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Direct noninvasive ^{1}H NMR analysis of stream water DOM: Insights into the effects of lyophilization compared with whole water

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Abstract

NMR spectroscopy is widely used in the field of aquatic biogeochemistry to examine the chemical structure of dissolved organic matter (DOM). Most aquatic DOM analyzed by proton NMR (^{1}H NMR) is concentrated mainly by freeze-drying prior to analysis to combat low concentrations, frequently <100 μM C, and eliminate interference from water. This study examines stream water with low dissolved organic carbon content by ^{1}H NMR with a direct noninvasive analysis of whole water using a water-suppression technique. Surface waters, collected from the headwaters of the Rio Tempisquito, Costa Rica, were examined directly, and the spectral characteristics were compared with those of the traditional preanalysis freeze-drying approach revealing significant differences in the relative intensity of peaks between the whole water and freeze-dried DOM. The freeze-dried DOM required less time to obtain quality spectra, but several peaks were missing compared with the spectra of whole water DOM; notably the most dominant peak in the spectrum constituting roughly 10% of the DOM. The stream water DOM showed an increase in the relative intensity of aliphatic methyl and methylene groups and a decrease in carbonyl, carboxyl, and carbohydrate functionalities after freeze-drying. The results of this study show that freeze-drying alters the original composition of DOM and thus freeze-dried DOM may not represent the original DOM. The information gained from whole water analysis of stream water DOM in a noninvasive fashion outweighs the attraction of reduced analysis times for preconcentrated samples, particularly for studies interested in investigating the low molecular weight fraction of DOM.

KEYWORDS

dissolved organic matter, freeze-dried DOM, headwater streams, lyophilization, proton NMR, stream water DOM, whole water analysis

1 | INTRODUCTION

Dissolved organic matter (DOM) is a heterogeneous mixture of high molecular weight and low molecular weight (LMW) organic compounds that fuels microbial metabolism in natural waters.[1] The complex heterogeneous nature of DOM has made characterization difficult.[2] About 40 years of research in the field of organic

geochemistry have focused on the characterization of DOM to better understand the driving forces behind microbial metabolism.[3] Many of these studies have employed NMR spectroscopy to obtain information on the average structural characteristics of the DOM, primarily high molecular weight-DOM.[4]

NMR spectroscopy is a highly versatile tool that can characterize complex molecular structures and interactions within a variety of sample matrices.[5] The nondestructive nature of this technique makes it ideal for environmental samples. The chemical structure of natural DOM has been analyzed extensively with NMR using a variety of solvents and protocols with great success due to its comprehensive analysis of functional group composition.[6] In studies analyzing aquatic DOM, solution-state ^{1}H NMR and ^{13}C NMR techniques are commonly applied as they are best suited to analyzing water samples in their native state.[6c, d, 7] However, these studies are limited by sensitivity due to low DOM concentrations. ^{1}H NMR can potentially address the issue of low concentrations, as it is more sensitive than ^{13}C NMR but is often limited by signal interference from water.

As a result, most studies analyzing aquatic DOM often use extensive sample preconcentration of the DOM and water removal consisting of several rounds of evaporation and redissolution in an aprotic solvent.[6b, 8] In addition to preconcentration, techniques such as ultrafiltration,[9] solid phase extraction,[10] and reverse osmosis coupled to electrodialysis[11] are used to increase signal from DOM and decrease signal from water. It is well recognized that isolation and/or concentration procedures alter the composition and properties of DOM.[12] Fractions lost during isolation or concentration may include polar LMW compounds that do not adsorb to XAD resins and C_{18} sorbents.[13] Some of the resins used for solid phase extraction, as in the styrene divinyl copolymer found in PPL cartridges, are selective toward nonpolar compounds while only retaining a portion of polar molecules resulting in a partially biased sample. Furthermore, these resins only retain approximately 40–60% of the initial carbon content and exclude a significant portion of the DOM from analysis. In the case of reverse osmosis coupled to electrodialysis or ultrafiltration, these small molecules can be adsorbed onto ion-exchange dialysis membranes during desalting or pass through the dialysis membranes and are not recovered.[13]

Of these methods, lyophilization, or freeze-drying, is one of the most commonly used techniques to preconcentrate aquatic DOM as it is considered to result in the least alteration of DOM composition compared with other preconcentration and isolation methods.[12b] Unfortunately, testing the effects of lyophilization on DOM composition is difficult given that most methods require preconcentration prior to analysis of DOM. Thus, there is very little information on the effects of lyophilization on DOM composition. Evidence to date suggests that lyophilization of natural waters may result in alteration of the structure and/or conformation of DOM, as well as the formation of aggregates during the lyophilization process.[5,14]

The ideal strategy to examine aquatic DOM composition involves direct analysis without preconcentration. Using a water-suppression technique originally proposed by Lam and Simpson (2008), direct analysis of freshwater DOM can be achieved at low concentration (<100 μM C) by ^{1}H NMR without the introduction of artifacts or other possible contaminants. Despite the benefits of direct analysis of DOM, drawbacks to this approach have limited its widespread use. The primary drawbacks to direct analysis using the water-suppression technique are decreased signal to noise and 10–100× longer analysis time, thus limiting the number of samples that could be analyzed by this approach.

