
Supporting Information

Insight into citric acid-enhanced abiotic degradation of benzo[a]pyrene: transformation of iron species and hydroxyl radical production

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Text S1. Chemicals

Benzo(a)anthracene (BaA, ≥98.0%), Benzo(a)pyrene (BaP, ≥95.0%), Indeno(1,2,3-cd)pyrene (IcdP, ≥95.0%) and Dibenzo(a,h)anthracene (DaA, ≥95.0%) were obtained from Aladdin Biochemical Technology Co., Ltd. Citric acid (CA, ≥97.0%), Oxalic acid (OA, ≥97.0%), Malic acid (MA, ≥97.0%) and Tartaric acid (TA, ≥97.0%) were all purchased from Aladdin Biochemical Technology Co., Ltd. All solvents (HPLC grade) used for sample processing and analysis, including dichloromethane, n-hexane, methanol and acetonitrile, were purchased from Sigma-Aldrich Corporation. Sodium bicarbonate ($NaHCO_3$, ≥98%), sodium hydroxide (NaOH, analytical grade), hydrochloric acid (HCl, ≥98%), phosphoric acid (H_3PO_4, ≥98%), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, ≥98%), 2,2′-bipyridine (BPY, ≥99%), were obtained from Shanghai Macklin Biochemical Co., Ltd. Potassium dichromate ($K_2Cr_2O_7$, ≥98%), sulfuric acid (H_2SO_4, analytical grade) ferrous sulfate ($FeSO_4$, ≥98%) were obtained from Shanghai Pharmaceutical Group Chemical Reagents Co., Ltd.

Text S2. Methods of soil physicochemical properties

Soil organic matter (SOM) was measured using $K_2Cr_2O_7$ oxidation external heating method. First, 1.0 g of soil was placed into a 50 mL centrifuge tube. Next, 5 mL of $K_2Cr_2O_7$ solution and 5 mL of H_2SO_4 were added to the tube to facilitate mixing. The mixture was then placed in a 100 °C incubator and heated for 90 minutes. After this, the volume was adjusted to 50 mL by adding distilled water. The sample was subsequently centrifuged, and the supernatant was collected. The absorbance of the supernatant was measured at a wavelength of 590 nm using a UV-vis spectrophotometer[1].

Soil Electrical Conductivity (EC) was measured using water extraction method. Both oven-dried and air-dried soil samples were first sieved through a 2-mm mesh. Exactly 50.00 ± 0.01 g of each soil sample was weighed into a wide-mouth bottle. Then, 250 mL of CO_2-free deionized water was added to the bottle, which was immediately sealed to prevent atmospheric CO_2 absorption. The mixture was vigorously shaken for 3 minutes to ensure complete soil-water interaction. After shaking, the suspension was rapidly filtered through a Buchner funnel under vacuum to obtain a clear extract. The filtrate was promptly analyzed for electrical conductivity using a calibrated conductivity meter at room temperature (25 ± 1°C). All extractions were performed in triplicate to ensure reproducibility.

Text S3. Purification method of soil samples

Purification column was used to purify the extract, the column was pre-washed with 10 mL n-hexane and dichloromethane, respectively. The leaching rate was controlled at 2 mL/min. Before the anhydrous sodium sulfate at the top was exposed to air, the polytetrafluoroethylene piston at the bottom of the chromatographic column was closed and the effluent was discarded. To purify the extract, about 1 mL of the concentrated extract was transferred to the chromatographic column, and the concentrator was washed three times with 2 mL n-hexane. All the washing liquid was transferred to the chromatographic column, and the target substance was eluted with 25 mL n-hexane solution before the anhydrous sodium sulfate at the top was exposed to the air. The eluent was collected in the concentrator. The eluent was concentrated to about 1 mL by nitrogen blowing enrichment method, added to about 3 mL acetonitrile, and then concentrated to less than 1 mL, the solvent was completely converted to acetonitrile, and the volume was determined to be 1 mL to be measured.

Text S4. Quantification of ·OH

At the predetermined time intervals, 0.5 g of soil was taken into a 50 mL centrifuge tube and 10 mL methanol was added to extract 7 - hydroxycoumarin (7 - COU). The sample was ultrasound and centrifuged at 3000 r/min for 5 min, the supernatant was collected into a nitrogen blow tube, nitrogen was blown to near dryness and then 1 mL of methanol was added. The supernatant was filtered through 0.22 μm filter membrane. The filtered supernatant was injected into a liquid phase vial to determine the concentration of 7 - COU. The instrument of HPLC (Thermo Fisher Ultra 3000) equipped with a ultraviolet detector was used to analyze 7-COU at λ = 228 nm. The mobile phase was a mixture of aqueous phosphate and acetonitrile (v/v = 85:15) at a flow rate of 1.0 mL/min [2].

