

## Supporting Information

### Characterizing Molecular Fingerprint of Dissolved Organic Matter Shaped by Natural and Anthropogenic Factors in a Large Macrophytic Shallow Lake Wetland

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This supporting information consists of 14 pages, including 2 sections of Text, 4 Tables, and 3 Figures.

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### *Text S1. Sampling and Extraction methods for DOM*

Surface water samples were collected at a depth of 0.5 m in 1000 mL amber glass bottles (Sichuan Shubo Co., Ltd, China) that had been previously acid-washed and rinsed twice with sample water. After sampling, all samples were transported to the laboratory on ice in 24 hours, immediately filtered through a 0.7  $\mu\text{m}$  pore size glass fiber filter (Whatman GF/F, USA), and stored in the dark at 4°C. The following DOM sample preparation and FT-ICR MS (Bruker Daltonics, Germany) measurement was carried out as soon as possible.

The DOM content in water was extracted with a PPL SPE cartridge using a modified method adopted from previous works (Dittmar et al., 2008; Yang et al., 2017). The 6 mL/500 mg Bond Elut PPL SPE cartridge (Agilent, USA) was preconditioned with 20 mL of HPLC-grade methanol, followed by 20 mL of acidified (pH 2) Milli-Q water (Millipore, USA). A sufficient volume of acidified (pH 2) sample (to obtain 100  $\mu\text{g}$  C, informed by the predetermined DOC content of the sample) was then gravity-filtered through the PPL cartridge. The cartridge was then rinsed with 20 mL of acidified Milli-Q water (pH 2) and dried with ultrapure nitrogen gas. The bound sample on the cartridge was eluted with 5 mL of HPLC-grade methanol (Thermo-Fisher, USA). The eluent was dried with nitrogen gas flow, redissolved with 1 mL of HPLC-grade methanol, and kept in the dark in a refrigerator ( $-20\text{ }^{\circ}\text{C}$ ) prior to analysis. The recovery efficiencies of PPL SPE extraction ranged from 46 to 62% (53% on average,  $n = 25$ ) on a DOC basis (Table S2)

### *Text S2. Additional information for FT-ICR-MS data processing*

We divided the van Krevelen (VK) diagrams into regions corresponding to seven compound classes: lipids, peptide-like compounds, carbohydrates, unsaturated hydrocarbons, lignins, condensed aromatics, and tannins, according to previously published reports (Kim et al., 2003; Kellerman et al., 2015). The stoichiometric ranges used to establish the classification boundaries follow:

- lipids ( $\text{O/C}=0\text{--}0.3$ ,  $\text{H/C}=1.5\text{--}2.0$ ),
- peptide-like compounds ( $\text{O/C}=0.3\text{--}0.67$ ,  $\text{H/C}=1.5\text{--}2.2$ ,  $\text{N/C}\geq 0.05$ ),
- carbohydrates ( $\text{O/C}=0.67\text{--}1.2$ ,  $\text{H/C}=1.5\text{--}2$ ),
- unsaturated hydrocarbons ( $\text{O/C}=0\text{--}0.1$ ,  $\text{H/C}=0.7\text{--}1$ ),
- lignins ( $\text{O/C}=0.1\text{--}0.67$ ,  $\text{H/C}=0.7\text{--}1.5$ ,  $\text{AI}<0.67$ ),
- condensed aromatics ( $\text{O/C}=0\text{--}0.67$ ,  $\text{H/C}=0.2\text{--}0.7$ ,  $\text{AI}\geq 0.67$ ),
- tannins ( $\text{O/C}=0.67\text{--}1.2$ ,  $\text{H/C}=0.5\text{--}1.5$ ,  $\text{AI}<0.67$ ).

*Supplementary Tables*

Table S1 General characteristics of each sampling site

Sample	Site <sup>a</sup>	Longitude (°)	Latitude (°)	Runoff	Macrophyte	Total pollution discharge
<b>S1</b>	CPT	116.010	38.827	Y	Moderate	Low
<b>S2</b>	DC	115.950	38.850	N	Poor	Low
<b>S3</b>	GDD	115.842	38.920	Y	Poor	High
<b>S4</b>	GDZZ	116.030	38.900	N	Moderate	Moderate
<b>S5</b>	NLZ	115.946	38.904	N	Moderate	Low
<b>S6</b>	QT	116.031	38.862	N	Poor	Low
<b>S7</b>	SCD	116.000	38.942	N	Poor	Low
<b>S8</b>	ZLZ	116.090	38.897	Y	Rich	High
<b>S9</b>	BGHK	116.014	38.981	Y	Poor	Low
<b>S10</b>	BY41	115.958	38.950	N	Poor	Moderate
<b>S11</b>	BY51	115.984	38.933	N	Rich	Low
<b>S12</b>	BY61	116.019	38.933	N	Rich	Moderate
<b>S13</b>	BY87	116.066	38.906	N	Rich	High
<b>S14</b>	BY96	116.056	38.892	N	Rich	High
<b>S15</b>	BY171	116.005	38.963	Y	Moderate	Low
<b>S16</b>	BY200	116.023	38.907	N	Rich	Moderate
<b>S17</b>	BY215	116.073	38.892	Y	Moderate	High
<b>S18</b>	BY268	116.063	38.874	N	Poor	Low
<b>S19</b>	BY280	116.047	38.851	N	Poor	Low
<b>S20</b>	BY291	115.989	38.830	N	Poor	Low
<b>S21</b>	BY304	115.995	38.810	Y	Moderate	Low
<b>S22</b>	BY321	116.027	38.807	Y	Moderate	Moderate
<b>S23</b>	WJZ	116.007	38.916	N	Moderate	Moderate
<b>S24</b>	ZLHK	115.885	38.810	Y	Poor	High
<b>S25</b>	ZWXHK	116.096	38.889	Y	Poor	High

