SUPPLEMENTARY MATERIALS

Unraveling the Coupled Dynamics between DOM Transformation and Arsenic Mobilization in Aquifer Systems during Microbial Sulfate Reduction: Evidence from Sediment Incubation Experiment

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Contents:

1.1 Set up of the microcosm experiment.

1.2 SPE solid phase extraction of DOM samples for FT-ICR-MS analysis.

2. Supplementary figures

Figure S1. Spectra of three DOM components during the incubation.

Figure S2. The variations of different fluorescence indexes of dissolved organic matter.

Figure S3. Van Krevelen diagram of DOM molecular responding to the different NOSC values in STR and JHP sediments.

Figure S4. The comparison of common and unique DOM molecules from the STR and JHP sediments before and after the incubation.

Figure S5. The comparison of carboxylic-rich alicyclic molecules (CRAM) and carboxyl-containing aliphatic molecules (CCAM) values in the STR and JHP sediments before and after the incubation.

3. Supplementary table

Table S1. The molecular number of different DOM components before and after the incubation.

Table S2. The variations in the bioproduction index (I_bioprod) from the JHP and STR sediments before and after the incubation.

Table S3. The variations in the terrestrial index (I_terr) from the JHP and STR sediments before and after the incubation.

Table S4. Sequential extraction results indicating the different As fractions in the STR and JHP sediments before the incubation.

Table S5. Variations in the nominal oxidation state of carbon (NOSC) and modified aromaticity index (AI_{mod}) values in the STR and JHP sediments before and after the incubation.

1. Supplementary methods

1.1 Set up of the microcosm experiment

The ionic compositions and pH of the artificial groundwater were carefully adjusted to match the groundwater chemistry in situ. For the sediments from the Jianghan Plain, the artificial groundwater consisted of $CaCl_2$ (2.3 mmol/L), $MgCl_2$ (0.6 mmol/L), and PIPES buffer (5 mmol/L), with a pH condition of 6.95. In contrast, for the sediments from the Singe Tsangpo River, the artificial groundwater comprised $CaCl_2$ (0.75 mmol/L), Na_2SO_4 (0.39 mmol/L), and PIPES buffer (5 mmol/L), with a pH of 8.3. Before mixing with the sediment, the artificial groundwater underwent a purging process using high-purity nitrogen (> 99%) for a minimum of 1 hour to eliminate oxygen. Subsequently, it was autoclaved at 120°C for 20 minutes to sterilize and prevent microbial interference. The serum bottles containing the artificial groundwater were then assembled with the sediment samples inside an anaerobic glove box maintained under a nitrogen atmosphere. The prepared serum bottles, grouped accordingly, were incubated in dark conditions at a constant temperature of 20°C for 95 days. This temperature was chosen to closely mimic the average temperature of the groundwater in the aquifers.

1.2 SPE solid phase extraction of DOM samples for FT-ICR-MS analysis

The molecular weight distribution of DOM was performed using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS, Bruker Solari X). The sediment slurry samples were collected in 50 ml sterilized centrifuge tubes and then were vibrated for 8 hours at 25 °C anaerobically. Then the slurries were harvested by centrifugation for 20 min at 4200 rpm and were subjected to solid phase extraction (SPE). Mass spectrometer analysis of SPE-DOM was performed using a negative ion Apollo II ESI coupled with a Solarix 7.0 T Bruker Ultra FT-ICR mass spectrometer at Tianjin University.

SPE solid phase extraction procedures are as follows (extraction column type is Agilent Bond Elut PPL): the samples were filtered through 0.22 μm filter membrane first, and then

formic acid was introduced into the samples for adjusting the pH to 2. Before the solid phase extraction, the PPL column was activated by methanol and HCl effluent, respectively. Then the samples were flowed through the column at a speed of ~ 5ml/min for concentrating the target compounds, the final dissolved organic carbon in the DOM was about 100 mg/L under a loss ratio of 50% during the extraction. After the flowing through of the samples, 18 ml HCl of 0.01 mmol/L was used to remove the dissolved salts in the column. Then the preserved DOM in the column was discharged by 12 ml methanol, and then methanol in the samples was removed by a nitrogen blowing method. The final samples were frozen at a -20°C conditions until further analysis.

