Supporting Information: The Effect of Advanced Secondary Municipal Wastewater Treatment on the Molecular Composition of Dissolved Organic Matter

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S1. Sample characterization

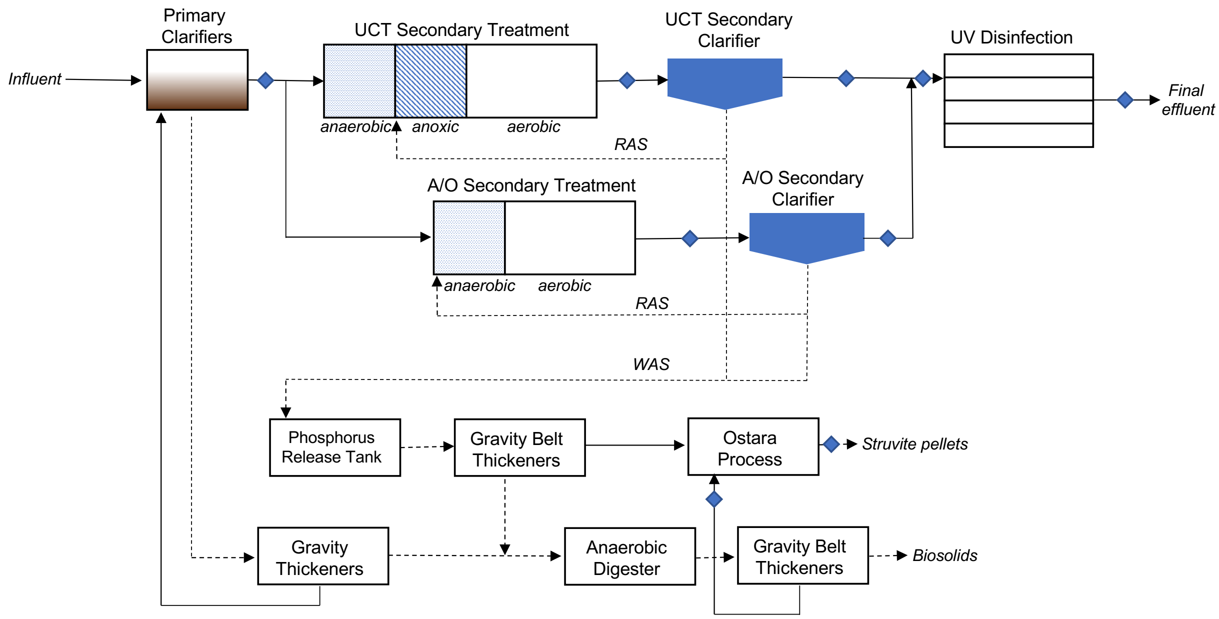

Figure S1. Schematic of liquid (solid lines) and solid (dashed lines) handling in the Nine Springs Wastewater Treatment Plant. RAS indicates return activated sludge. WAS indicates waste activated sludge. Sampling locations are indicated by diamonds.

Bulk analysis methods

Dissolved organic carbon ([DOC]) was determined as the difference between total dissolved carbon and dissolved inorganic carbon with a GE Sievers M5310C TOC analyzer, calibrated daily with potassium hydrogen phthalate. pH was determined with a Mettler Toledo EL20 pH probe, which was calibrated daily. Alkalinity was determined by titration with 0.1 N H_2SO_4 using a Mettler Toledo G20 automatic titrator, which was calibrated daily with $CaCO_3$. Anion concentrations were determined by anion exchange chromatography with a Dionex ICS-2100 instrument utilizing conductivity detection, an AS22 4 x 250 mm column, and 30 mM NaOH eluent. Cation concentrations were quantified by inductively coupled plasma-optical emission spectroscopy with a Perkin Elmer Optima 4300 DV instrument. All anion and cation analytical measurements included calibrations with certified standards. Note that reliable DOC and alkalinity measurements could not be made for the Ostara influent or effluent due to the very high inorganic carbon concentrations in the two samples.

Table S1. Summary of anion concentrations in the main treatment train of the Nine Springs Wastewater Treatment Plant. BDL indicates that the anion concentration was below the detection limit.

Sample	**[Cl^-] (μM)**	**[NO_3^-] (μM)**	**[NO_2^-] (μM)**	**[SO_4^{2-}] (μM)**
Primary Effluent	10736	BDL	BDL	1309
A/O Effluent	9122	1172	BDL	1427
A/O Secondary Clarifier	8465	841	BDL	1407
UCT Effluent	8868	1079	BDL	1359
UCT Secondary Clarifier	7973	1152	BDL	1425
UV Influent	8747	1124	BDL	1403
UV Effluent	8582	1166	BDL	1417

Table S2. Summary of cation concentrations in the main treatment train of the Nine Springs Wastewater Treatment Plant. BDL indicates that the cation concentration was below the detection limit.

Sample	**[Ca^{2+}] (μM)**	**[Fe(III)] (μM)**	**[K^+] (μM)**	**[Mg^{2+}] (μM)**	**[Na^+] (μM)**
Primary Effluent	2332 ± 21	3.4 ± 0.03	5.8 ± 0.6	1983 ± 16	10465 ± 82
A/O Effluent	2453 ± 19	1.8 ± 0.01	3.6 ± 0.7	1789 ± 7	8780 ± 63
A/O Secondary Clarifier	2172 ± 15	BDL	3.5 ± 1.0	1779 ± 11	9020 ± 83
UCT Effluent	2123 ± 13	BDL	2.8 ± 0.6	1804 ± 17	8938 ± 62
UCT Secondary Clarifier	2129 ± 17	BDL	5.0 ± 0.5	1790 ± 12	8781 ± 51
UV Influent	2168 ± 22	BDL	4.5 ± 0.5	1803 ± 11	8851 ± 118
UV Effluent	2095 ±27	BDL	3.5 ± 0.6	1758 ± 12	8470 ± 92

S2. FT-ICR MS analysis

For comparison of molecular composition before and after wastewater treatment processes, the formula lists were examined for formulas that were present in only the influent or the effluent. For common formulas, their change in intensity was compared using a relative intensity index:

$$Relative\ intensity\ index = \frac{I_{m/z,1}/\sum I_1}{I_{m/z,2}/\sum I_2} \qquad \textbf{(S1)}$$

where $I_{m/z,1}$ is the intensity of the ion in the influent and $\sum I_1$ is the sum of the intensities of all identified formulas in the influent. The analogous parameters with the subscript of 2 correspond to the effluent. Formulas with a relative intensity index >1 are designated as having a higher relative intensity in the influent, while samples with an index <1 have a higher relative intensity in the effluent.

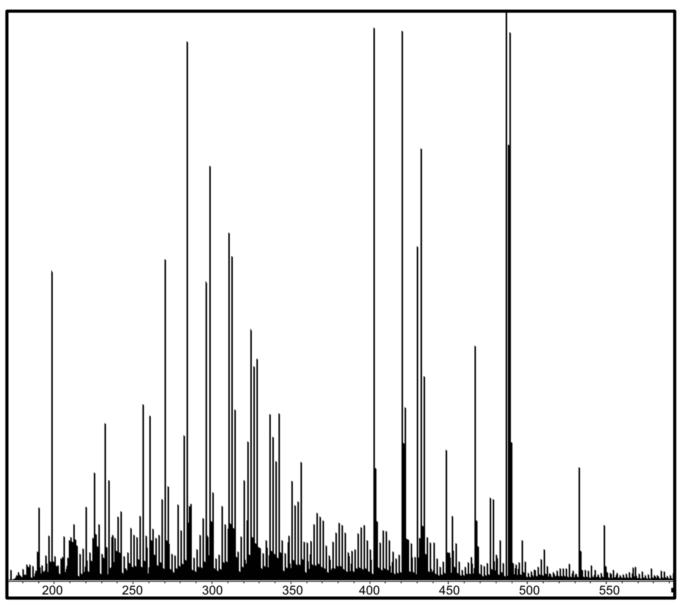

Figure S2. Representative negative mode electrospray ionization FT-ICR mass spectrum for UCT secondary effluent (170 – 600 *m/z*).

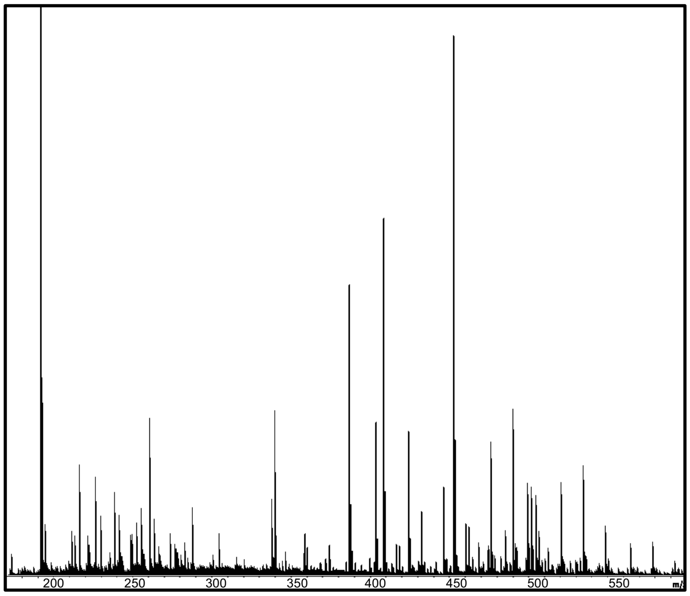

Figure S3. Representative positive mode electrospray ionization FT-ICR mass spectrum for A/O secondary effluent (170 – 600 *m/z*).

Table S3. The number of formulas in each compound class and the total number of formulas identified by FT-ICR MS in negative mode for each sample.

Sample	CHO	CHON	CHOS	CHOP	CHOCl	Assigned formulas	Total # of peaks
Primary Effluent	953	212	704	180	178	2227	18500
A/O Effluent	1430	535	672	229	129	2995	19679
A/O Secondary	1365	470	621	221	77	2754	20416
UCT Effluent	1533	545	676	32	227	3013	19738
UCT Secondary	1294	414	629	217	114	2668	20422
UV Influent	1382	501	674	240	95	2892	20406
UV Effluent	1442	487	684	202	109	2924	19925
Ostara Influent	1118	349	558	19	62	2106	20007
Ostara Effluent	1254	633	439	49	100	2475	17568

Table S4. The number of unique formulas in each compound class and the total number of formulas identified by FT-ICR MS in positive mode for each sample, as well as the average (± the standard deviation) of the H:C, O:C, DBE, AI, MLB_L, and mass accuracy of all assigned formulas.

Sample	$H:C_{avg}$	$O:C_{avg}$	DBE_{avg}	AI_{avg}	MLB_L (%)	error (ppm)	CHO	CHON	Assigned formulas	Total # of peaks
Primary Effluent	1.65 ± 0.32	0.35 ± 0.14	4.89 ± 4.17	0.06 ± 0.23	72.5	-0.02 ± 0.11	586	229	815	16565
A/O Effluent	1.46 ± 0.33	0.43 ± 0.18	6.36 ± 3.46	0.14 ± 0.25	46.7	-0.05 ± 0.09	1038	911	1949	19478
A/O Secondary	1.47 ± 0.31	0.43 ± 0.17	6.40 ± 3.39	0.12 ± 0.25	48.4	-0.03 ± 0.10	1196	608	1804	19415
UCT Effluent	1.44 ± 0.33	0.39 ± 0.16	6.19 ± 3.51	0.17 ± 0.24	45.9	-0.05 ± 0.10	834	351	1185	17719
UCT Secondary	1.50 ± 0.32	0.43 ± 0.16	6.05 ± 3.40	0.11 ± 0.25	50.6	-0.04 ± 0.09	1368	539	1907	17816
UV Influent	1.55 ± 0.30	0.43 ± 0.16	5.79 ± 3.31	0.07 ± 0.25	57.1	-0.04 ± 0.09	1053	633	1686	18764
UV Effluent	1.52 ± 0.31	0.42 ± 0.16	6.04 ± 3.44	0.10 ± 0.24	52.8	-0.03 ± 0.10	1260	467	1727	18605
Ostara Influent	1.60 ± 0.30	0.41 ± 0.13	5.41 ± 3.60	0.05 ± 0.23	65.5	0.00 ± 0.11	920	184	1104	17794
Ostara Effluent	1.59 ± 0.29	0.40 ± 0.16	5.23 ± 3.15	0.06 ± 0.23	64.2	0.00 ± 0.10	1017	283	1300	17786

Table S5. Ranges used to define compound classes in van Krevelen diagrams.

Class	H:C	O:C	Reference
1. Lipids	1.5-2.2	0-0.3	(Hockaday et al. 2009, Sleighter and Hatcher 2007)
2. Proteins	1.5-2.2	0.3-0.6	(Hockaday et al. 2009, Kujawinski et al. 2009)
3. Aminosugars	1.5-1.7	0.6-0.7	(Sleighter and Hatcher 2007)
4. Carbohydrates	>1.5	>0.67	(Hockaday et al. 2009)
5. Condensed hydrocarbons	0.5-1.1	0-0.25	(Kujawinski et al. 2009)
6. Lignin	0.7-1.5	0.3-0.67	(Hockaday et al. 2009, Kujawinski et al. 2009)
7. Tannin	0.5-1.3	0.6-0.9	(Sleighter and Hatcher 2007)

S3. van Krevelen diagrams of all Nine Springs samples

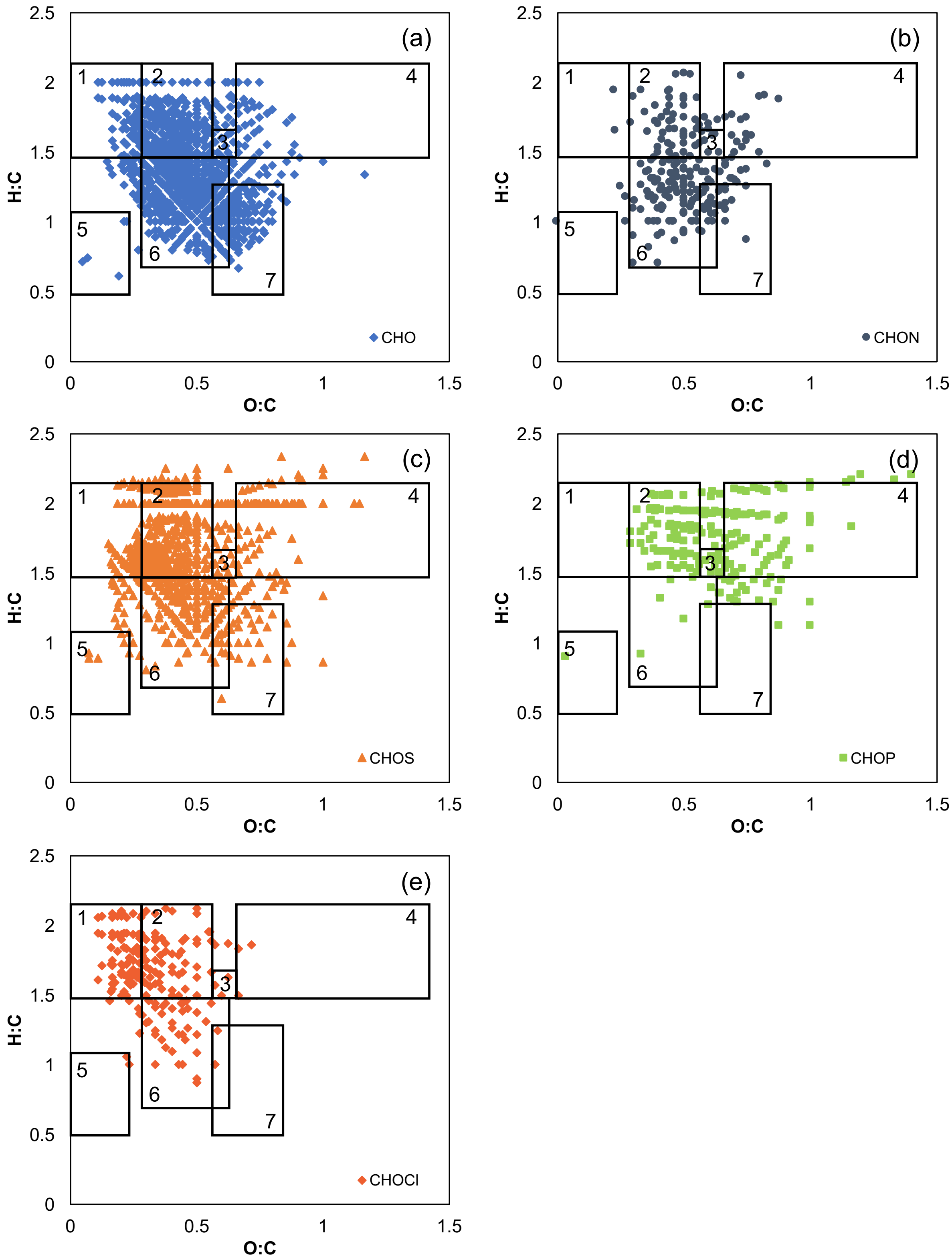

Figure S4. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the Nine Springs primary effluent determined using negative mode FT-ICR MS.

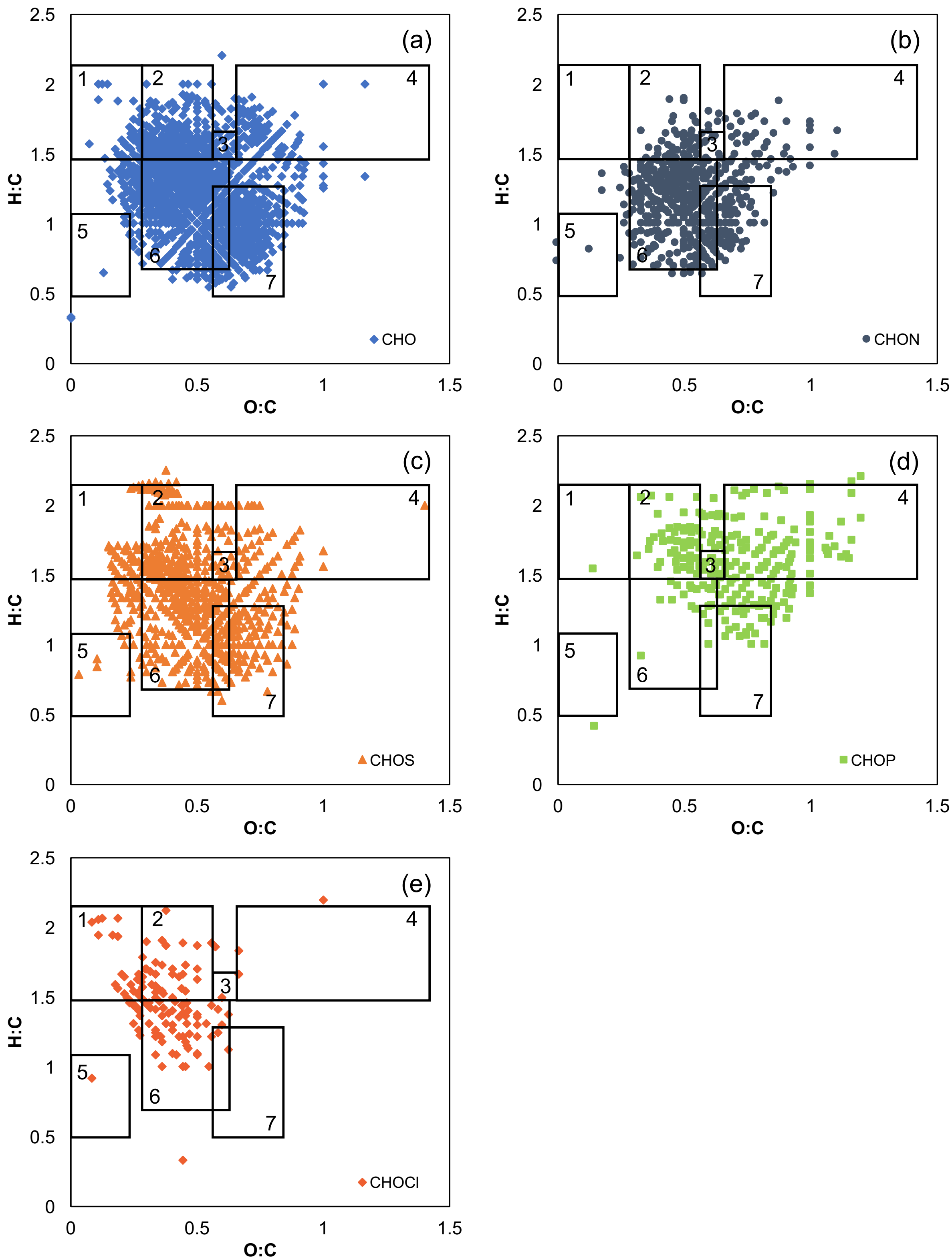

Figure S5. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the A/O secondary effluent determined using negative mode FT-ICR MS.