a) Sampling sites include 8 nation-controlled stations (S1–S8) and 17 other sampling sites (S9–S25).

Table S2 Recovery efficiencies of PPL-SPE for 25 DOM samples

Sample	Recovery (%)	Sample	Recovery (%)
S1	46.16	S14	49.49
S2	59.87	S15	60.95
S3	61.13	S16	51.27
S4	49.28	S17	49.68
S5	47.49	S18	45.94
S6	46.68	S19	47.93
S7	61.13	S20	53.81
S8	51.61	S21	53.47
S9	59.07	S22	62.27
S10	47.24	S23	60.58
S11	55.85	S24	47.70
S12	47.55	S25	54.27
S13	58.88		

Table S3 Water chemical properties of samples in the Baiyangdian wetland

Sample	DOC (mg/L)	pH	DO (mg/L)	TP (mg/L)	TN (mg/L)	NH4-N (mg/L)
S1	4.920	8.0	9.7	0.03	1.05	0.03
S2	3.446	8.0	7.1	0.05	1.59	0.33
S3	2.700	7.0	5.8	0.07	0.88	0.34
S4	5.031	8.0	5.0	0.04	0.49	0.04
S5	5.232	7.0	3.4	0.08	0.74	0.04
S6	5.653	8.0	8.0	0.04	0.40	0.03
S7	2.700	8.0	6.9	0.03	1.63	0.04
S8	4.341	7.0	5.5	0.04	0.93	0.34
S9	2.589	7.0	12.7	0.04	2.97	0.11
S10	6.170	8.6	10.6	0.06	3.12	0.14
S11	3.793	8.4	14.1	0.04	2.61	0.34
S12	3.727	9.4	12.7	0.05	3.20	0.45
S13	3.823	8.2	12.1	0.05	3.25	0.15
S14	5.331	8.9	14.0	0.04	3.24	0.55
S15	2.597	8.2	12.4	0.03	1.58	0.06
S16	5.401	8.1	14.3	0.03	2.31	0.07
S17	5.462	8.1	11.3	0.01	1.82	0.62
S18	5.959	8.1	6.7	0.01	2.69	0.38
S19	5.612	7.9	11.3	0.02	3.32	0.06
S20	3.746	7.9	12.7	0.02	3.44	0.06
S21	6.959	8.0	9.1	0.03	1.23	0.10
S22	5.082	8.0	11.9	0.01	6.08	0.06
S23	3.267	8.0	8.9	0.03	2.83	0.11
S24	4.064	8.1	11.1	0.04	1.30	0.21
S25	5.092	8.0	8.3	0.03	2.52	0.09