Text S5. Electron paramagnetic resonance (EPR) analysis

The electron paramagnetic resonance (EPR) spectra were collected using a Bruker EMX/plus EPR spectrometer (E500-9.5/12, Germany). The parameters of the EPR spectrometer were set as: center field at 3510 G, X-band microwave frequency of 9.85 GHz, microwave power of 1.70 mW, spectral window of 100 G, modulation amplitude of 1.00 G, modulation frequency of 100 kHz, time constant of 163.84 ms, conversion time 40.00 ms, sweep time 40.96 s and 5 times of X-scans.

Text S6. Determination of dissolved and adsorbed Fe in soil

At predetermined time intervals, 0.5 g of soil was taken into 5 mL centrifuge tube, 4 mL of deionized water was added. The sample was shaken for 30 min and centrifuged at 3000 r/min for 5 min. The supernatant was collected to obtain dissolved Fe. The residual soil was treated with 3 mL H_2SO_4 and 1 mL HF and digested at 120 °C for 30 min,and then 1 mL H_3BO_4 solution was added to stand for 10 min. The sample was filtered to obtain adsorbed Fe. The dissolved Fe and adsorbed Fe were determined by 1,10-o-phenanthroline colorimetric method [3].

Text S7. Cyclic voltammetry (CV) test

Cyclic voltammetry (CV) was performed on a computer-controlled CHI-660C electrochemical workstation using a three-electrode cell, using a platinum electrode as the working electrode, an Ag/AgCl electrode (saturated with KCl) as the reference electrode, and a saturated calomel reference electrode (SCE) as the auxiliary electrode between −0.10 and +1.30V. CV were recorded at a scan rate (v) of 50 mV/s in a 0.05 M KCl solution (pH 6) containing 20 mM of Fe(II) or Fe(II)-CA. These electrolyte solutions were purged with argon for 30 min for removal of dissolved oxygen before starting the measurements.

Text S8. Determination of the intermedidates of BaP

To analyze the possible intermediates of BaP degradation, the sampled solution was measured by Ultra-High Performance Liquid Chromatography coupled with Quadrupole-Orbitrap High-Resolution Mass Spectrometry (UHPLC-Q-Orbitrap HRMS, Thermo Scientific, USA) with a Hypersil GOLD C18 column (150 × 2.1 mm, 3 μm). The following chromatographic conditions were used: flow rate of 0.3 mL/min, injection volume of 5 μL, mobile phase A consisting of a 0.1 % formic acid aqueous solution, and mobile phase B consisting of acetonitrile. The gradient elution program started with 10 % mobile phase B for 1 min, followed by a linear increase to 99 % mobile phase B over 3 min, and then returned to the initial ratio and maintained for 6 min. The mass spectrometry conditions included a mass scanning range of 50–800 m/z in the positive mode, with a source temperature of 350 °C and a sheath gas flow rate of 12 L/min [4].

Table S1. Physicochemical Properties of the Soil in this Study.

Parameter	Soil-1	Soil-2	Soil-3	Soil-4	Soil-5	Soil-6	Soil-7
Location	Hainan	Chongqing	Jilin	Hebei	Heilongjiang	Yunnan	Liaoning
pH	6.89	5.5	6.24	5.74	5.09	8.45	7.62
Organic matter content (g/kg)	15.96	11.20	39.68	25.71	22.75	172.5	104.3
Fe $(II)_{dis}$ (g/kg)	1.354	0.794	0.421	0.040	0.026	0.004	1.232
Fe $(II)_{ad}$ (g/kg)	2.804	2.173	1.921	1.139	0.320	0.250	1.275
EC (us/cm)	66.70	22.18	122.75	107.10	22.75	172.50	104.3

Table S2. Physicochemical properties of the selected LMWOAs in this study.

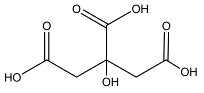
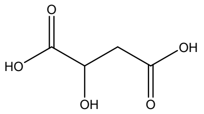
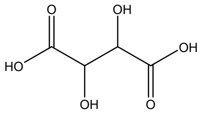
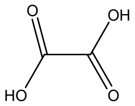
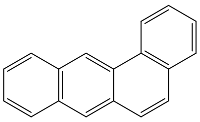
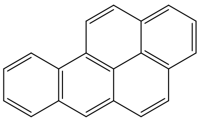
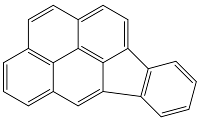
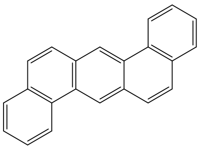
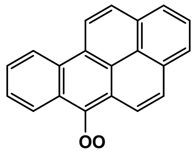
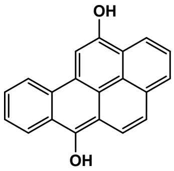
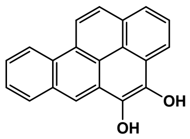
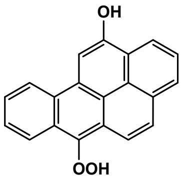
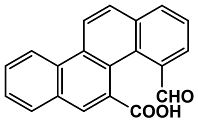
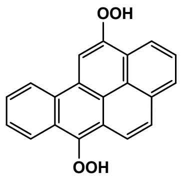
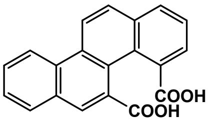
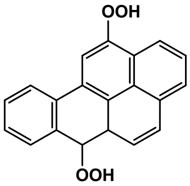
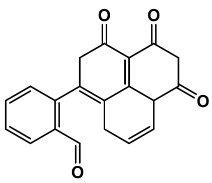
	Name	Structure	α-OH	-COOH
LMWOAs	Citric acid (CA)		1	3
	Malic acid (MA)		1	2
	Tartaric acid (TA)		2	2
	Oxalic acid (OA)		2	2