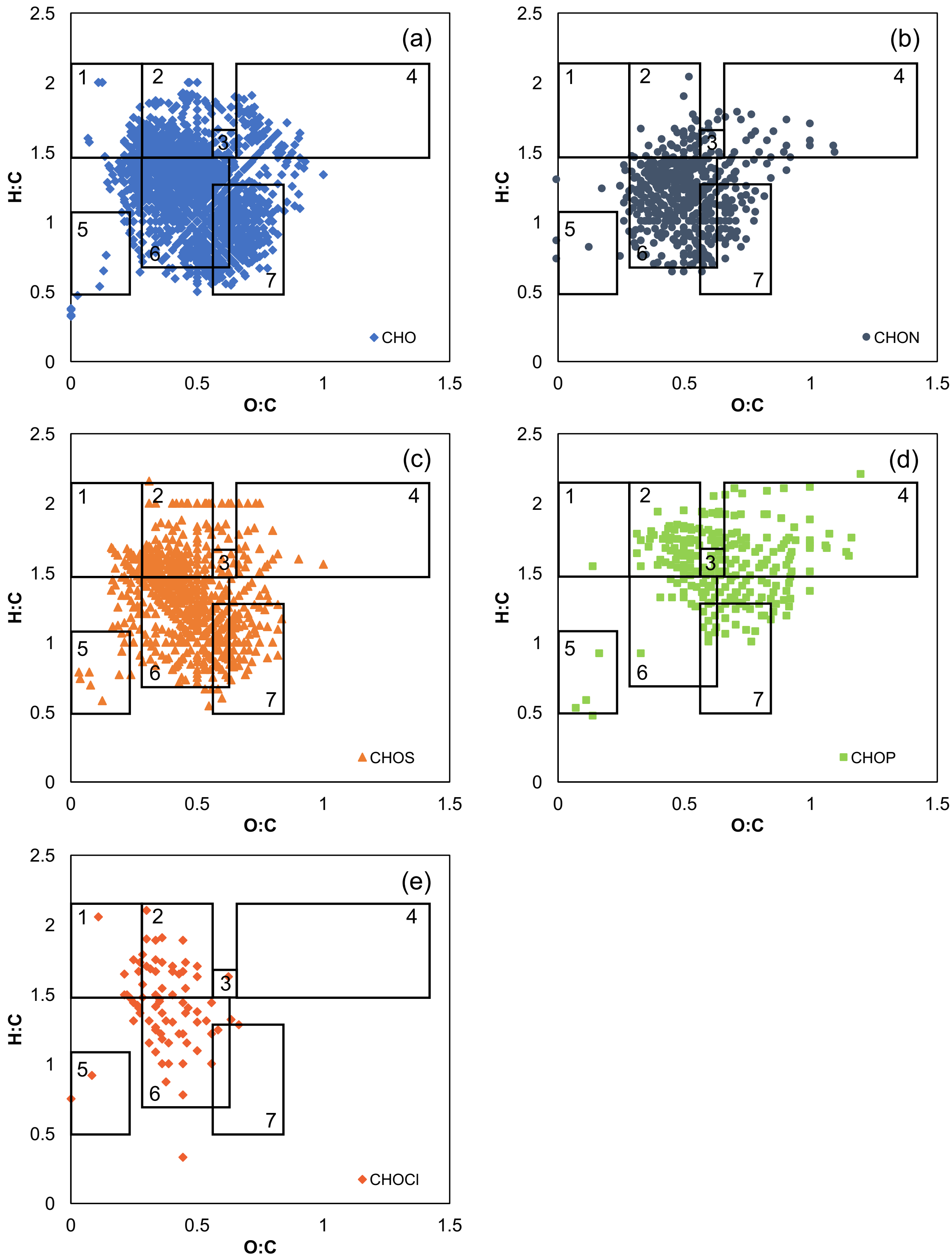

Figure S6. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the A/O secondary clarifier effluent determined using negative mode FT-ICR MS.

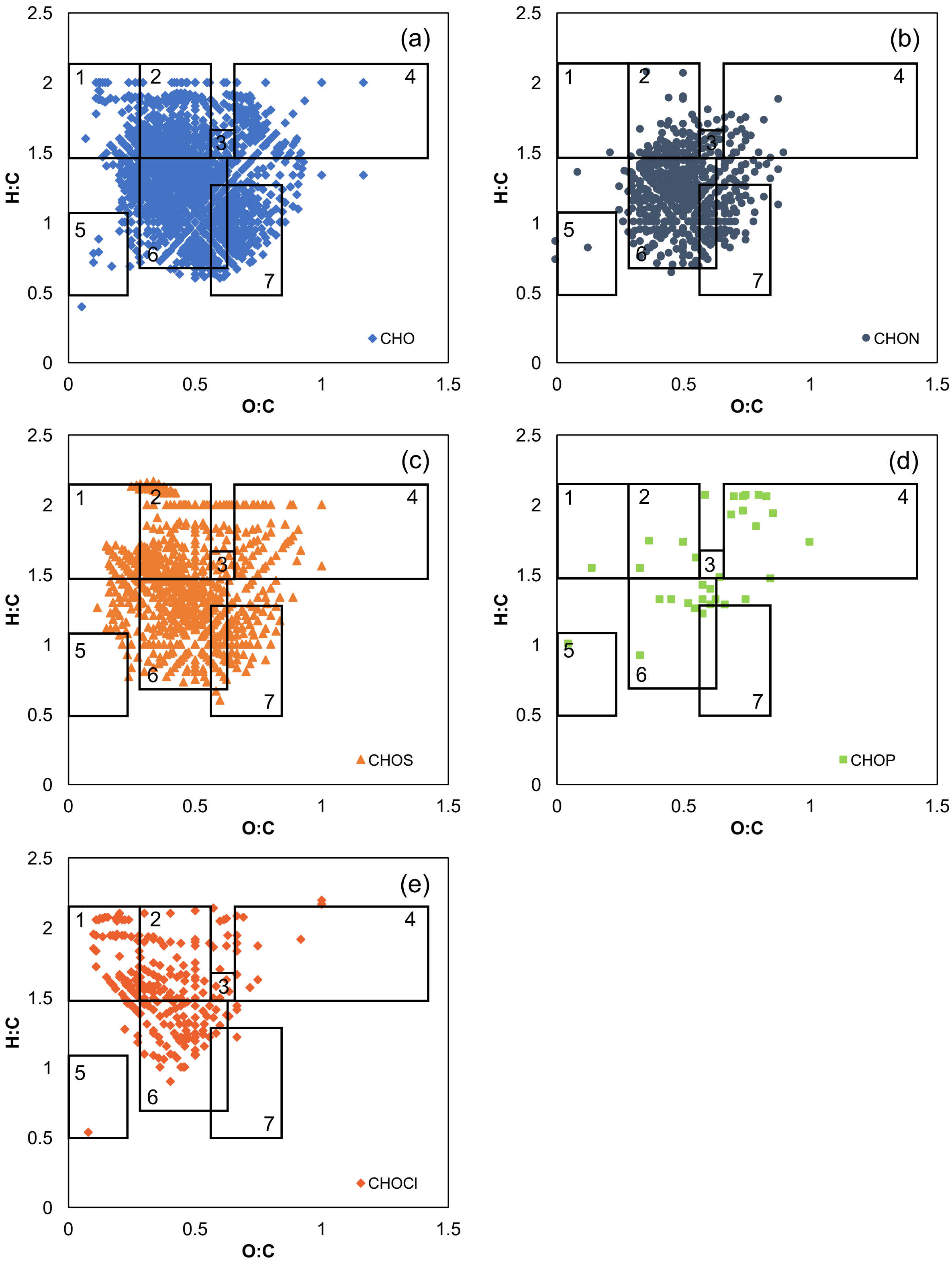

Figure S7. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the Nine Springs UCT secondary effluent determined using negative mode FT-ICR MS.

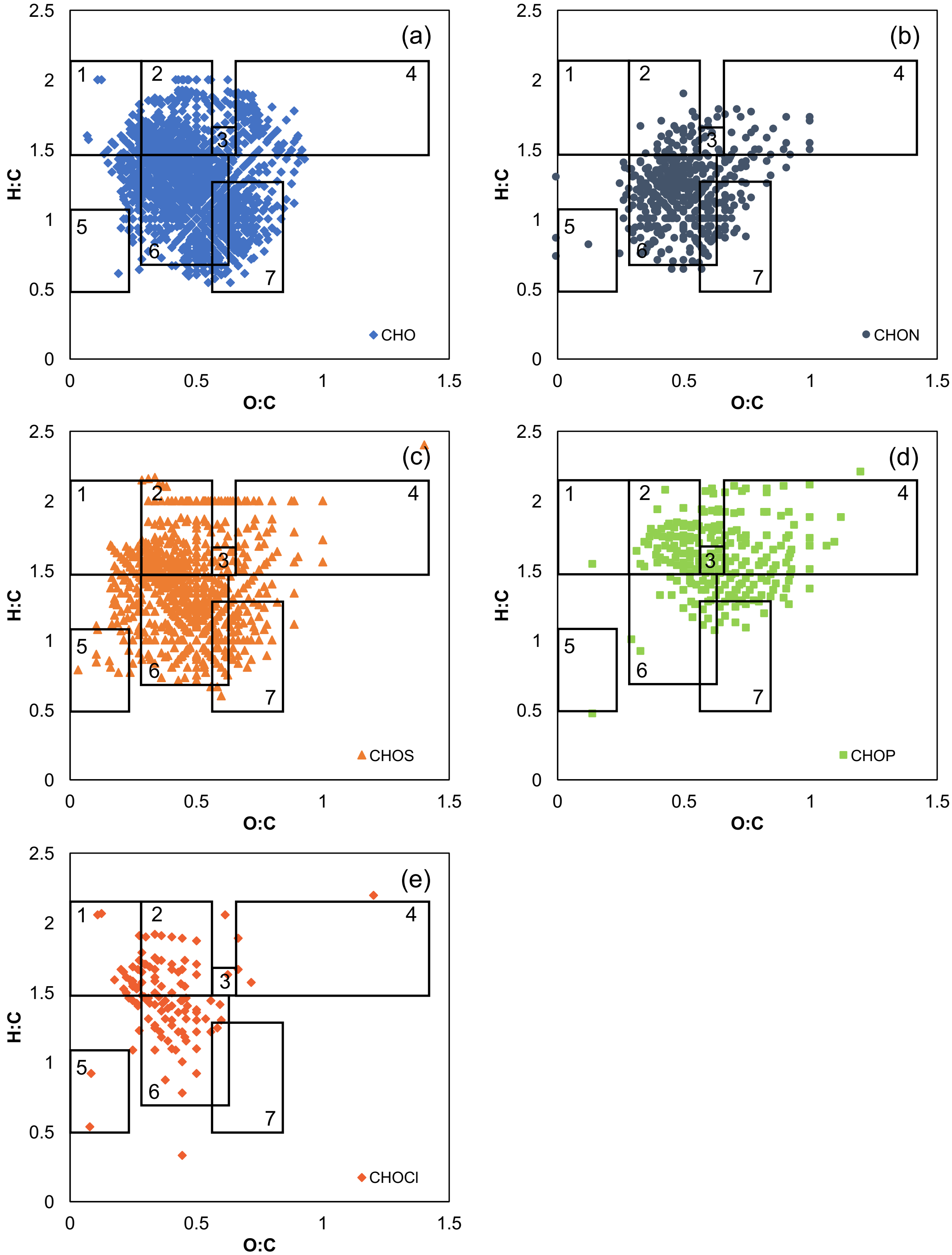

Figure S8. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the UCT secondary clarifier effluent determined using negative mode FT-ICR MS.

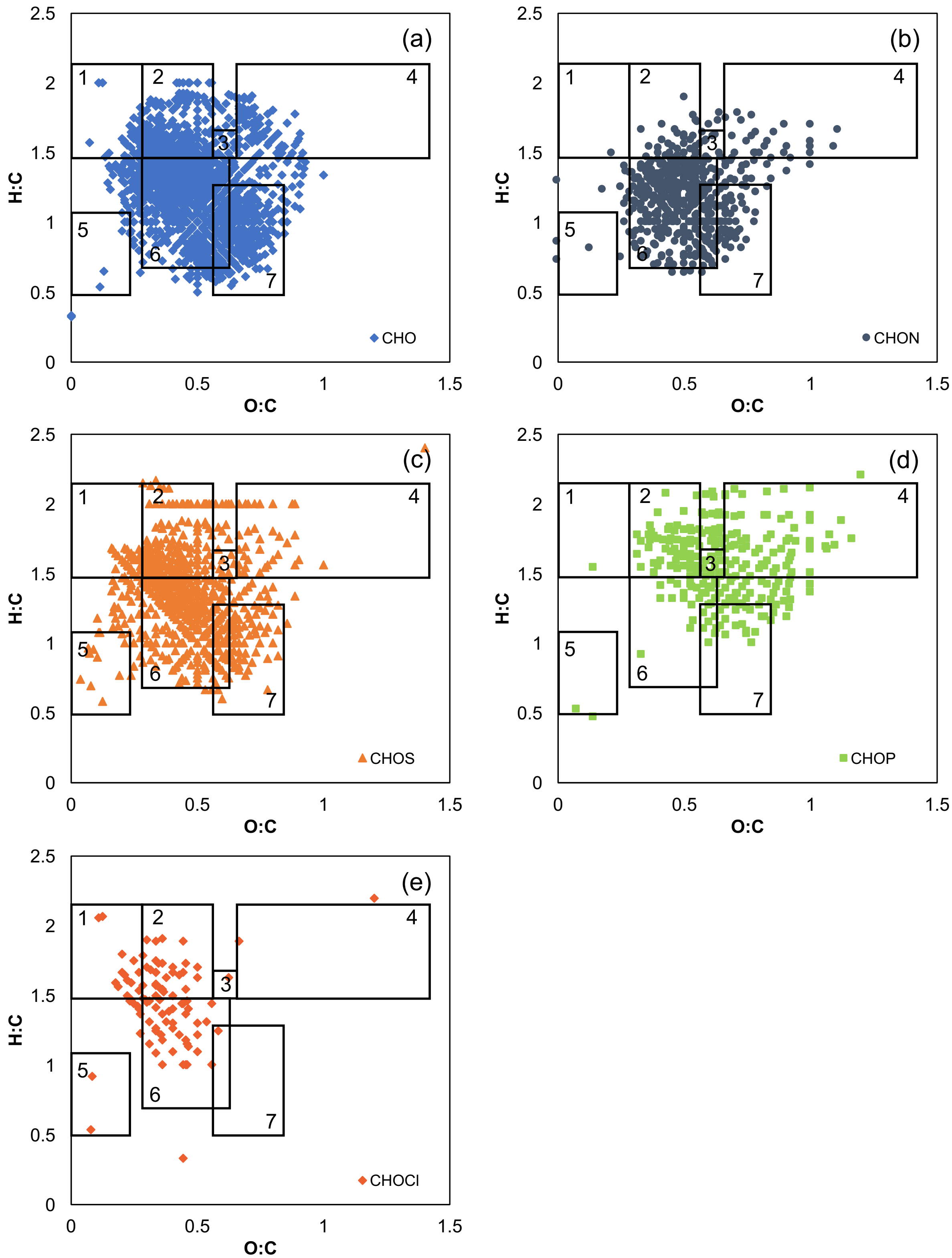

Figure S9. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the Nine Springs UV influent determined using negative mode FT-ICR MS.

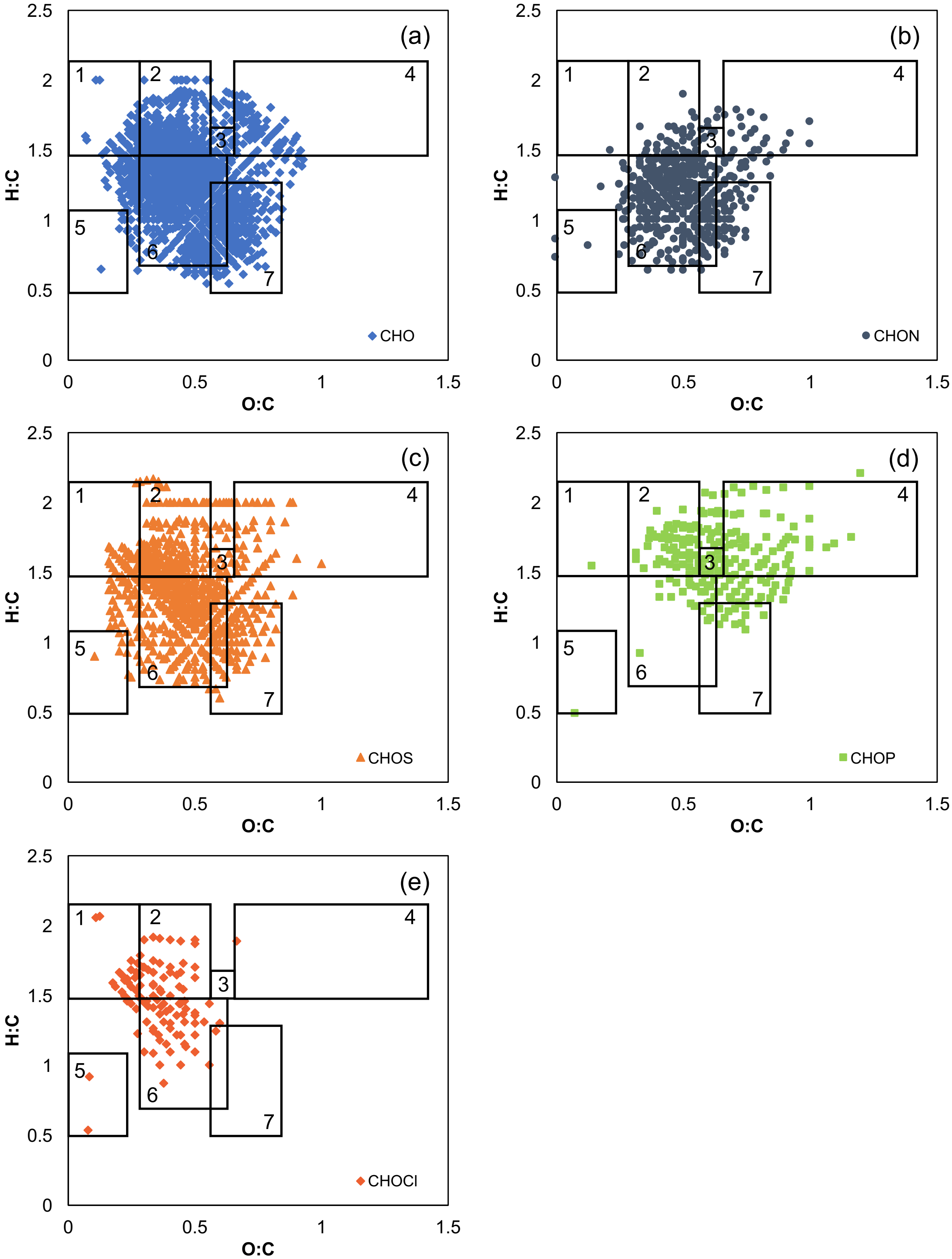

Figure S10. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the Nine Springs UV effluent determined using negative mode FT-ICR MS.

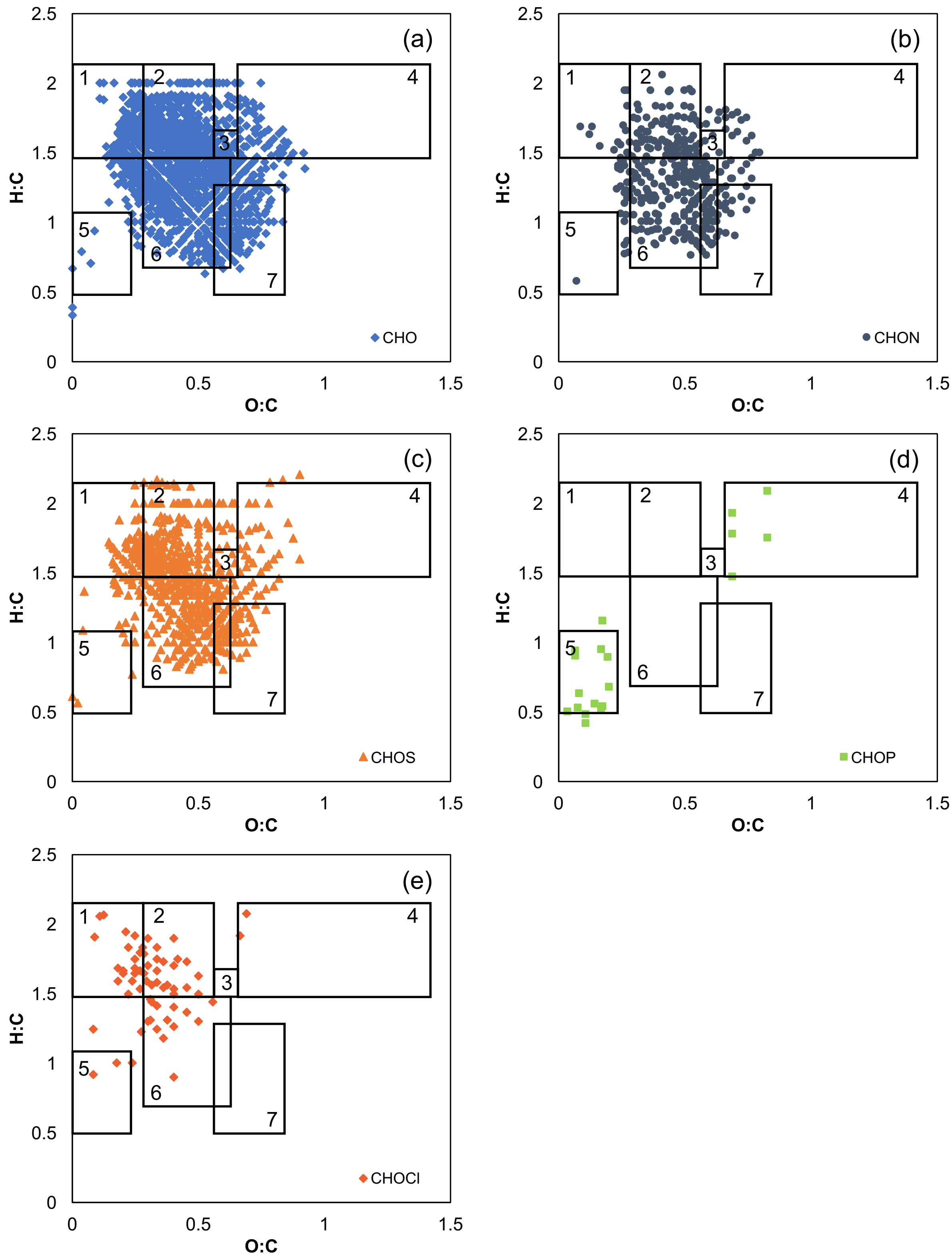

Figure S11. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the Nine Springs Ostara influent determined using negative mode FT-ICR MS.