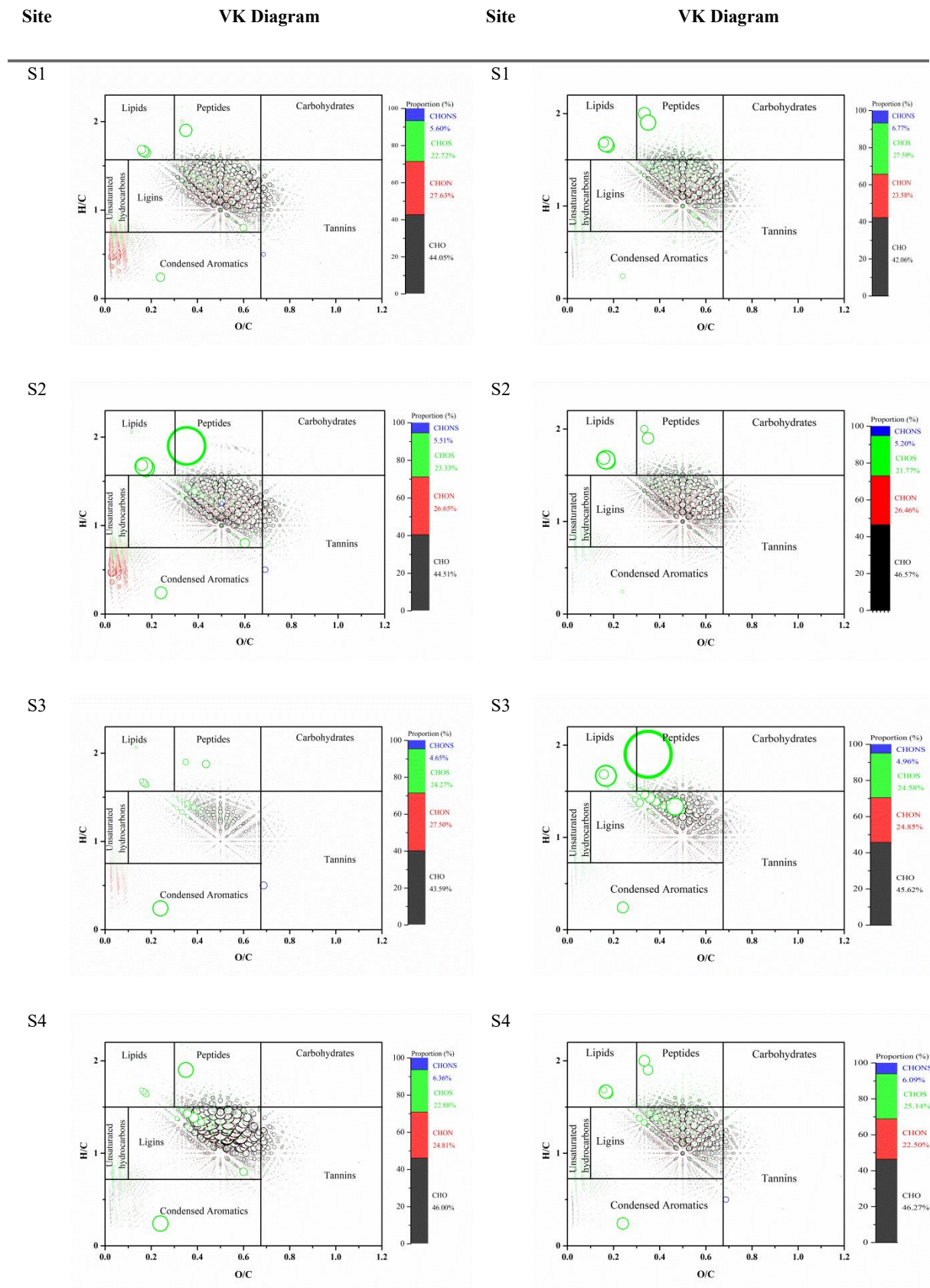
Table S4 Input natural variables and anthropogenic variables in RDA: Habitat characteristics, anthropogenic activity indices, and total discharge of pollutants in Baiyangdian wetland

Sample	Depth of water (m)	submerged macrophyte quality index, SMQI <sup>a</sup>	Vegetation cover (%) <sup>b</sup>	Impervious layer ratio (%) <sup>b</sup>	Construction land (%) <sup>b</sup>	Agricultural land (%) <sup>b</sup>	dCOD (t/a) <sup>c</sup>	dNH4-N (t/a) <sup>c</sup>	dTN (t/a) <sup>c</sup>	dTP (t/a) <sup>c</sup>
S1	2.7	5.2	12.23	97.75	87.69	99.99	12.81	0.66	0.98	0.08
S2	2.6	1.0	9.38	95.13	78.09	99.56	10.15	0.50	0.86	0.08
S3	1.8	1.4	8.89	93.72	92.31	19.86	87.94	14.62	21.77	5.42
S4	1.2	5.2	35.17	91.87	73.38	89.68	31.82	2.59	3.73	0.55
S5	2.0	5.0	15.51	96.64	74.76	93.42	24.27	1.63	2.45	0.31
S6	3.0	4.0	38.96	97.65	92.20	99.37	5.98	0.28	0.50	0.05
S7	1.8	1.4	27.00	91.87	80.57	92.99	4.47	0.26	0.45	0.06
S8	2.3	8.7	36.67	91.93	82.47	75.38	44.85	4.67	6.77	1.28
