

*Supporting Information for*

**Comparison of the chemical composition of dissolved organic matter in three lakes in Minnesota, USA**

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Submitted to **Special issue of *Environmental Science & Technology* honoring the contributions of George Aiken**

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**NMR analysis.** The multiple-cross polarization (multiCP) technique,<sup>1</sup> was applied to all the samples to obtain quantitative <sup>13</sup>C NMR spectra. The spectra were measured at a spinning speed of 14 kHz, where spinning sidebands are fairly small (< 3%) and have little overlap with centerbands. The 90° pulse lengths were 4.3 μs for <sup>1</sup>H and 4 μs for <sup>13</sup>C. All the spectra were recorded with a Hahn echo generated by a 180° pulse with EXORCYCLE phase cycling<sup>2</sup> applied one rotation period ( $t_r$ ) after the end of cross polarization, in order to achieve dead-time-free detection. The <sup>1</sup>H decoupling field strength was  $|\gamma B_1|/2\pi = 65$  kHz during the period of  $2 t_r = 0.14$  ms duration before the Hahn echo, and approximately 55 kHz during signal detection. After a recycle delay of 0.35 s, the ramp for CP (90-100%) on the <sup>1</sup>H channel was implemented with seven 1.1-ms and one 0.55-ms ramp CP periods. The duration of the repolarization period  $t_z$  in multiCP was 0.3 s. Corresponding multiCP spectra of nonprotonated C and mobile groups such as rotating CH<sub>3</sub> were obtained after a dipolar dephasing time of 68 μs (multiCP/DD). The number of scans collected was 2048 per spectrum for HPOA isolates and 2560 for TPIA isolates. The total time for multiCP and multiCP/DD experiments was 3-4 h per sample, tremendously less than the measuring time required by quantitative direct polarization experiments.

Both acetal/ketal (O-C-O) and aromatic carbons resonate between 120 and 90 ppm. To resolve the overlapping resonances, the aromatic carbon signals were selectively suppressed by inserting a three-pulse <sup>13</sup>C chemical-shift-anisotropy (CSA) filter with a CSA-filter time of 68 μs into the <sup>13</sup>C cross polarization and total suppression of sidebands (CP/TOSS) NMR experiments.<sup>3</sup> The number of scans averaged was 1536 or 2048 for each spectrum. The CSA filter was also combined with CP/TOSS and dipolar dephasing (48 μs) to select signals from ketal

(nonprotonated OCO) carbons. The number of scans averaged for the spectrum of CSA filter coupled with dipolar dephasing was 2560 or 3072 for each sample.

$^1\text{H}$ - $^{13}\text{C}$  two dimensional heteronuclear correlation (2D HETCOR) NMR experiments<sup>4</sup> were performed at a spinning speed of 7.5 kHz. Standard Hartmann-Hahn CP (HH-CP) with 80- $\mu\text{s}$  CP time reveals primarily one-bond  $^1\text{H}$ - $^{13}\text{C}$  connectivities. HH-CP of 0.5 ms allows for some  $^1\text{H}$  spin diffusion and therefore correlates the carbons with protons within  $\sim 0.5$  nm radius. In addition, a 40- $\mu\text{s}$  dipolar dephasing delay was inserted in the HH-CP HETCOR to reveal multi-bond  $^1\text{H}$ - $^{13}\text{C}$  proximities for nonprotonated carbons and mobile groups such as  $\text{CH}_3$ . The recycle delay was 0.5 s. Longer-range proximities were probed by  $^1\text{H}$  spin diffusion during a mixing time of 1 ms, correlating the carbons with protons within  $\sim 1.6$  nm radius.