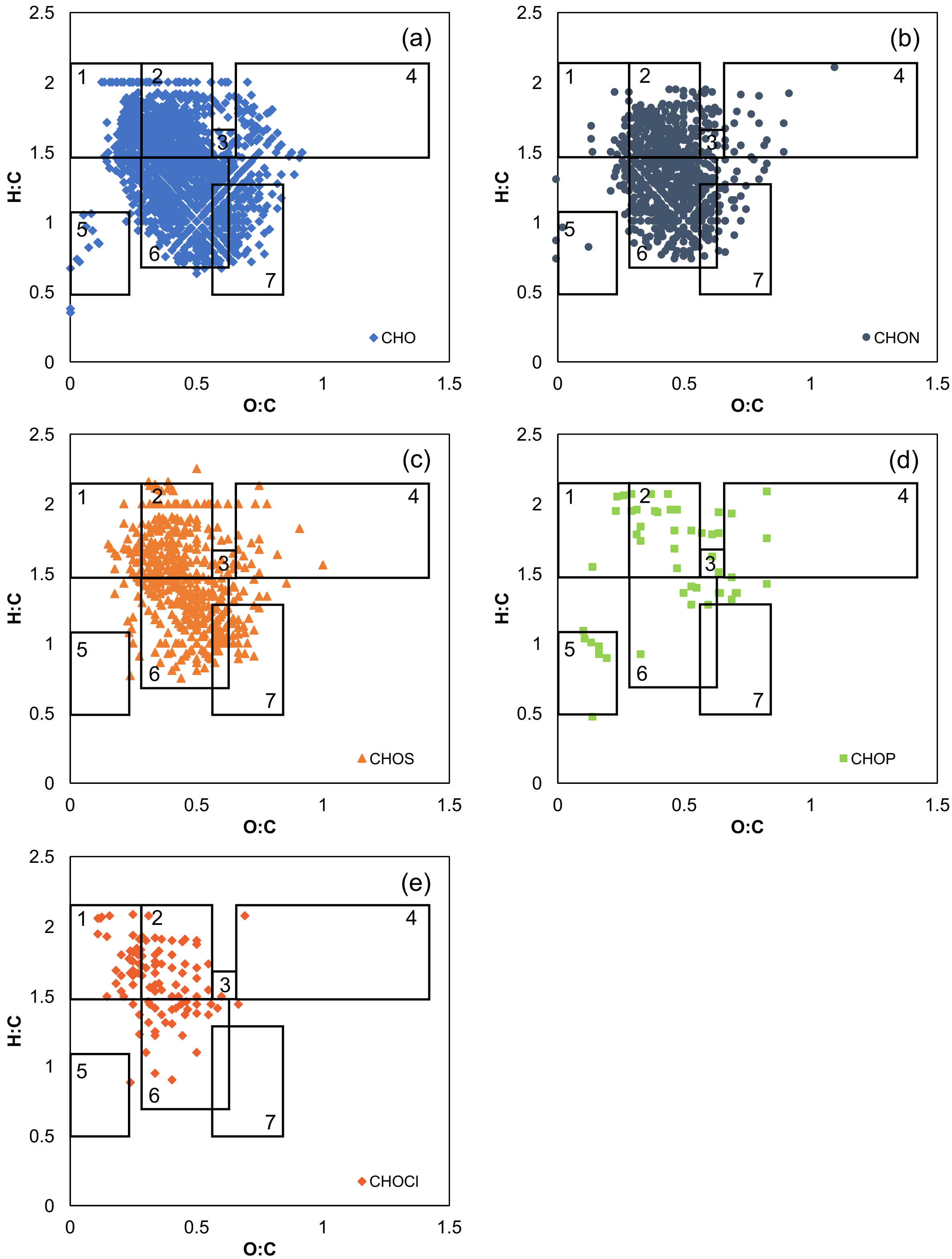

Figure S12. van Krevelen diagrams for (a) CHO, (b) CHON, (c) CHOS, (d) CHOP, and (e) CHOCl formulas in the Nine Springs Ostara effluent determined using negative mode FT-ICR MS.

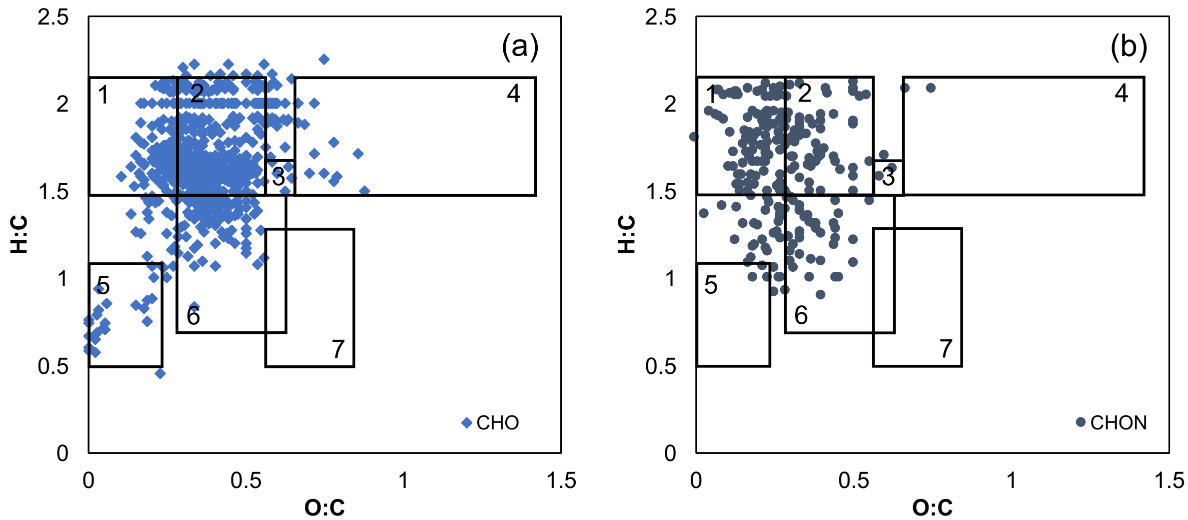

Figure S13. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs primary effluent determined using positive mode FT-ICR MS.

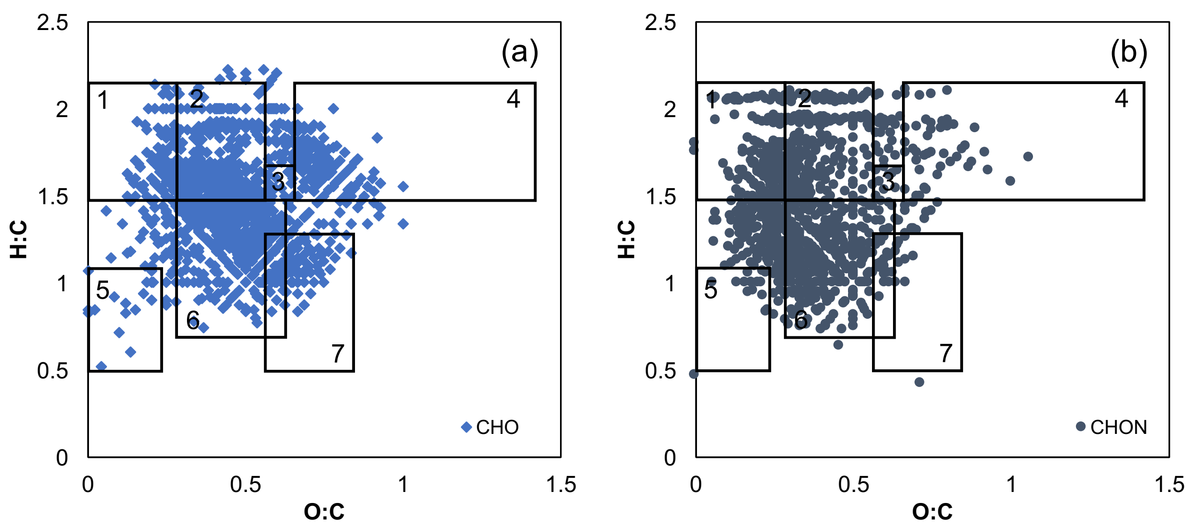

Figure S14. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs A/O secondary effluent determined using positive mode FT-ICR MS.

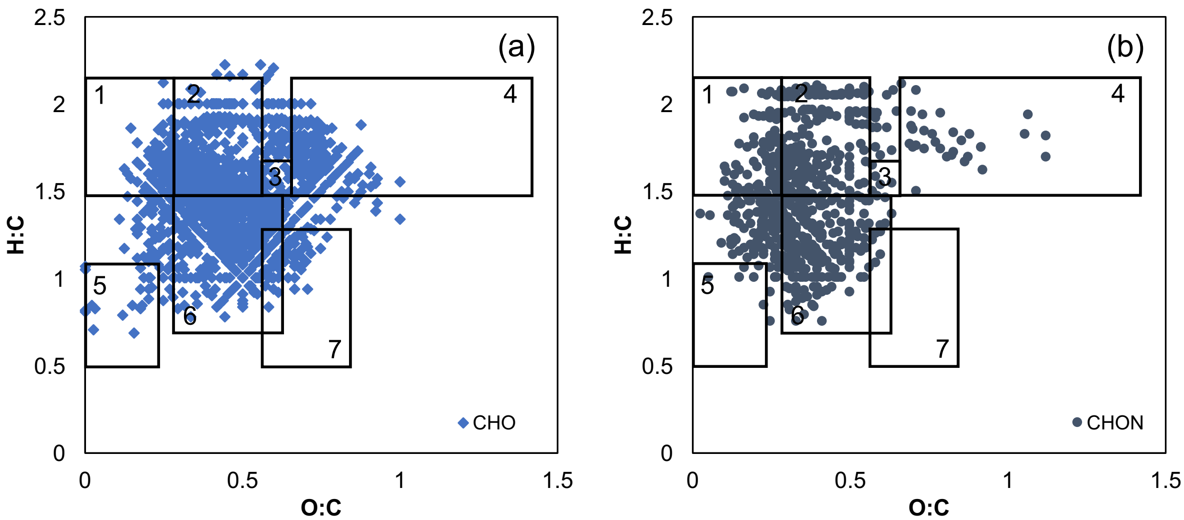

Figure S15. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs A/O secondary clarifier effluent determined using positive mode FT-ICR MS.

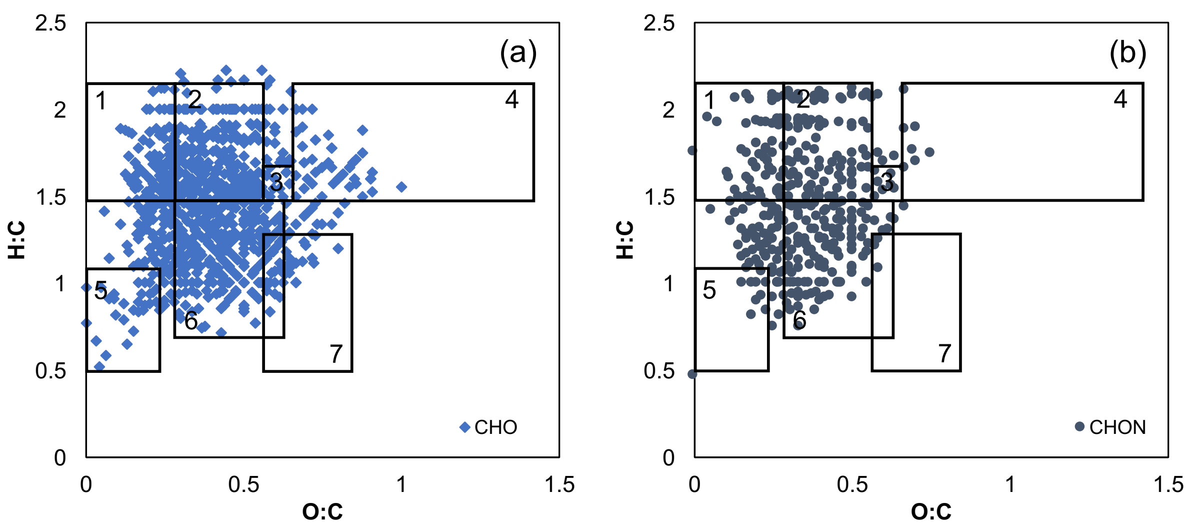

Figure S16. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs UCT secondary effluent determined using positive mode FT-ICR MS.

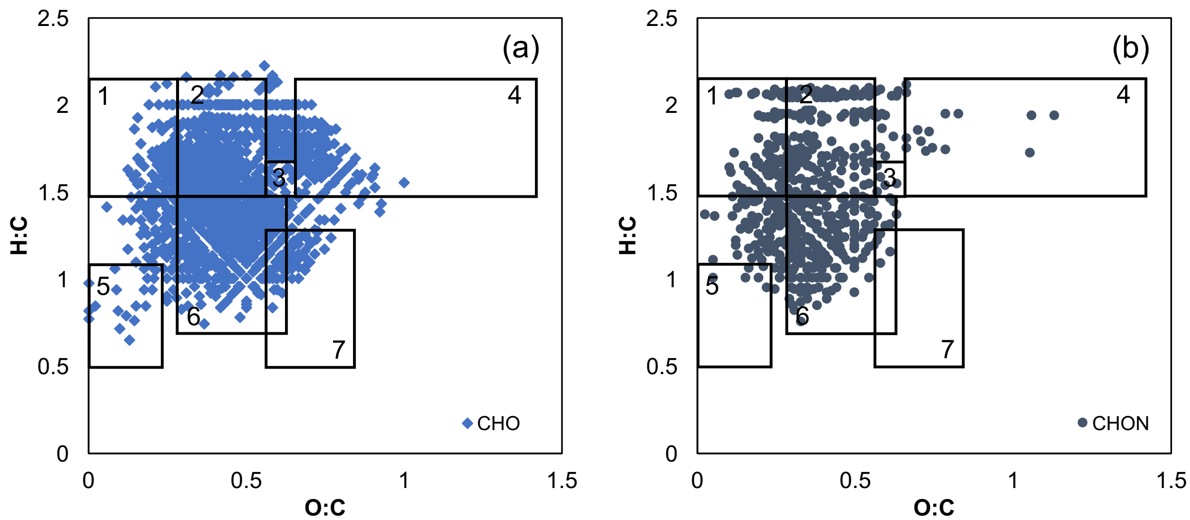

Figure S17. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs UCT secondary clarifier effluent determined using positive mode FT-ICR MS.

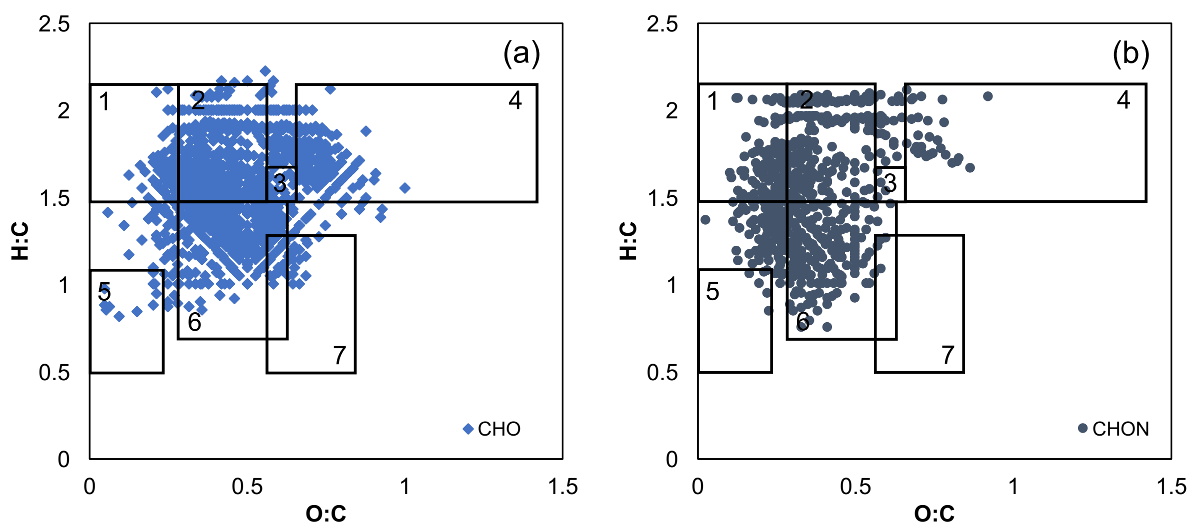

Figure S18. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs UV influent determined using positive mode FT-ICR MS.

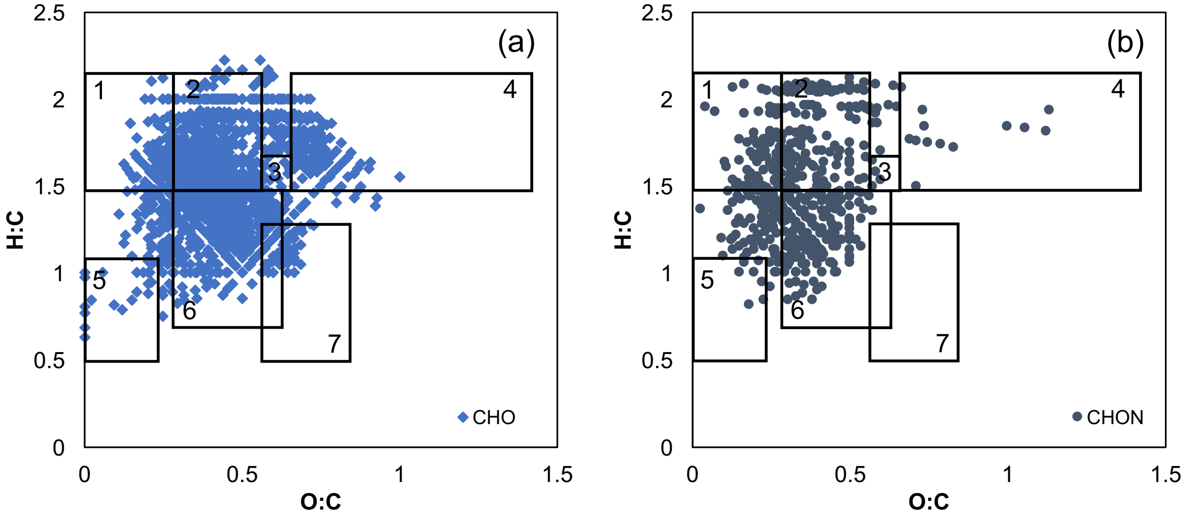

Figure S19. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs UV effluent determined using positive mode FT-ICR MS.

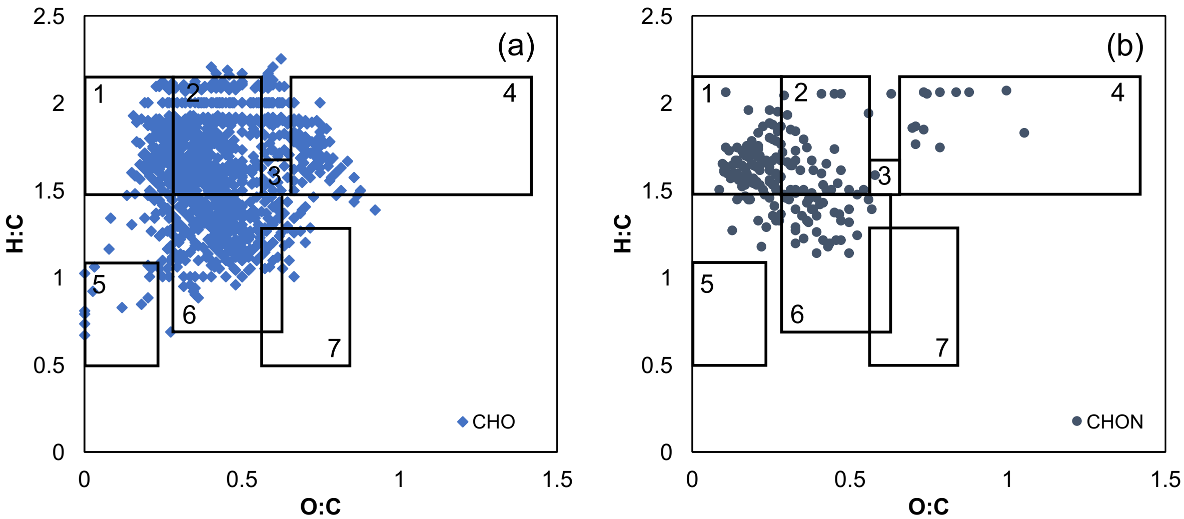

Figure S20. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs Ostara influent determined using positive mode FT-ICR MS.

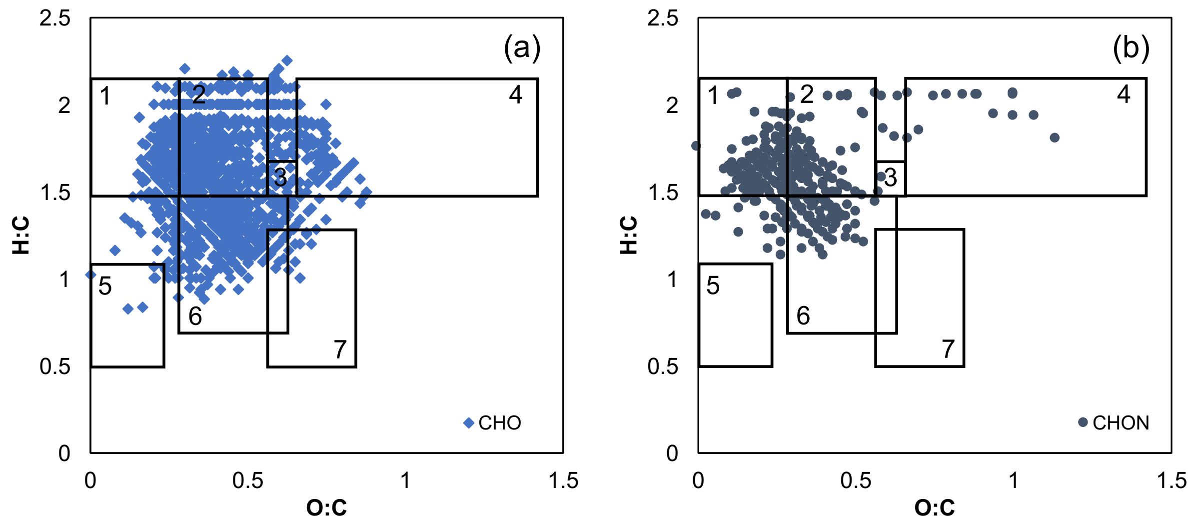

Figure S21. van Krevelen diagrams for (a) CHO and (b) CHON formulas in the Nine Springs Ostara effluent determined using positive mode FT-ICR MS.