The details of FT-ICR-MS analysis and the data analysis are as follows: the ion source is an electrospray ionization source (ESI) in negative ion mode. The main detection parameters include continuous sampling at a speed of 120 μL/h, capillary entrance voltage of -4.0 kV, ion accumulation time of 0.2s, acquisition mass range of 100-1600 Da, sampling point number of 4 M 32-bit data, and time-domain signal superposition for 300 times to improve signal-to-noise ratio. Before sample detection, the instrument is calibrated with 10 mmol/L sodium formate, and after sample detection, internal standard correction is performed using soluble organic matter (known molecular formula). After calibration, the mass error of detection is less than 1 ppm.

3. Supplementary figures

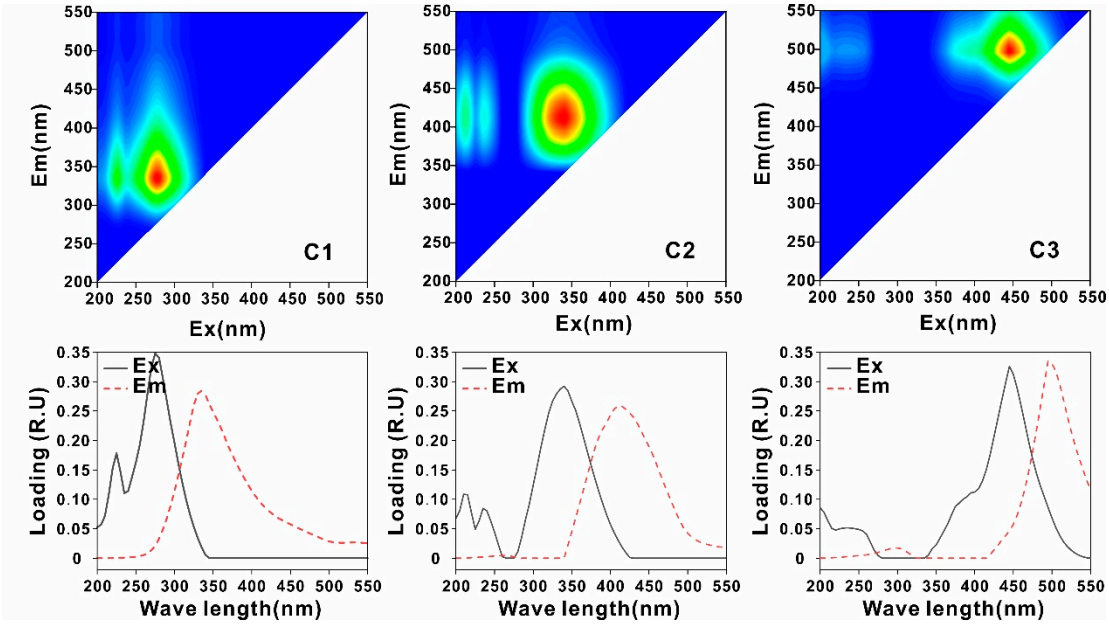

Figure S1. Spectra of three DOM components during the incubation.