S9	1.0	0.0	27.00	91.87	80.57	92.99	4.47	0.26	0.45	0.06
S10	2.1	0.0	27.00	91.87	80.57	92.99	25.24	2.59	3.65	0.61
S11	2.4	8.0	35.17	91.87	73.38	89.68	4.47	0.26	0.45	0.06
S12	2.2	8.7	36.67	91.93	82.47	75.38	31.82	2.59	3.73	0.55
S13	1.0	10	38.96	97.65	92.20	99.37	44.85	4.67	6.77	1.28
S14	2.6	7.0	38.96	97.65	92.20	99.37	44.85	4.67	6.77	1.28
S15	2.8	5.2	12.23	97.75	87.69	99.99	4.47	0.26	0.45	0.06
S16	2.2	7.2	43.66	96.00	87.22	99.80	31.82	2.59	3.73	0.55
S17	2.2	5.7	34.14	92.38	89.79	80.00	44.85	4.67	6.77	1.28
S18	1.6	0.0	9.08	97.63	98.68	95.23	5.98	0.28	0.50	0.05
S19	1.0	1.4	27.00	91.87	80.57	92.99	5.98	0.28	0.50	0.05
S20	1.6	0.0	35.17	91.87	73.38	89.68	12.81	0.66	0.98	0.08
S21	2.7	7.0	36.67	91.93	82.47	75.38	12.41	0.57	0.96	0.07
S22	1.0	5.7	36.67	91.93	82.47	75.38	20.02	1.75	2.79	0.53
S23	3.0	8.0	35.17	91.87	73.38	89.68	31.82	2.59	3.73	0.55
S24	3.0	1.4	4.01	86.98	85.53	9.21	191.26	34.24	51.66	13.40
S25	1.5	0.0	36.67	91.93	82.47	75.38	44.85	4.67	6.77	1.28