S4. Comparison of ESI polarity

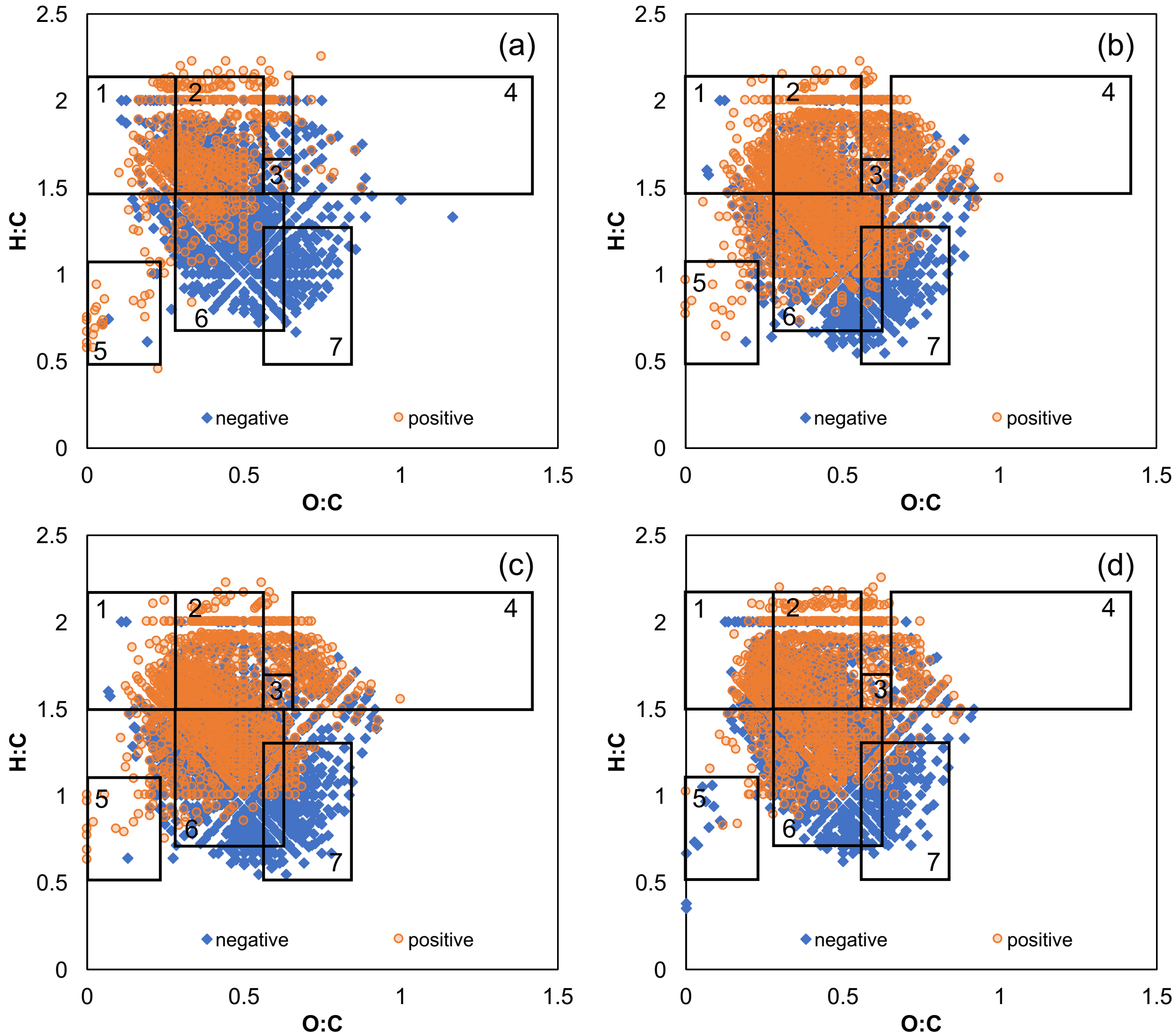

Figure S22. Representative van Krevelen diagrams showing CHO formulas for (a) primary effluent, (b) UCT secondary clarifier effluent, (c) final effluent, and (d) Ostara effluent samples determined using negative mode and positive mode FT-ICR MS.

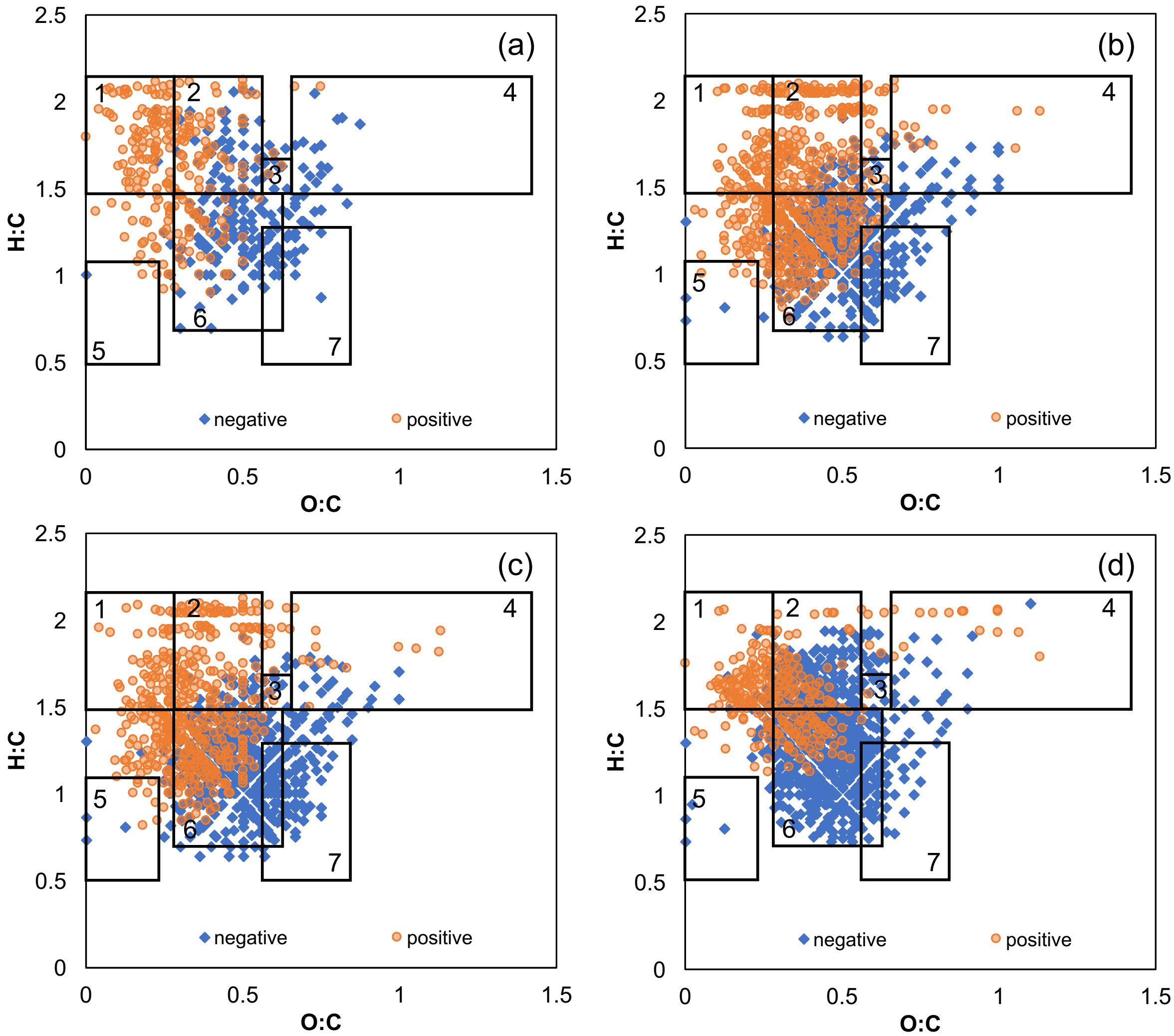

Figure S23. Representative van Krevelen diagrams showing CHON formulas for (a) primary effluent, (b) UCT secondary clarifier effluent, (c) final effluent, and (d) Ostara effluent samples determined using negative mode and positive mode FT-ICR MS.

S5. Process comparison

Primary effluent vs. A/O secondary effluent

Table S6. The number, H:C, O:C, and DBE of formulas that were only present in primary effluent or had increased relative intensity in primary effluent compared to A/O secondary effluent as determined using FT-ICR MS in negative mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in primary eff.	139	1.60	0.40	5.21	Only in A/O effluent	616	1.17	0.54	9.36
	Increased I in primary eff.	152	1.61	0.42	4.07	Increased I in A/O effluent	662	1.22	0.50	7.91
CHON	Only in primary eff.	49	1.61	0.48	4.33	Only in A/O effluent	372	1.16	0.54	9.22
	Increased I in primary eff.	41	1.48	0.52	4.34	Increased I in A/O effluent	122	1.24	0.53	7.05
CHOS	Only in primary eff.	298	1.74	0.45	3.86	Only in A/O effluent	267	1.16	0.54	8.00
	Increased I in primary eff.	176	1.65	0.43	3.40	Increased I in A/O effluent	230	1.38	0.49	5.34
CHOP	Only in primary eff.	81	1.77	0.65	3.16	Only in A/O effluent	130	1.49	0.76	5.56
	Increased I in primary eff.	29	1.92	0.65	2.17	Increased I in A/O effluent	70	1.62	0.66	4.49
CHOCl	Only in primary eff.	104	1.71	0.31	2.55	Only in A/O effluent	55	1.38	0.38	5.25
	Increased I in primary eff.	37	1.65	0.37	2.32	Increased I in A/O effluent	37	1.47	0.37	4.22

Table S7. The number, H:C, O:C, and DBE of formulas that were only present in primary effluent or had increased relative intensity in primary effluent compared to A/O secondary effluent as determined using FT-ICR MS in positive mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in primary eff.	289	1.69	0.34	5.21	Only in A/O effluent	741	1.41	0.50	6.62
	Increased I in primary eff.	183	1.70	0.39	4.01	Increased I in A/O effluent	114	1.48	0.41	5.91
CHON	Only in primary eff.	95	1.45	0.37	6.66	Only in A/O effluent	777	1.43	0.39	6.95
	Increased I in primary eff.	61	1.76	0.31	3.54	Increased I in A/O effluent	73	1.44	0.31	6.22

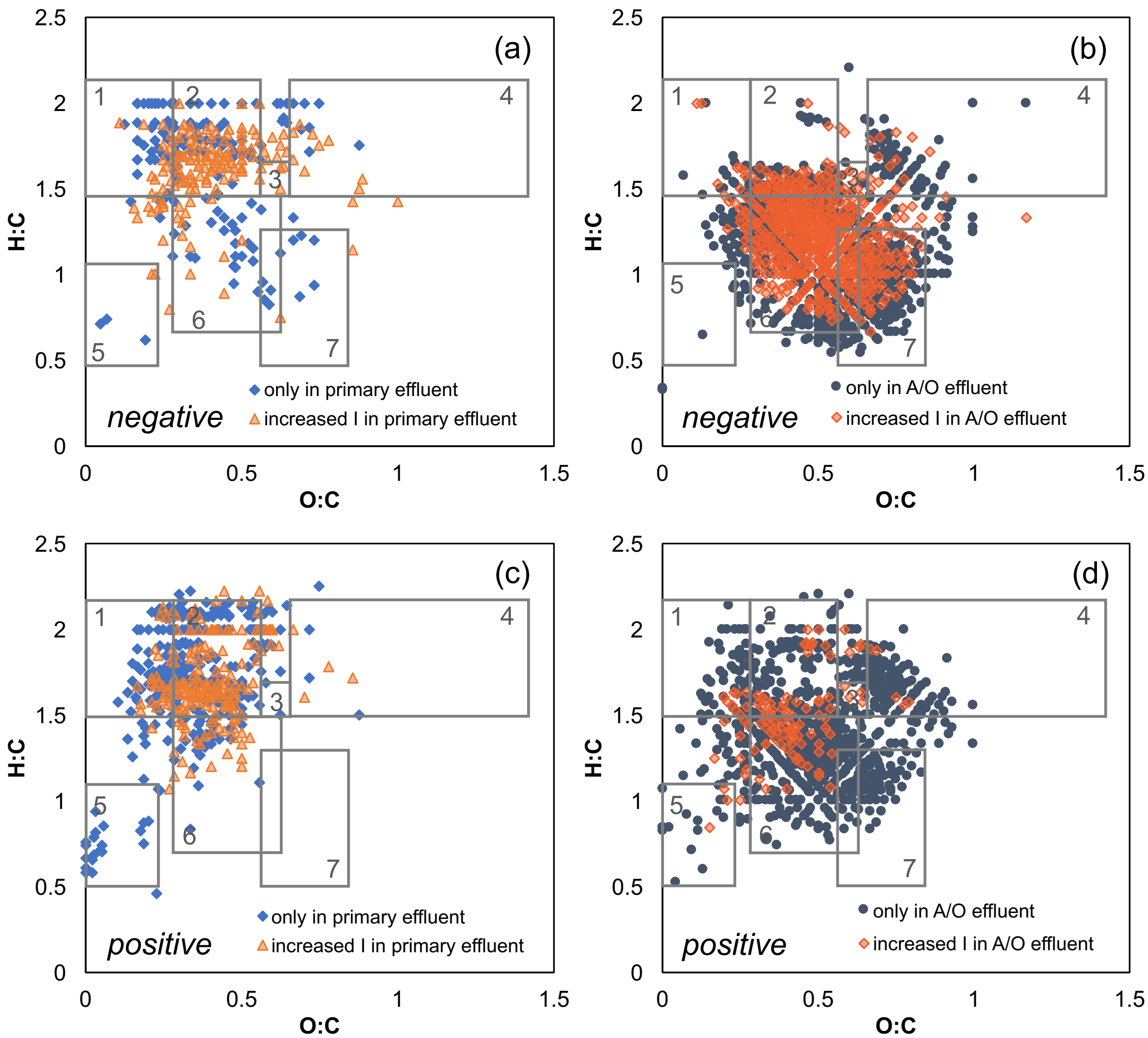

Figure S24. Negative and positive mode CHO formulas that were (a, c) only present in primary effluent or had increased relative intensity in primary effluent compared to A/O secondary effluent and (b, d) only present in A/O secondary effluent or had increased relative in intensity in A/O secondary effluent compared to primary effluent.

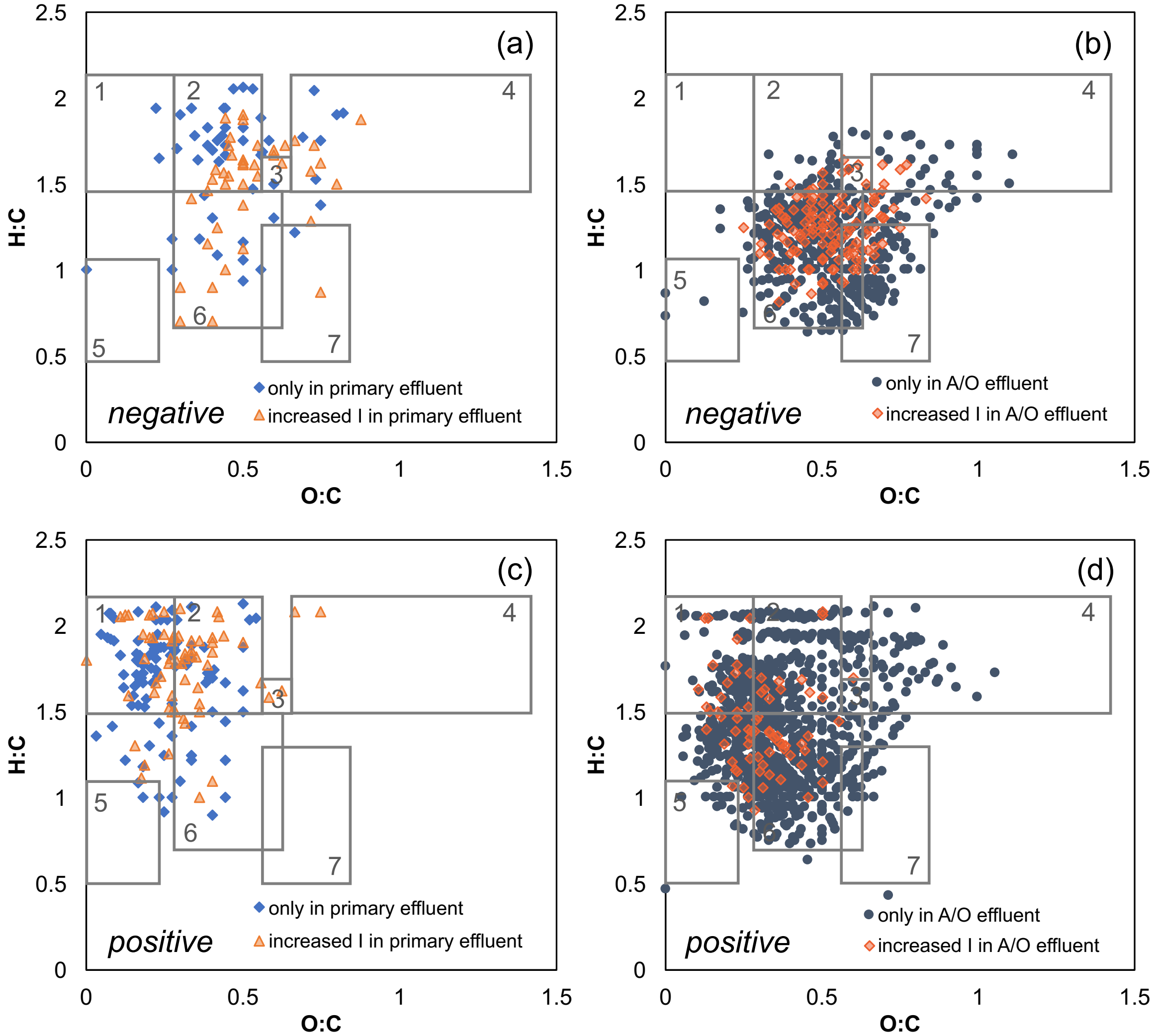

Figure S25. Negative and positive mode CHON formulas that were (a, c) only present in primary effluent or had increased relative intensity in primary effluent compared to A/O secondary effluent and (b, d) only present in A/O secondary effluent or had increased relative in intensity in A/O secondary effluent compared to primary effluent.

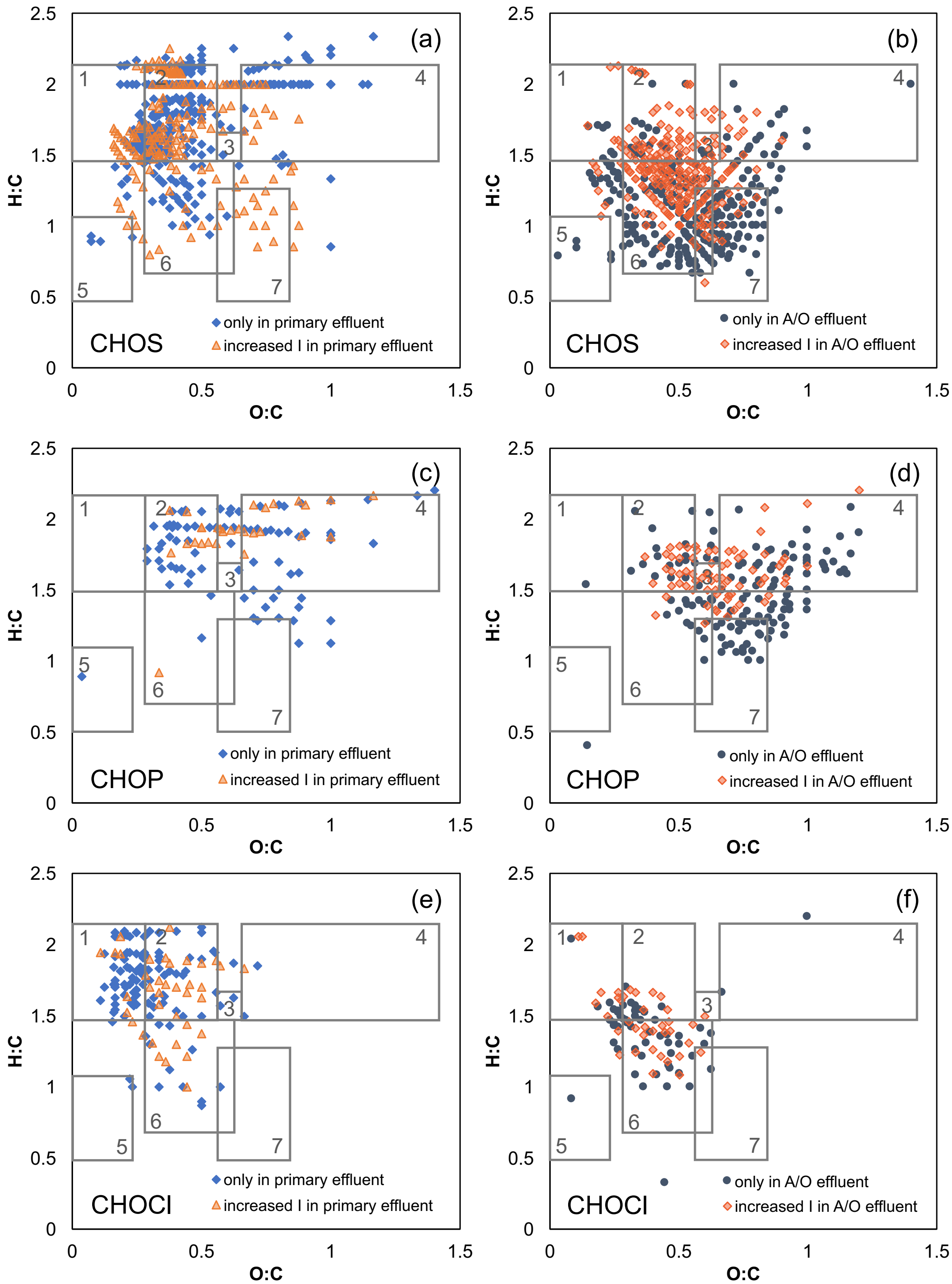

Figure S26. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in primary effluent or had increased relative intensity in primary effluent compared to A/O secondary effluent and (b, d, f) only present in A/O secondary effluent or had increased relative in intensity in A/O secondary effluent compared to primary effluent.