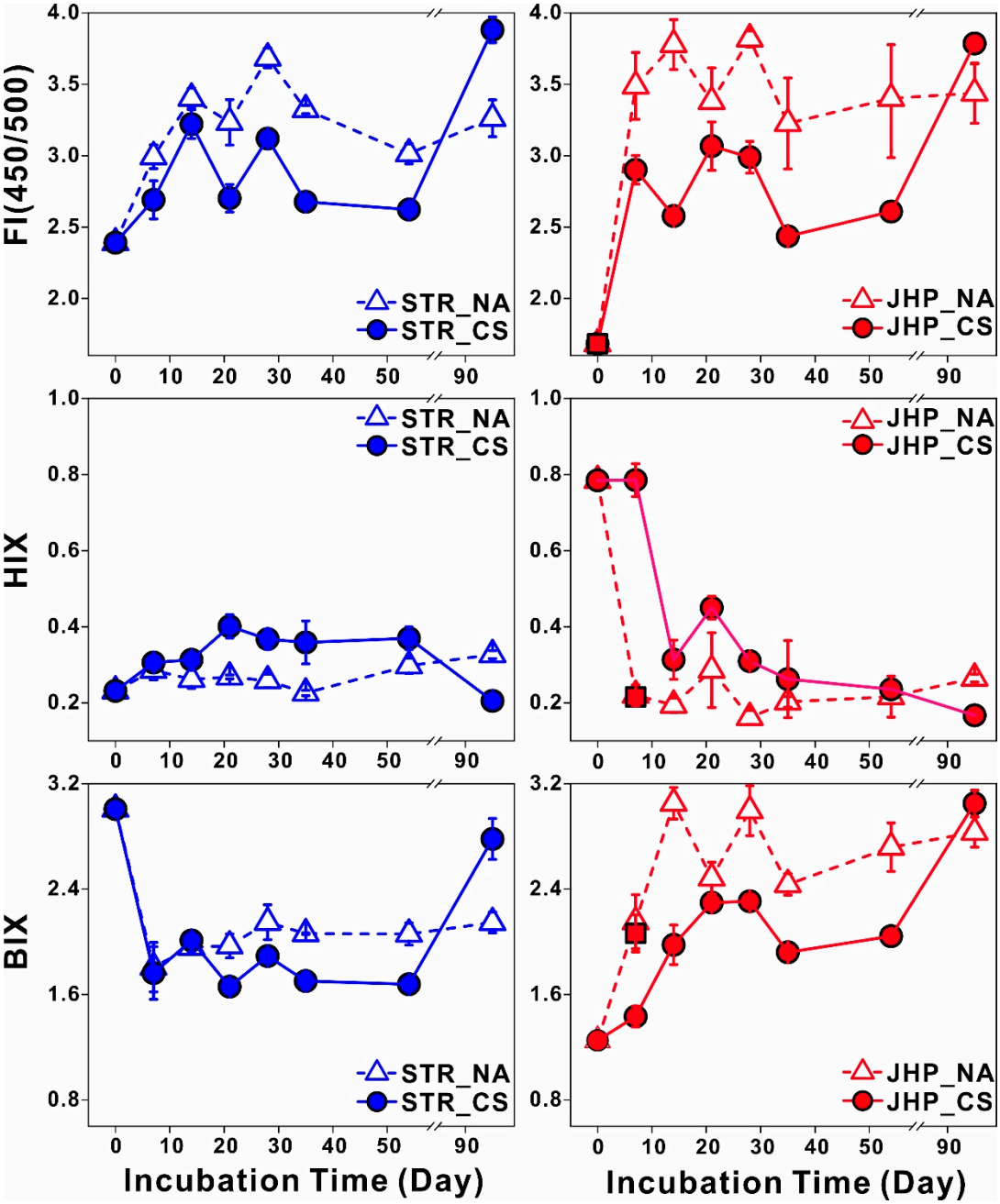

Figure S2. The variations of different fluorescence indexes of dissolved organic matter.