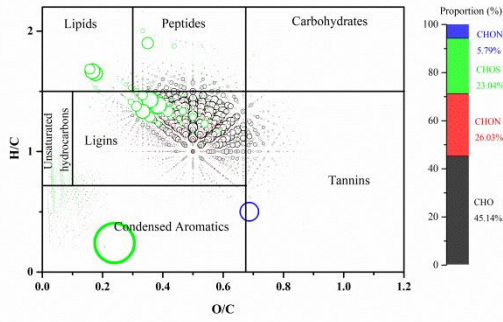
a) The quality of submersed macrophyte community was evaluated using the submersed macrophyte quality index (SMQI) collected from Xu et al. (Xu et al., 2022);

- b) The anthropogenic activity index (vegetation cover percentage (VP), construction land percentage (CP), agricultural land percentage (AP) and impervious layer ratio (ILR)) data were collected from the literature (Cai et al., 2023) and calculated according to the method reported by Fu et al. (Fu et al., 2021);
- c) The total discharge of pollutants into BYD from point sources and non-point sources (dCOD, dNH<sub>4</sub>-N, dTN, dTP) were collected from Han and Bu (Han and Bu, 2023).

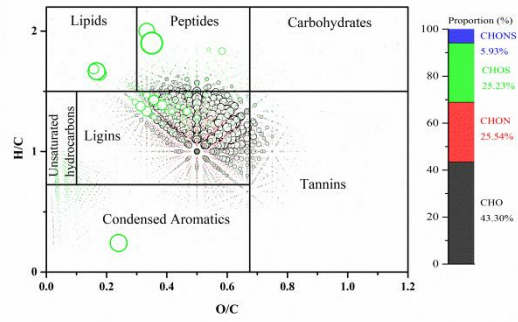
Supplementary Figures



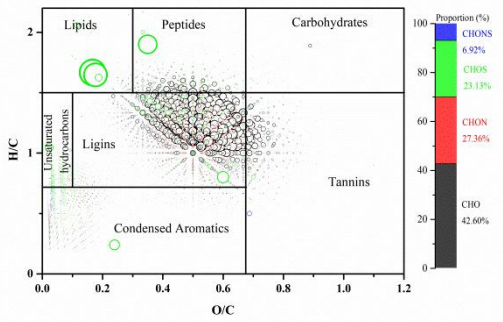
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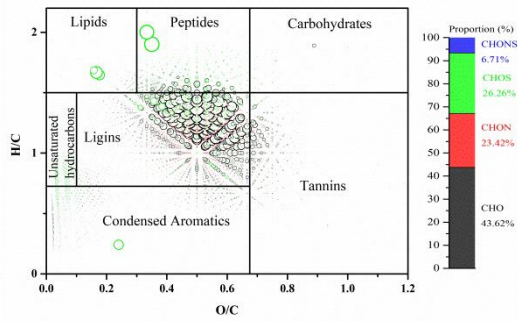
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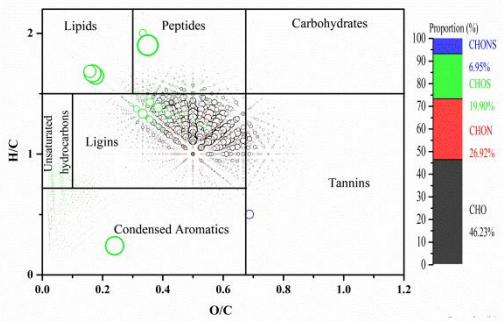
