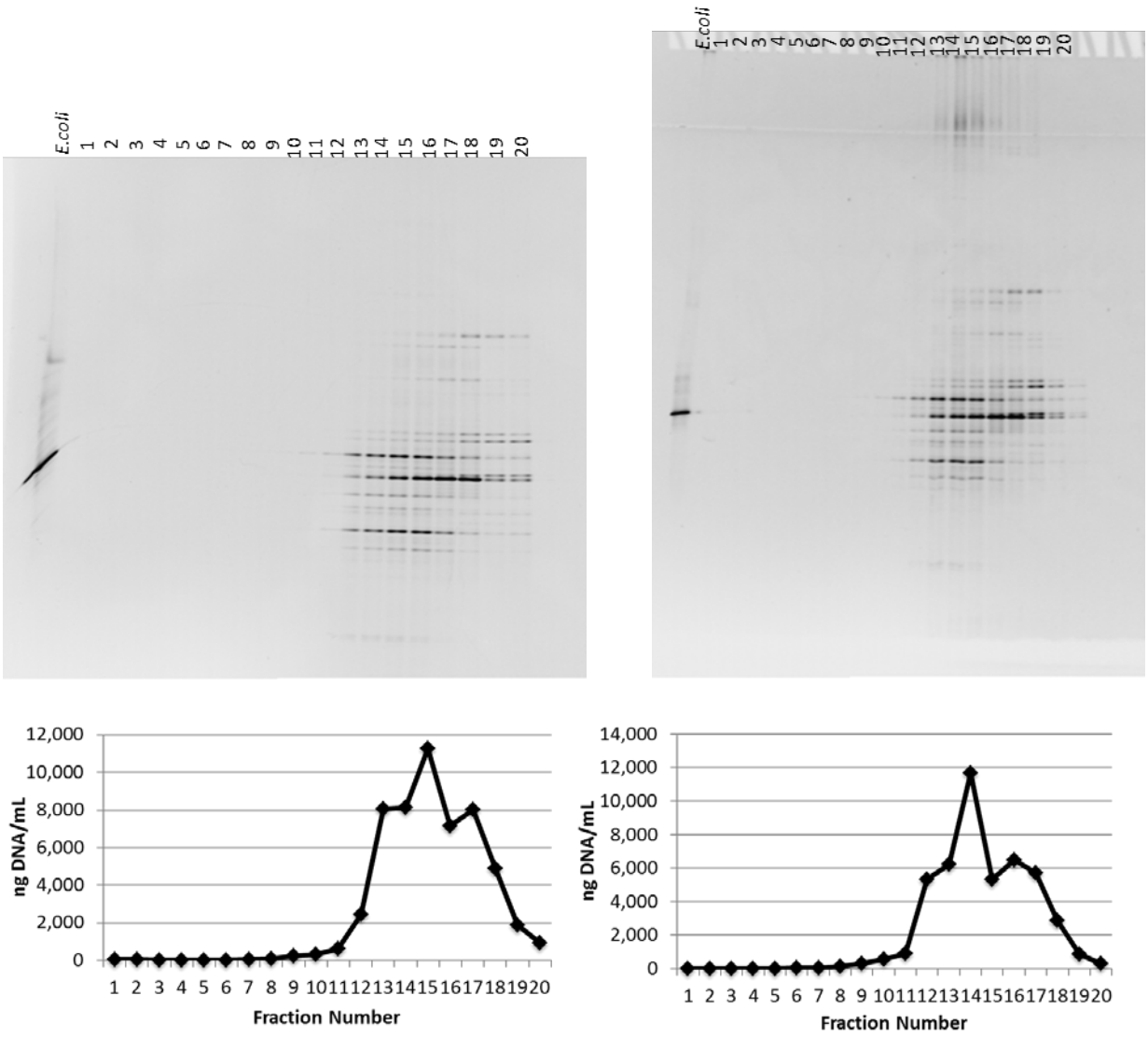

FIG. S7. DGGE reverse image and DNA concentration in each fraction from duplicate incubations of **unlabeled anthraquinone in bioreactor-treated soil**.