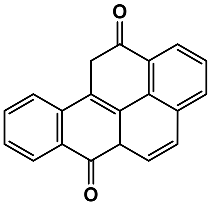
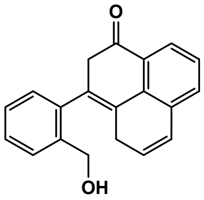
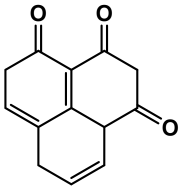
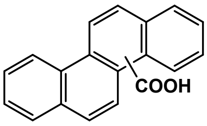
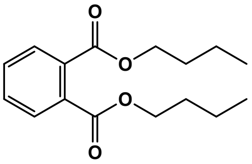
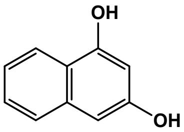
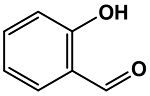
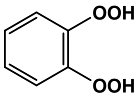
Table S3. Physicochemical properties of the selected PAHs in this study.

	Name	Structure	Formula	IP (ev)[c]
PAHs	Benzo(a)anthracene (BaA)		$C_{18}H_{12}$	7.45
	Benzo(a)pyrene (BaP)		$C_{20}H_{12}$	7.12
	Indeno(1,2,3-cd)pyrene (IcdP)		$C_{22}H_{12}$	7.84
	Dibenzo(a,h)anthracene (DaA)		$C_{22}H_{14}$	7.40

The relative information of PAHs were obtained from the National Insti tute of Standards and Technology (NIST) Chemistry Web Book (http://webbook.nist.gov/chemistry/ion-ser.html).

Table S4. Identified degradation products of BaP degradation by the CA group.

Products NO.	RT (min)	m/z (M+H)	Chemical Formula	Structural Formula	NL
P1	5.82	284.0832	$C_{20}H_{11}O_2$		2.38E5
P2	7.23	285.0910	$C_{20}H_{12}O_2$	 	1.55E7
P3	6.53	301.0859	$C_{20}H_{12}O_3$	 	3.60E5
P4	5.25	317.0808	$C_{20}H_{12}O_4$	 	9.01E4
P5	7.91	319.0965	$C_{20}H_{14}O_4$	 	5.60E5

P6	7.23	283.0744	$C_{20}H_{10}O_2$		3.1E5
P7	5.41	275.1067	$C_{19}H_{14}O_2$		5.60E4
P8	4.92	215.0703	$C_{13}H_{10}O_3$		1.31E5
P9	5.41	273.0910	$C_{19}H_{12}O_2$		1.08E4
P10	8.97	279.1591	$C_{16}H_{22}O_4$		7.71E6
P11	5.14	161.0597	$C_{10}H_8O_2$		1.64E5
P12	2.00	123.0441	$C_7H_6O_2$		4.34E5
P13	1.40	143.03389	$C_6H_6O_4$		8.45E5