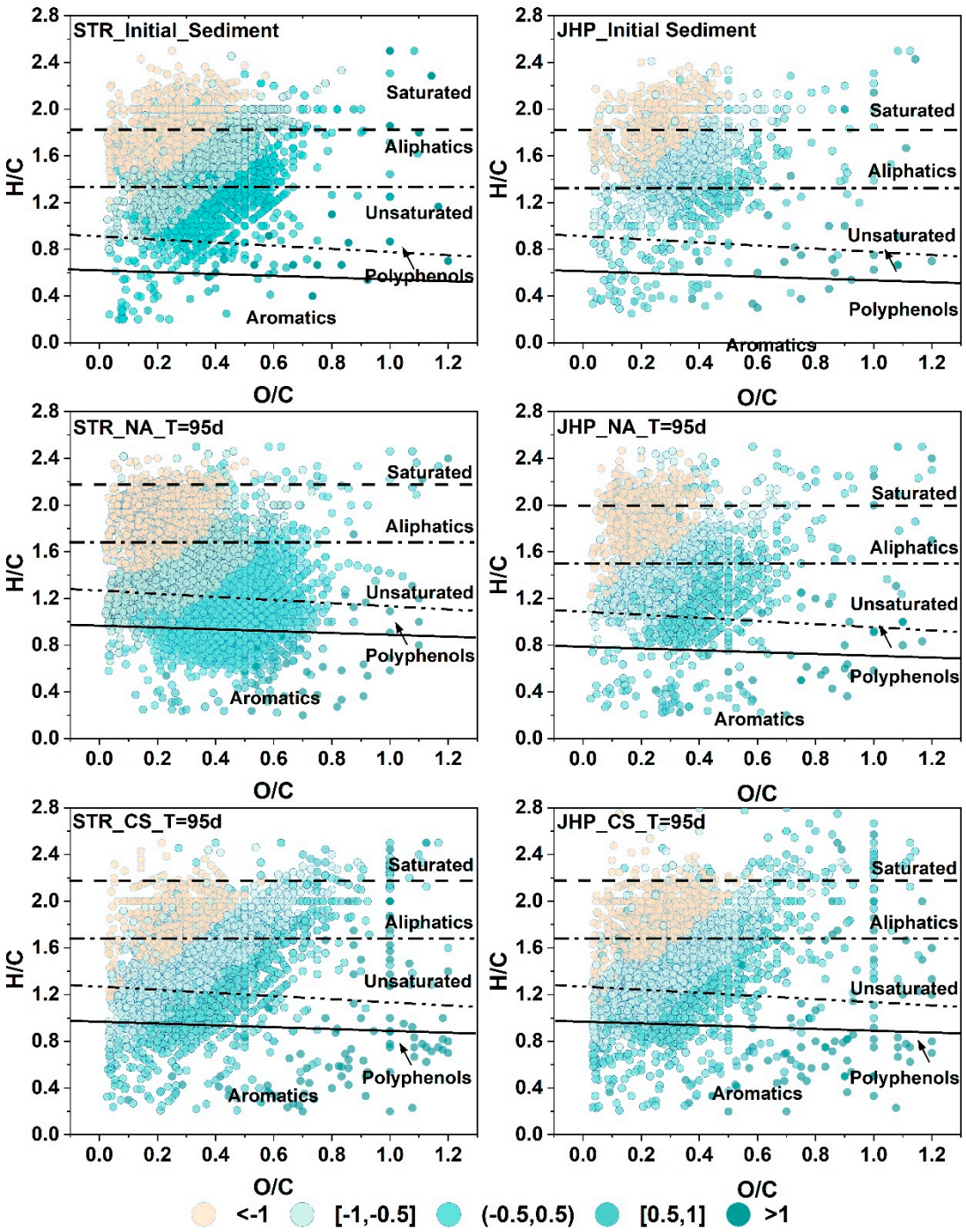

Figure S3. Van Krevelen diagram of DOM molecular responding to the different NOSC values in STR and JHP sediments.