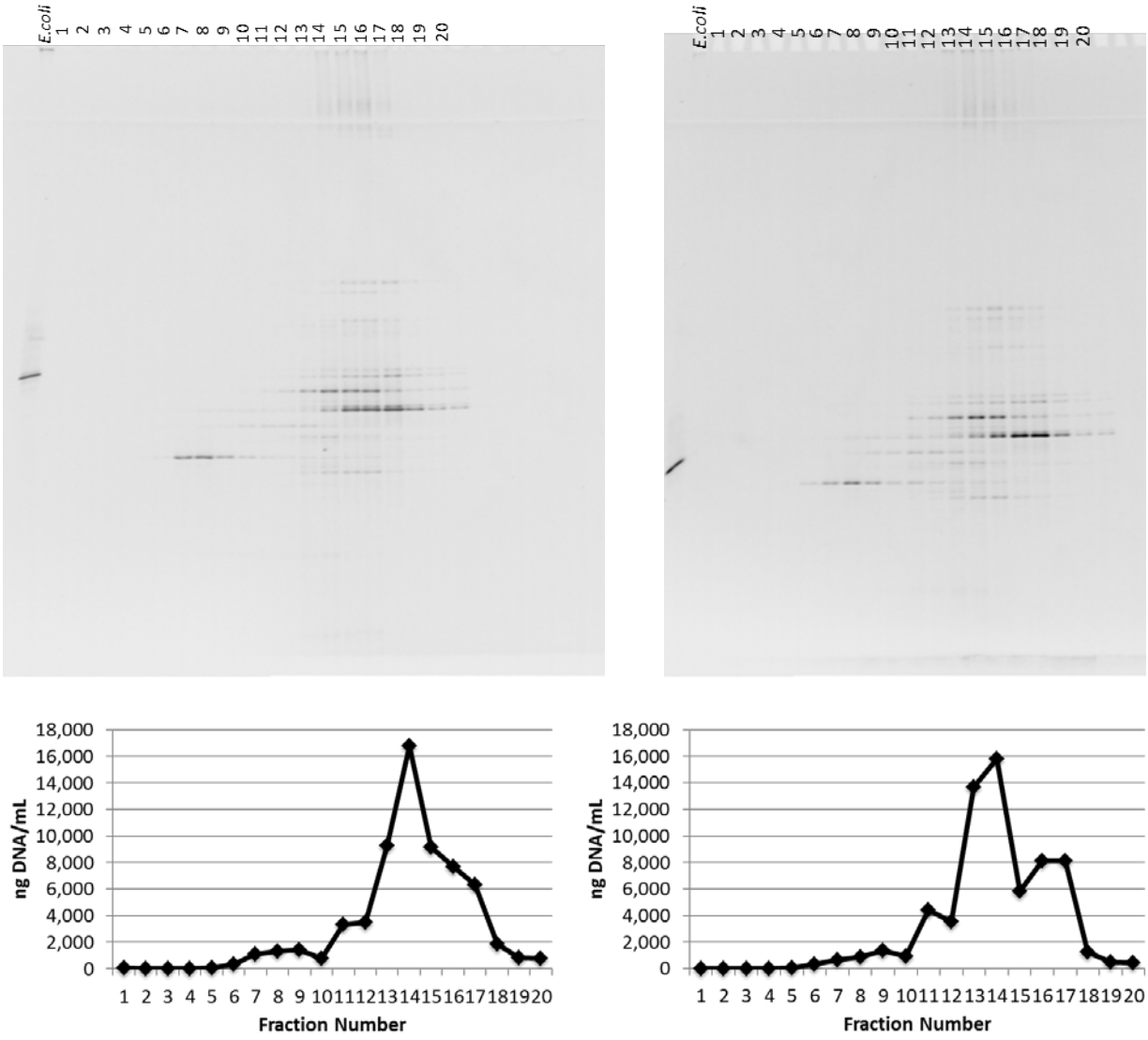

FIG. S8. DGGE reverse image and DNA concentration in each fraction from duplicate incubations of **^{13}C-labeled anthraquinone in bioreactor-treated soil**. From these data, fractions 7,8, and 9 were selected as the fractions containing ^{13}C-enriched ("heavy") DNA in each of the incubations.