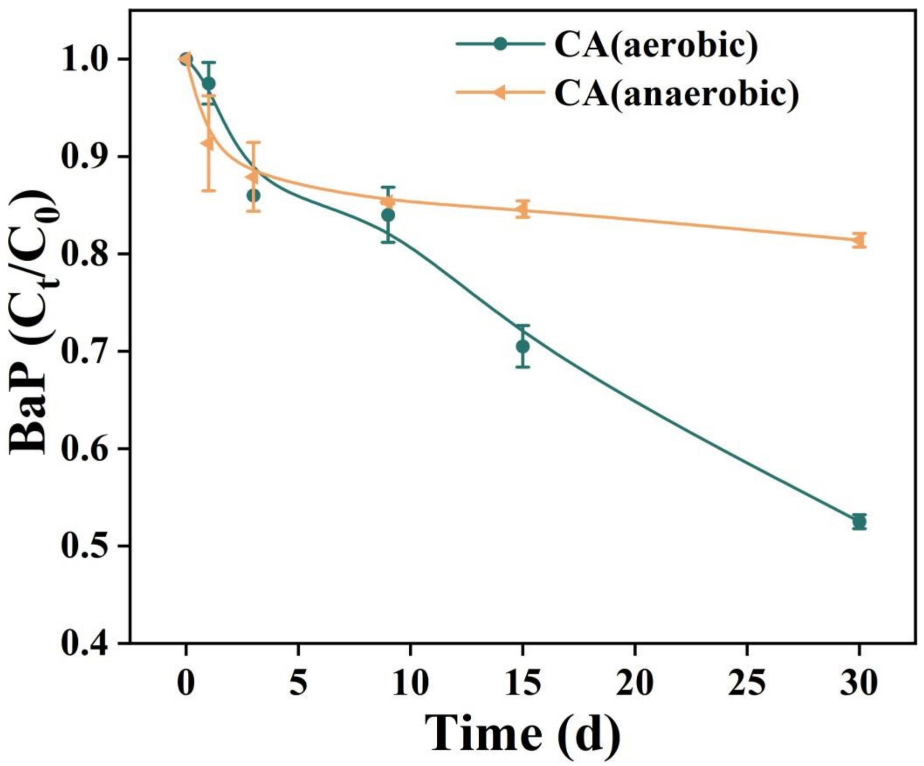


Figure S1. Degradation rates of BaP with N_2 in sterilized soil. Conditions: T = 25℃, RH ~ 50 ± 5%, initial soil pH = 7.0, [CA] = 40 mM.

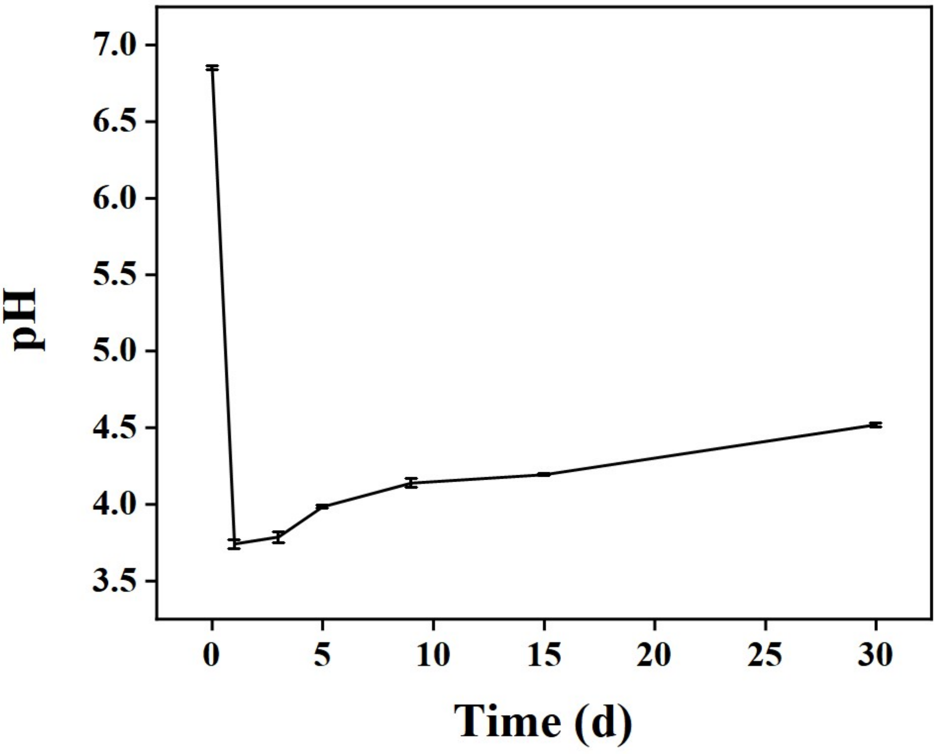
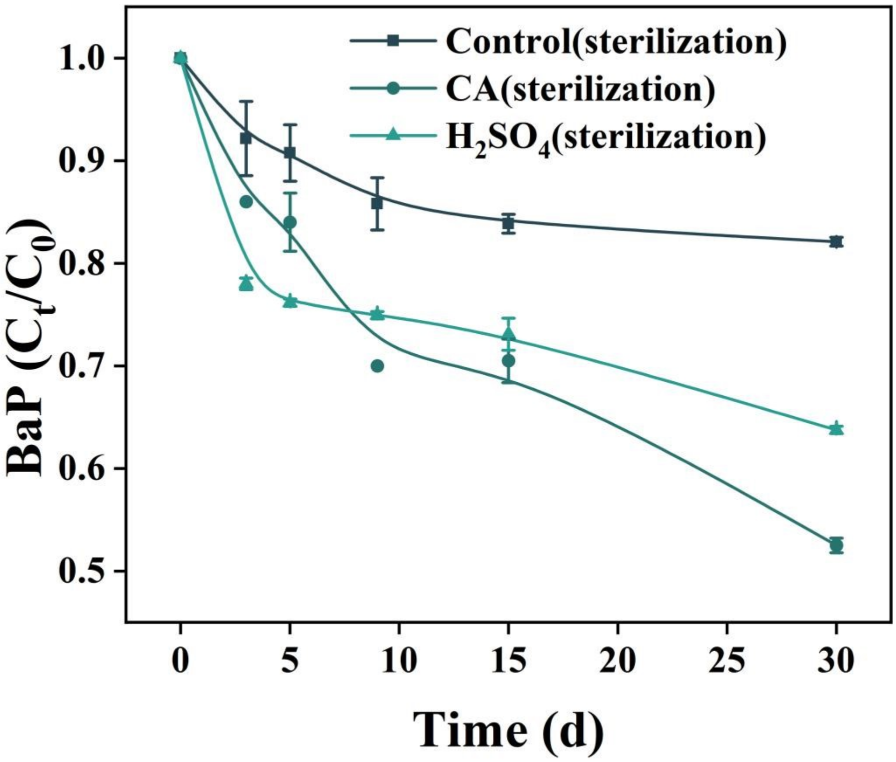


Figure S2. Changes of soil pH in the presence of CA. Conditions: T = 25℃, RH ~ 50 ± 5%, initial soil pH = 7.0, [CA] = 40 mM.