In the current study, a modified version of the WATERGATE water-suppression pulse sequence originally outlined by Adams et al. (2013) was used to saturate the water signal at a chemical shift of ~4.8 ppm thereby eliminating interference from water and enabling quality ^{1}H NMR spectra to be obtained on whole water DOM without sample pretreatment.[7b, 15] This approach may allow for analysis of DOM without sacrificing sensitivity and while preserving the original composition of DOM. Unfortunately, this technique can result in either the loss or suppression of other signals in and around the resonance region of water (4.2–5.5 ppm). However, there are only a few structural entities in DOM, such as anomeric protons in carbohydrates and olefins, that resonate in this region of the spectrum. Signals upfield from the resonance region of water (3.5–4.2 ppm), such as carbohydrates and methoxyl groups in lignin, may also be suppressed using this approach.[12b]

Our use of direct noninvasive ^{1}H NMR, used for the first time in studies of stream water DOM, has led to detection of LMW compounds in these Costa Rican streams, including acetate and other small organic molecules. This approach provides a stark contrast with other stream water DOM studies that preconcentrate the DOM prior to ^{1}H NMR analysis, thereby leading to the loss of some, but possibly not all, LMW components of the DOM. In addition, freeze-drying potentially introduces artifacts from contamination that can be easily avoided using the direct whole water approach. We demonstrate these effects by comparing whole water analyses with analyses of DOM concentrated by freeze-drying.

2 | RESULTS

2.1 | Characterization of DOM by ^{1}H NMR

Figure 1 displays the ^{1}H NMR spectra of the freeze-dried DOM compared with the whole water DOM. The spectra of stream water DOM show the majority of signal between 0.6 and 4.2 ppm (aliphatic and carbohydrate regions) with minimal signal (<7%) at 6–9 ppm corresponding to olefinic and aromatic hydrogens. Thus, the 0.0 to 4.2 ppm range is isolated and amplified in Figure 1; the full spectra (0–10 ppm) can be found in Figures S1 and S2. The peak at 4.78 ppm is residual water after water suppression and the peak just before 0.0 ppm is the internal standard (TSP).

Stream water spectra are dominated by narrow sharp peaks indicating the presence of small molecules in

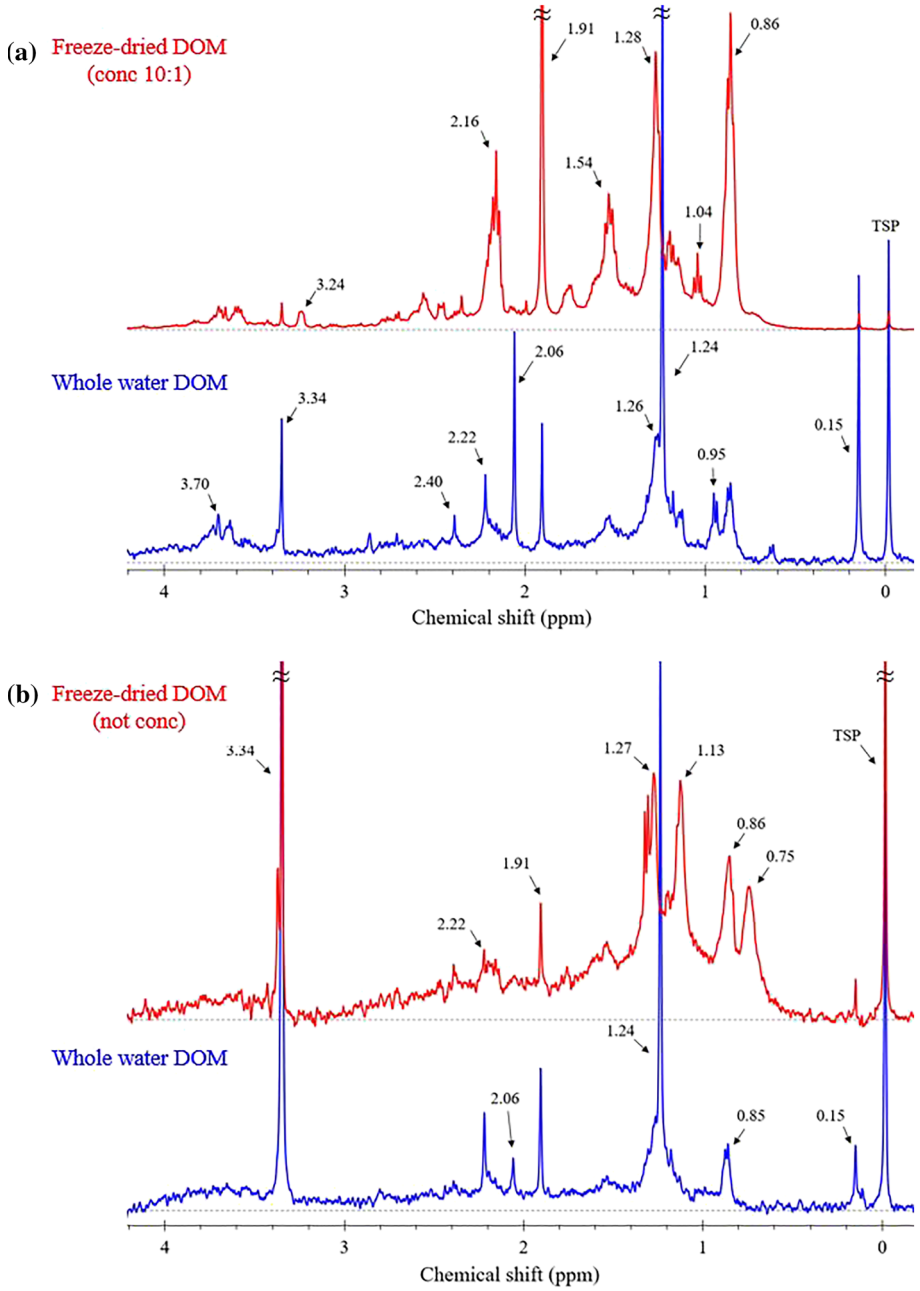

FIGURE 1 ^{1}H NMR spectra of stream water DOM analyzed as whole water DOM (blue spectral lines) and after subject to freeze-drying (red spectral lines): (a) stream water collected from Quebrada Kathia and concentrated 10:1 by freeze-drying in H_2O:D_2O, due to the difference in concentrations between the whole water DOM and freeze-dried DOM, the whole water DOM spectrum was amplified for ease of viewing; and (b) stream water collected from Quebrada Rosa and freeze-dried but not concentrated in H_2O:D_2O, these spectra are normalized to the internal standard because they have the same concentration. Chemical shifts of notable peaks are indicated. TSP is the internal standard. The gray dotted line represents the baseline. DOM, dissolved organic matter