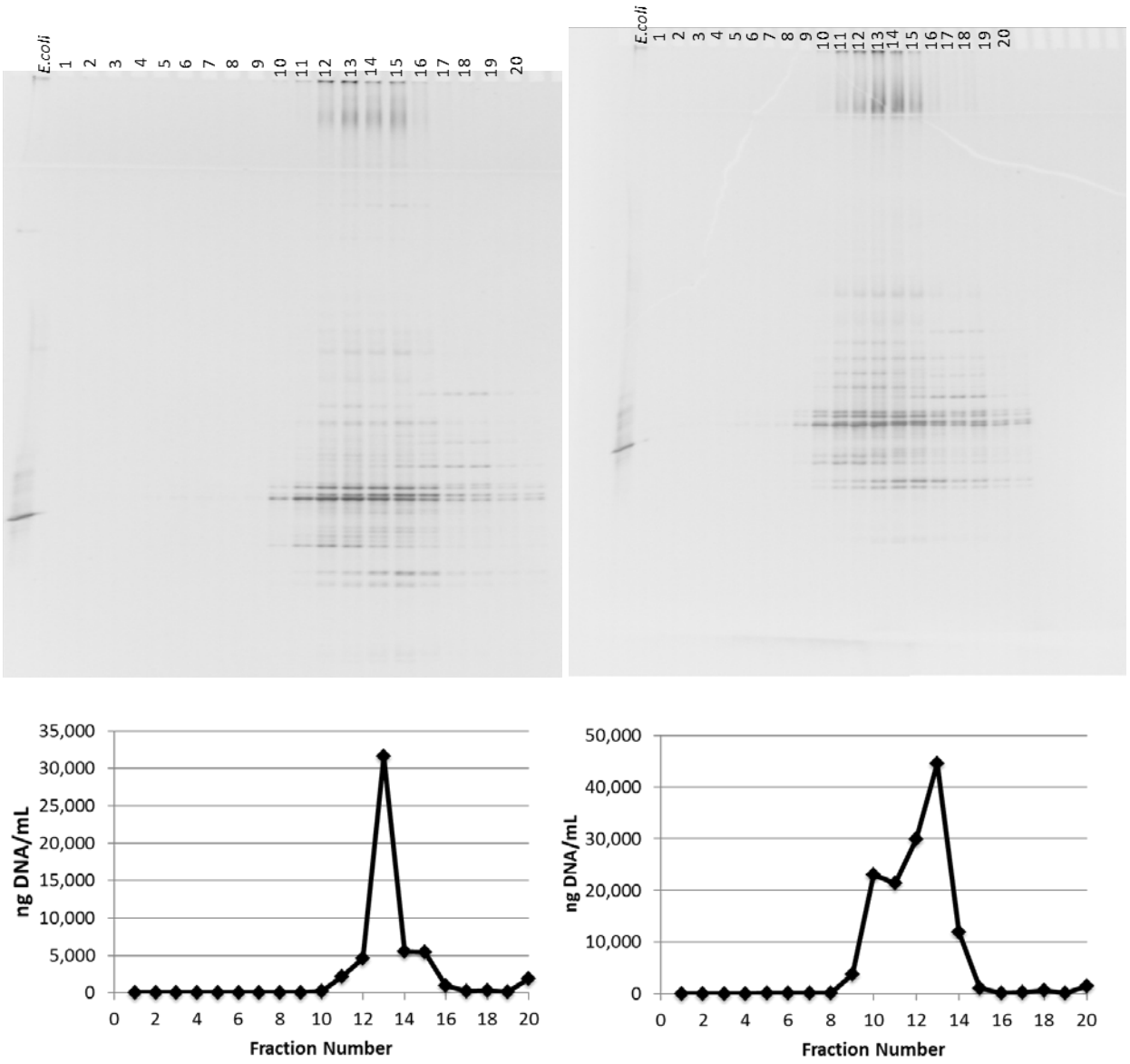

FIG. S9. DGGE reverse image and DNA concentration in each fraction from duplicate incubations of **unlabeled anthracene in feed soil**.