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222 **Figure S3.** Degradation rates of BaP with H_2SO_4 in sterilized soil. Conditions: T = 223 25℃, RH ~ 50 ± 5%, initial soil pH = 7.0, [CA] = 40 mM.

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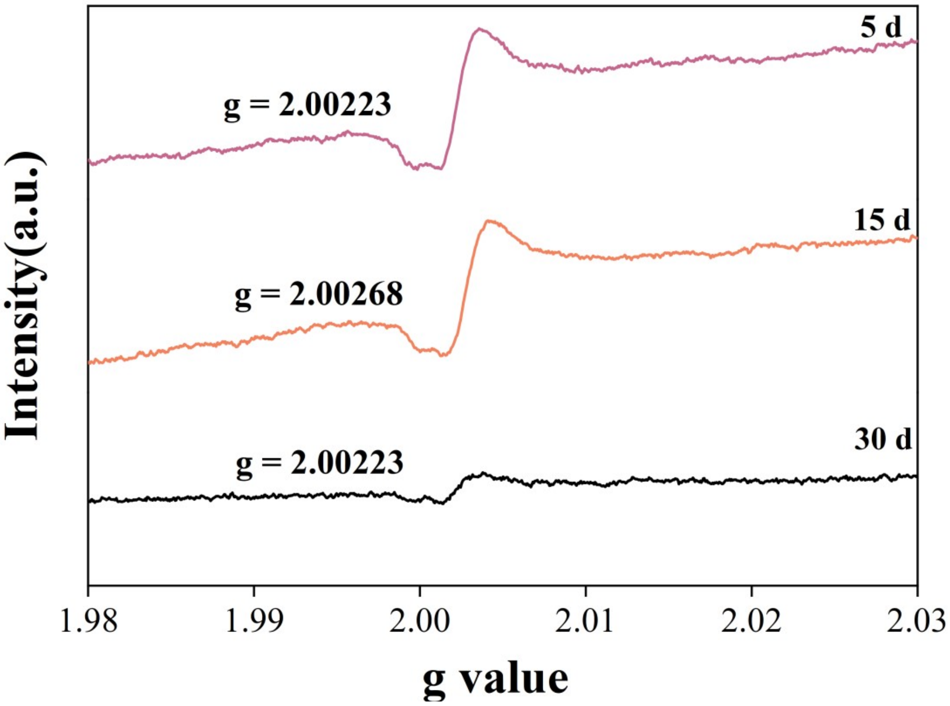

Figure S4. Persistent free radicals (PFRs) generation with the addition of CA in BaP-contaminated soil. Conditions: T = 25℃, RH ~ 50 ± 5%, initial soil pH = 7.0, [CA] = 40 mM.