Primary effluent vs. UCT secondary effluent

Table S8. The number, H:C, O:C, and DBE of formulas that were only present in primary effluent or had increased relative intensity in primary effluent compared to UCT secondary effluent as determined using FT-ICR MS in negative mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in primary eff.	91	1.65	0.38	4.95	Only in UCT eff.	671	1.28	0.49	8.65
	Increased I in primary eff.	148	1.61	0.42	3.89	Increased I in UCT eff.	714	1.25	0.49	7.78
CHON	Only in primary eff.	47	1.59	0.46	4.40	Only in UCT eff.	380	1.18	0.50	9.24
	Increased I in primary eff.	39	1.45	0.52	4.44	Increased I in UCT eff.	126	1.27	0.53	6.90
CHOS	Only in primary eff.	270	1.75	0.45	3.70	Only in UCT eff.	242	1.21	0.50	7.74
	Increased I in primary eff.	185	1.65	0.42	3.54	Increased I in UCT eff.	219	1.39	0.50	5.31
CHOP	Only in primary eff.	171	1.75	0.66	3.41	Only in UCT eff.	23	1.60	0.62	5.87
	Increased I in primary eff.	5	1.63	0.56	4.80	Increased I in UCT eff.	4	1.35	0.54	6.50
CHOCl	Only in primary eff.	82	1.66	0.31	2.84	Only in UCT eff.	131	1.53	0.42	3.87
	Increased I in primary eff.	47	1.75	0.35	1.96	Increased I in UCT eff.	47	1.53	0.36	3.71

Table S9. The number, H:C, O:C, and DBE of formulas that were only present in primary effluent or had increased relative intensity in primary effluent compared to UCT secondary effluent as determined using FT-ICR MS in positive mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in primary eff.	312	1.67	0.36	5.54	Only in UCT eff.	560	1.34	0.42	7.55
	Increased I in primary eff.	119	1.76	0.37	3.35	Increased I in UCT eff.	155	1.55	0.41	5.06
CHON	Only in primary eff.	120	1.75	0.26	4.30	Only in UCT eff.	242	1.44	0.37	5.63
	Increased I in primary eff.	29	1.65	0.32	3.79	Increased I in UCT eff.	80	1.43	0.31	5.58

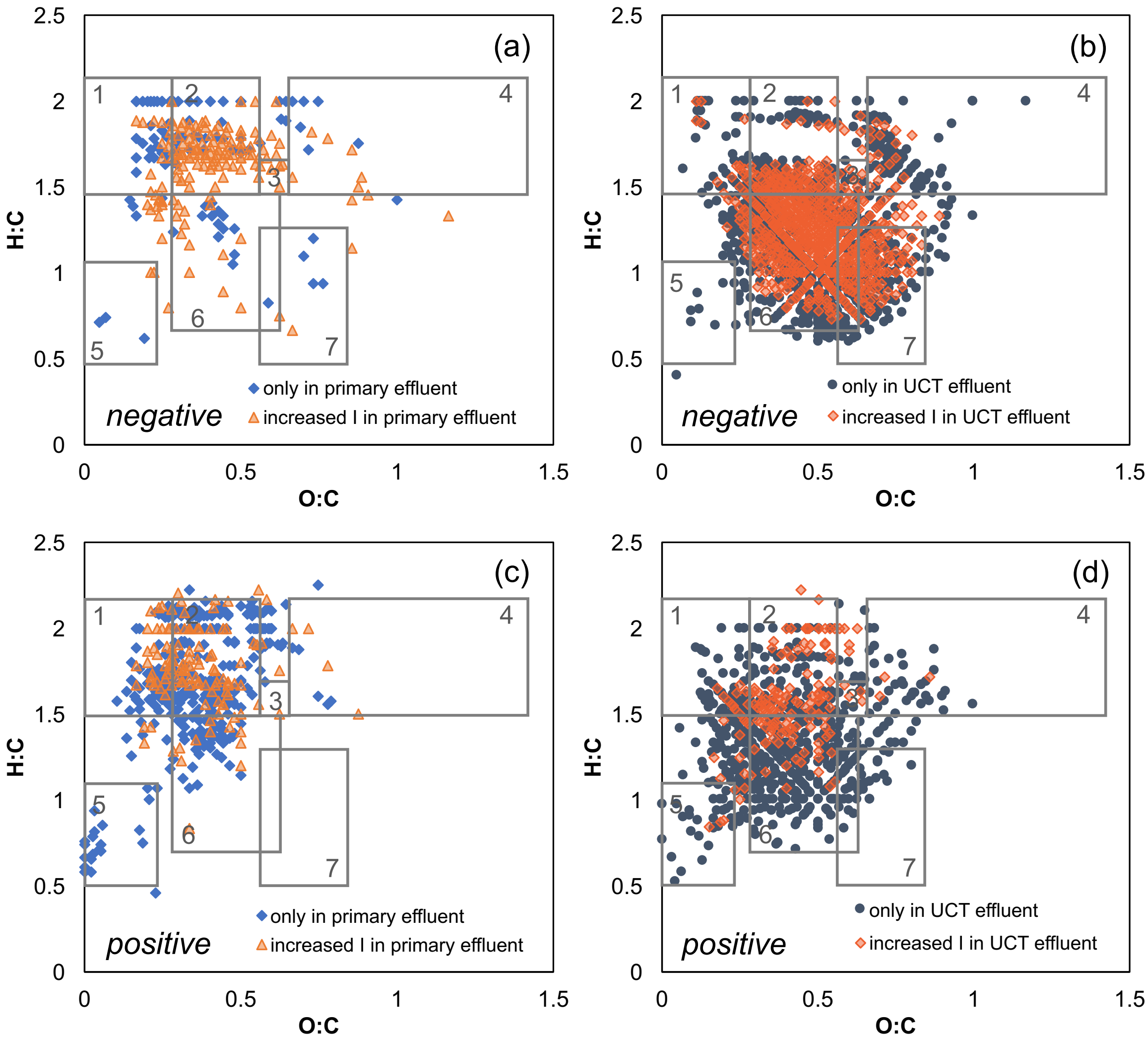

Figure S27. Negative and positive mode CHO formulas that were (a, c) only present in primary effluent or had increased relative intensity in primary effluent compared to UCT secondary effluent and (b, d) only present in UCT secondary effluent or had increased relative in intensity in UCT secondary effluent compared to primary effluent.

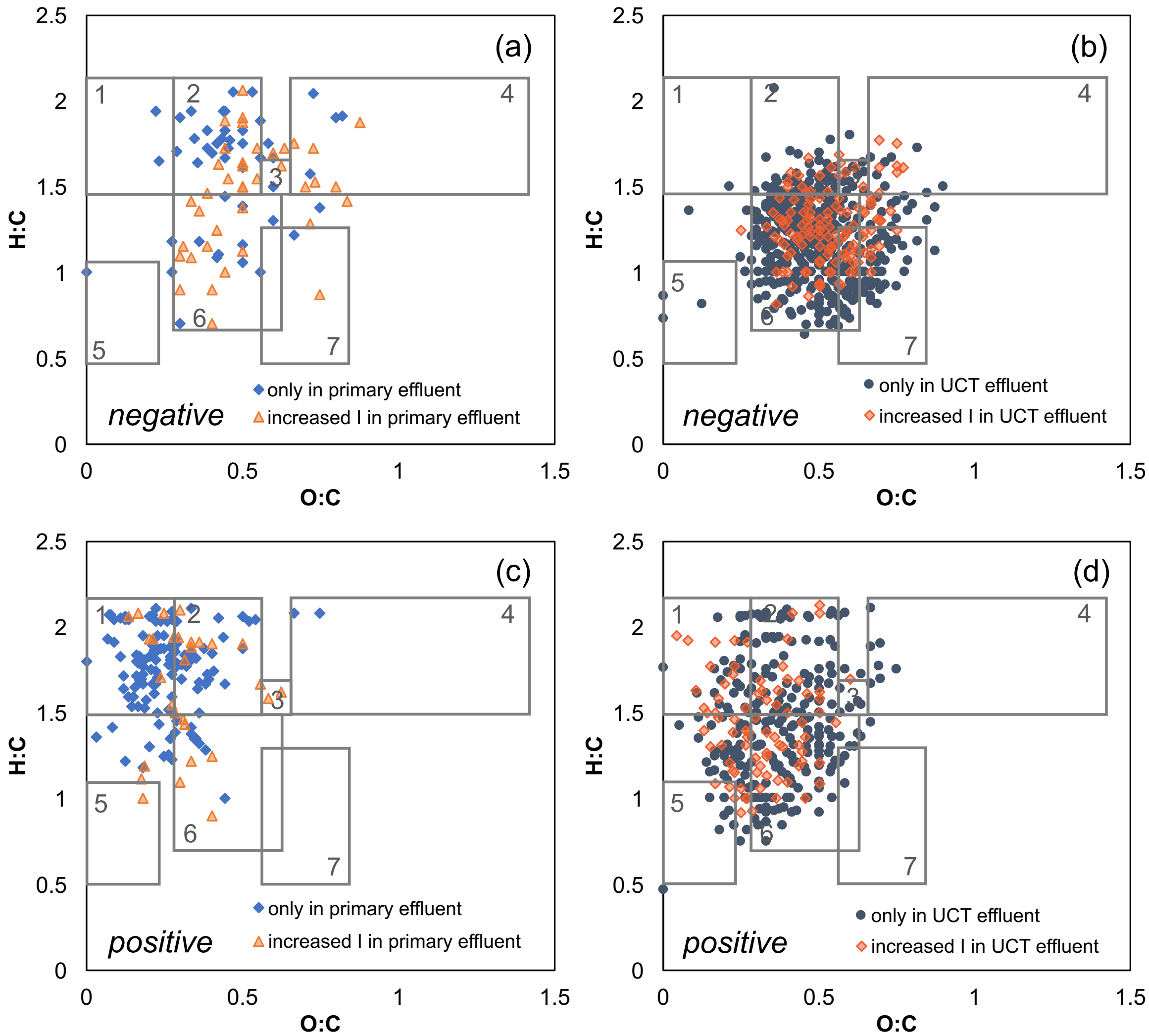

Figure S28. Negative and positive mode CHON formulas that were (a, c) only present in primary effluent or had increased relative intensity in primary effluent compared to UCT secondary effluent and (b, d) only present in UCT secondary effluent or had increased relative in intensity in UCT secondary effluent compared to primary effluent.

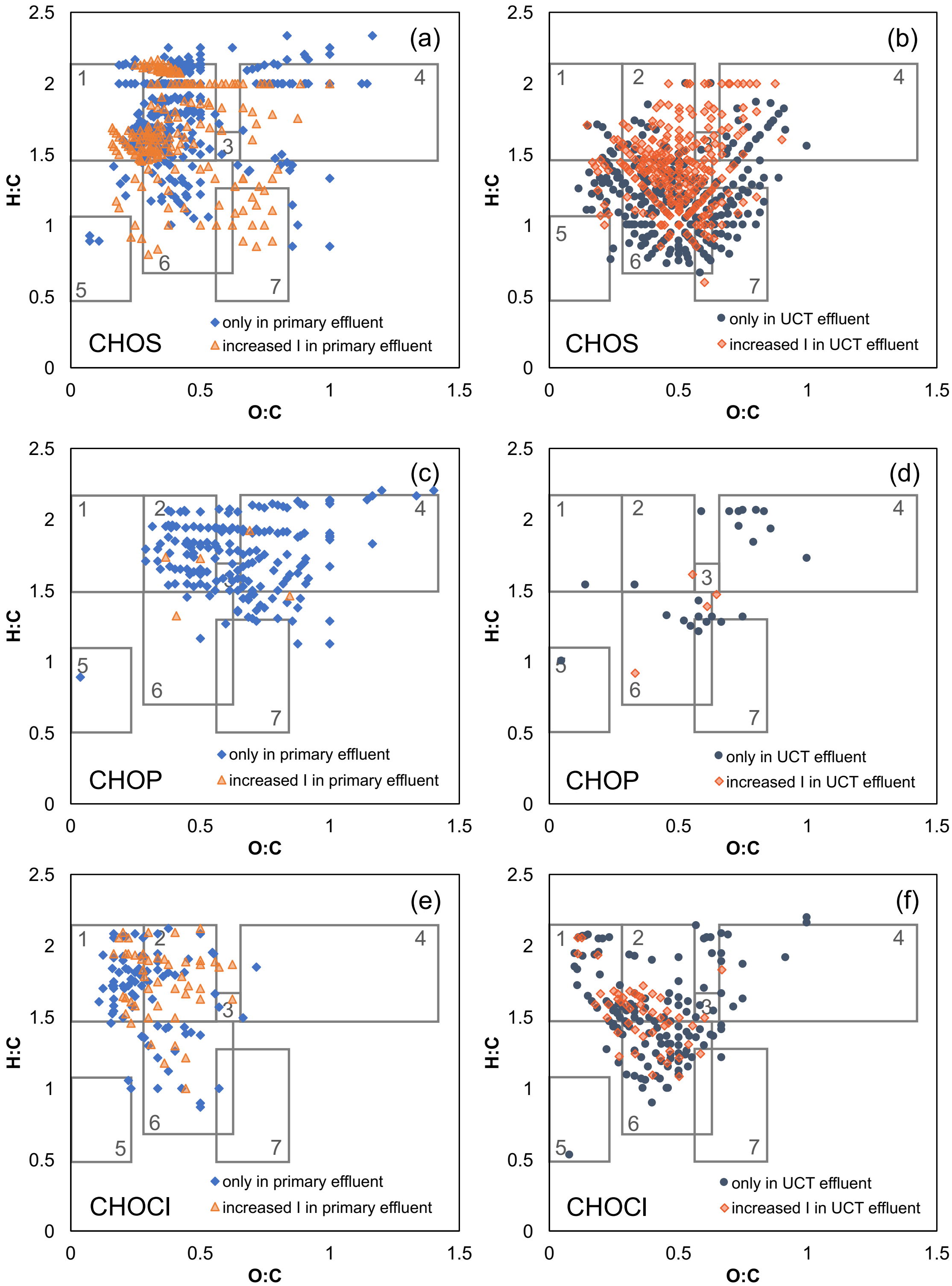

Figure S29. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in primary effluent or had increased relative intensity in primary effluent compared to UCT secondary effluent and (b, d, f) only present in UCT secondary effluent or had increased relative in intensity in UCT secondary effluent compared to primary effluent.

A/O secondary effluent vs. UCT secondary effluent

Table S10. The number, H:C, O:C, and DBE of formulas that were only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to UCT secondary effluent as determined using FT-ICR MS in negative mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in A/O	154	1.06	0.59	10.38	Only in UCT	257	1.45	0.42	7.42
	Increased I in A/O	697	1.11	0.55	8.50	Increased I in UCT	579	1.45	0.43	7.08
CHON	Only in A/O	123	1.20	0.58	8.88	Only in UCT	133	1.28	0.48	8.85
	Increased I in A/O	273	1.13	0.54	8.36	Increased I in UCT	139	1.35	0.47	7.87
CHOS	Only in A/O	120	1.26	0.56	7.45	Only in UCT	124	1.46	0.47	6.35
	Increased I in A/O	313	1.32	0.53	5.84	Increased I in UCT	239	1.48	0.41	5.15
CHOP	Only in A/O	213	1.60	0.73	4.66	Only in UCT	16	1.73	0.64	4.81
	Increased I in A/O	14	1.44	0.60	6.57	Increased I in UCT	2	1.23	0.24	8.00
CHOCl	Only in A/O	24	1.37	0.36	5.54	Only in UCT	122	1.63	0.40	3.14
	Increased I in A/O	25	1.29	0.47	5.00	Increased I in UCT	80	1.58	0.35	1.58

Table S11. The number, H:C, O:C, and DBE of formulas that were only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to UCT secondary effluent as determined using FT-ICR MS in positive mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in A/O	553	1.49	0.50	6.38	Only in UCT	349	1.42	0.35	7.54
	Increased I in A/O	33	1.30	0.66	6.97	Increased I in UCT	452	1.46	0.43	5.65
CHON	Only in A/O	656	1.45	0.38	7.12	Only in UCT	96	1.47	0.35	5.34
	Increased I in A/O	89	1.31	0.45	6.61	Increased I in UCT	166	1.54	0.31	4.92

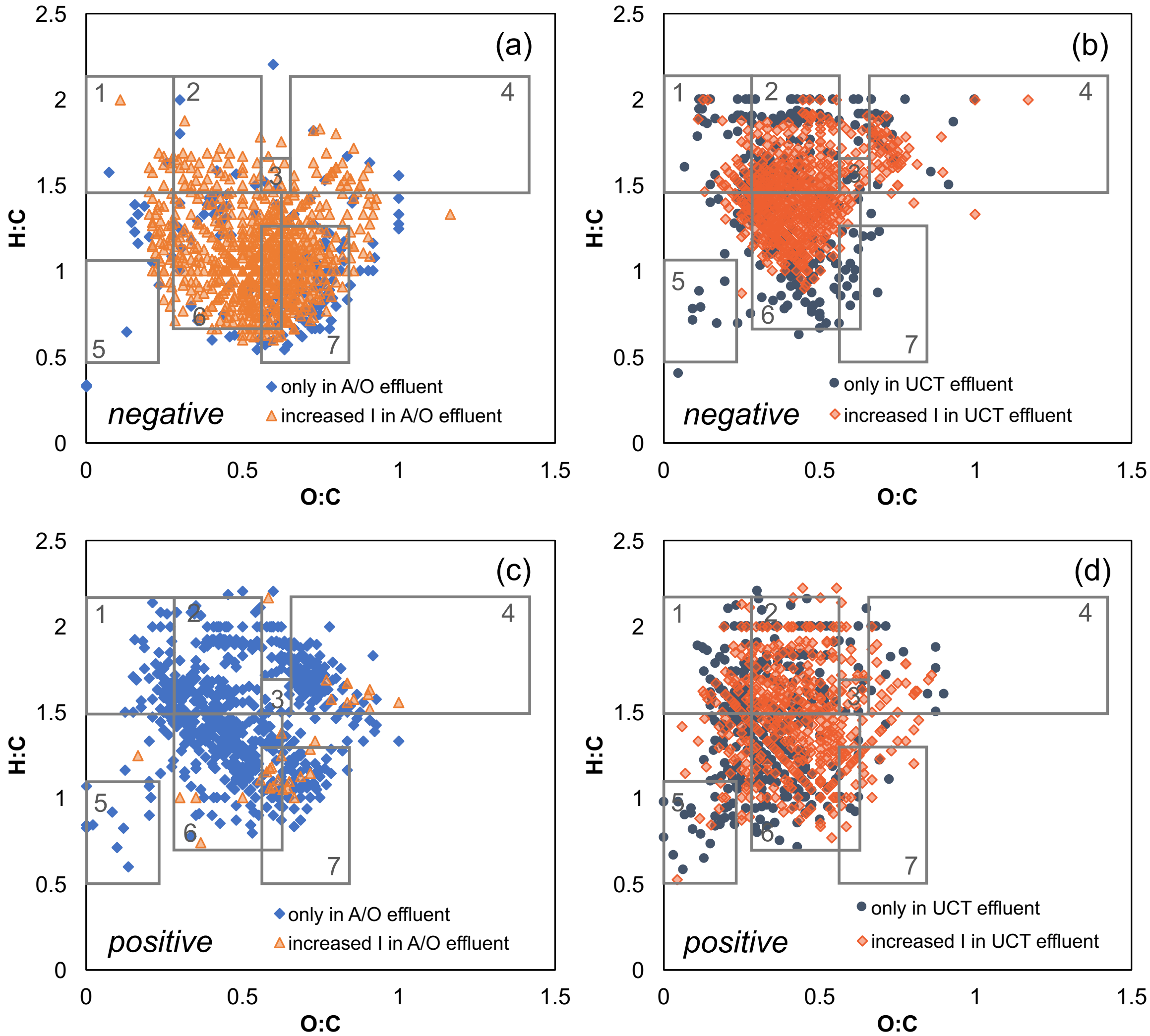

Figure S30. Negative and positive mode CHO formulas that were (a, c) only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to UCT secondary effluent and (b, d) only present in UCT secondary effluent or had increased relative in intensity in UCT secondary effluent compared to A/O secondary effluent.