**Table S1.** Summary of NMR methods used in this study for DOM characterization.

<b>NMR Experiment</b>	<b>Acronym</b>	<b>Purpose</b>
Multiple cross polarization	multiCP	Provides quantitative information about all C atoms, with good signal-to-noise ratio
Multiple cross polarization after dipolar dephasing	multiCP/DD	Provides quantitative information about nonprotonated C and mobile segments, such as rotating –CH <sub>3</sub> groups
Cross polarization/total suppression of sidebands	CP/TOSS	Provides qualitative information about all C atoms, at moderate spinning frequencies
Chemical shift anisotropy filter	CSA filter	Selects sp <sup>3</sup> -hybridized C signals and helps differentiate between overlapping resonances of aromatic and acetal/ketal (O–C–O) carbons
Chemical shift anisotropy filter after dipolar dephasing	CSA filter with DD	Identifies ketal (nonprotonated O–C–O) carbons
Two-dimensional <sup>1</sup> H- <sup>13</sup> C heteronuclear correlation	2D HETCOR	Provides <sup>1</sup> H- <sup>13</sup> C proximities
Two-dimensional <sup>1</sup> H- <sup>13</sup> C heteronuclear correlation with dipolar dephasing	2D HETCOR with dipolar dephasing	Reveals <sup>1</sup> H- <sup>13</sup> C proximities for nonprotonated C (multi-bond) and mobile groups like –CH <sub>3</sub>
Two-dimensional <sup>1</sup> H- <sup>13</sup> C heteronuclear correlation with <sup>1</sup> H spin diffusion	2D HETCOR with spin diffusion	Reveals long-range <sup>1</sup> H- <sup>13</sup> C proximities and detects domains and heterogeneities

**Table S2.** Peak areas (in %) in  $^{13}\text{C}$  multiCP NMR spectra of HPOA and TPIA isolates from different lakes, and the assigned structural moieties associated with the spectral regions.

Samples		ppm												
		220-190	190-160	160-143	143-100		123-100 (OCO)		100-64 (OC)		64-0 (Alkyl C)			
		Ketone C	COO/NC=O	Arom. C-O	Arom. C-C <sup>a</sup>	Arom. C-H	OC <sub>np</sub> O <sup>b</sup>	OCHO <sup>b</sup>	OC <sub>np</sub> <sup>a</sup>	OCH <sub>n</sub>	C <sub>q</sub> <sup>a</sup>	OCH <sub>3</sub> <sup>a</sup>	CH <sub>2</sub> /CH/NCH	CH <sub>3</sub> <sup>a</sup>
HPOA	Williams	1.9 ± 0.4	17.4 ± 0.5	3.0 ± 0.3	5.6 ± 1.0	4.1 ± 1.3	1.8 ± 0.4	0.9 ± 0.7	6.4 ± 0.7	11.0 ± 1.0	5.2 ± 0.8	0.1 ± 0.6	29.0 ± 1.9	13.5 ± 1.0
	Shingobee	2.6 ± 0.5	18.3 ± 0.4	4.5 ± 0.2	10.0 ± 0.7	5.0 ± 1.1	2.1 ± 0.3	0.8 ± 0.5	7.0 ± 0.6	9.8 ± 0.9	4.4 ± 0.5	0.9 ± 0.6	23.1 ± 1.5	11.5 ± 0.8
	Manganika	2.5 ± 0.3	16.9 ± 0.4	6.7 ± 0.2	13.2 ± 0.6	7.2 ± 0.9	2.1 ± 0.3	0.8 ± 0.4	6.1 ± 0.4	10.3 ± 0.6	3.1 ± 0.3	1.6 ± 0.3	20.4 ± 1.1	9.3 ± 0.5
TPIA	Williams	1.3 ± 0.3	20.8 ± 0.3	2.4 ± 0.2	3.6 ± 0.7	2.4 ± 0.9	2.4 ± 0.4	1.1 ± 0.5	8.9 ± 0.5	15.9 ± 0.8	4.0 ± 0.4	< 0.1	26.8 ± 1.2	10.4 ± 0.6
	Shingobee	2.4 ± 0.3	21.3 ± 0.3	3.2 ± 0.2	5.9 ± 0.7	3.2 ± 1.0	2.9 ± 0.3	0.9 ± 0.4	9.3 ± 0.5	14.9 ± 0.7	3.8 ± 0.4	< 0.1	23.3 ± 1.1	8.9 ± 0.5
	Manganika	2.6 ± 0.4	20.0 ± 0.4	4.2 ± 0.3	8.6 ± 0.9	3.2 ± 1.2	2.8 ± 0.5	1.4 ± 0.6	9.4 ± 0.6	14.2 ± 0.8	3.6 ± 0.5	< 0.1	21.2 ± 1.3	8.8 ± 0.8

Uncertainty is based on signal to noise ratio and is reported as one standard deviation.