addition to larger molecules likely associated with broader signals in the spectra. The observation of multiple sharp signals was surprising given that ^{1}H NMR studies of aquatic DOM typically display broad overlapping peaks with only traces of sharp signals that have been difficult to interpret.[4, 6a, d, 12b, 16] We have previously used the same water-suppression technique for solution state ^{1}H NMR to analyze DOM from other nonriverine sources, such as terrestrial aquatic DOM and water-soluble aerosol organic matter,[7b, 15, 17] and have observed the predominantly broad peaks characteristic of DOM along with sharp peaks. These sharp peaks may indicate high abundances of small molecules present in this Costa Rican stream water. The observation of sharp peaks in spectra of other stream water samples indicate that sharp peaks are commonly observed in other stream systems (Figure S3).

Most ^{1}H NMR spectra of DOM lack well-defined sharp peaks and instead have broad unresolved peaks with overlapping signals. These broad and overlapping peaks have often been attributed to the formation of aggregates during the preconcentration process prior to analysis.[4b, 6c, 10b, 16a] Aggregates likely exhibit an increased apparent molecular weight that imparts decreased spin–spin coupling (T_2) that inversely affects signal linewidth. Examining the spectra in Figure 1, the presence of broad peaks in whole water DOM is apparent at natural abundance without preconcentration. This suggests that the broadness observed by others in ^{1}H NMR spectra of DOM is not solely from aggregation during preconcentration; however, there is an overall increase in signal broadness after freeze-drying, see Figure 1. Signal broadness has been suggested to result from heterogeneity of DOM and the interaction of DOM with paramagnetic metals or a combination of these processes.[12b] The spectra of the freeze-dried DOM, both concentrated 10:1 and nonconcentrated, contain sharp peaks but there was an increase in signal broadness within the 0.6 to 1.8 ppm region of the ^{1}H NMR spectra. This broadening may be due to the formation of aggregates during freeze-drying that impart higher molecular weight or interactions between DOM and metals during the freeze-drying process.[6e, 10b, 14b]

2.2 | Verification of peaks in ^{1}H NMR spectra

Given the presence of sharp peaks in the spectra of DOM, it was possible to resolve specific compounds present in the stream water and, in some cases, the identity of these compounds was verified by standards. All identified peaks were required to have a signal-to-noise ratio greater than 4. From higher to lower field (right to left), the peak at 1.91 ppm is assigned to acetate, the peak at 3.34 ppm is methanol, and the peak at 8.44 ppm is formate.[7b, 18]

In addition to verification using standards, we modified the pH of the stream water using diluted trace metal grade hydrochloric acid and sodium hydroxide to test the assignment of peaks to small organic acids and alcohols. The stream water had a pH of approximately 7.5, thus any small organic acid present should be in conjugate base form. When the stream water was acidified to pH 3 to protonate conjugate bases such as acetate, we would expect a chemical shift to 2.08 ppm. Likewise, adjusting the pH of the stream water to pH 10 should not result in protonation of conjugate bases of acids like acetate and thus no shift is expected at high pH. As expected, the acetate peak moved from 1.91 to 2.08 ppm at pH 3 and did not shift at pH 10, thus strengthening our interpretation of this peak as acetic acid/acetate (Figure S4). In contrast to organic acids, methanol is not influenced by pH and therefore should have the same chemical shift upon pH adjustment. Consistently, the methanol peak position did not shift with change in pH of the stream water.

The peak at 2.06 ppm was initially attributed to acetic acid. However, upon acidification, the peak at 2.06 ppm did not shift in position or intensity (Figure S4). Instead, acetate (at 1.91 ppm) disappeared and a new peak appeared at 2.08 ppm creating a split peak with the peak at 2.06 ppm. This change in the spectrum as a function of pH indicates that the peak at 2.06 ppm is not acetic acid. A difference of 0.02 ppm is significant given that the instrument collects 16,384 data points per scan yielding 1 data point every 0.0008 ppm. Thus, there is sufficient resolution to clearly separate these two peaks into acetic acid at 2.08 ppm and a separate unknown compound at 2.06 ppm. The 2.06 ppm peak was not prominent in the freeze-dried stream water, suggesting that freeze-drying altered its abundance.