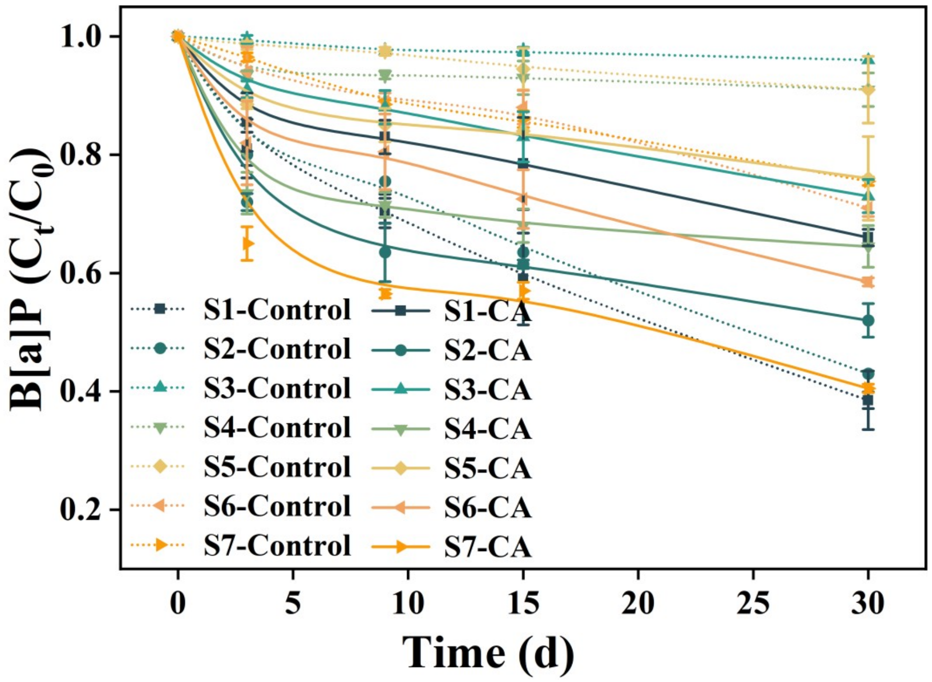

Figure S5. The BaP degradation in different kinds of soil with CA; S1, S2, S3, S4, S5, S6 and S7 represented the soils of areas Hainan, Chongqing, Jilin, Hebei, Heilongjiang Yunnan and Liaoning, respectively.

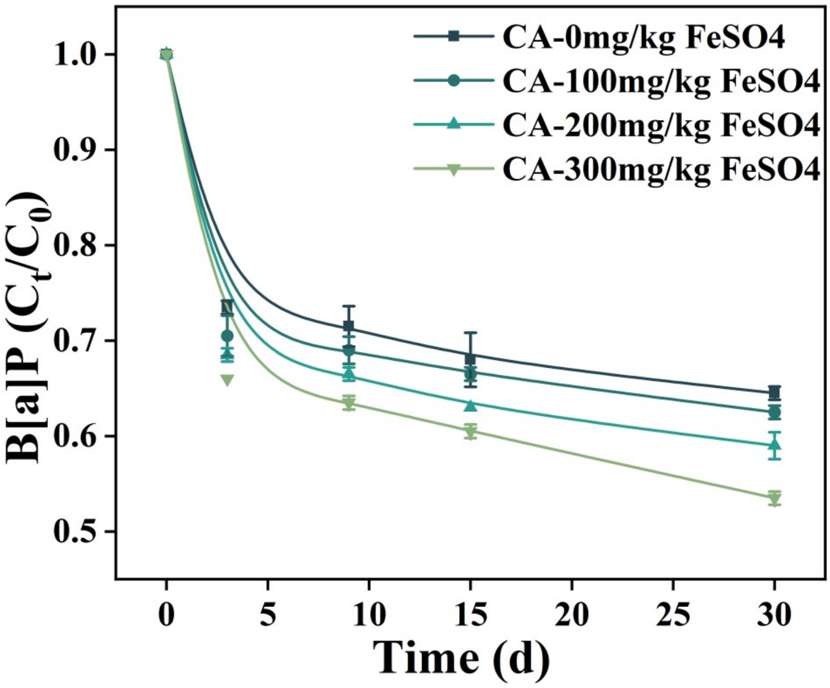

Figure S6. The BaP degradation in different concentration of $FeSO_4$ with CA. Conditions: T = 25°C, RH ~ 50 ± 5%, initial soil pH = 7.0, [CA] = 40 mM.

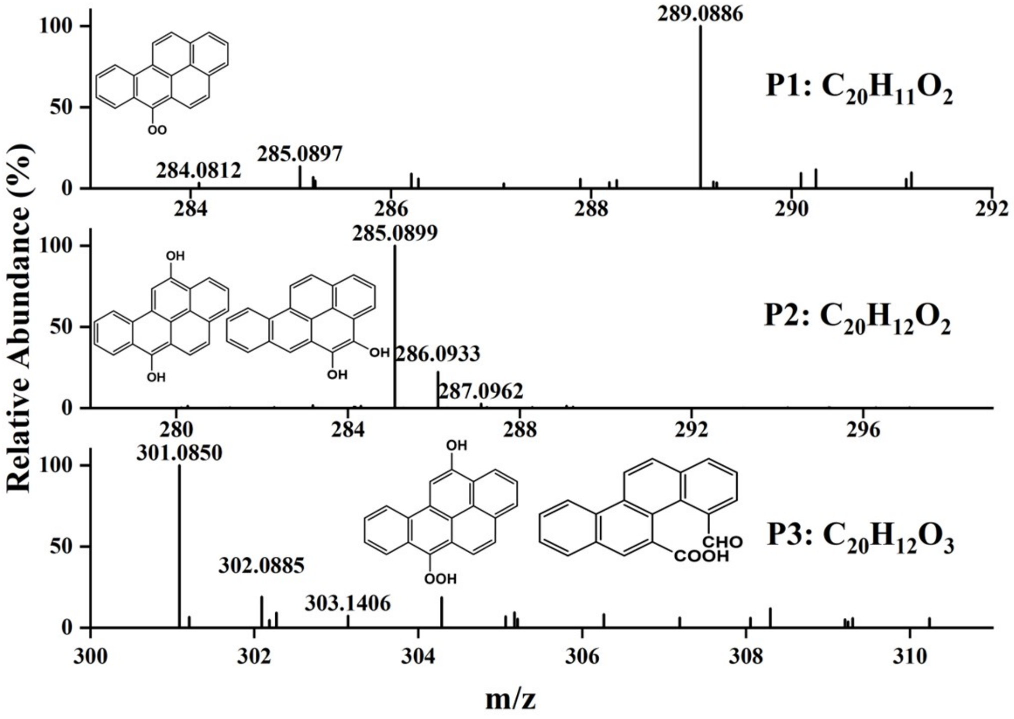

Figure S7. Mass spectra of P1, P2, and P3 products identified in the BaP degradation process.