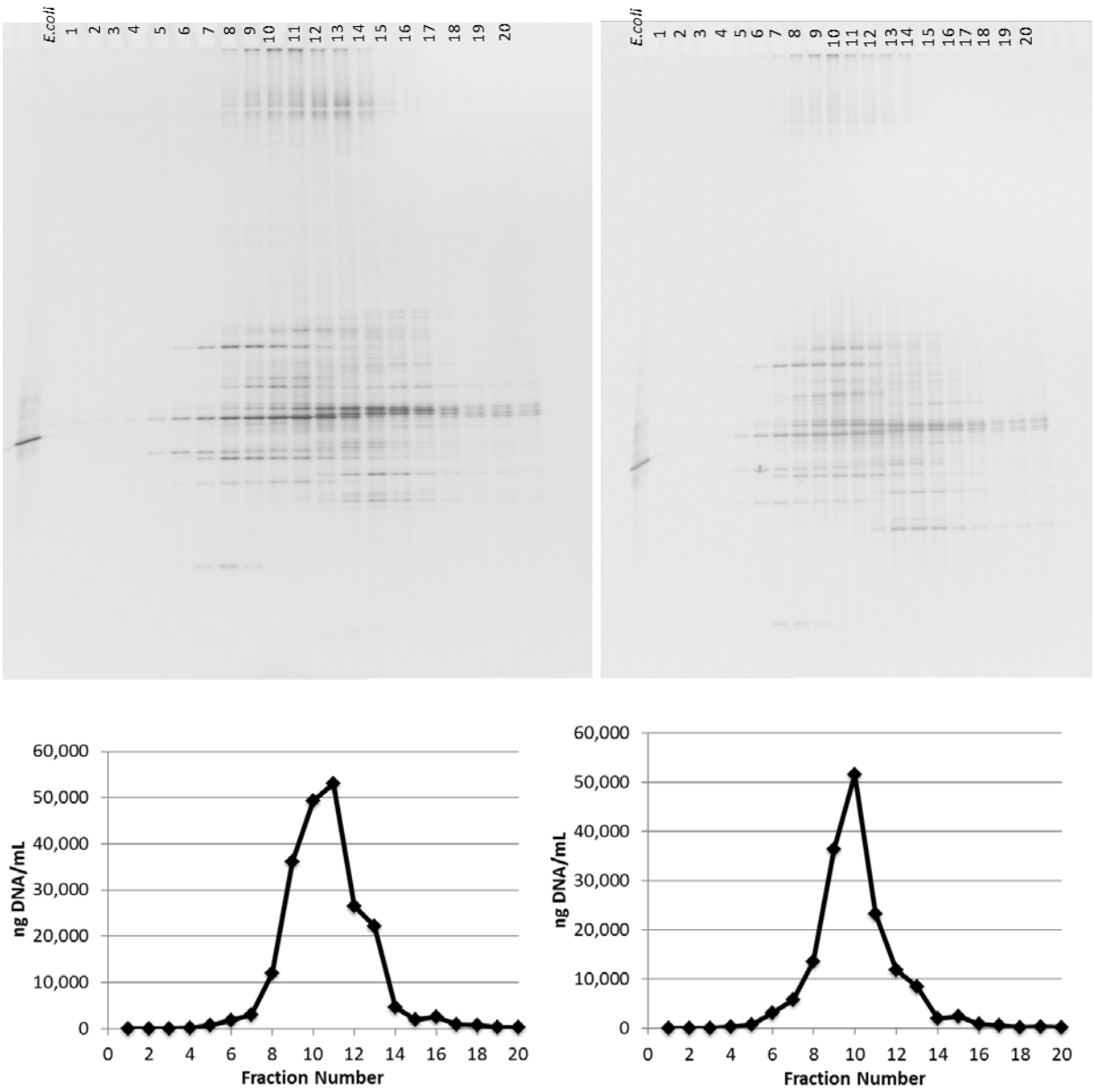

FIG. S10. DGGE reverse image and DNA concentration in each fraction from duplicate incubations of **^{13}C-labeled anthracene in feed soil**. From these data, fractions 5, 6, 7, and 8 were selected as the fractions containing ^{13}C-enriched ("heavy") DNA in each of the incubations.

SIP-Identified Groups

The number of sequences assigned to each group in each of the clone libraries for duplicate incubations with a given ^{13}C-labeled substrate are summarized in Table S1.