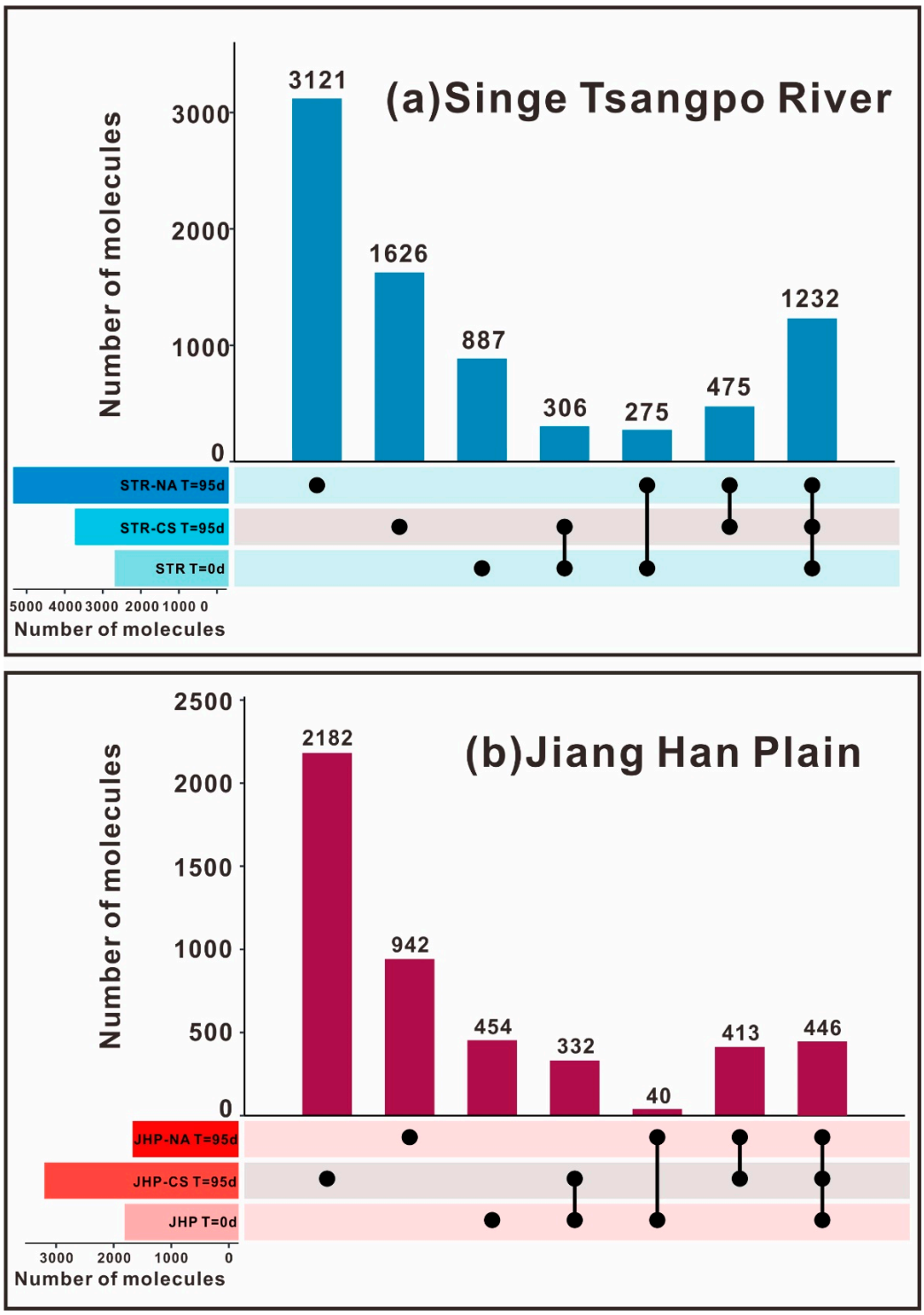

Figure S4. The comparison of common and unique DOM molecules from the STR and JHP sediments before and after the incubation.