We can speculate as to the identity of other resonances observed in the spectra based on ^{1}H NMR spectra of DOM available in the literature. The sharp peak at 1.24 ppm dominates the ^{1}H NMR spectra of the whole water DOM and is attributed to the $C\mathbf{H}_2$ proton in a long-chain methylene.[17b, 19] Short-chain organic acids, likely C3 to C10 carbons in length, resonate as broad signals at 0.86 ppm ($C\mathbf{H}_3$), 1.53 ppm ($C\mathbf{H}_2$), and 2.16 ppm ($C\mathbf{H}_2$).[18b, 20] Exact assignment of these short-chain organic acids cannot be determined due to the low dissolved organic carbon (DOC) concentration and overlapping resonances. The sharp peak at 2.22 ppm is assigned to acetone, a by-product from the photochemical degradation of DOM.[21] One peak that appears to be consistent in ^{1}H NMR spectra of whole water DOM is a sharp single peak

at 0.15 ppm. This peak has been observed in other ^{1}H NMR studies of aquatic DOM, though not always acknowledged, and is attributed to a naturally occurring silicate.[6a, 12b] It has been suggested that this peak results from the increased concentration of silicate species upon freeze-drying.[12b] However, we see this peak at near constant concentration before and after freeze-drying suggesting that there is a fair amount of dissolved silicates detectable in aquatic DOM at natural abundance.

Although there is greater uncertainty in the exact assignment of other peaks in the spectra, information regarding the chemical environment of hydrogens in the stream water can be obtained based on chemical shift assignments of likely functional groups observed in DOM. Protons sharing the same chemical environment have similar chemical shifts and, as a result, can be described using the following six regions of the spectrum.[6d, f, 15a, 22] From higher to lower field, we identify the following assignments: Region 1: 0.6–1.3 ppm, hydrogens in aliphatic chains with 0.6–1.0 ppm being terminal methyl groups (C**H**$_3$) and 1.0–1.3 ppm corresponding to chain methylene groups (C**H**$_2$); Region 2: 1.3–1.85 ppm, protons bound to carbon that are at least four bonds away from the nearest heteroatom (**H**C—C—C—X) and protons on alicyclic rings with a large number of methyl moieties; Region 3: 1.85–2.1 ppm, acetate (C**H**$_3$COO$^-$) and acetate derivatives (C**H**$_3$COO—R); Region 4: 2.1–3.2 ppm, (a) aliphatic protons in α-position to carbonyl and carboxylic moieties (**H**C—C═Y) and (b) protons bound to carbon that are adjacent to a nitrogen, sulfur, or halogen atom, (**H**C—X); Region 5: 3.2–4.2 ppm, protons in carbohydrate, ether, and alcohol functionalities; and Region 6: 6.0–9.0 ppm, olefinic and aromatic protons (Ar—**H**).

2.3 | Quantification of ^{1}H NMR spectral assignments

Unlike other pulse sequences used in ^{1}H NMR, water suppression yields varying degrees of signal attenuation throughout the ^{1}H NMR spectrum due to J-modulation line-shape distortions and T_2 relaxation losses.[23] As a result, ^{1}H NMR spectra collected using water suppression are considered nonquantitative with the majority of signal suppression occurring near the water region, up to 1.1 ppm on either side.[12b] However, the streams analyzed in this study are similar to each other in DOC concentration and each displayed a sharp acetate peak at 1.91 ppm. Due to their similar chemical composition (e.g., pH, conductivity, and DOC concentration, see Table S1), ^{1}H NMR can still give semiquantitative information. To demonstrate that stream water DOM analyzed without preconcentration by ^{1}H NMR can yield quantitative information, the acetate peak at 1.91 ppm in the whole water spectrum was quantified for one of the streams using standard additions, see Figure 2.

Standard additions were performed using varying concentrations of calcium acetate (0.5–4.0 μM) on the initial stream water and then analyzed by ^{1}H NMR using the same instrument parameters. The peak area of acetate increased linearly with each standard addition. The acetate peak increased in intensity and remained a single sharp peak, see Figure 2b. Addition of acetate did not influence other peak intensities, shape, or chemical shift in the spectrum. The peak area of the CH_2 peak remained constant in all of the spiked

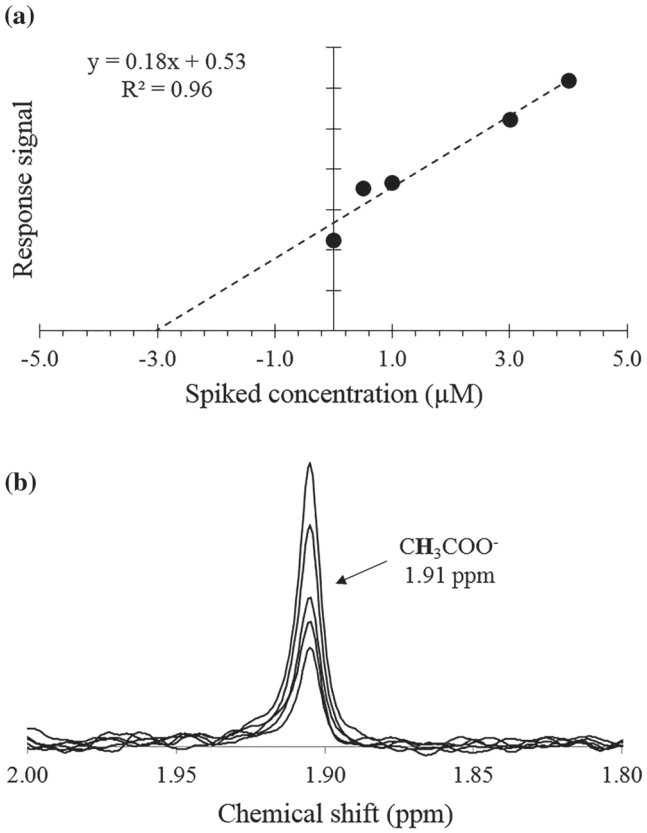

FIGURE 2 Standard additions of stream water dissolved organic matter, collected from Quebrada Kathia, analyzed as whole water by ^{1}H NMR with water suppression. (a) Standard curve of the calcium acetate additions. The black data points are the standard additions and the dotted line is the extrapolated linear regression used to determine the acetate concentration in the stream water. (b) ^{1}H NMR spectra of the acetate peak in the stream water overlaid with each addition of calcium acetate, full spectra for the matrix spikes are shown in Figure S5. Due to limited volume of stream water available for analysis, the stream water used for standard additions was collected from the same stream displayed in Figure 1 but 7 days later under similar baseflow conditions. Both stream water samples have similar pH, conductivity, and dissolved organic carbon concentrations

samples. Acetate concentrations in the initial stream water were calculated to be 3.0 ± 0.44 μM (R^2 = 0.96). The successful quantification of acetate using this technique demonstrates that despite the use of water suppression, semiquantitative spectra can still be obtained.

