Supporting Information

Using ESI FT-ICR MS to Characterize Dissolved Organic Matter in Salt Lakes with Different Salinity

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Number of Figures: 4

Number of tables: 4

1. Experimental Methods

Sample extraction for the FT-ICR MS measurement. 500 mL filtered water samples were acidified with hydrochloric acid (p.a. grade, Merck, Germany) to pH 2 and pumped at 5 mL/min through Bond Elut-PPL solid phase extraction cartridges (500 mg, 6 mL, Agilent, USA). The cartridges were previously rinsed with methanol and acidified (pH 2) Milli-Q water. To achieve complete salt removal, the cartridges were rinsed with 50 mL acidified Milli-Q water before elution. Upon the completion of extraction, DOM water samples were eluted with 10 mL LC-MS grade methanol immediately. The eluted samples were stored at -20 °C in the dark before MS analysis, which would be completed within 48 h.

Microbial Community Composition. (1) DNA extraction and PCR amplification. Water samples from QHL and DHL were filtered onto a 0.2 μm Supor filter and frozen at -80 °C. DNA extraction was done using the Water DNA Kit (Omega Bio-tek, U.S.) according to manufacturer's protocols. PCR amplification was carried out by using the primers (338F 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3') in V3-V4 hypervariable regions of the bacteria 16S rRNA gene. The PCR reactions were performed under the following protocal: 3 min at 95 °C (denaturation), 27 cycles of 30 s at 95 °C, 30 s at 55 °C (annealing), and 45 s at 72 °C (elongation), and 10 min at 72 °C (final extension). Triplicate PCR reactions were pooled for each sample, and the amplification products were further purified. **(2) Sequencing analysis.** The purified amplicons were sequenced by Majorbio (Shanghai, China). The raw reads were uploaded to the NCBI

Sequence Read Archive database (Accession Number: SRP265086). Clustering of operational taxonomic units (OTUs) at 97% similarity was done using UPARSE (version7.1). Removal of chimeric sequences was done using UCHIME. Sequence taxonomy was analyzed by Ribosomal Database Project Classifier algorithm using the Silva (SSU123) 16S rRNA database.

2. FT-ICR MS Data Processing

Van Krevelen diagrams boundary regions. In this study, we divided the van Krevelen diagrams into regions corresponding to seven compound classes: lipids, aliphatic/proteins, lignins/carboxylic rich alicyclic molecules (CRAM)-like structures, carbohydrates, unsaturated hydrocarbons, aromatic structures, and tannins, according to previously published reports[1, 2]. The stoichiometric ranges used to establish the classification boundaries follow:

- lipids, H/C = 1.5–2.0, O/C = 0–0.3
- aliphatic/proteins, H/C = 1.5–2.2, O/C = 0.3–0.67
- lignins/CRAM-like structures, H/C = 0.7–1.5, O/C = 0.1–0.67
- carbohydrates, H/C = 1.5–2.4, O/C = 0.67–1.2
- unsaturated hydrocarbons, H/C = 0.7–1.5, O/C = 0–0.1
- aromatic structures, H/C = 0.2–0.7, O/C = 0–0.67
- tannins, H/C = 0.6–1.5, O/C = 0.67–1.0

Equations for average molecular mass, weighted averaged parameters, and aromaticity index (AI). Molecular formulas were used to calculate the weighted averaged values[3, 4] and aromaticity index (AI)[5, 6] for molecular composition with the equations below, where C, H, O, N, and S refer to the stoichiometric number of carbon, hydrogen, oxygen, nitrogen, and sulfur atoms in each formula, respectively. Relative magnitude (M_i) is calculated by dividing the peak magnitude (I_i) by the summed total peak magnitude for each sample.

- $M_i = I_i / \sum I_i$
- Average molecular mass = $\sum m/z_i \times M_i$
- $X/C = \sum X/C_i \times M_i$; X = H, O, N, S
- $DBE = \sum(C + 0.5N - 0.5H + 1)_i \times M_i$
- $AI = \sum[(1 + C - O - S - 0.5H) / (C - O - N - S)]_i \times M_i$

Kendrick Mass Defect (KMD) analysis. The KMD calculation equations for carboxyl group (COO) follow[7-9]:

- Kendrick Mass (COO) = exact m/z of peak × (nominal mass of COO/ exact mass of COO)
- KMD (COO) = Observed nominal mass - Kendrick Mass (COO)

where nominal mass of nominal mass of COO = 44.00000, exact mass of COO = 43.98983, and observed nominal mass is the integer value of the observed mass.