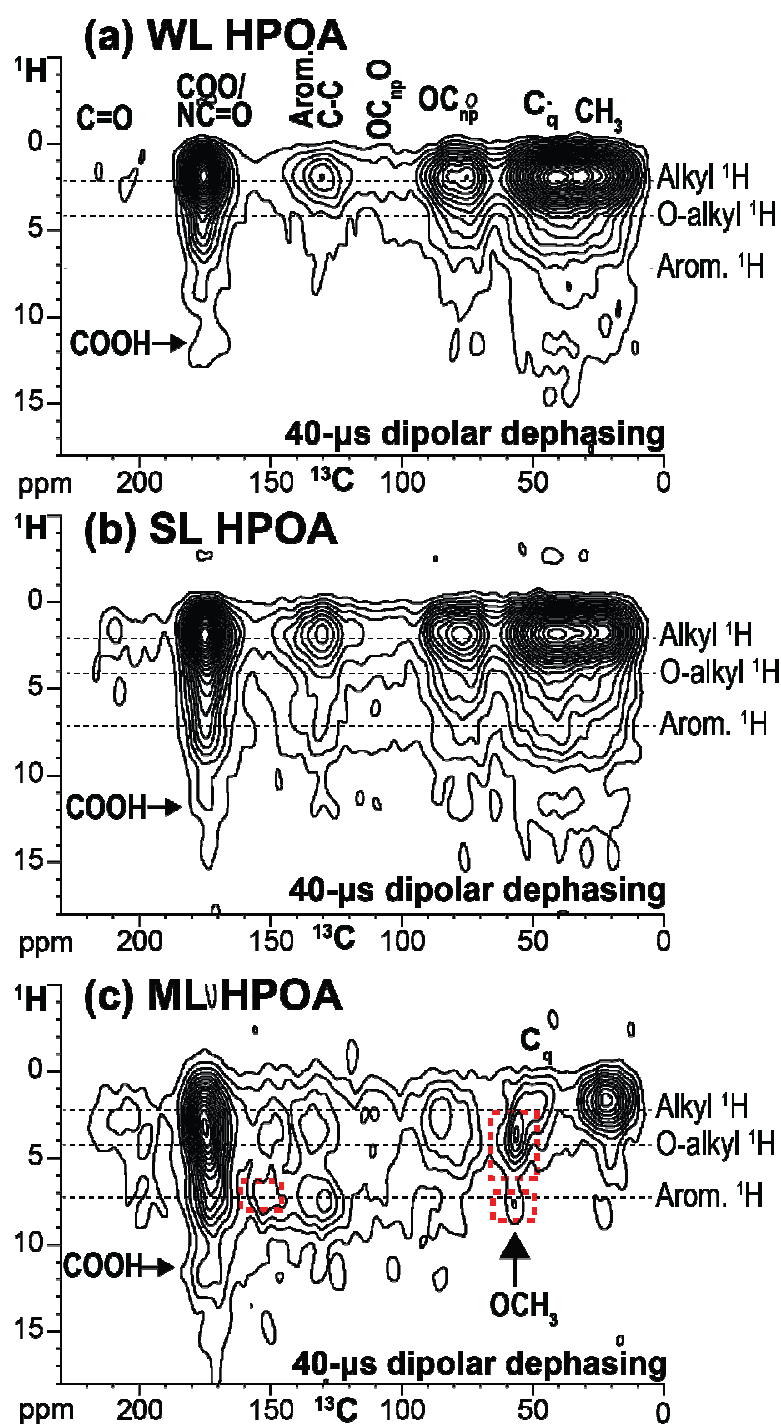
<sup>a</sup> Based on a multiCP spectrum with 40-μs dipolar dephasing.