2.4 | Comparison of ^{1}H NMR spectra: Freeze-dried DOM versus whole water DOM

All ^{1}H NMR spectra were integrated and normalized to the sum of their total peak areas, not the internal standard, for both the whole water DOM and freeze-dried DOM; see Figure 3. There was a 16% total change in the relative intensity of integrated regions between the concentrated freeze-dried DOM collected from Quebrada Kathia and its corresponding whole water DOM. The concentrated freeze-dried DOM had a 7% increase in the relative intensity in Region 1, 6% increase in Region 2, 2% increase in Region 3, and 1% increase in Region 6; the corresponding whole water DOM lost 4% of its relative intensity in Region 4 and 12% in Region 5 after freeze-drying. Similarly, there was a 26% total change in the relative intensity of integrated regions between the nonconcentrated freeze-dried DOM collected from Quebrada Rosa and its corresponding whole water. The nonconcentrated freeze-dried DOM showed a 17% increase in Region 1, 7% increase in Region 2, and 2% increase in Region 6, whereas the whole water DOM lost 1% of its relative intensity in Region 3, 3% in Region 4, and 22% in Region 5. We attribute the larger percent change shown by the Quebrada Rosa whole water DOM due to Quebrada Rosa being a smaller stream with a different stream chemistry (i.e., pH, conductivity, and DOC) and DOM composition.

Based on the area integrations, both the concentrated and nonconcentrated freeze-dried DOM experienced the same trends in relative intensity over the six regions of the ^{1}H NMR spectrum. The shared increases in relative intensity indicate that the freeze-dried DOM is more enriched in aliphatic methyl moieties than the original whole water DOM. The overall loss of relative intensity in Regions 4 and 5 for both whole water samples suggests that the original whole water DOM has a higher composition of carbonyl, carboxylic, and carbohydrate structural components that may be lost or altered after freeze-drying. The loss of these oxygenated groups is unexpected as these functionalities are not considered to be inherently volatile. Oxygenated species usually facilitate the solubilization of

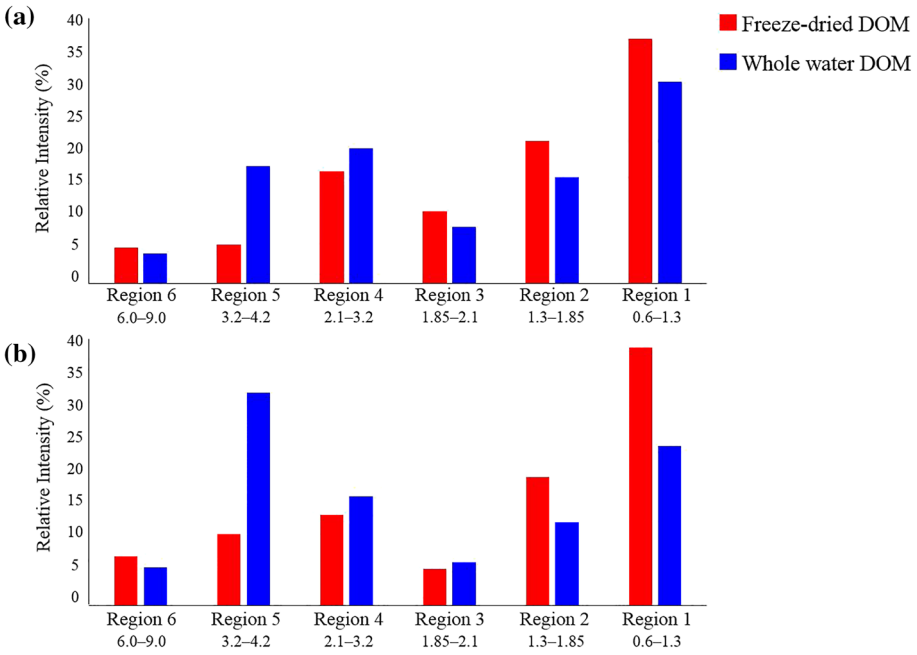

FIGURE 3 Comparison of peak area integrations over the six spectral regions of the ^{1}H NMR spectra shown in Figure 1 of (a) the concentrated 10:1 freeze-dried DOM and corresponding whole water collected from Quebrada Kathia and (b) the nonconcentrated freeze-dried DOM and corresponding whole water collected from Quebrada Rosa. The regions are organized by chemical shift (ppm). All spectra were normalized to their sum of integrals. DOM, dissolved organic matter

DOM in water; however, lyophilization has the potential to collapse the structural network of DOM thereby altering its ability to redissolve in water. It is possible that these oxygenated functionalities undergo an alternative form of aggregation creating new tightly agglomerated compounds that may express different solubility characteristics. Free biopolymers in marine DOM can spontaneously aggregate forming polymeric gel-like structures with a carbohydrate, protein, and lipid matrix.[24] Although polymeric structures are less commonly studied in freshwater environments, transparent exopolymer particles have been found in freshwater lakes, streams, and rivers.[25] It is possible that the carbonyl, carboxylic, and carbohydrate structural components in these stream waters may exhibit similar aggregate characteristics and are not fully redissolving in water.