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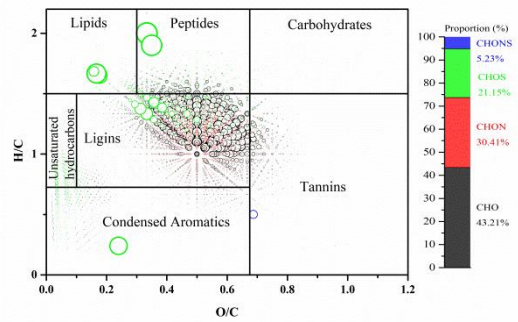
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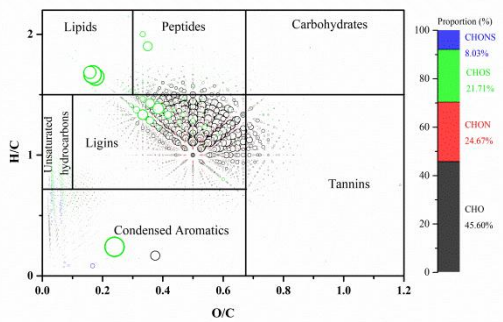
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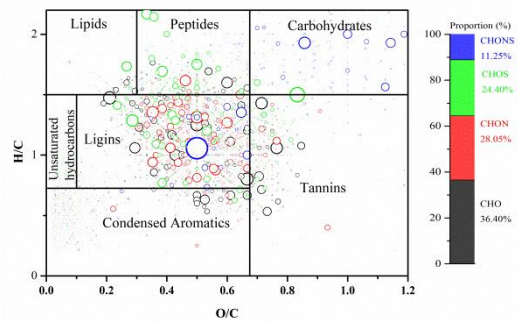
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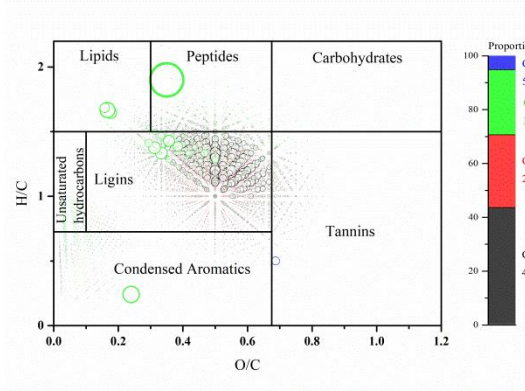
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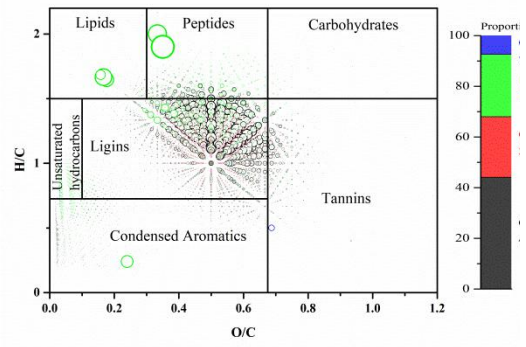
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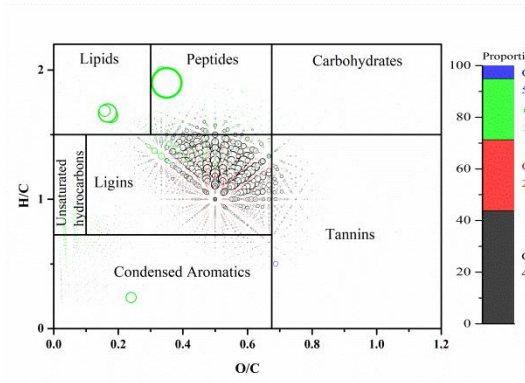
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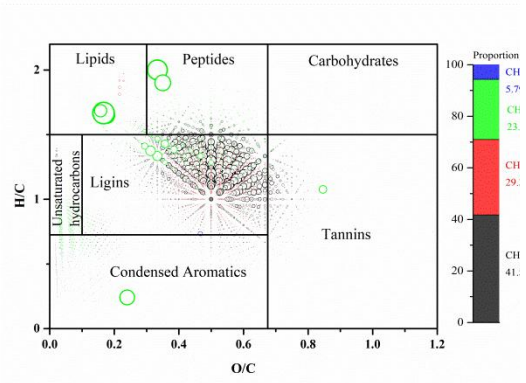
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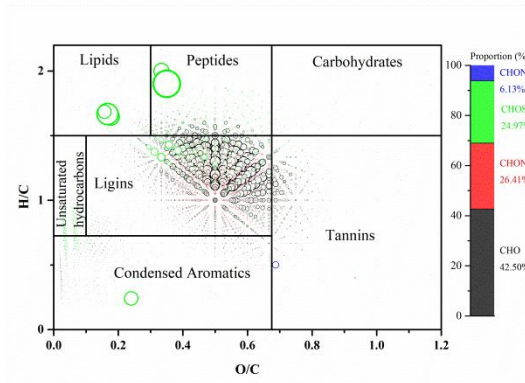
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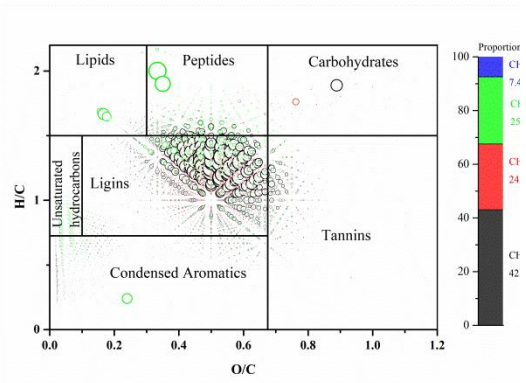
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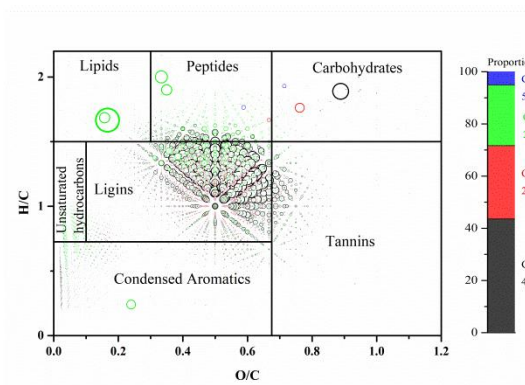