<sup>b</sup> Based on a CSA-filtered CP/TOSS spectrum and a CSA-filtered CP/TOSS spectrum with dipolar dephasing.

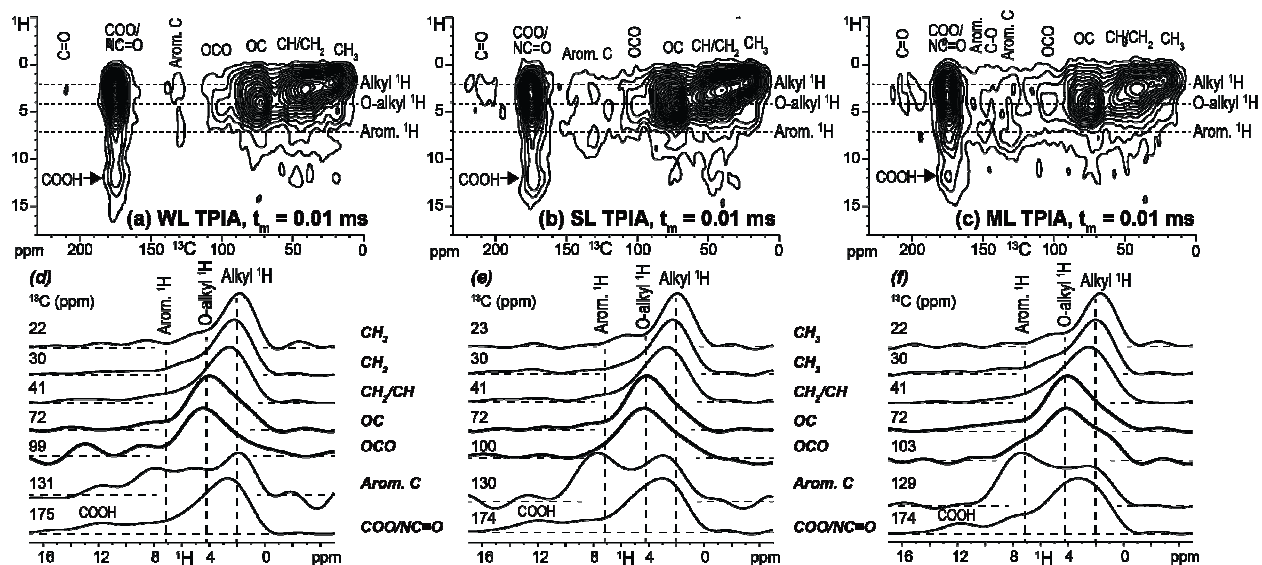
**Table S3.** DOC concentrations, SUVA<sub>254</sub>, and major chemical fractions of DOM.

Date	Sample	DOC (mg C L <sup>-1</sup> )	Fraction	% of DOC	SUVA <sub>254</sub> (L mg C <sup>-1</sup> m <sup>-1</sup> )	Ash-free elemental contents (%) and C isotopic data (‰)						
						C	H	O	N	S	δ <sup>13</sup> C	Age (ybp)
9/18/2013	Williams Lake (WL)	7.2	HPOA	30	1.9	56.10	5.62	35.71	1.83	0.75	-26.1	>Modern
			TPIA	21	1.2	50.81	5.21	39.59	3.26	1.12	ND	ND
9/4/2013	Shingobee Lake (SL)	5.2	HPOA	37	3.1	55.13	5.03	37.15	1.76	0.93	-29.1	315
			TPIA	22	2.2	49.89	4.64	41.74	2.61	1.12	ND	ND
6/26/2012	Manganika Lake (ML)	10.5	HPOA	40	4.2	52.21	4.62	40.39	1.66	1.13	ND	ND
			TPIA	17	2.9	45.52	4.60	45.44	2.36	2.08	ND	ND

DOC is dissolved organic carbon, and SUVA<sub>254</sub> is specific UV absorbance determined at 254 nm. HPOA is hydrophobic acid, and TPIA is transphilic acid. ybp, years before present. ND, not determined.