The shift in the relative intensity of regions, shared between the concentrated and nonconcentrated freeze-dried DOM, suggests that these differences are attributed to changes in DOM composition during the freeze-drying process and not from an increase in concentration of DOC from freeze-drying. If the freeze-drying process had no effect on the structural composition of DOM, then there would be no change in the relative intensity of peaks between the whole water and freeze-dried samples.

Area integrations give useful information regarding compound class but not individual peaks. In addition to the area integrations discussed above, individual peaks were integrated and compared. Comparison of peaks between the whole water and the freeze-dried samples was straightforward because both were analyzed in the same solvent. Thus, a difference in the chemical shift of peaks was not expected. The relative intensity and signal-to-noise ratio of noticeable peaks in the freeze-dried DOM and whole water spectra are listed in Tables 1 and 2. Table 1 compares the whole water DOM and the concentrated 10:1 freeze-dried DOM collected from Quebrada Kathia. Table 2 compares peaks found in the whole water DOM and nonconcentrated freeze-dried DOM collected from Quebrada Rosa. Only peaks that had a signal-to-noise ratio ≥ 4 and are clearly identified in the spectra are listed.

Comparison of peaks in the spectra reveals that a number of peaks were lost or substantially decreased in intensity when the stream water DOM was freeze-dried. The majority of peaks lost upon freeze-drying are in the 0.6 to 1.3 ppm region corresponding to aliphatic hydrogens in terminal methyl and chain methylene functional groups. Of these, the most intense signal in the whole water DOM, 1.24 ppm, disappears in both of the freeze-dried stream waters. Other peaks that share similar behavior are the peaks at 2.06 and 2.22 ppm in the whole water DOM. Their loss after freeze-drying suggests that these compounds are volatile. For example, methanol is a prominent peak at 3.34 ppm in the whole water spectrum but is significantly attenuated in the freeze-dried spectra due to its high volatility. The peaks for acetate and formate, both small molecules, exhibit different behavior compared with methanol. The peak for formate, 8.44 ppm, shows an increase in intensity upon freeze-drying in both the concentrated 10:1 and nonconcentrated freeze-dried DOM. Meanwhile, acetate only increased in the concentrated freeze-dried DOM and remained relatively constant in the nonconcentrated freeze-dried sample (see full ^{1}H NMR spectra in Figures S1 and S2). We suspect that these small molecules did not decrease upon freeze-drying possibly because they exist as anions complexed to metal cations in these streams[26] and, as such, would not be volatilized as acetic acid and formic acid, respectively, during freeze-drying.

In addition to the loss of peaks during freeze-drying, there were also new peaks present in the freeze-dried spectra that were not observed in the whole water spectra. Peaks unique to the freeze-dried spectra may not have been detectable due to the lower concentration in the whole water DOM. These peaks may have been masked by more intense signals in the whole water spectrum. For example, the triplet at 1.04 ppm in the concentrated freeze-dried sample is not observed with confidence in the whole water spectrum, but there appears to be a small peak in the whole water spectrum centered at 1.04 ppm but was not assigned as a peak due to its low signal-to-noise ratio. In contrast, the peak at 0.75 ppm in the nonconcentrated DOM cannot be the result of increased concentration. It is possible that this peak could be an artifact of the lyophilization process as a very small amount is also present in the process blank, see Figure S7. Another possibility for new peaks in the freeze-dried spectrum is that loss of volatile species can alter the molecular structure of the DOM. This alteration may cause protons to resonate at different frequencies. When DOM is freeze-dried, it is concentrated and compacted into a solid; it is possible that this forced interaction may cause the DOM to further aggregate forming the new peaks observed in the freeze-dried DOM spectrum. Additionally, any hydrated compound has the potential to be altered once the water is removed during lyophilization.

As proof of concept, we have included ^{1}H NMR spectra of three additional freshwater streams all analyzed as whole water DOM without preconcentration in 90:10 H_2O:D_2O, see Figure 4. Each ^{1}H NMR spectrum displayed in Figure 4 correlates to a stream of low DOC concentration and is arranged according to stream size, smallest to largest, following Quebrada Rosa and Quebrada Kathia

TABLE 1 ^{1}H NMR peaks found in the whole water DOM and concentrated 10:1 freeze-dried DOM collected from Quebrada Kathia