TABLE S1. Summary of clone library results

	Number of clones in library	
Substrate and soil sample	**Duplicate 1**	**Duplicate 2**
Anthracene, feed soil		
Pyrene Group 2	19	19
Anthracene Group 1	9	6
Variovorax	5	8
Bradyrhizobium	0	4
Pigmentiphaga	2	0
unclassified	2	0
Anthraquinone, feed soil		
Phenylobacterium	41	46
Anthraquinone, treated soil		
Sphingomonas	38	39
Phenylobacterium	2	2
Sphingomonadaceae	3	0

Each major group associated with assimilation of ^{13}C from incubations with [U-^{13}C]anthraquinone or [U-^{13}C]anthracene was quantified in the ultracentrifuge fractions collected from incubations with ^{13}C-labeled substrates and incubations with unlabeled substrates. Comparisons between the incubations with ^{13}C-labeled substrate and the corresponding unlabeled substrate are shown in Fig. S11.

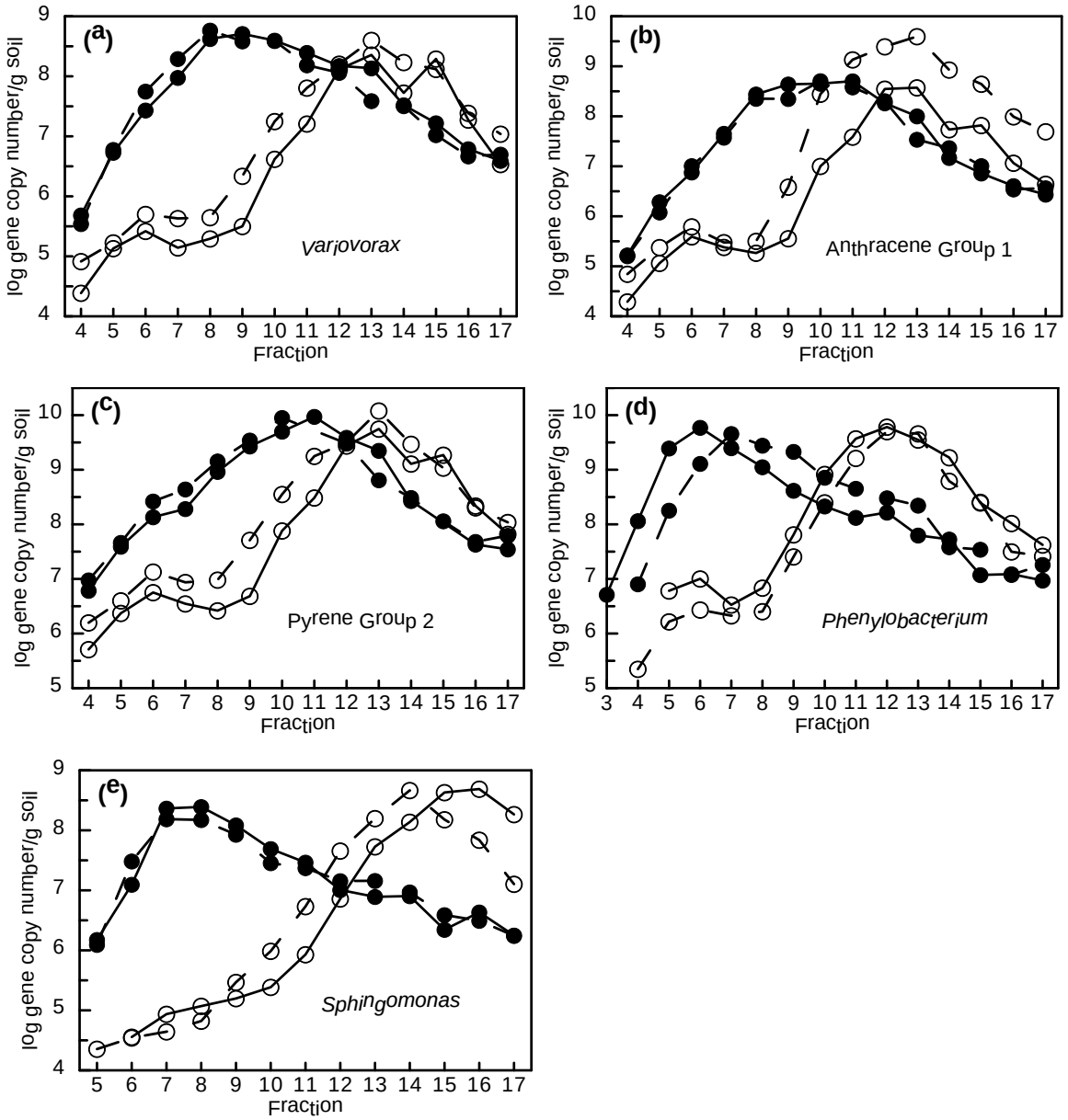

FIG. S11. Quantification of major anthracene- or anthraquinone-degrading groups in ultracentrifuge fractions for incubations with ^{13}C-labeled substrates (closed symbols) or unlabeled substrates (open symbols). Data from each duplicate incubation with the respective substrate are shown. **(a)** – **(c)** Incubations with anthracene in feed soil; **(d)** incubations of anthraquinone in feed soil; **(e)** incubations with anthraquinone in treated soil. For panels (a) – (d), the "heavy" fractions used to create clone libraries were 5, 6, 7 and 8. For panel (e) the heavy fractions used to create clone libraries were 7, 8, and 9.

Pyrosequencing Results.

The representation of major SIP-identified groups in pyrosequencing libraries from the present study and from Singleton et al (3) is summarized in Table S2. The large change in abundance of *Phenylobacterium* sequences in feed soil between 2010 and 2013 is striking. Although the DNA was extracted from the feed soil samples using different kits (FastDNA Spin Kit for Soil in 2010 and PowerSoil DNA Extraction Kit in 2013), it is likely that the large difference in *Phenylobacterium* sequence abundance between samples is due to growth during long-term storage of the soil.