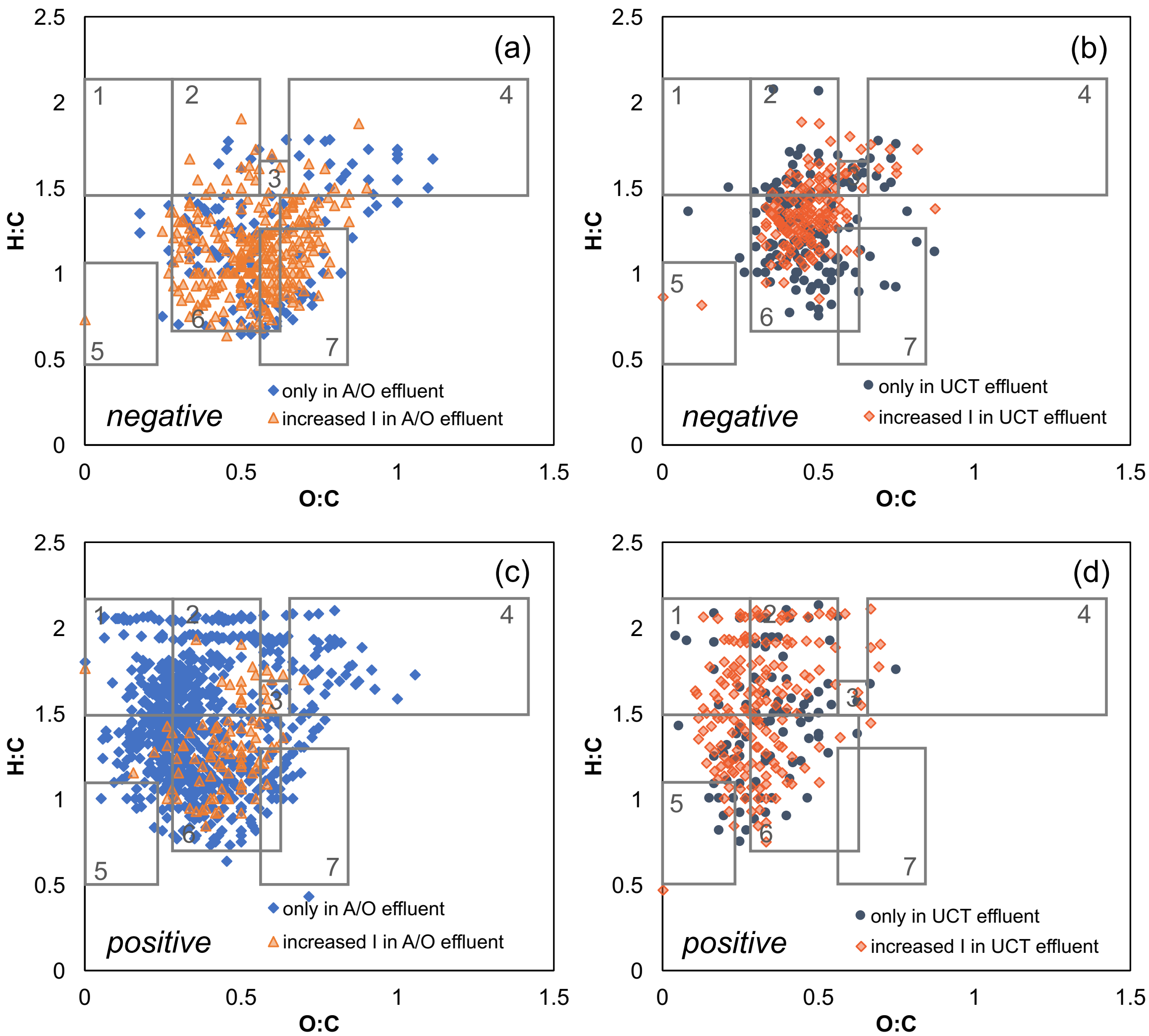

Figure S31. Negative and positive mode CHON formulas that were (a, c) only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to UCT secondary effluent and (b, d) only present in UCT secondary effluent or had increased relative in intensity in UCT secondary effluent compared to A/O secondary effluent.

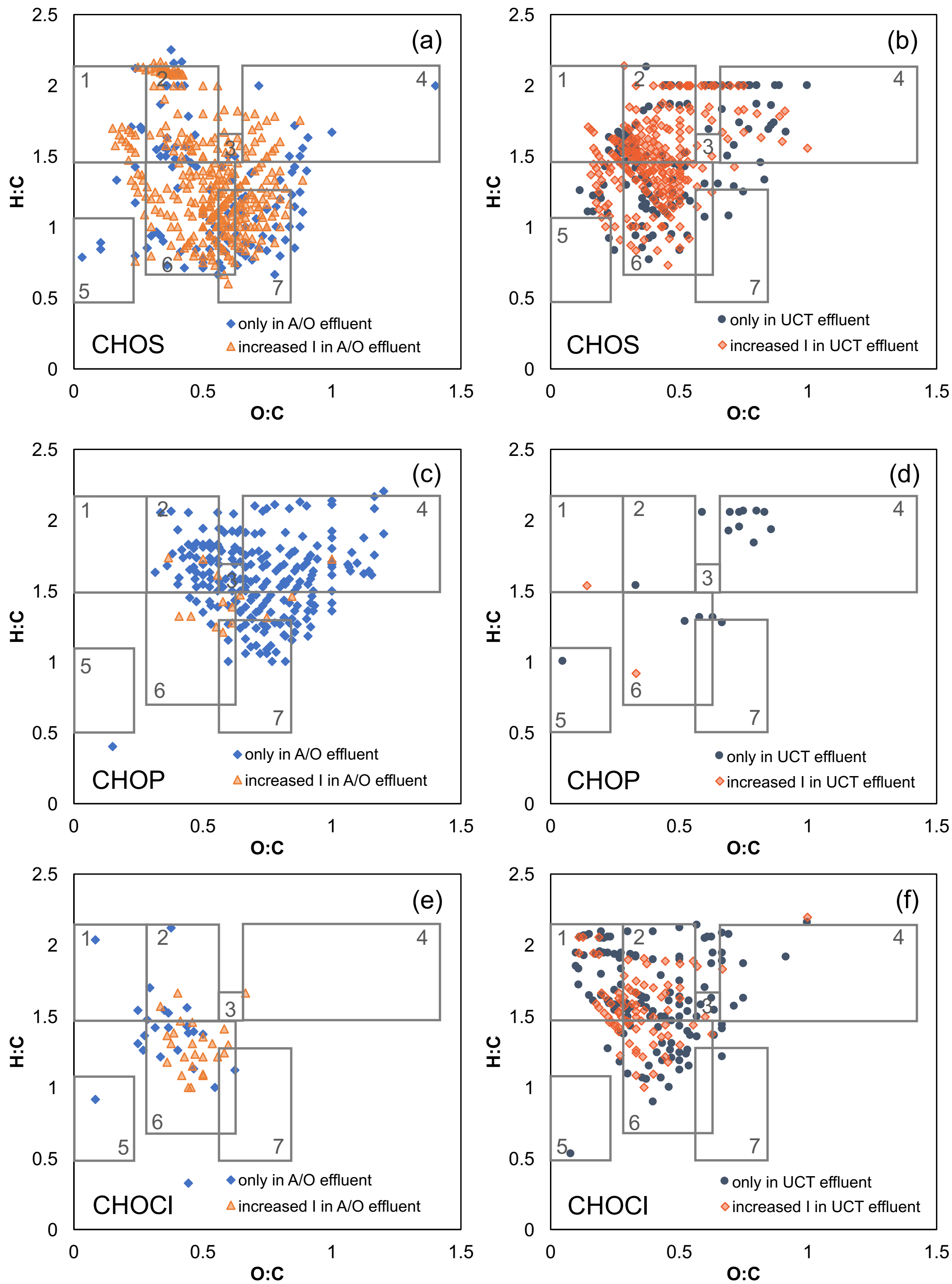

Figure S32. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to UCT secondary effluent and (b, d, f) only present in UCT secondary effluent or had increased relative in intensity in UCT secondary effluent compared to A/O secondary effluent.

A/O secondary effluent vs. A/O secondary clarifier effluent

Table S12. The number, H:C, O:C, and DBE of formulas that were only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to A/O secondary clarifier effluent as determined using FT-ICR MS in negative mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in secondary eff.	238	1.29	0.55	6.68	Only in clarifier eff.	173	1.15	0.40	11.59
	Increased I in secondary eff.	155	1.27	0.66	5.49	Increased I in clarifier eff.	1037	1.23	0.48	8.85
CHON	Only in secondary eff.	162	1.21	0.55	8.33	Only in clarifier eff.	97	1.17	0.47	10.80
	Increased I in secondary eff.	117	1.30	0.63	6.07	Increased I in clarifier eff.	256	1.15	0.48	9.41
CHOS	Only in secondary eff.	186	1.49	0.51	4.69	Only in clarifier eff.	135	1.27	0.41	9.05
	Increased I in secondary eff.	90	1.36	0.59	4.67	Increased I in clarifier eff.	396	1.31	0.46	6.72
CHOP	Only in secondary eff.	48	1.65	0.78	4.50	Only in clarifier eff.	40	1.41	0.52	7.33
	Increased I in secondary eff.	121	1.55	0.73	4.88	Increased I in clarifier eff.	60	1.59	0.62	4.88
CHOCl	Only in secondary eff.	69	1.52	0.38	4.03	Only in clarifier eff.	16	1.33	0.38	4.75
	Increased I in secondary eff.	33	1.48	0.38	3.88	Increased I in clarifier eff.	27	1.39	0.36	4.63

Table S13. The number, H:C, O:C, and DBE of formulas that were only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to A/O secondary clarifier effluent as determined using FT-ICR MS in positive mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in secondary eff.	194	1.41	0.46	6.76	Only in clarifier eff.	352	1.44	0.42	7.45
	Increased I in secondary eff.	179	1.36	0.59	6.37	Increased I in clarifier eff.	665	1.52	0.45	5.80
CHON	Only in secondary eff.	421	1.44	0.39	6.83	Only in clarifier eff.	118	1.57	0.41	6.15
	Increased I in secondary eff.	422	1.42	0.36	6.75	Increased I in clarifier eff.	68	1.67	0.39	5.15

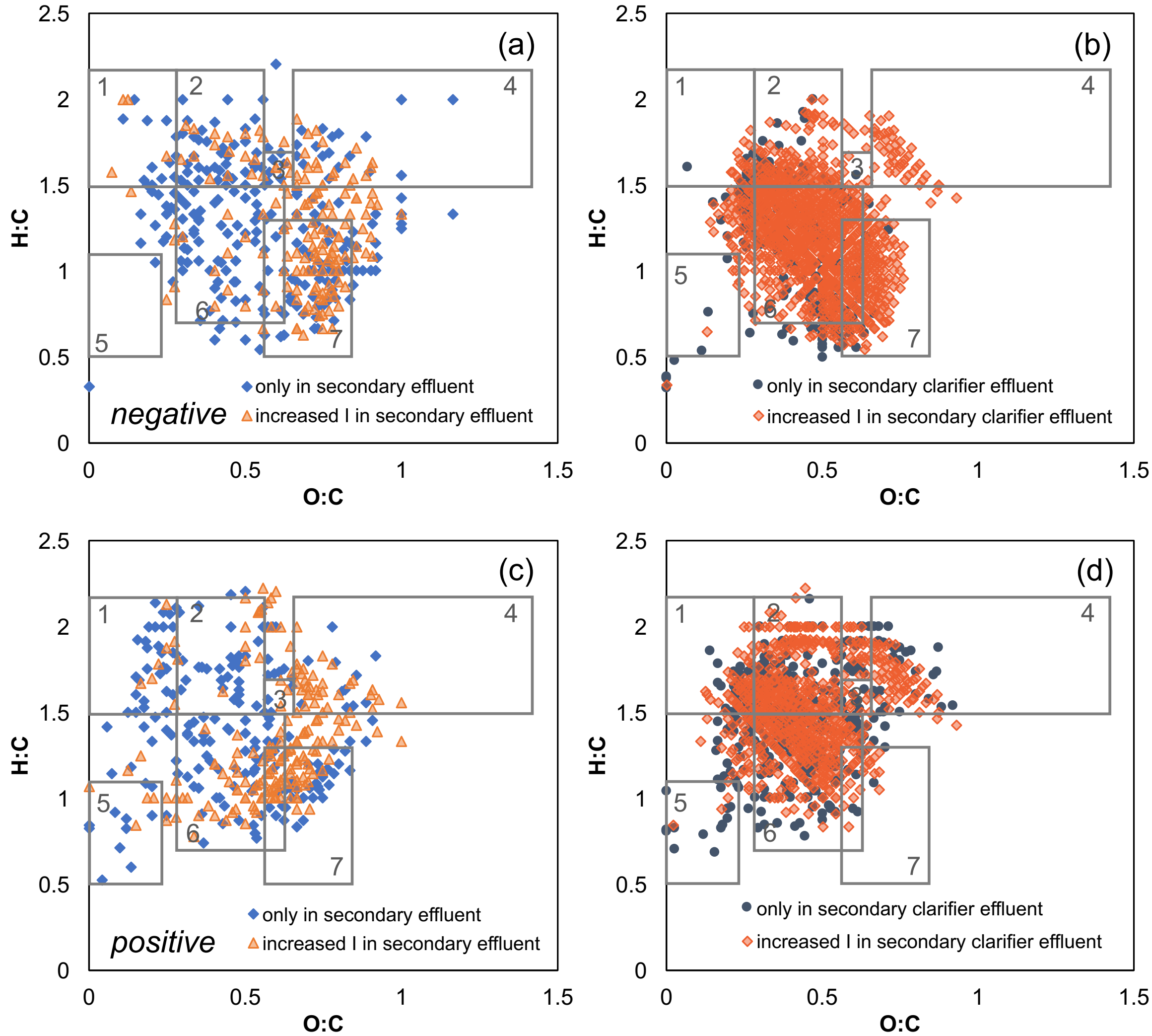

Figure S33. Negative and positive mode CHO formulas that were (a, c) only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to A/O secondary clarifier effluent and (b, d) only present in A/O secondary clarifier effluent or had increased relative in intensity in A/O secondary clarifier effluent compared to A/O secondary effluent.

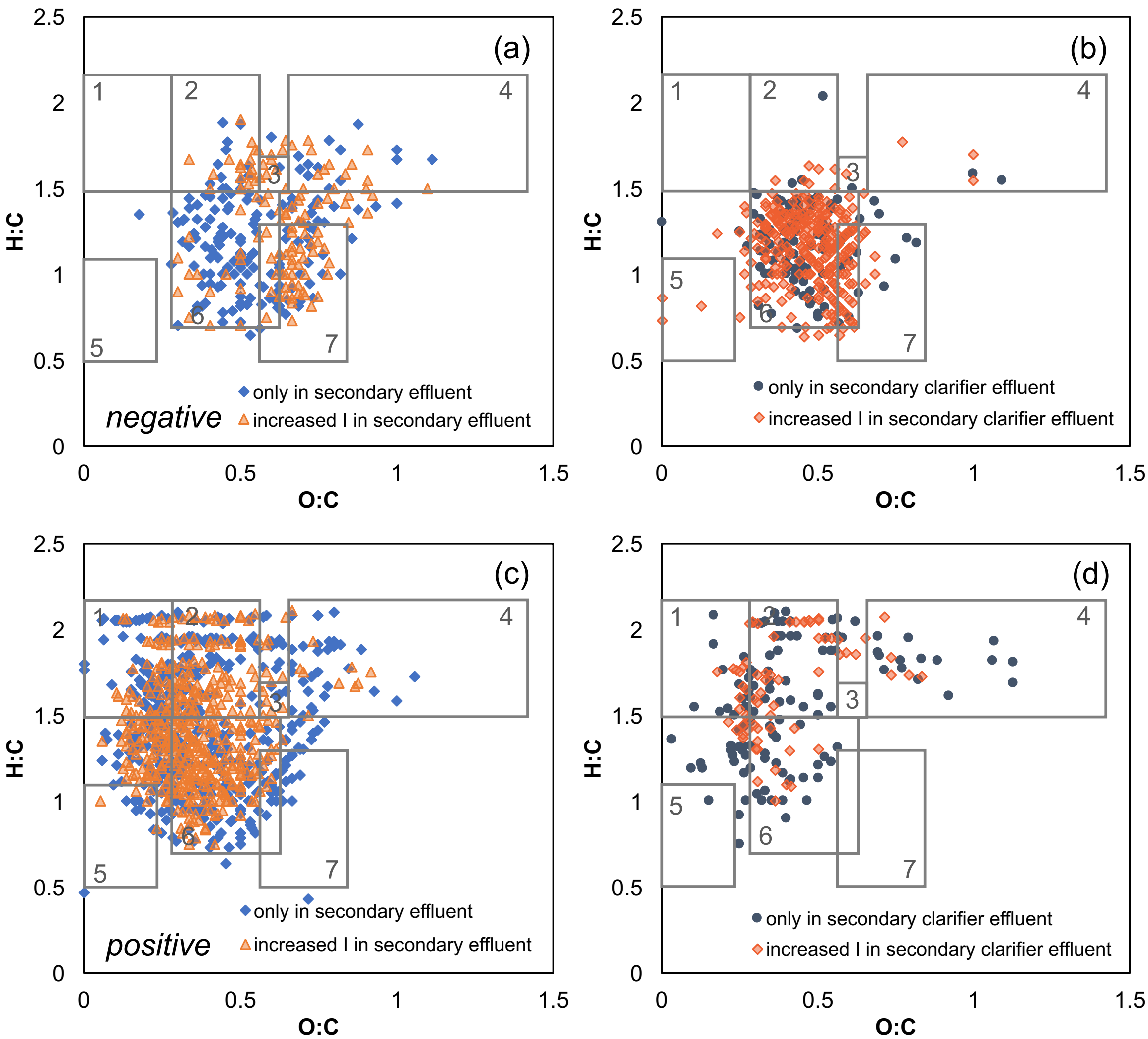

Figure S34. Negative and positive mode CHON formulas that were (a, c) only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to A/O secondary clarifier effluent and (b, d) only present in A/O secondary clarifier effluent or had increased relative in intensity in A/O secondary clarifier effluent compared to A/O secondary effluent.

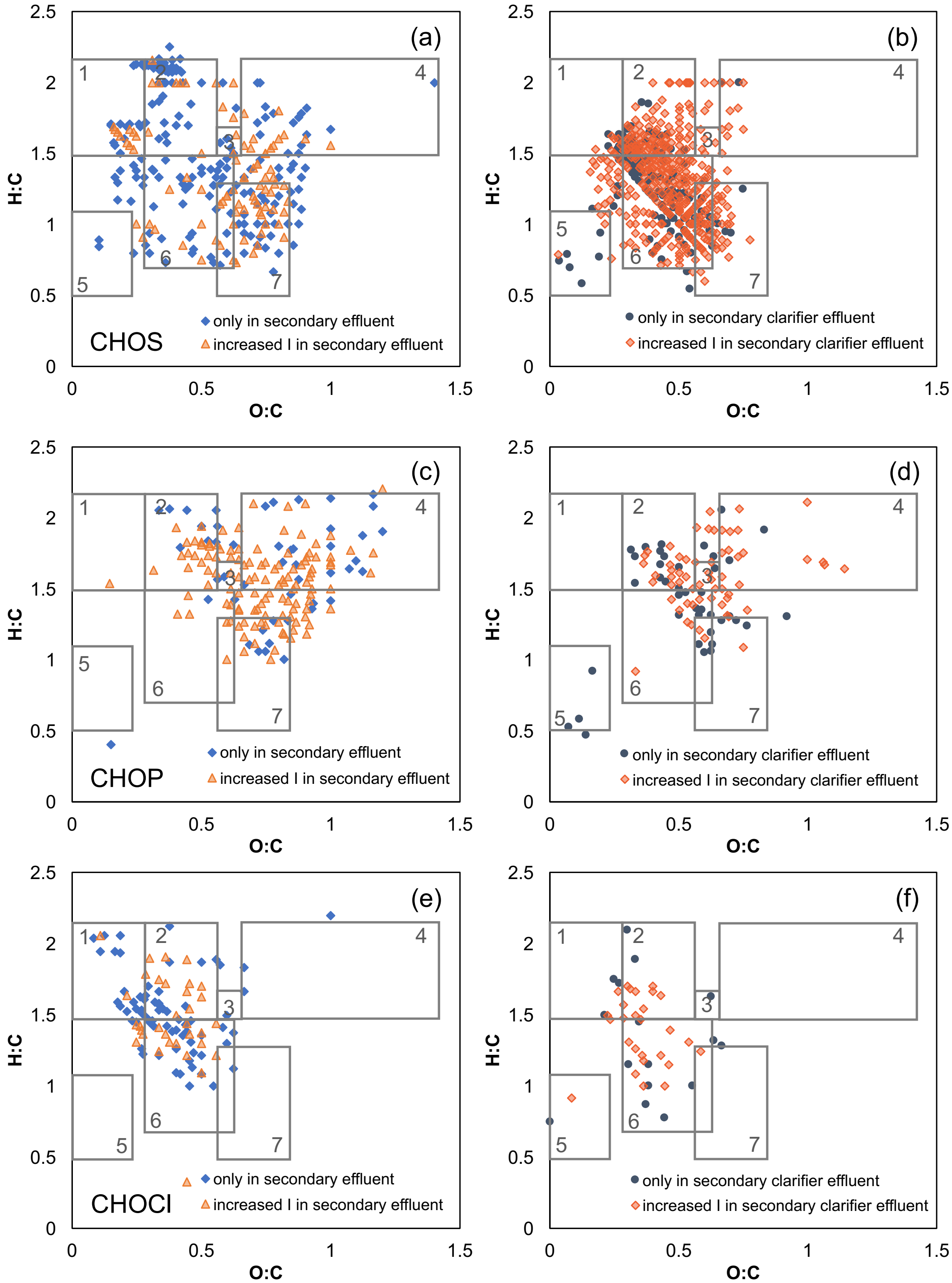

Figure S35. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in A/O secondary effluent or had increased relative intensity in A/O secondary effluent compared to A/O secondary clarifier effluent and (b, d, f) only present in A/O secondary clarifier effluent or had increased relative in intensity in A/O secondary clarifier effluent compared to A/O secondary effluent.