		Whole water DOM			Freeze-dried DOM (conc 10:1)		
Region. (ppm)	**Functional group**	**Chemical shift (ppm)**	**Relative intensity (%)**	**S/N**	**Chemical shift (ppm)**	**Relative intensity (%)**	**S/N**
0.6–1.3	R—C**H**$_3$	0.62	0.3	4			
	R—C**H**$_2$—R	0.64	0.2	4	0.74	0.9	33
		0.86	1.9	20	0.85	2.1	428
		0.88	1.3	18	0.86	5.0	625
		0.94	0.8	15	0.88	2.9	496
		0.95	1.0	17	1.02	0.7	106
		1.04	0.4	5	1.04	0.9	151
		1.13	1.0	13	1.06	0.7	102
		1.15	1.0	12	1.18	1.2	167
		1.18	1.1	18	1.20	1.1	194
		1.24	13.5	151	1.28	12.0	548
		1.26	5.3	32			
1.3–1.85	H—C—C—X				1.50	1.1	155
	alicyclic C**H**$_2$	1.33	1.0	17	1.52	1.8	238
		1.53	2.0	11	1.53	2.1	268
					1.55	1.6	210
					1.76	2.0	87
1.85–2.1	C**H**$_3$COO$^-$	1.91	3.1	34	1.91	8.7	1,515
	C**H**$_3$COO–R	2.06	5.2	57	1.99	0.4	56
2.1–3.2	H—C=Y				2.14	1.5	234
	H—C—X				2.16	2.3	352
					2.18	1.7	262
		2.22	2.2	22	2.20	1.0	188
		2.39	0.9	11	2.21	1.4	137
		2.86	0.8	7	2.35	0.5	66
					2.47	1.0	53
					2.57	0.6	72
					2.70	0.2	35
					2.72	0.2	29
3.2–4.2	H—C—O—R				3.24	0.6	36
					3.35	0.3	52
		3.35	2.0	2.0	3.58	0.3	39
		3.64	2.2	2.2	3.59	0.3	45
		3.70	1.3	1.3	3.61	0.3	44
					3.66	0.3	44
					3.68	0.2	38
					3.70	0.4	45
6.0–9.0	Ar—H	8.44	0.2	4	8.44	3.8	733

Note. Peaks are arranged according to which region they are found in the ^{1}H NMR spectrum. The chemical shift, relative intensity, and signal-to-noise ratio (S/N) are given for each peak. Only peaks with an S/N > 4 are displayed.

Abbreviation: DOM, dissolved organic matter.

(streams shown in Figure 1). Spectra have been analyzed under identical instrumental parameters and are normalized to TSP, the internal standard just before 0.0 ppm. Despite low DOC concentrations, the ^{1}H NMR spectra acquired using this method show clear resolved signals and distinct differences in the spectral characteristics of these Costa Rican streams. The structural diversity of the DOM among these streams represents a critical part of a larger question concerning biogeochemical alterations of DOM across a longitudinal transect and as a function of biodegradation but is beyond the scope of the current assessment of a direct noninvasive ^{1}H NMR analysis.

3 | DISCUSSION

The characterization of aquatic DOM is a complex analytical challenge that has been ongoing for decades. The

TABLE 2 ^{1}H NMR peaks found in the whole water DOM and nonconcentrated freeze-dried DOM collected from Quebrada Rosa

		Whole water DOM			Freeze-dried DOM (not conc)		
Region (ppm)	Functional group	Chemical shift (ppm)	Relative intensity (%)	S/N	Chemical shift (ppm)	Relative intensity (%)	S/N
0.6–1.3	R—C**H**$_3$	0.85	1.5	13			
	R—C**H**$_2$—R	0.88	0.9	12	0.75	5.0	35
		1.18	1.4	12	0.86	1.4	52
		1.24	9.9	113	1.13	2.6	72
		1.27	1.4	19	1.19	1.0	41
		1.28	1.1	17	1.27	6.0	78
1.3–1.85	**H**C—C—C—X	1.31	0.9	13	1.31	2.0	64
	alicyclic C**H**$_2$	1.33	0.7	10	1.32	1.8	58
		1.39	0.6	6	1.53	0.8	25
		1.53	0.7	7	1.76	1.1	16
1.85–2.1	C**H**$_3$COO$^-$	1.91	2.5	29	1.91	1.4	30
	C**H**$_3$COO—R	2.06	1.3	10	2.05	0.3	13
2.1–3.2	**H**C—C=Y	2.16	0.4	6	2.16	0.7	16
	HC—X	2.20	0.8	8	2.19	0.8	22
		2.22	2.0	20	2.22	0.6	25
		2.39	0.6	6	2.47	0.3	12
3.2–4.2	**H**C—O—R	3.34	19.2	243	3.34	2.4	105
					3.37	1.1	38
6.0–9.0	Ar—**H**	8.44	0.6	7	7.63	0.4	11
					8.09	0.3	8
					8.10	0.3	9
					8.44	0.8	33

Note. Peaks are arranged according to which region they are found in the ^{1}H NMR spectrum. The chemical shift, relative intensity, and signal-to-noise ratio (S/N) are given for each peak. Only peaks with a signal-to-noise ratio ≥ 4 are displayed.

Abbreviation: DOM, dissolved organic matter.

noninvasive approach presented here allows for the direct analysis of stream water DOM, analyzed as whole water and without preconcentration. Whole water DOM yielded ^{1}H NMR spectra with well-defined peaks that can be identified and quantified without difficulty despite having a lower DOC concentration. Concentrating stream water DOM prior to ^{1}H NMR analysis revealed that freeze-drying influences the NMR spectral characteristics. Comparison of the spectra shows that a number of peaks present in the whole water DOM were either lost and/or altered after freeze-drying demonstrating the effect freeze-drying has on stream water DOM. In contrast to our findings, Lam and Simpson (2008) have suggested that lyophilization has no impact on aquatic DOM composition. However, the results of this study along with more recent NMR studies have suggested that any preconcentration of DOM could influence NMR spectra by aggregation of the DOM.[5, 14b]

The primary drawback to whole water DOM analysis is the extended instrument run time. However, this drawback is offset by lack of alteration of DOM during preconcentration steps such as freeze-drying. This study suggests that the extra time is worth it especially if the goal of the study includes characterization of small, volatile fractions of DOM. Even though DOM concentration by freeze-drying significantly reduces analysis time and increases the signal-to-noise ratio, the data generated in this study suggest that freeze-drying DOM results in the loss of an unknown number of volatile compounds and/or alteration to the molecular composition of the original DOM.