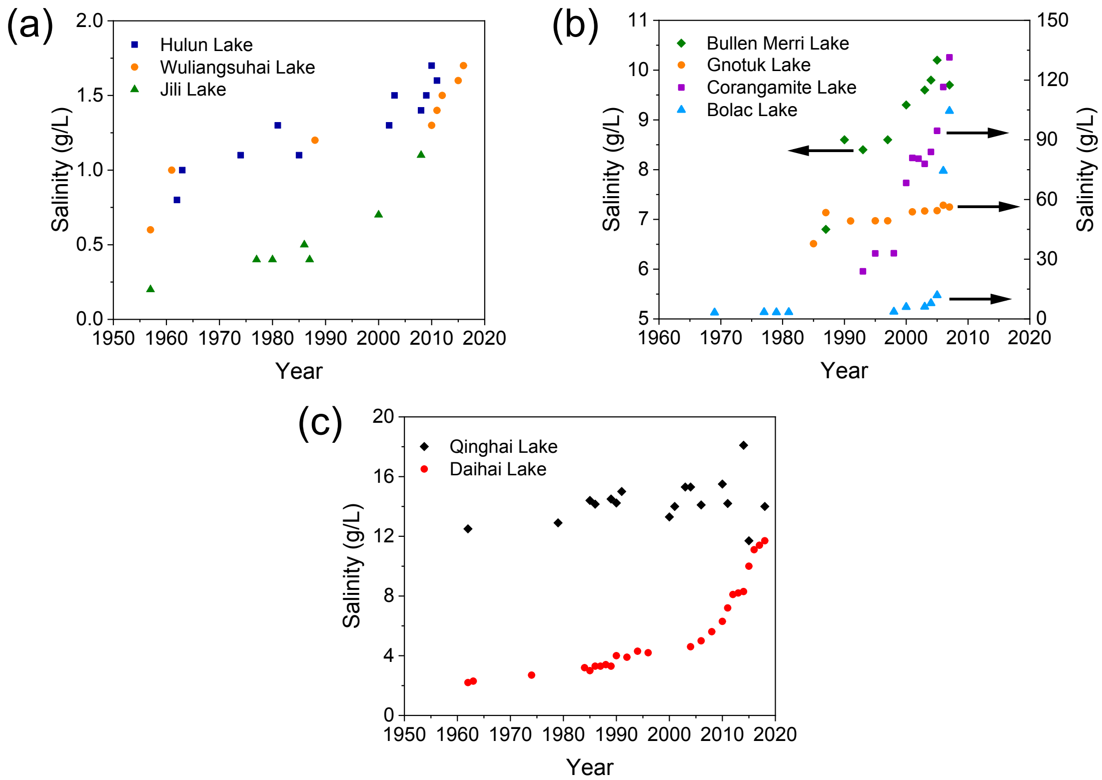

Figure S1. Salinity variation of salt lakes in recent decades. (a) Hunlun Lake, Wuliangsuhai Lake, and Jili Lake from China in Asia[10-12]; (b) Bullen Merri Lake, Gnotuk Lake, Corangamite Lake, and Bolac Lake from Australia in Oceanica[13]; (c) Lakes in the present study: Qinghai Lake and Daihai Lake[14-18].