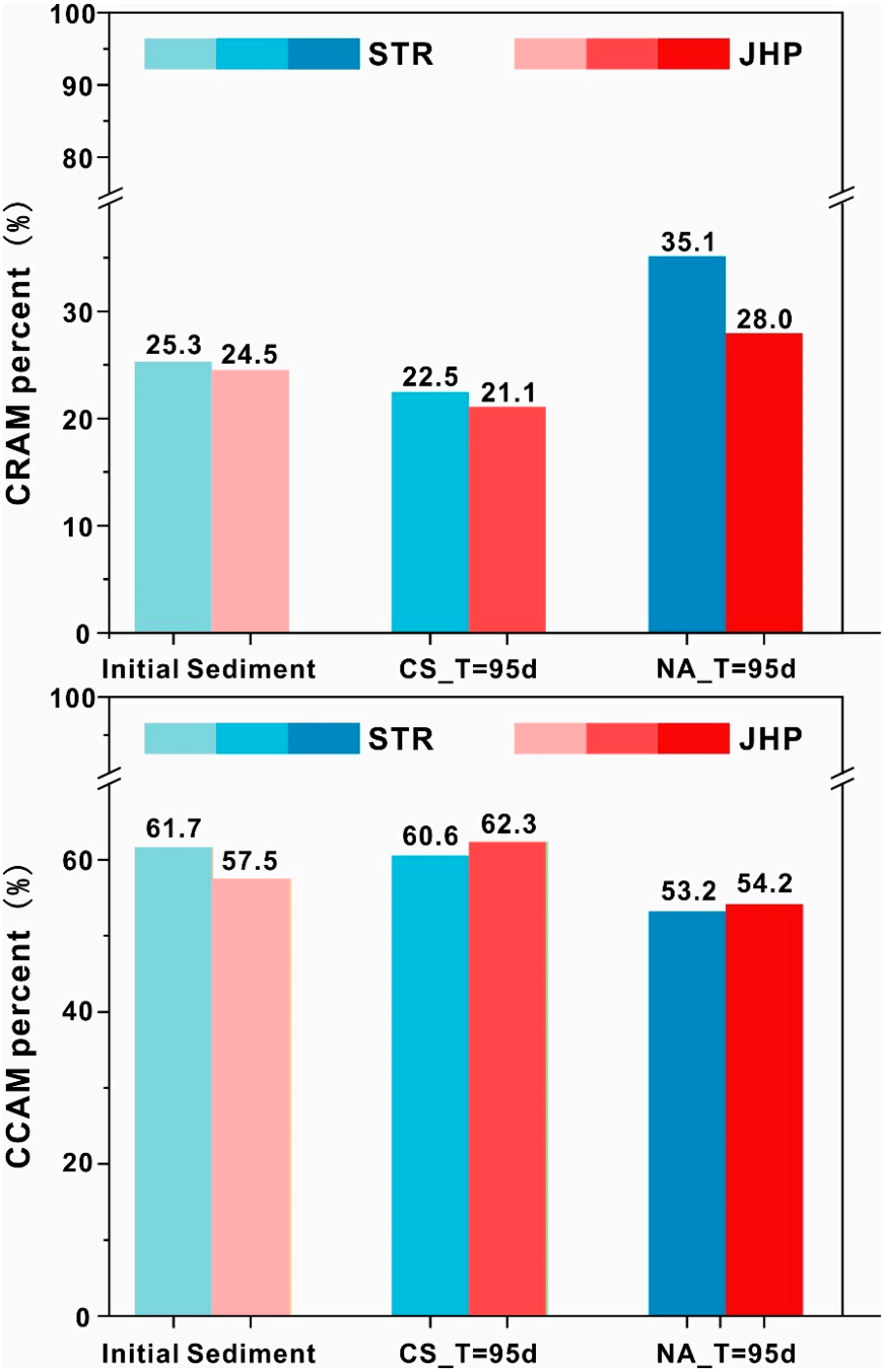

Figure S5. The comparison of carboxylic-rich alicyclic molecules (CRAM) and carboxyl-containing aliphatic molecules (CCAM) values in the STR and JHP sediments before and after the incubation.

4. Supplementary table

Table S1. The molecular number of different DOM components before and after the incubation.

Organic matter type	STR T=0d	STR CS T=95d	STR NA T=95d	JHP T=0d	JHP CS T=95d	JHP NA T=95d
Amino sugar	79	160	107	37	110	40
Carb	27	92	27	17	52	19
ConHC	96	171	307	76	157	99
Lignin	1126	1503	2832	406	1335	771
Lipid	839	768	1579	374	791	613
Protein	851	1160	765	368	953	295
Tannin	14	33	123	16	36	21
UnsatHC	58	163	146	27	193	69
Other	17	72	30	16	101	29
Total	3107	4122	5916	1337	3728	1956

Table S2. The variations in the bioproduction index (I_bioprod) from the JHP and STR sediments before and after the incubation.