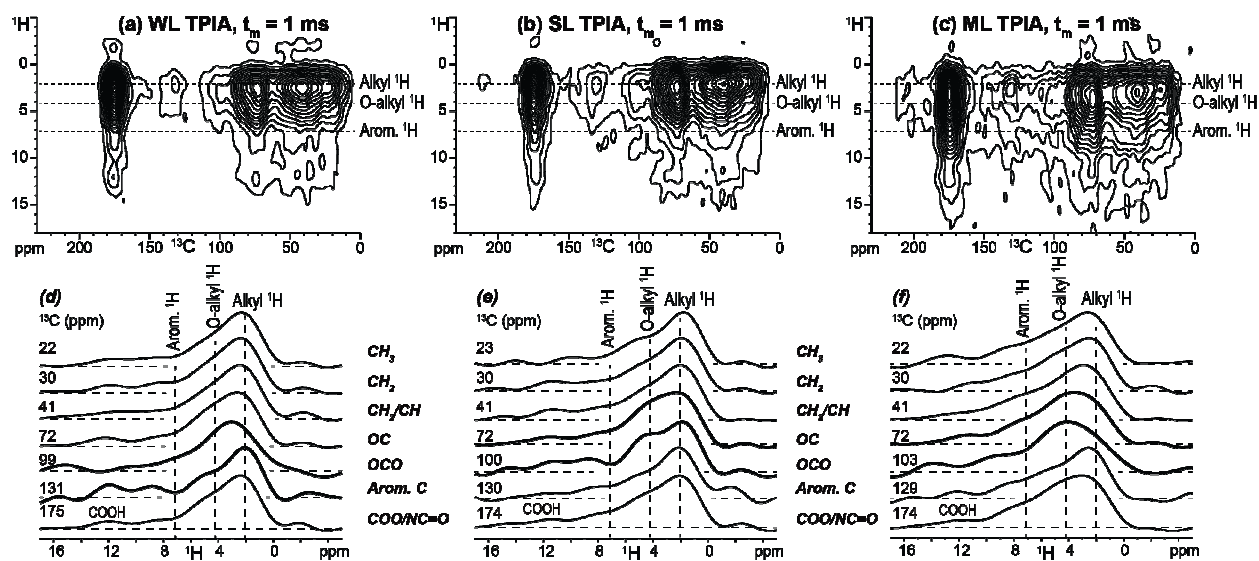


**Figure S1.** 2D  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectrum with 0.5-ms HH-CP and 40- $\mu\text{s}$  dipolar dephasing of HPOA isolates from (a) Williams Lake, (b) Shingobee Lake, and (c) Manganika Lake. Distinct cross peaks of  $\text{OCH}_3$  shown by red boxes.