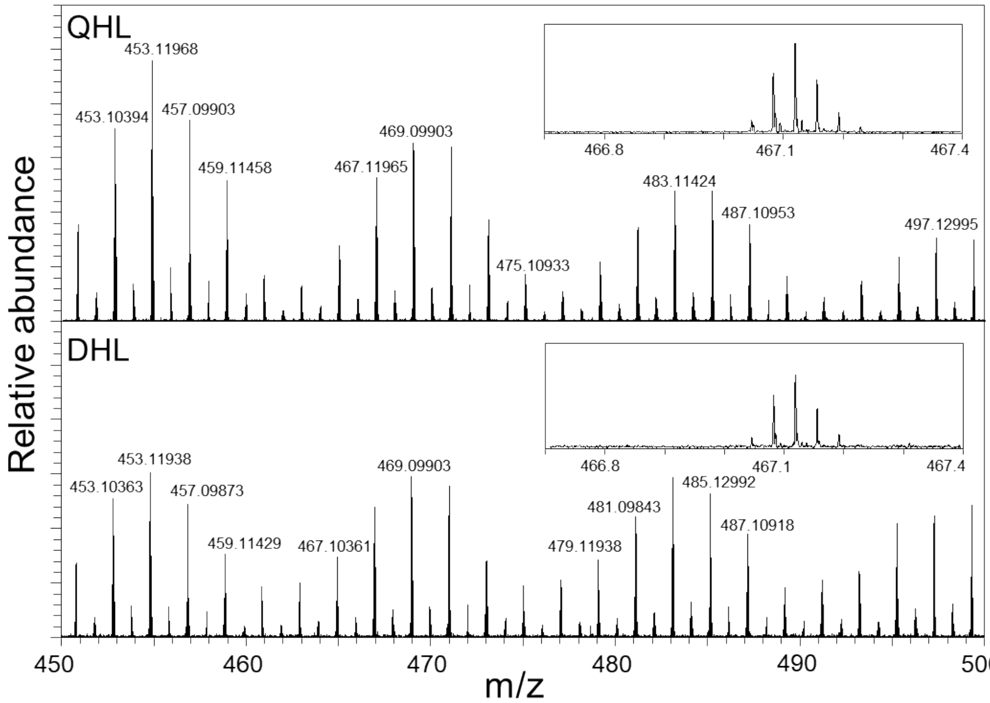

Figure S2. An expanded region (450–500 m/z) of the mass spectra of QHL and DHL. The insets expand the region of 466.7–467.4 m/z. The spectra have high magnitude peaks with a low mass defect (0.0–0.5) of organic matters, and have no obvious peaks with a high mass defect (0.7–0.8) of salts, confirming that PPL-SPE method used in this work effectively removed salts in samples[19].

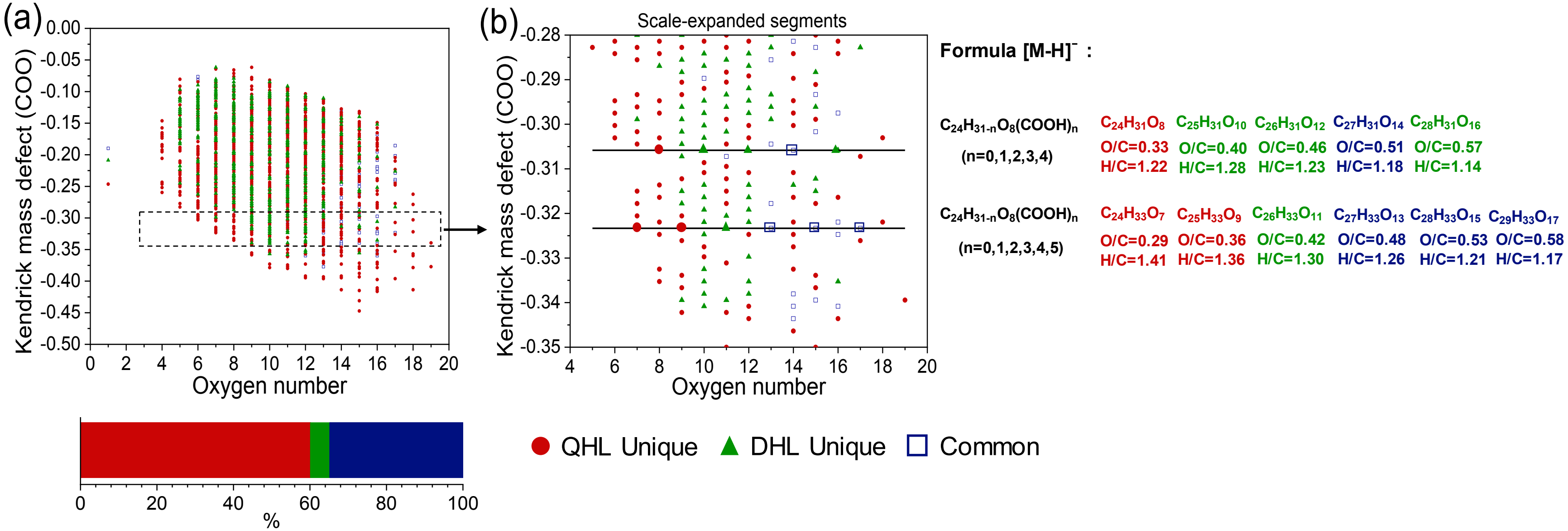

Figure S3. KMD (COO)-number of O in the formulas plots (a) and its scale-expanded segments (b) for lignins/CRAM-like structures of CHO formulas in the DOM of the two lakes. The assigned formulas (as [M-H]- ions) of some randomly selected points in the segments are also displayed. QHL Unique, the unique molecular formulas of QHL; DHL Unique, the unique molecular formulas of DHL; Common, the common molecular formulas of both lakes.