Sample	*I_bioprod*
STR_T=0	3.30
STR_CS_T=95	5.96
STR_NA_T=95	2.00
JHP_T=0	3.55
JHP_CS_ T=95	6.59
JHP_NA_T=95	5.34

The bioproduction index is calculated as follows:

$$I_{\_bioprod} = \frac{C_{13}H_{18}O_5 + C_{13}H_{16}O_6 + C_{13}H_{19}NO_6 + C_{13}H_{17}NO_7 + C_{18}H_{28}O_7}{C_{14}H_{16}O_8 + C_{17}H_{24}O_8 + C_{16}H_{22}NO_9 + C_{19}H_{28}O_9 + C_{19}H_{28}O_{10}}$$

The numerator and denominator are the sum of the peak intensity of different molecular formulas.

Table S3. The variations in the terrestrial index (I_terr) from the JHP and STR sediments before and after the incubation.

Relative intensity	STR_T=0d	STR_CS_T=95d	STR_NA_T=95d	JHP_T=0d	JHP_CS_T=95d	JHP_NA_T=95d
NEG	0.003385	0.004583	0.008862	0.0009112	0.002772	0.005254
POS	0.002057	0.001919	0.005806	0.001411	0.001360	0.001396
I_terr	0.6220	0.7049	0.6042	0.3923	0.6708	0.7900

Note: The terrestrial index is calculated as follows:

$$I_{\_terr} = \frac{sum\ magnitudes\ NEG\ formuals}{sum\ magnitudes\ (NEG + POS)\ formuals}$$

The numerator and denominator are the sum of the relative intensity of different molecular formulas.

Table S4. Sequential extraction results indicating the different As fractions in the STR and JHP sediments before the incubation.

arsenic content (mg/kg)	As_{ion}	As_{ads}	As_{amp}	As_{cry}	As_{py}
STR_initial sediment	0.3	10.3	19.33	2.9	2.8
JHP_initial sediment	1.2	21.55	19.7	0.9	1.2

Note: As_{ion} represents ionically bound As, which is extracted using a 1 $mol \cdot L^{-1}$ $MgCl_2$ solution with a pH adjusted to 8.0±0.05. As_{ads} represents strongly adsorbed As, which is extracted using a 0.5 $mol \cdot L^{-1}$ NaH_2PO_4 solution with a pH adjusted to 5±0.05. As_{amp} represents the amorphous iron (hydr)oxide bound arsenic, which is extracted using a 0.2 $mol \cdot L^{-1}$ oxalic acid-ammonium ammonium oxalate solution with a pH adjusted to 3±0.05. As_{cry} represents the crystalline iron (hydr)oxide bound arsenic, which is extracted using a solution of 0.2 $mol \cdot L^{-1}$ ammonium oxalate + 0.1M ascorbic acid with a pH adjusted to 3±0.05. As_{py} represents the As that is co-precipitated by pyrite and As_4S_4/As_2S_3, which is extracted using a 16 $mol \cdot L^{-1}$ concentrated HNO_3 solution.

Table S5. Variations in the nominal oxidation state of carbon (NOSC) and modified aromaticity index (AI_{mod}) values in the STR and JHP sediments before and after the incubation.

Value		STR			JHP		
		Initial sediment	CS_T=0d	NA_T=0d	Initial sediment	CS_T=0d	NA_T=0d
NOSC	average	-0.78	-0.65	-0.64	-0.73	-0.68	-0.71
	maximum	3.75	4.25	2.80	4.50	4.25	3.29
	minimum	-1.88	-1.89	-1.93	-2.10	-1.91	-1.97
AI_{mod}	average	0	0.08	0.16	0.08	0.08	0.06
	maximum	3	17	7	15	19	7
	minimum	-11	-17	-10	-7	-19	-13