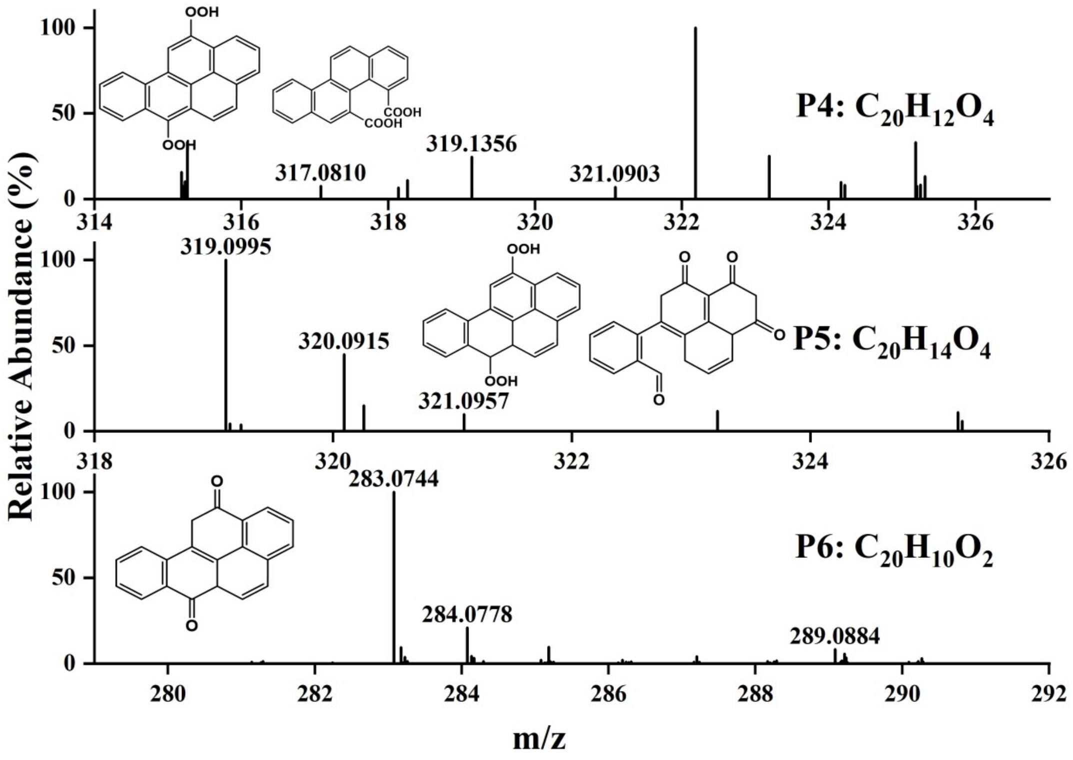

Figure S8. Mass spectra of P4, P5, and P6 products identified in the BaP degradation process.