TABLE S2. Percent relative abundances of sequences representing the major anthraquinone and anthracene degraders in pyrosequencing libraries of bioreactor-treated soil and untreated feed soil samples.

	Treated Soil Samples				Feed Soil Samples	
Genus or Group	**June 2012 [a]**	**April 2013 [a]**	**2010 Weekly [b]**	**2010 Monthly [b]**	**2010 [b]**	**2013 [a]**
Phenylobacterium [c]	5.5	8.2	0.9	0.8	0	17.1
Sphingomonas [c]	0.1	0	0.4	0.6	0	0
Pyrene Group 2 [d]	1.1	0.6	6.1	4.0	0.1	0
Anthracene Group 1 [d]	0.1	0.1	0.3	1.1	10.0	2.6
Variovorax [d]	0.1	0	0	0.1	0.9	0
Total # sequences	1,805	4,461	10,415	4,621	9,441	7,092

[a] Samples from this study.

[b] From Singleton et al., 2011 (3). In that previous study, two bioreactors were operated in parallel, each with an effective residence time of 35 days; in one reactor 20% of the slurry volume was removed and replaced with feed soil slurried in reactor buffer every week ("weekly-fed" reactor; same operating mode as in the present study) and in the second reactor 80% of the contents was removed and replaced every 28 days ("monthly-fed" reactor). Samples from the previous study were obtained 140 days after a common startup period for the two reactors.

[c] Identified as anthraquinone-degrader in feed soil (*Phenylobacterium*) or treated soil (*Sphingomonas*).

[d] Identified as anthracene-degrader in feed soil.

REFERENCES

1. **Sieckmann, R.** 1991. ^{13}C NMR study of derivatives of dibenzobarrelene, anthraquinone and anthracene. Magn. Reson. Chem. **29:**264-266.
2. **Hu, J., A. C. Adrion, J. Nakamura, D. Shea, and M. D. Aitken.** 2014. Bioavailability of (geno)toxic contaminants in polycyclic aromatic hydrocarbon–contaminated soil before and after biological treatment. Environ. Eng. Sci. **31:**176-182.
3. **Singleton, D. R., S. D. Richardson, and M. D. Aitken.** 2011. Pyrosequence analysis of bacterial communities in aerobic bioreactors treating polycyclic aromatic hydrocarbon-contaminated soil. Biodegradation **22:**1061-1073.