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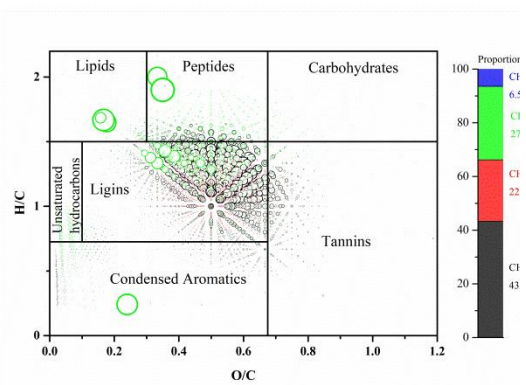
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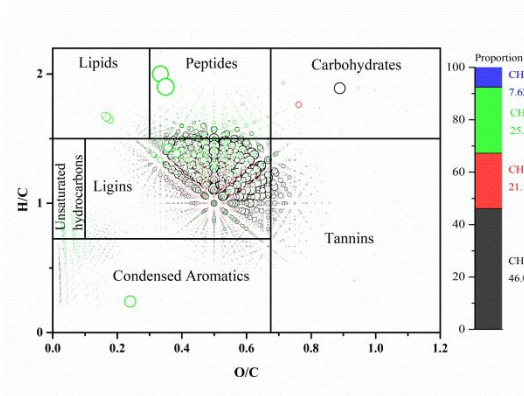
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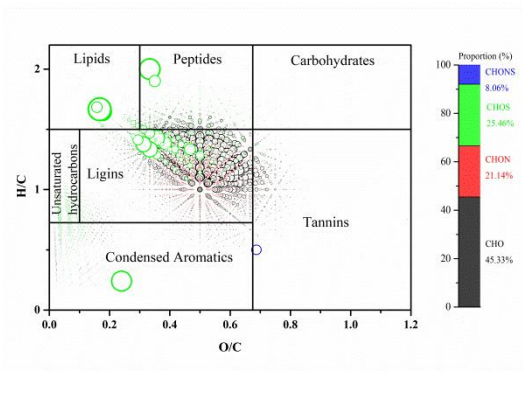
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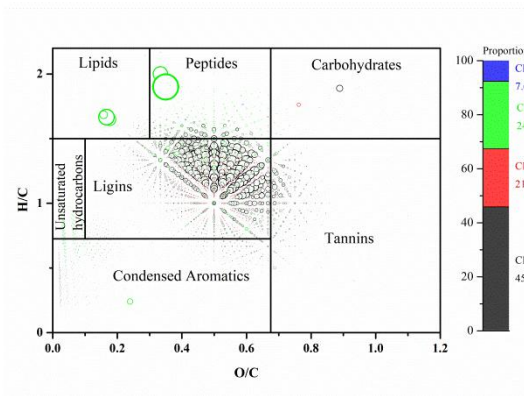
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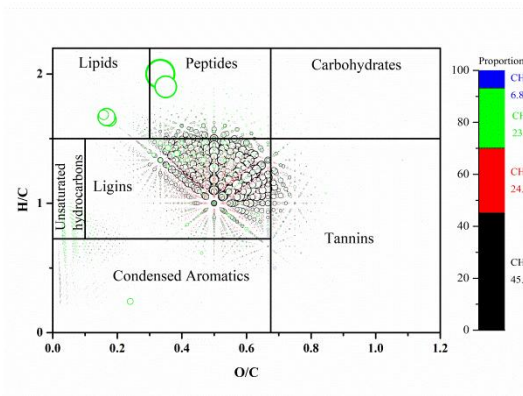
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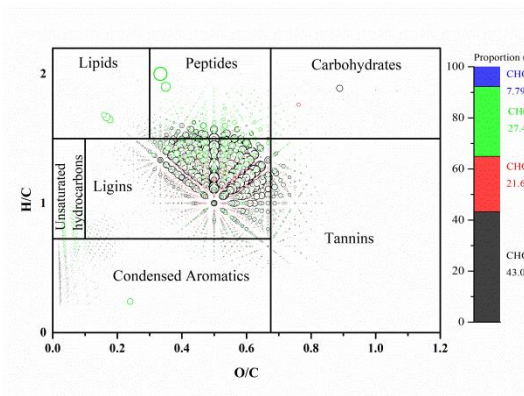
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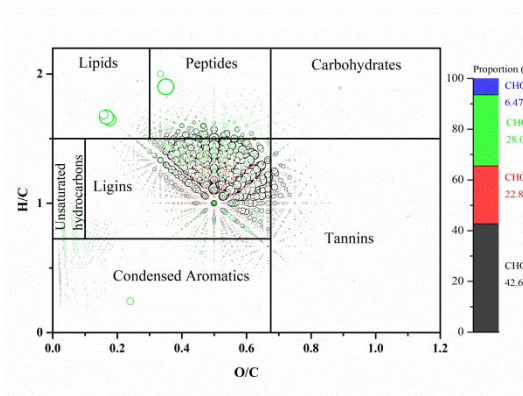
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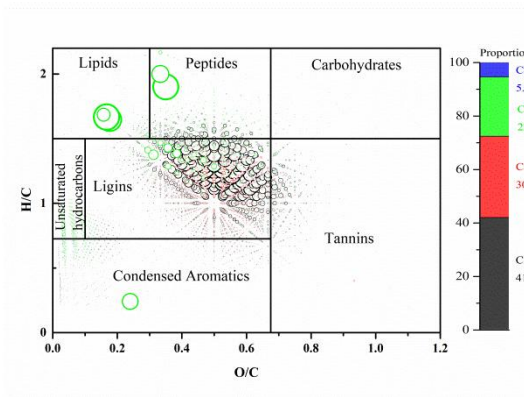
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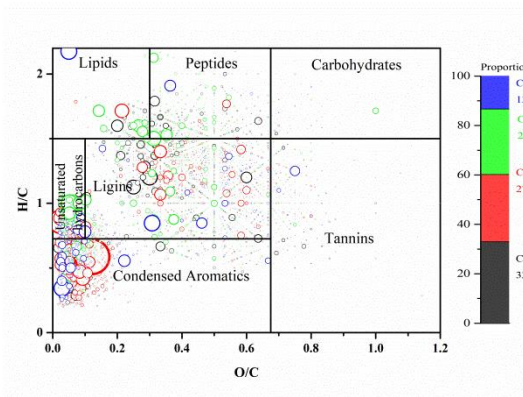
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S23