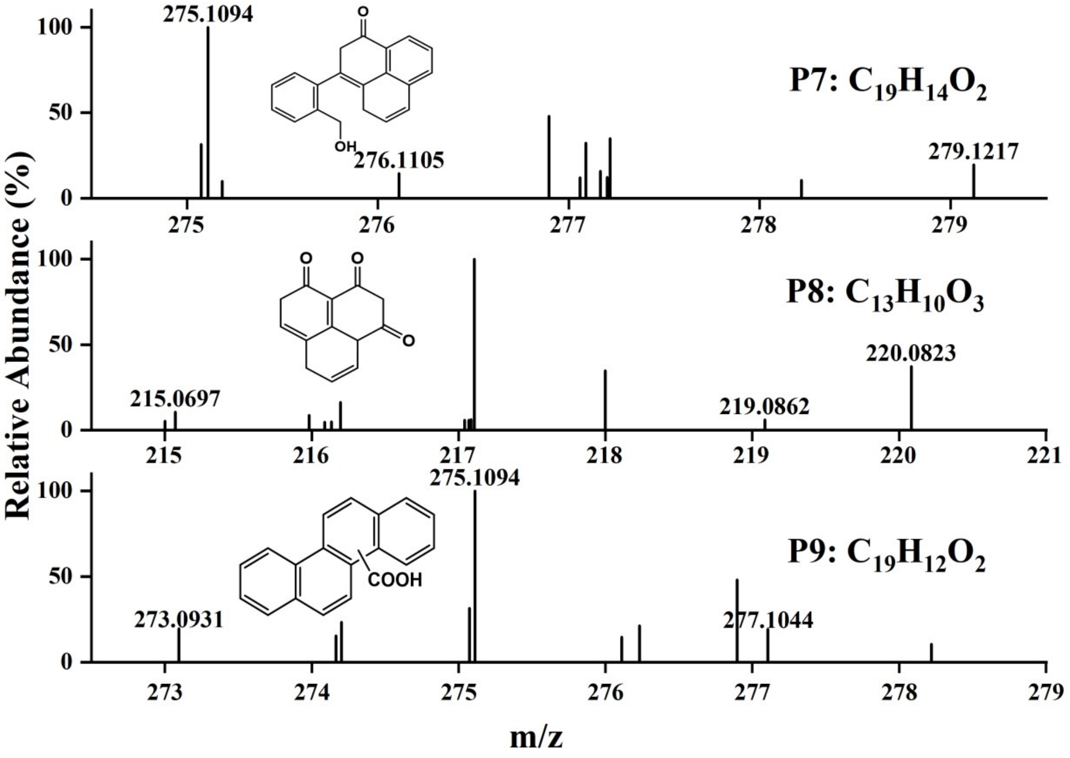

Figure S9. Mass spectra of P7, P8, and P9 products identified in the BaP degradation process.

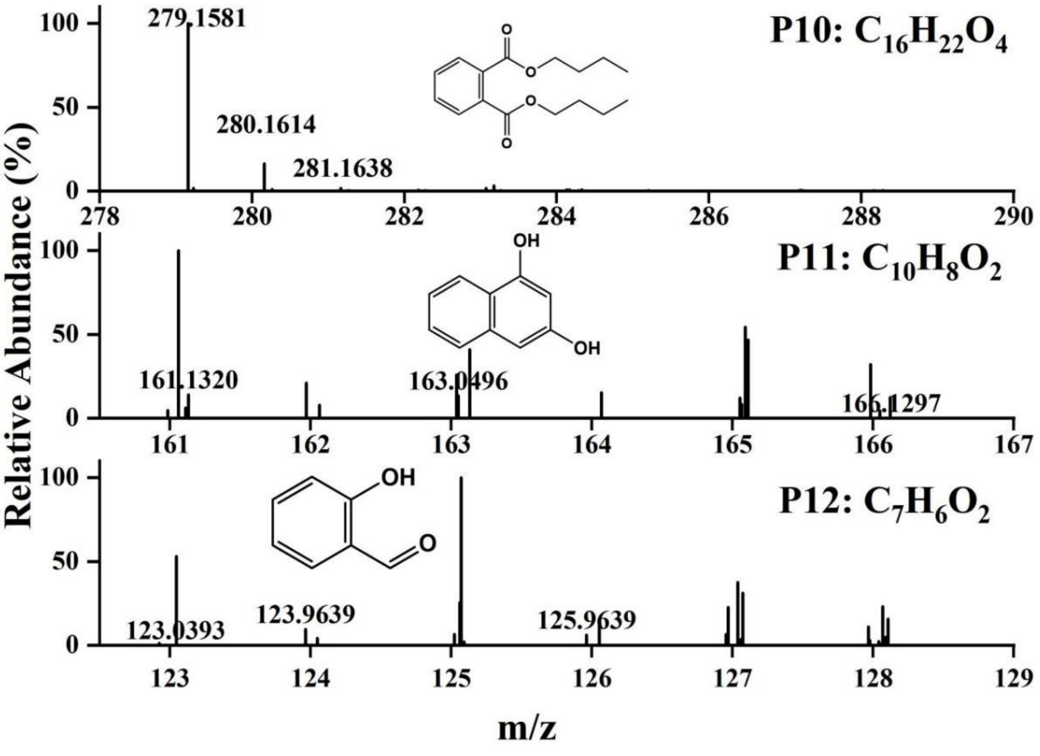

Figure S10. Mass spectra of P10, P11, and P12 products identified in the BaP degradation process.

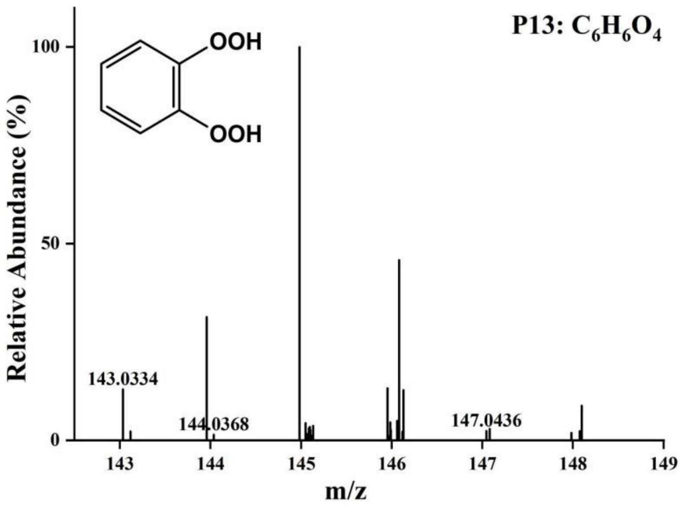

Figure S11. Mass spectra of P13 products identified in the BaP degradation process.

References

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