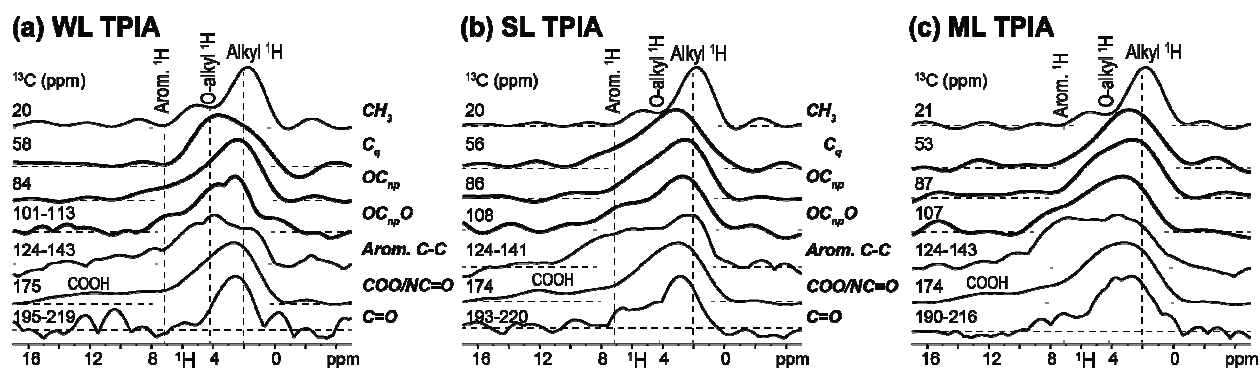


**Figure S2.** 2D  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectra with 0.5-ms HH-CP of TPIA isolates from (a) Williams Lake, (b) Shingobee Lake, and (c) Manganika Lake.  $^1\text{H}$  slices extracted from the 2D spectra: (d) refers to  $^1\text{H}$  slices of spectrum (a), (e) to spectrum (b), and (f) to spectrum (c).