S24



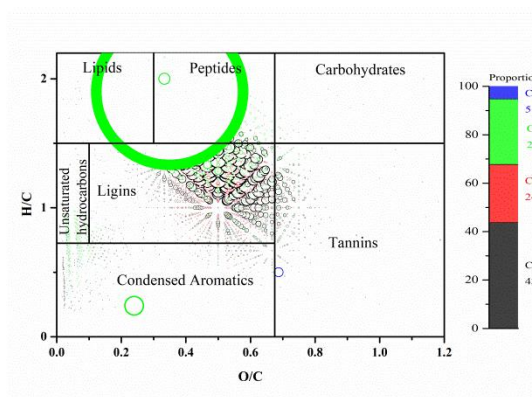
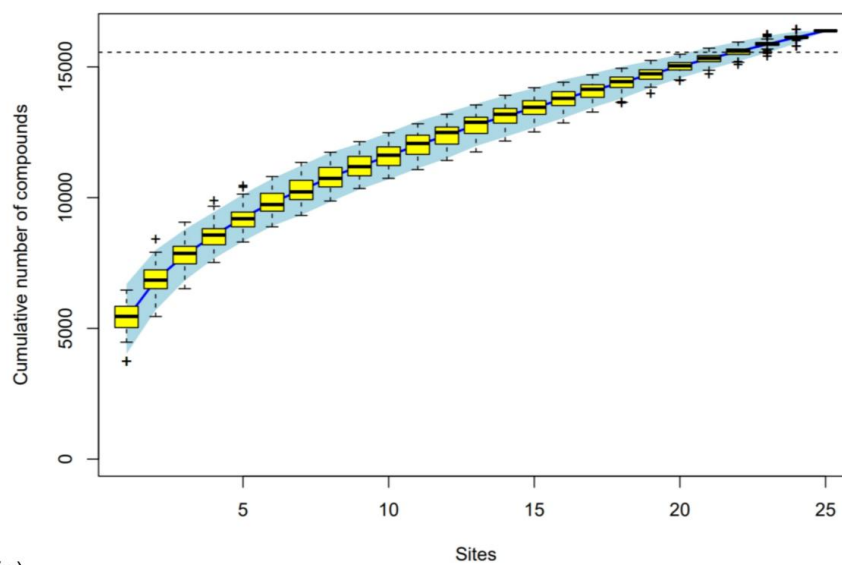
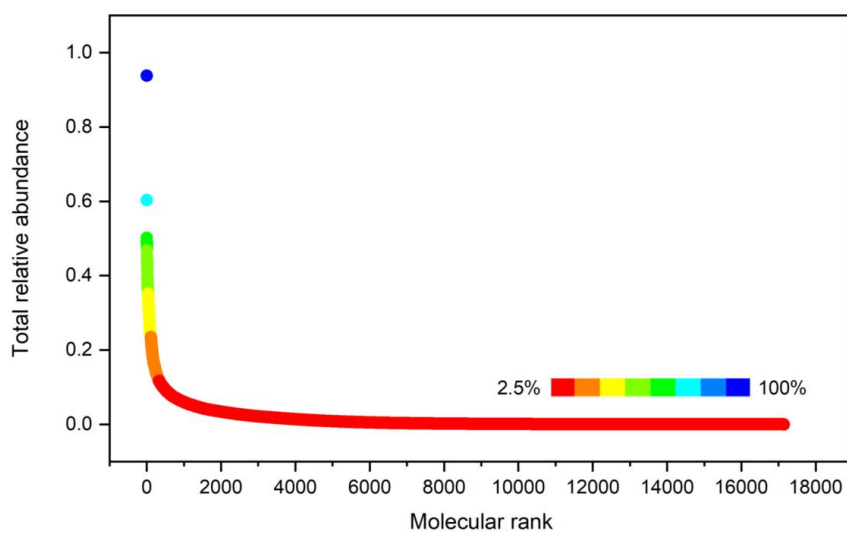


Fig. S1 The VK diagrams for all DOM samples in the Baiyangdian wetland



(a)



(b)

Fig. S2 Molecular distributions of FT-ICR-MS detected compounds in DOM samples across Baiyangdian wetland: (a) Number of unique molecules with each added sample. The black dotted line indicates 95% of compounds. (b) Rank abundance of the compounds across all DOM samples. Molecular compounds are color coded by the percentage of samples in which they occurred.

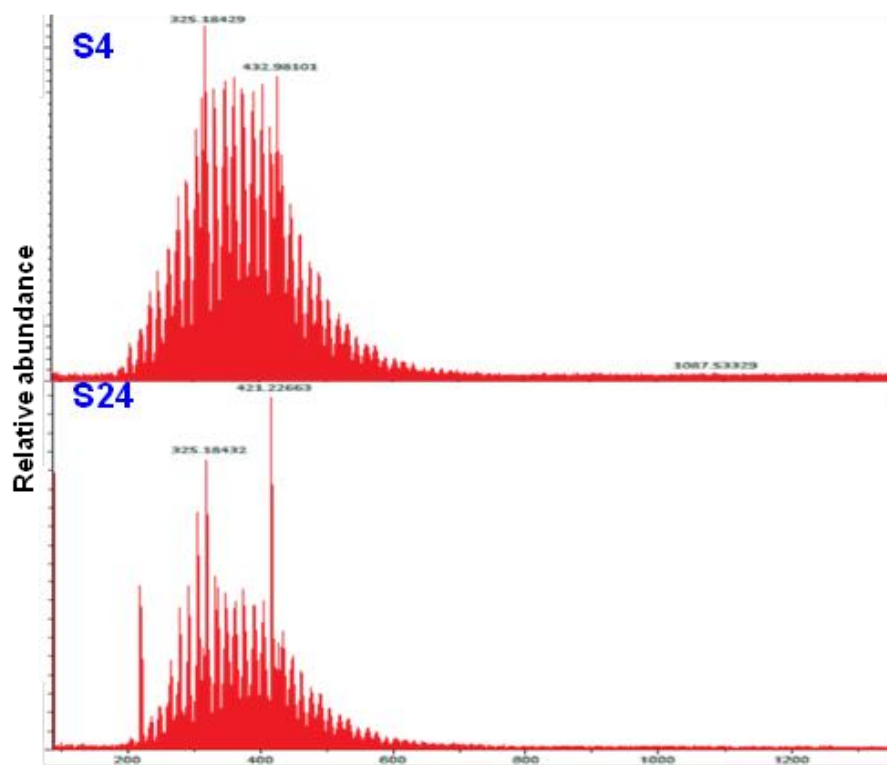


Fig. S3 Negative ion mass spectra of the DOM samples S4 and S24.

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