UCT secondary effluent vs. UCT secondary clarifier effluent

Table S14. The number, H:C, O:C, and DBE of formulas that were only present in UCT secondary effluent or had increased relative intensity in UCT secondary effluent compared to UCT secondary clarifier effluent as determined using FT-ICR MS in negative mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in secondary eff.	287	1.39	0.47	7.32	Only in clarifier eff.	48	1.09	0.41	10.67
	Increased I in secondary eff.	455	1.44	0.55	6.62	Increased I in clarifier eff.	791	1.18	0.45	8.62
CHON	Only in secondary eff.	192	1.29	0.52	8.39	Only in clarifier eff.	61	1.30	0.56	7.92
	Increased I in secondary eff.	192	1.26	0.56	8.09	Increased I in clarifier eff.	161	1.10	0.44	8.64
CHOS	Only in secondary eff.	136	1.48	0.48	5.60	Only in clarifier eff.	89	1.29	0.43	8.66
	Increased I in secondary eff.	102	1.45	0.53	5.03	Increased I in clarifier eff.	438	1.36	0.47	5.88
CHOP	Only in secondary eff.	12	1.73	0.66	5.00	Only in clarifier eff.	197	1.59	0.66	4.83
	Increased I in secondary eff.	1	1.54	0.33	7.00	Increased I in clarifier eff.	19	1.48	0.58	6.21
CHOCl	Only in secondary eff.	135	1.63	0.40	3.21	Only in clarifier eff.	22	1.35	0.41	5.73
	Increased I in secondary eff.	64	1.58	0.37	3.16	Increased I in clarifier eff.	28	1.32	0.39	5.18

Table S15. The number, H:C, O:C, and DBE of formulas that were only present in UCT secondary effluent or had increased relative intensity in UCT secondary effluent compared to UCT secondary clarifier effluent as determined using FT-ICR MS in positive mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in secondary eff.	192	1.42	0.36	7.40	Only in clarifier eff.	726	1.54	0.47	6.08
	Increased I in secondary eff.	636	1.45	0.42	6.19	Increased I in clarifier eff.	6	1.15	0.51	9.00
CHON	Only in secondary eff.	101	1.46	0.34	5.27	Only in clarifier eff.	289	1.55	0.38	6.02
	Increased I in secondary eff.	244	1.46	0.36	5.51	Increased I in clarifier eff.	6	1.39	0.44	6.83

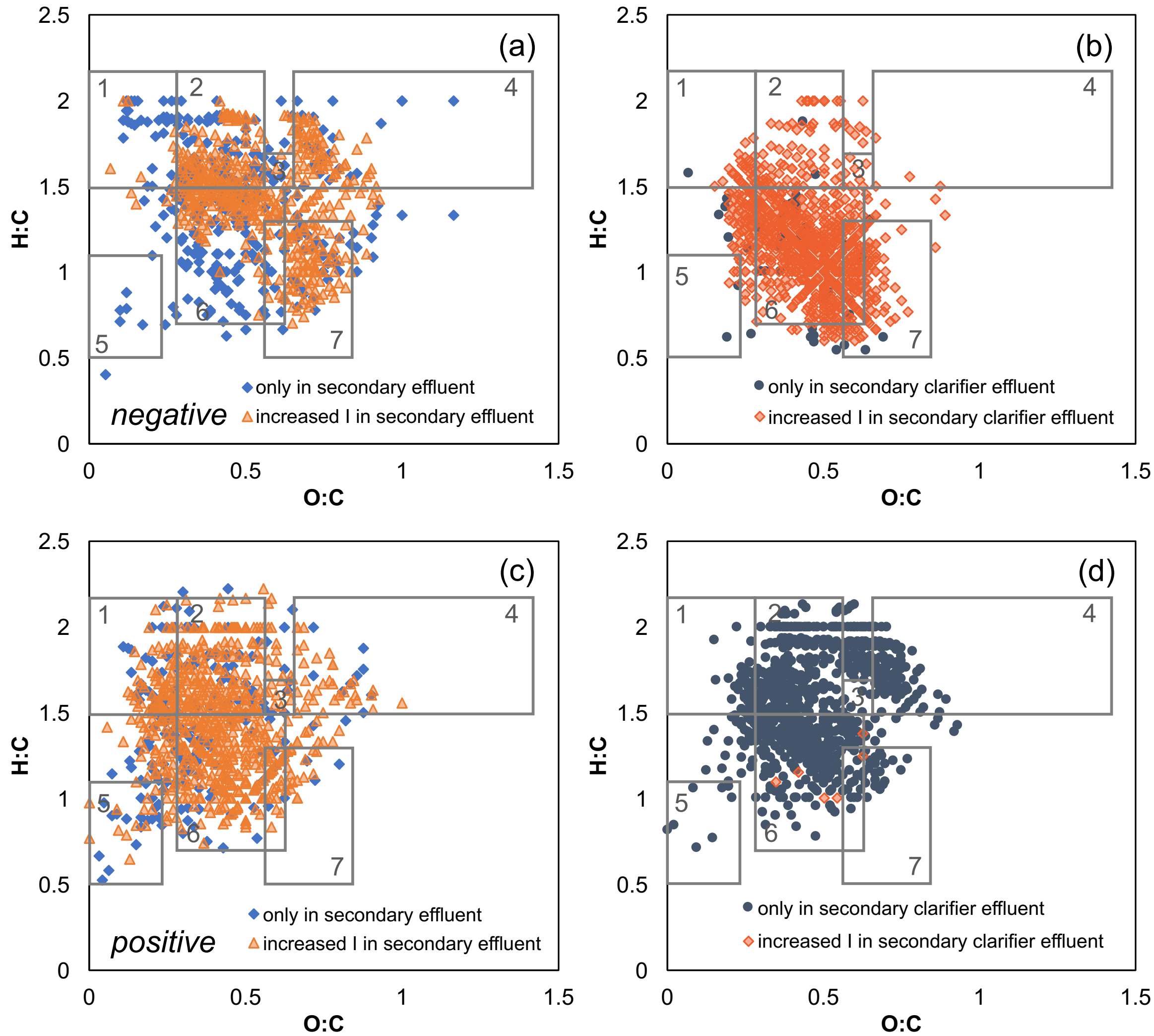

Figure S36. Negative and positive mode CHO formulas that were (a, c) only present in UCT secondary effluent or had increased relative intensity in UCT secondary effluent compared to UCT secondary clarifier effluent and (b, d) only present in UCT secondary clarifier effluent or had increased relative in intensity in UCT secondary clarifier effluent compared to UCT secondary effluent.

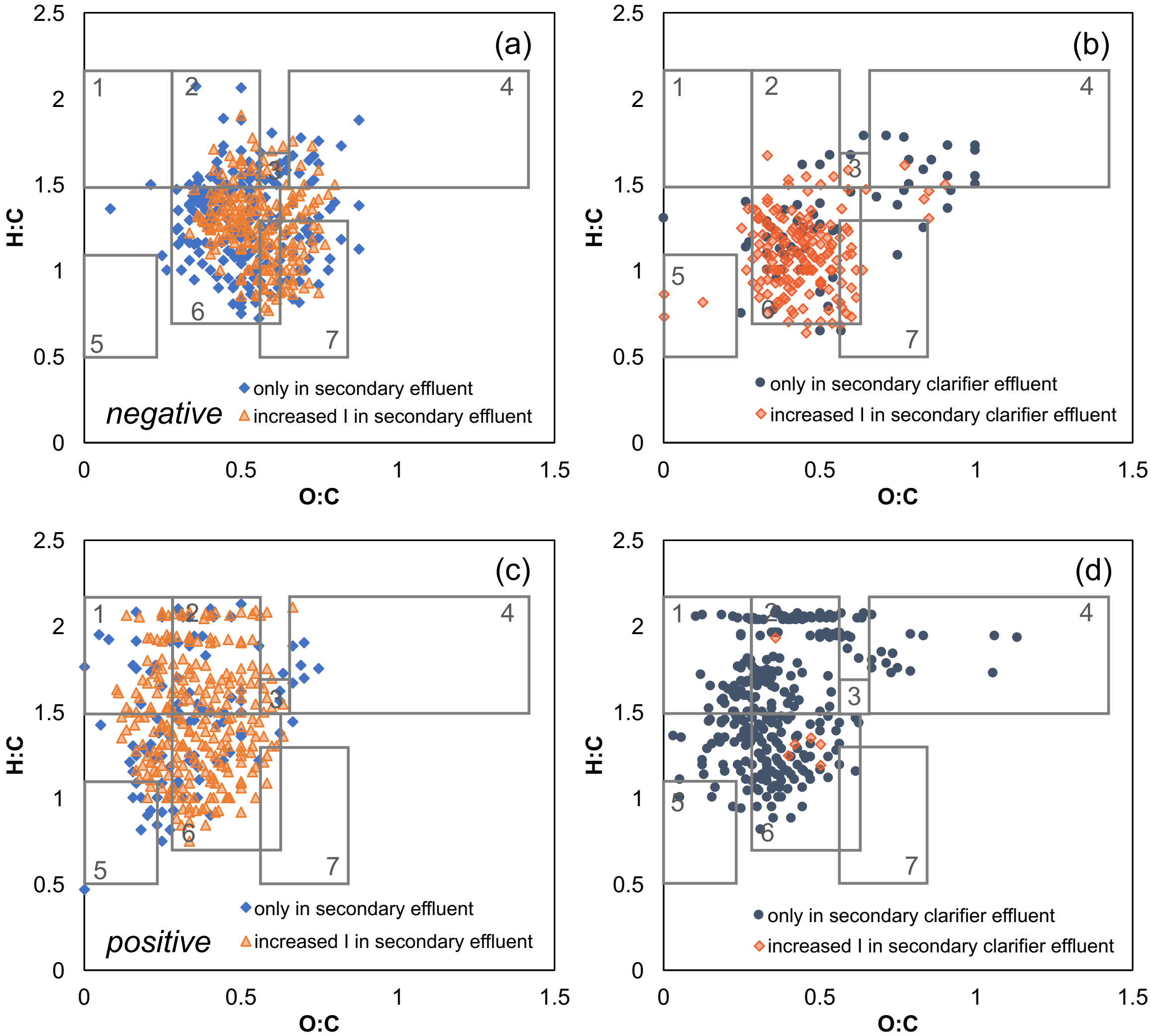

Figure S37. Negative and positive mode CHON formulas that were (a, c) only present in UCT secondary effluent or had increased relative intensity in UCT secondary effluent compared to UCT secondary clarifier effluent and (b, d) only present in UCT secondary clarifier effluent or had increased relative in intensity in UCT secondary clarifier effluent compared to UCT secondary effluent.

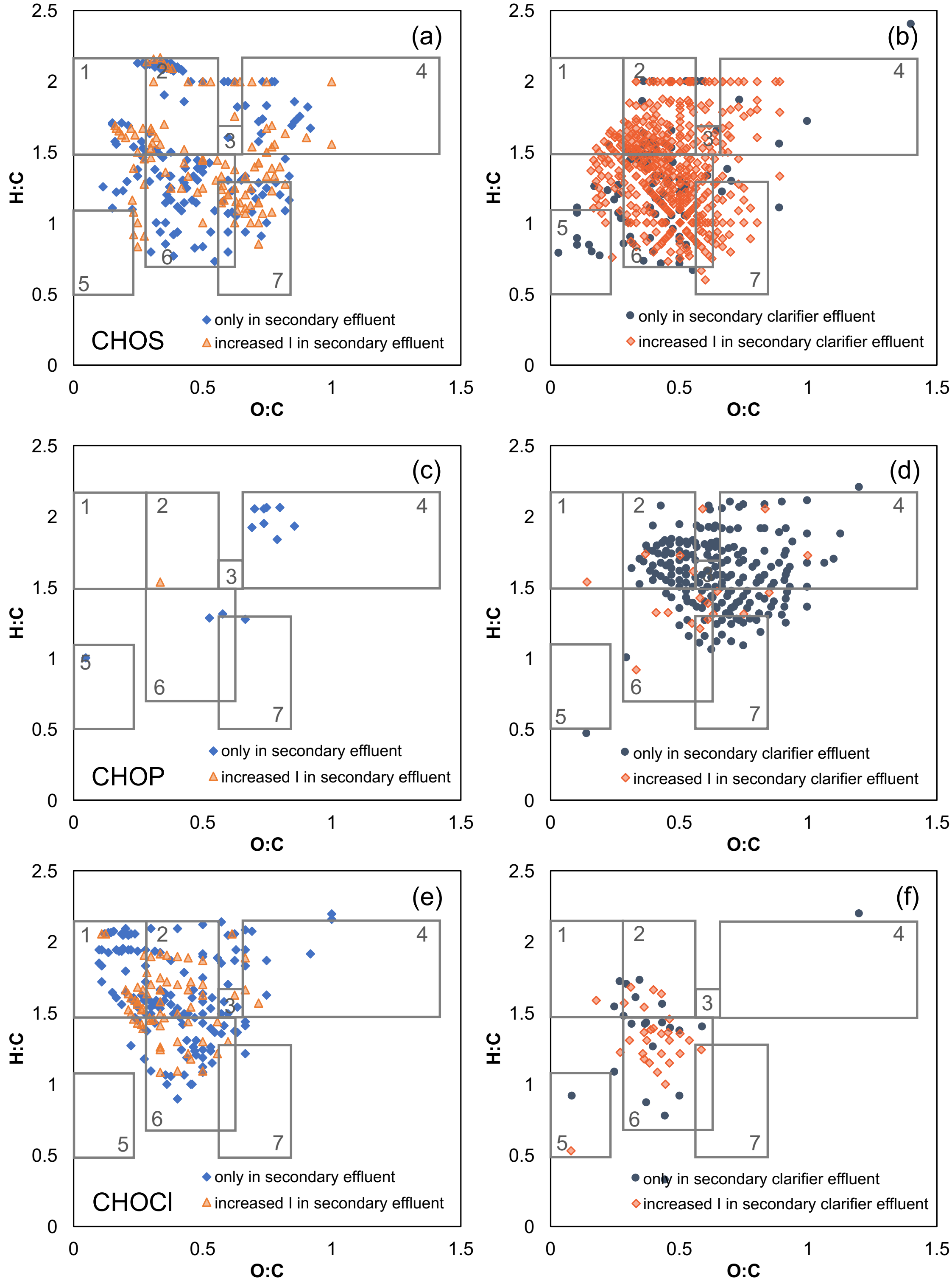

Figure S38. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in UCT secondary effluent or had increased relative intensity in UCT secondary effluent compared to UCT secondary clarifier effluent and (b, d, f) only present in UCT secondary clarifier effluent or had increased relative in intensity in UCT secondary clarifier effluent compared to UCT secondary effluent.

UV influent vs. UV effluent

Table S16. The number, H:C, O:C, and DBE of formulas that were only present in UV influent or had increased relative intensity in UV influent compared to final effluent as determined using FT-ICR MS in negative mode.

Formula		**n**	**H:C**	**O:C**	**DBE**		**n**	**H:C**	**O:C**	**DBE**
CHO	Only in influent	108	1.04	0.60	11.49	Only in effluent	166	1.29	0.45	7.44
	Increased I in influent	1111	1.23	0.51	8.63	Increased I in effluent	165	1.44	0.33	5.30
CHON	Only in influent	121	1.15	0.54	10.55	Only in effluent	107	1.15	0.50	8.78
	Increased I in influent	407	1.20	0.53	8.52	Increased I in effluent	36	1.19	0.33	6.92
CHOS	Only in influent	99	1.26	0.47	8.76	Only in effluent	109	1.36	0.46	6.05
	Increased I in influent	518	1.35	0.49	6.32	Increased I in effluent	47	1.47	0.28	5.23
CHOP	Only in influent	54	1.42	0.67	6.61	Only in effluent	16	1.49	0.56	7.13
	Increased I in influent	181	1.59	0.67	4.75	Increased I in effluent	5	1.59	0.54	5.20
CHOCl	Only in influent	13	1.40	0.44	5.46	Only in effluent	26	1.50	0.37	3.81
	Increased I in influent	74	1.48	0.35	4.04	Increased I in effluent	8	1.35	0.36	4.38

Table S17. The number, H:C, O:C, and DBE of formulas that were only present in UV influent or had increased relative intensity in UV influent compared to final effluent as determined using FT-ICR MS in positive mode.

Formula		**n**	**H:C**	**O:C**	**DBE**		**n**	**H:C**	**O:C**	**DBE**
CHO	Only in influent	89	1.54	0.48	6.40	Only in effluent	296	1.40	0.37	7.81
	Increased I in influent	175	1.81	0.55	2.80	Increased I in effluent	789	1.50	0.44	6.02
CHON	Only in influent	262	1.56	0.39	6.34	Only in effluent	96	1.47	0.37	6.09
	Increased I in influent	355	1.50	0.35	6.18	Increased I in effluent	16	1.52	0.40	5.81

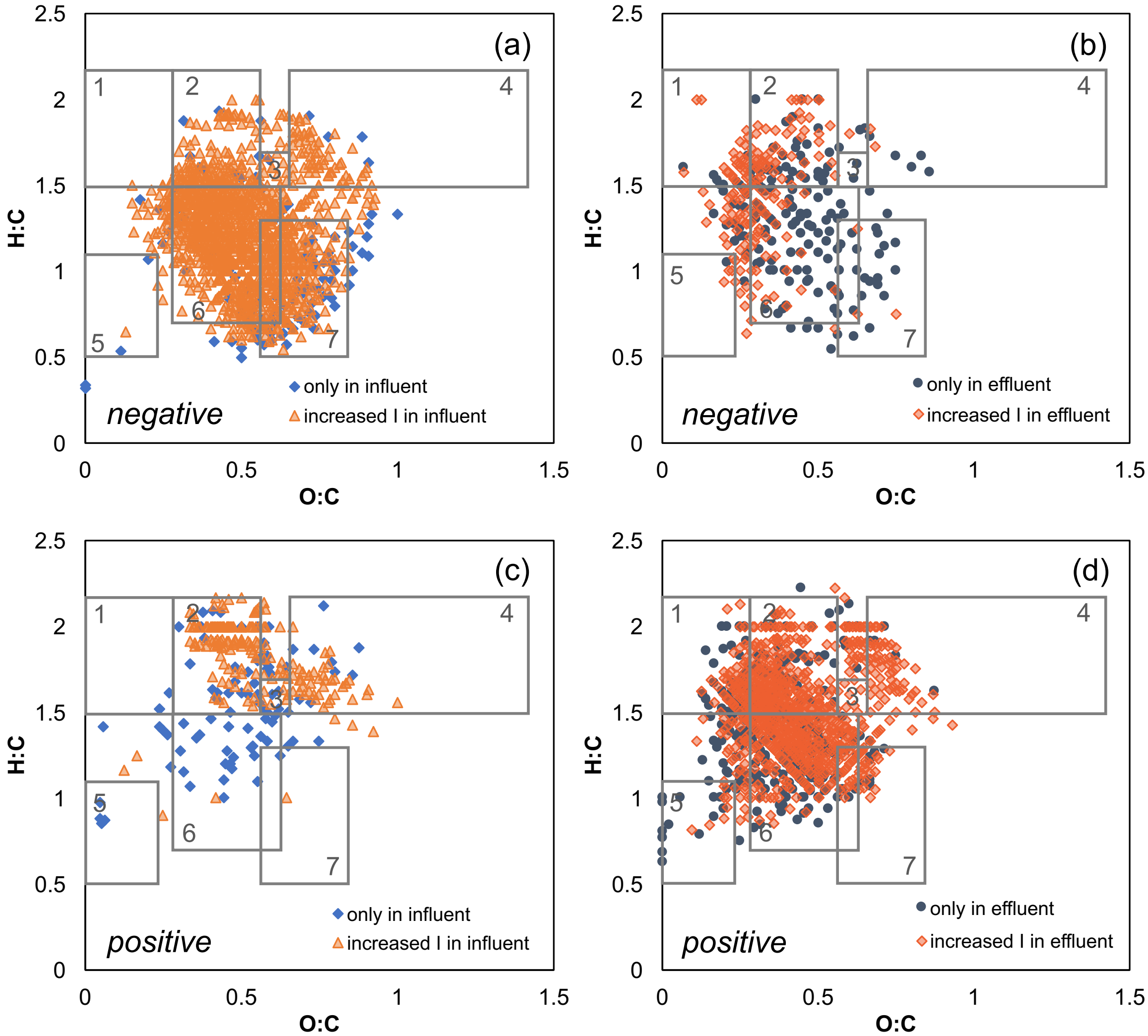

Figure S39. Negative and positive mode CHO formulas that were (a, c) only present in UV influent or had increased relative intensity in UV influent compared to final effluent and (b, d) only present in final effluent or had increased relative in intensity in final effluent compared to UV influent.

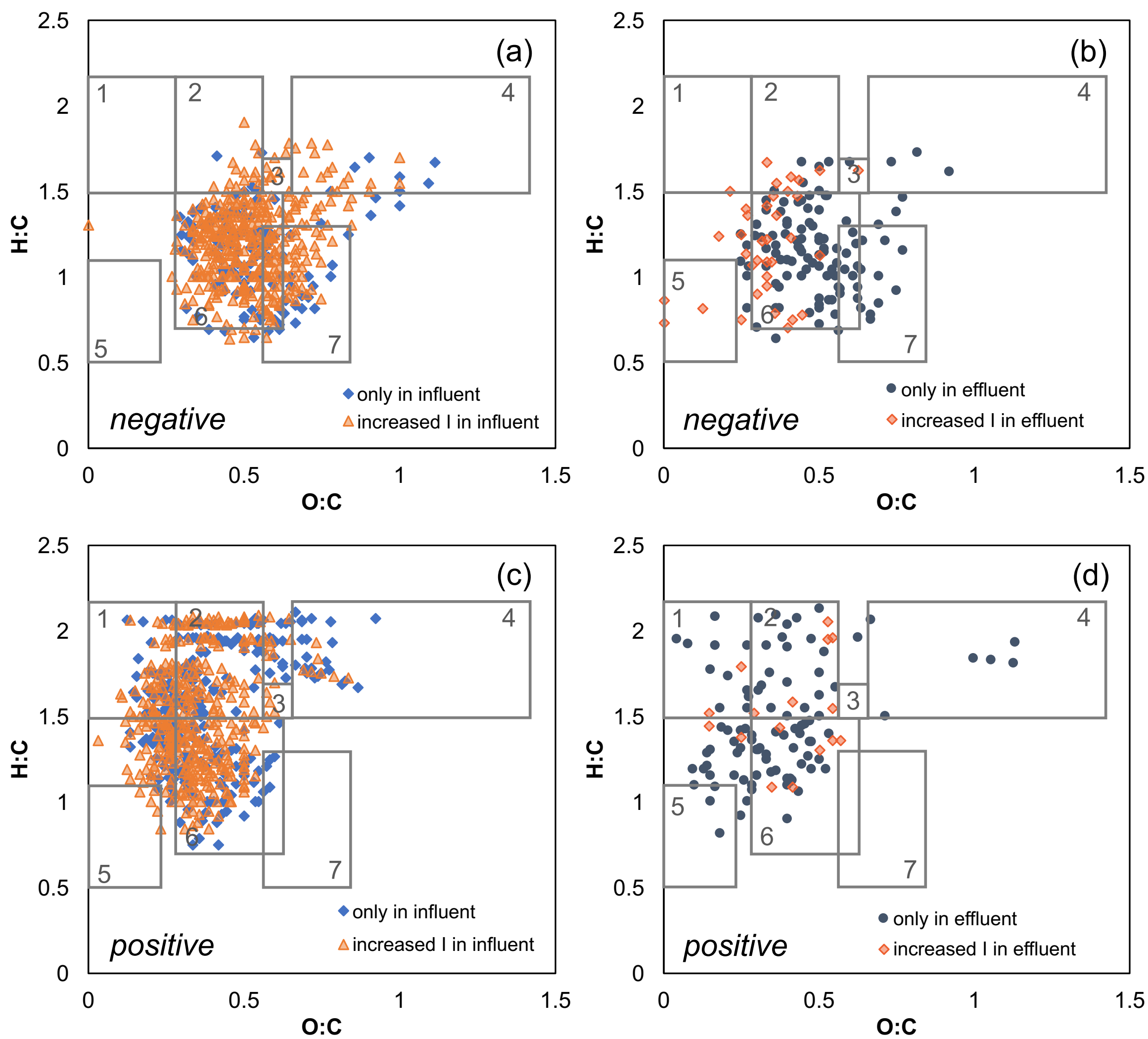

Figure S40. Negative and positive mode CHON formulas that were (a, c) only present in UV influent or had increased relative intensity in UV influent compared to final effluent and (b, d) only present in final effluent or had increased relative in intensity in final effluent compared to UV influent.