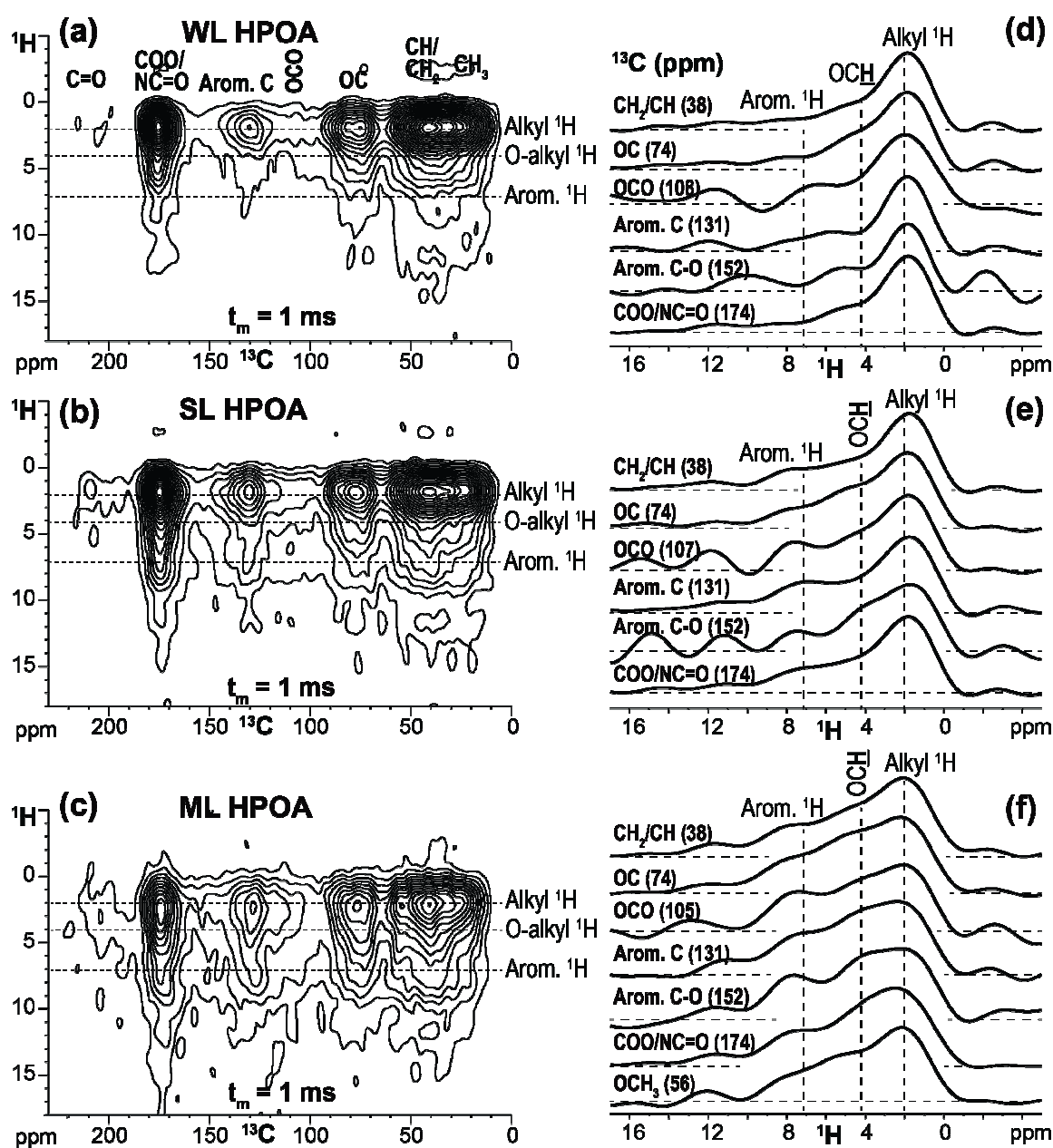




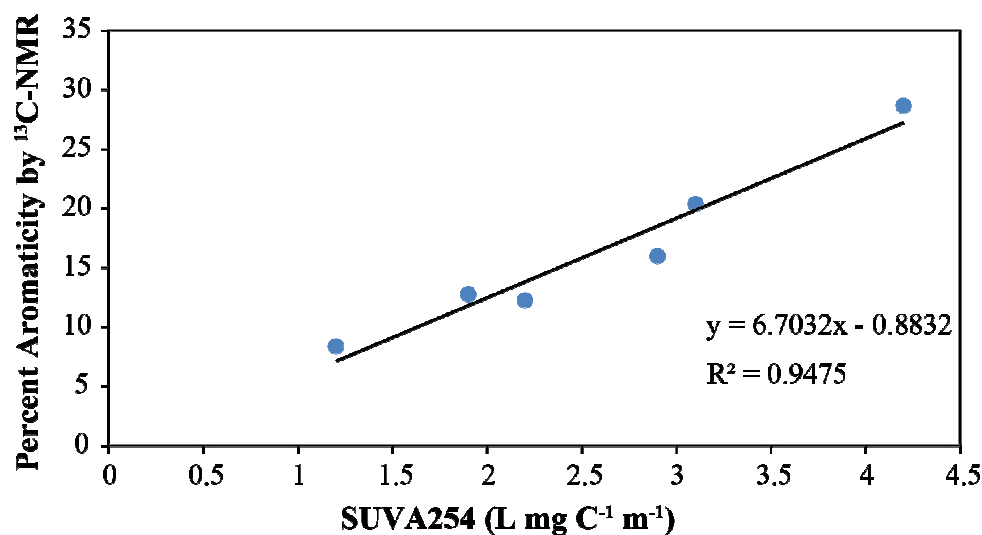
**Figure S3.** 2D  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectra with 0.5-ms HH-CP and 1-ms mixing time of TPIA isolates from (a) Williams Lake, (b) Shingobee Lake, and (c) Manganika Lake.  $^1\text{H}$  slices extracted from the 2D spectra: (d) refers to  $^1\text{H}$  slices of spectrum (a), (e) to spectrum (b), and (f) to spectrum (c).



**Figure S4.**  $^1\text{H}$  slices extracted from the 2D  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectrum with 0.5-ms HH-CP and 40- $\mu\text{s}$  dipolar dephasing of TPIA isolates from (a) Williams Lake, (b) Shingobee Lake, and (c) Manganika Lake.



**Figure S5.** 2D  $^1\text{H}$ - $^{13}\text{C}$  HETCOR spectra with 0.5-ms HH-CP and 1-ms mixing time of HPOA isolates from (a) Williams Lake, (b) Shingobee Lake, and (c) Manganika Lake.  $^1\text{H}$  slices extracted from the 2D spectra: (d) refers to  $^1\text{H}$  slices of spectrum (a), (e) to spectrum (b), and (f) to spectrum (c).



**Figure S6.** The relationship between percent aromaticity determined by <sup>13</sup>C NMR and SUVA<sub>254</sub>.

## References

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