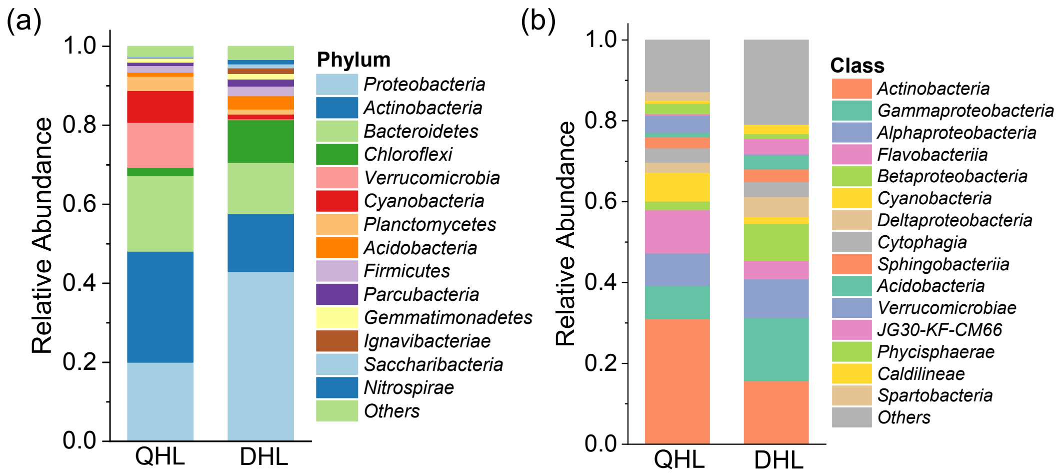

Figure S4. Microbial relative abundance of QHL and DHL at phylum level (a); class level (b).

Table S1. Geographical and climatic conditions of QHL and DHL.

Geographical and climatic conditions	QHL	DHL
Latitude and longitude	36°32′–37°15′N, 99°36′–100°47′E[20]	40°29′–40°37′N, 112°33′–112°46′E[21]
Altitude (m)	3193[20]	1221[21]
Surface area (km^2)	4400[22]	134[23]
Average annual temperature (°C)	0.9–2.7[22]	5.1[23]
Average annual precipitation (mm)	357[20]	423[23]
Average annual evaporation (mm)	800–1000[20]	1162[23]
Annual average solar radiation ($kcal/cm^2$)	~144.3[24]	~141.1[25]
Main soil type	Meadow soil[26]	Meadow soil[27]
Climate zone	East Asian Monsoon; semi-arid region[20]	East Asian Monsoon; semi-arid region[28]
Formation period	Late Tertiary[29]	Late Tertiary[30]

Table S2. Water properties of QHL and DHL.

Sample	pH	Temperature (°C)	DO (mg/L)	Electrical conductivity (mS/cm)	Salinity (g/L)	DOC (mg/L)
QHL	9.20	20.8	6.7	20.01	11.86	9.58
DHL	8.97	21.1	6.3	19.90	10.41	15.87

Table S3. CHONS-Formula (as $[M–H]^-$ ions) from mass scale-expanded segments (m/z = 319) in Figure 1 sorted by apparent molecular series.

Sample	m/z	Series	Formula	O/C	H/C	N/C	S/C
QHL	319.0461	CHO 1	C15H11O8	0.53	0.80	0.00	0.00
	319.0824	CHO 1	C16H15O7	0.44	1.00	0.00	0.00
	319.1188	CHO 1	C17H19O6	0.35	1.18	0.00	0.00
	319.1552	CHO 1	C18H23O5	0.28	1.33	0.00	0.00
	319.0672	CHO 2	C12H15O10	0.83	1.33	0.00	0.00
	319.1035	CHO 2	C13H19O9	0.69	1.54	0.00	0.00
	319.1399	CHO 2	C14H23O8	0.57	1.71	0.00	0.00
	319.0936	CHON 1	C15H15O6N2	0.40	1.07	0.13	0.00
	319.1299	CHON 1	C16H19O5N2	0.31	1.25	0.13	0.00
	319.0494	CHOS 1	C12H15O8S1	0.67	1.33	0.00	0.08
	319.0858	CHOS 1	C13H19O7S1	0.54	1.54	0.00	0.08
	319.1222	CHOS 1	C14H23O6S1	0.43	1.71	0.00	0.07
DHL	319.0459	CHO 1	C15H11O8	0.53	0.8	0	0
	319.0823	CHO 1	C16H15O7	0.44	1	0	0
	319.067	CHO 2	C12H15O10	0.83	1.33	0	0
	319.1033	CHO 2	C13H19O9	0.69	1.54	0	0
	319.1398	CHO 2	C14H23O8	0.57	1.71	0	0
	319.0492	CHOS 1	C12H15O8S1	0.67	1.33	0	0.08
	319.0856	CHOS 1	C13H19O7S1	0.54	1.54	0	0.08
	319.1219	CHOS 1	C14H23O6S1	0.43	1.71	0	0.07

Table S4. Total number of detected compounds, the contents of heteroatom, weighted averaged parameters, aromaticity index (AI) of two lakes.

Sample	Total number of compounds	Number of the O-compound	Number of the N-compound	Number of the S-compound	O/C	H/C	N/C	S/C	DBE	AI
QHL	3719	3639 (97.85%)	2024 (54.42%)	1497 (40.25%)	0.57	1.28	0.03	0.02	7.45	0.15
DHL	2058	1936 (94.07%)	1222 (59.38%)	919 (44.66%)	0.47	1.20	0.23	0.03	10.35	0.19

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