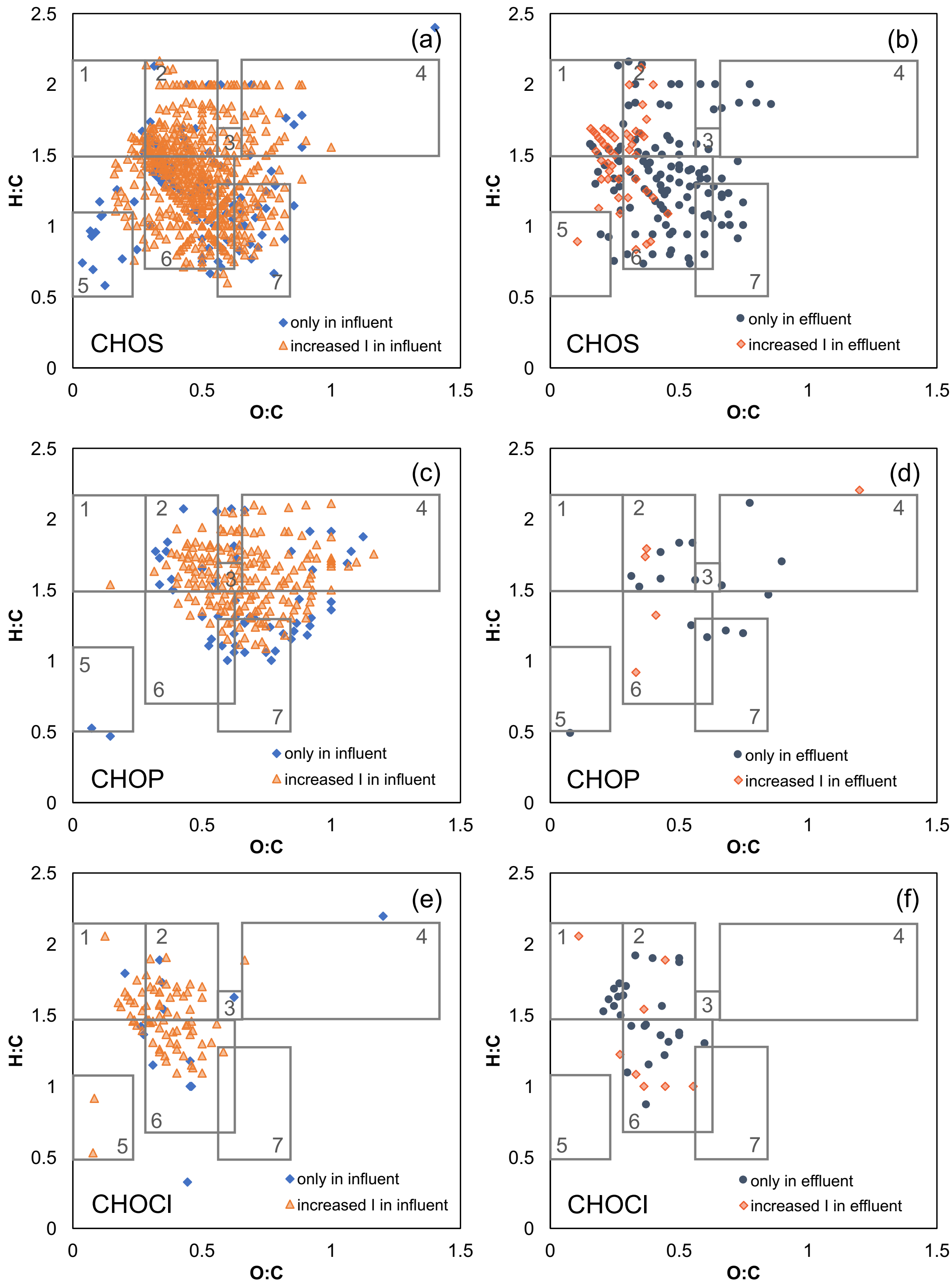

Figure S41. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in UV influent or had increased relative intensity in UV influent compared to final effluent and (b, d, f) only present in final effluent or had increased relative in intensity in final effluent compared to UV influent.

Ostara influent vs. Ostara effluent

Table S18. The number, H:C, O:C, and DBE of formulas that were only present in Ostara influent or had increased relative intensity in Ostara influent compared to Ostara effluent as determined using FT-ICR MS in negative mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in influent	383	1.41	0.45	6.60	Only in effluent	518	1.36	0.42	7.42
	Increased I in influent	517	1.40	0.41	7.27	Increased I in effluent	218	1.31	0.57	7.71
CHON	Only in influent	200	1.32	0.47	7.53	Only in effluent	484	1.34	0.45	7.46
	Increased I in influent	88	1.27	0.43	8.76	Increased I in effluent	61	1.37	0.56	7.59
CHOS	Only in influent	308	1.42	0.44	6.19	Only in effluent	189	1.46	0.45	5.35
	Increased I in influent	131	1.37	0.43	6.78	Increased I in effluent	119	1.47	0.51	5.58
CHOP	Only in influent	14	0.75	0.16	19.07	Only in effluent	44	1.57	0.43	6.27
	Increased I in influent	0	NA	NA	NA	Increased I in effluent	5	1.62	0.65	6.40
CHOCl	Only in influent	22	1.51	0.29	3.95	Only in effluent	61	1.63	0.35	2.92
	Increased I in influent	1	1.23	0.27	9.00	Increased I in effluent	39	1.62	0.34	3.00

Table S19. The number, H:C, O:C, and DBE of formulas that were only present in Ostara influent or had increased relative intensity in Ostara influent compared to Ostara effluent as determined using FT-ICR MS in positive mode.

Formula		n	H:C	O:C	DBE		n	H:C	O:C	DBE
CHO	Only in influent	135	1.52	0.40	7.19	Only in effluent	232	1.54	0.40	5.48
	Increased I in influent	80	1.60	0.50	5.85	Increased I in effluent	705	1.61	0.42	4.92
CHON	Only in influent	63	1.56	0.35	6.57	Only in effluent	162	1.58	0.35	5.85
	Increased I in influent	51	1.64	0.31	5.33	Increased I in effluent	70	1.60	0.31	5.39

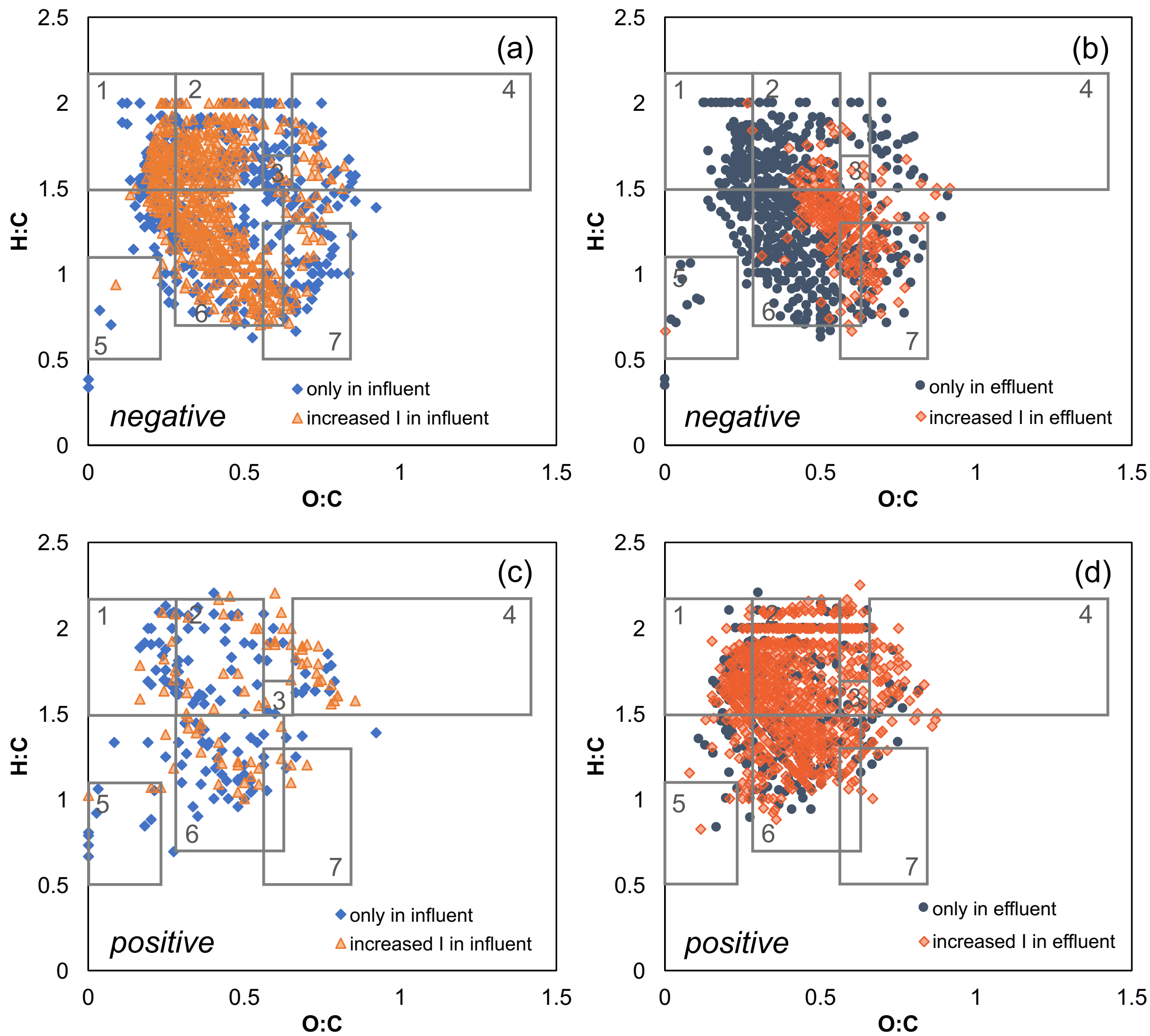

Figure S42. Negative and positive mode CHO formulas that were (a, c) only present in Ostara influent or had increased relative intensity in Ostara influent compared to Ostara effluent and (b, d) only present in Ostara effluent or had increased relative in intensity in Ostara effluent compared to Ostara influent.

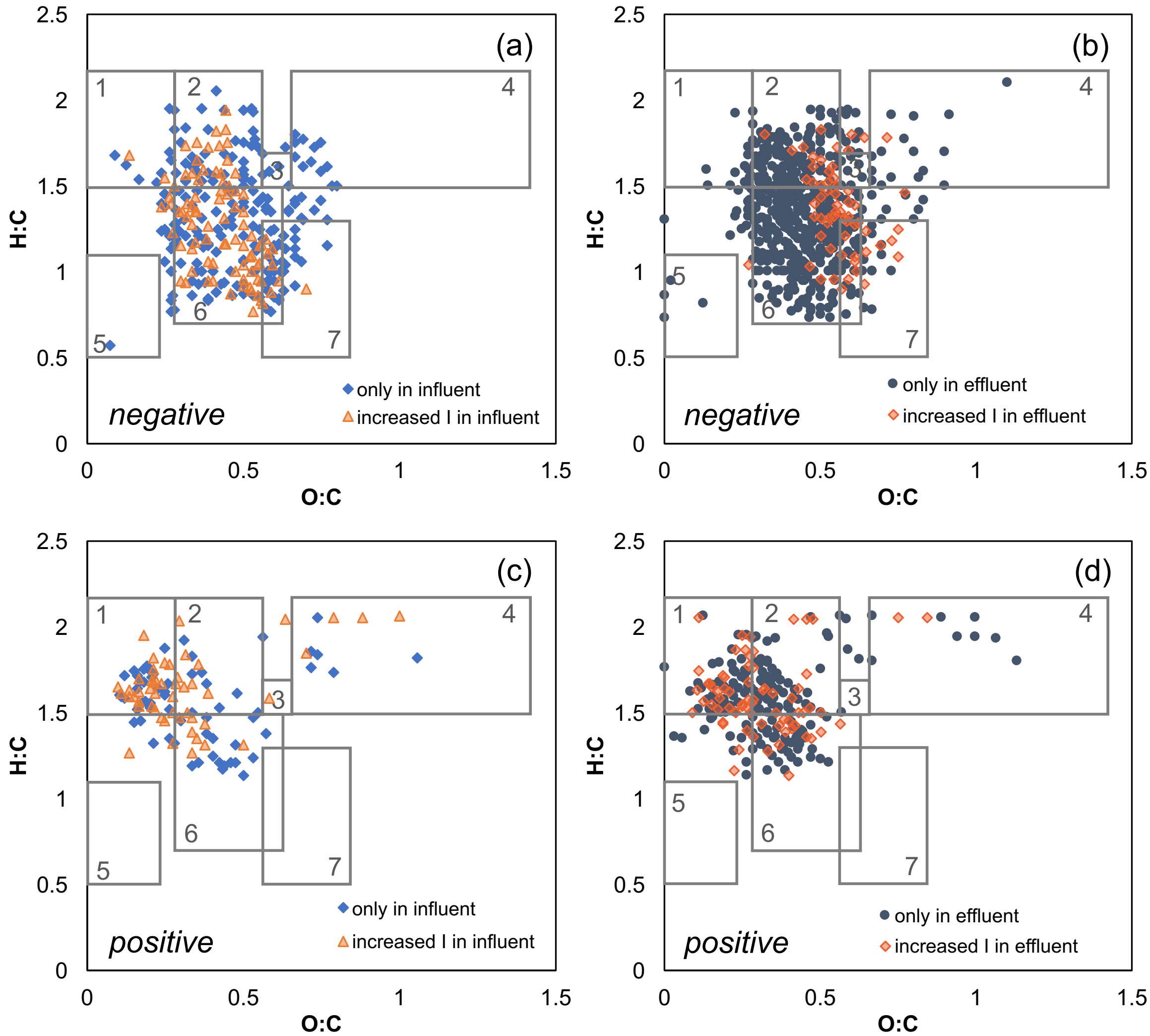

Figure S43. Negative and positive mode CHON formulas that were (a, c) only present in Ostara influent or had increased relative intensity in Ostara influent compared to Ostara effluent and (b, d) only present in Ostara effluent or had increased relative in intensity in Ostara effluent compared to Ostara influent.

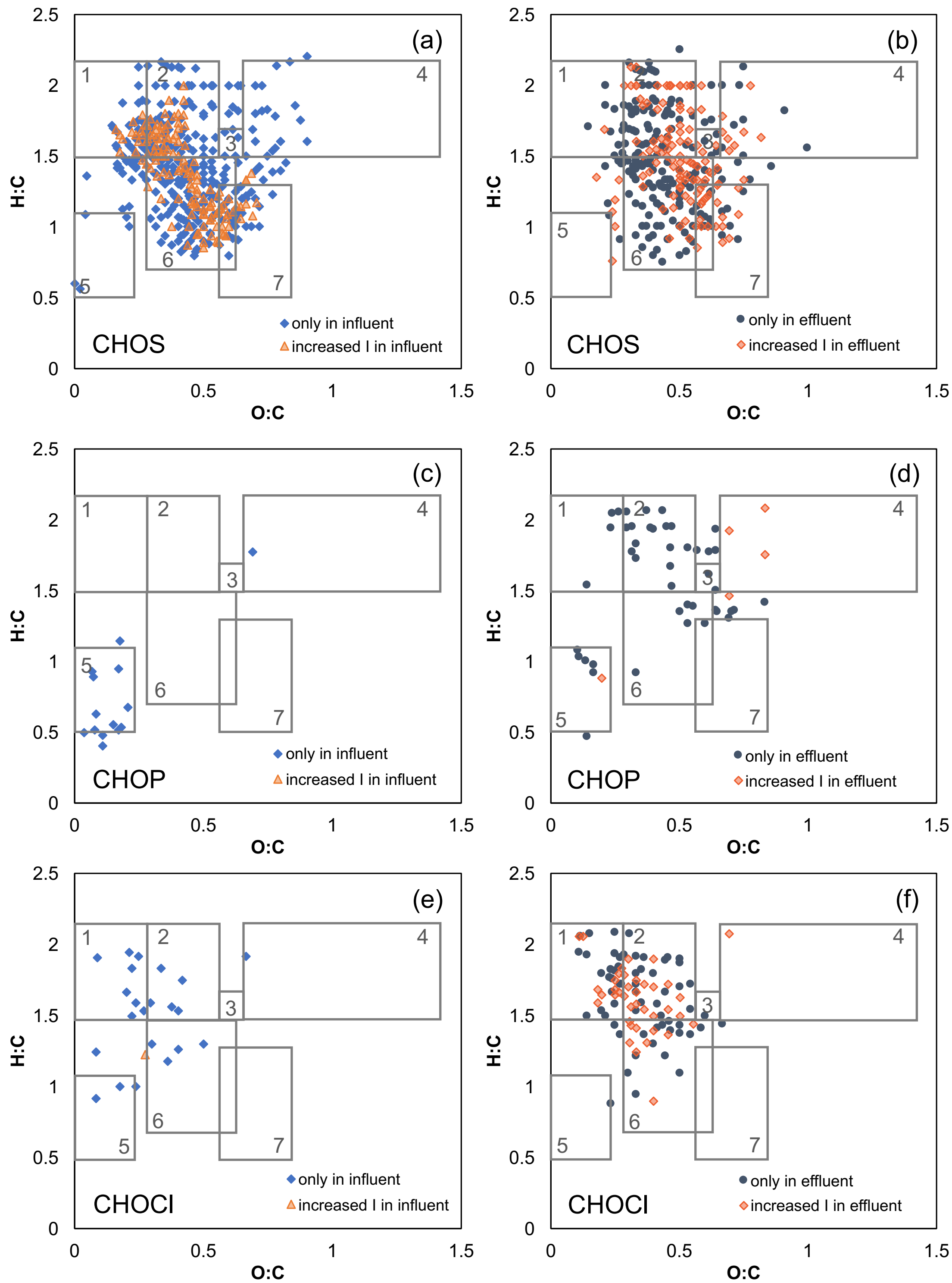

Figure S44. Negative mode CHOS, CHOP, and CHOCl formulas that were (a, c, e) only present in Ostara influent or had increased relative intensity in Ostara influent compared to Ostara effluent and (b, d, f) only present in Ostara effluent or had increased relative in intensity in Ostara effluent compared to Ostara influent.

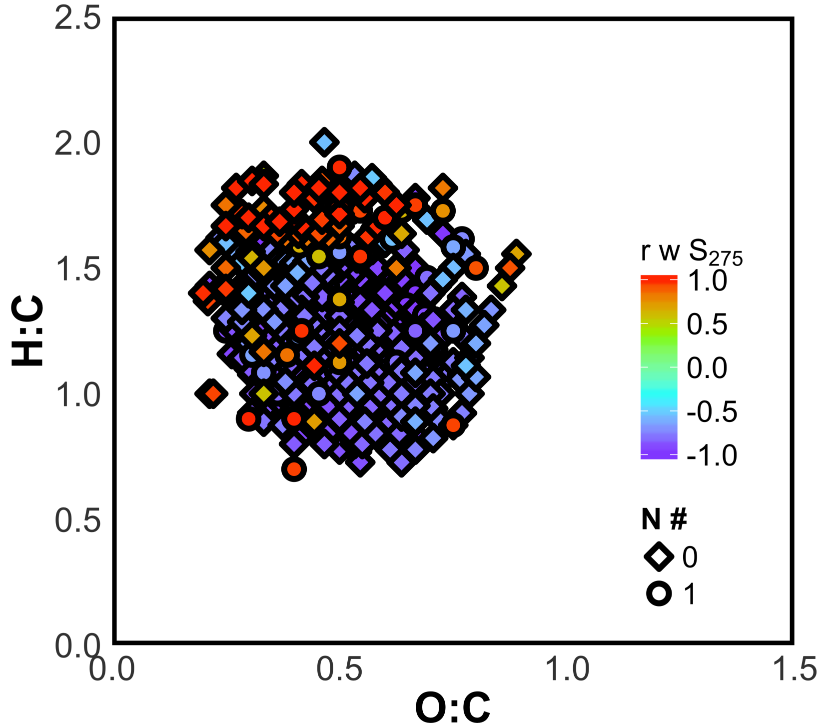

Figure S45. Pearson correlation coefficients calculated between the relative intensity of common FT-ICR MS CHO and CHON formulas and S275-295. The analysis was conducted using negative mode formulas in all samples excepting the Ostara influent and effluent. Only formulas with r ≤ -0.5 or ≥ 0.5 are shown.

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