Increasing the widespread application of direct ^{1}H NMR analysis of whole water DOM will enhance the accuracy of DOM characterization in natural aquatic systems. Although preconcentrating DOM may produce superior spectral qualities in a fraction of the time, one must consider the study objective. We have shown evidence that the structural information gained from preconcentrated DOM is not completely representative of the DOM in the stream water and could represent a significant portion of DOM that remains either

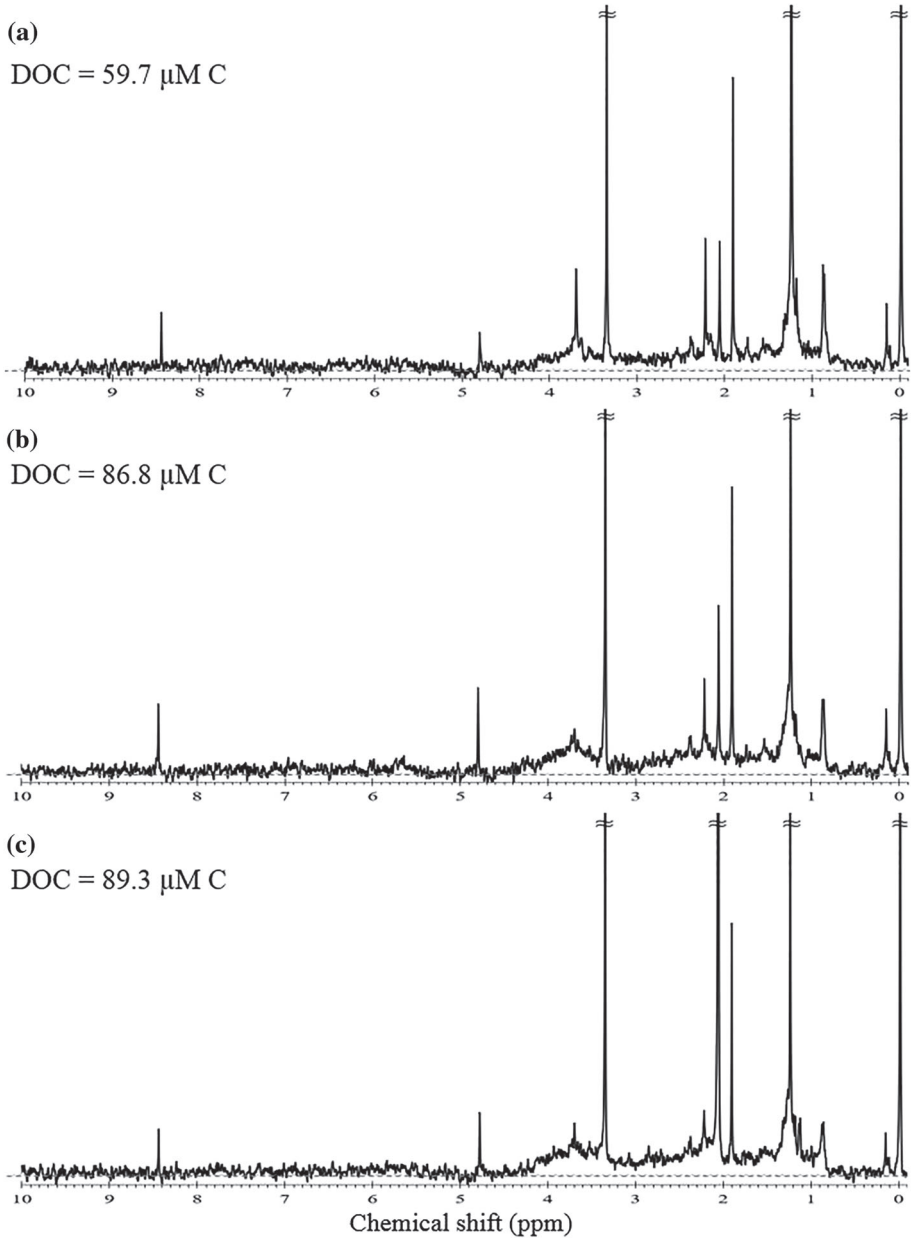

FIGURE 4 Full ^{1}H NMR spectra (0–10 ppm) of stream water dissolved organic matter collected from (a) Rio Tempisquito, (b) Rio Tempisquito Sur, and (c) confluence of the Rio Tempisquito and Rio Tempisquito Sur. Stream waters were analyzed as whole water without preconcentration in 90:10 H_2O:D_2O. Spectra have been analyzed under identical instrument parameters and are normalized to TSP, the internal standard just before 0.0 ppm. The gray dotted line represents the baseline. DOC, dissolved organic carbon

uncharacterized or mischaracterized in studies that preconcentrate DOM prior to ^{1}H NMR analysis. As such, this misrepresentation could have significant impact on the current knowledge surrounding the composition of aquatic DOM with overarching implications for understanding carbon cycling in natural waters. Future work utilizing 2-D NMR techniques would give useful insight into the alteration of DOM during freeze-drying but samples must have a higher DOC concentration than the ones used in this study.

4 | COMMENTS AND RECOMMENDATIONS

This technique is not only applicable to stream water DOM but can be applied to any freshwater system. We examined five headwaters streams originating from one watershed; the spectral characteristics of DOM are likely to change from one system to another depending on the freshwater composition. Employing this technique to multiple freshwater systems will provide a more comprehensive understanding of DOM as it exists in natural

waters. Unfortunately, this method is not ideal for saltwater matrices. We have tested this method using water samples from the open ocean with a low DOC concentration (<100 μM C) and a high salinity matrix (~30 ppt). We found that there was interference from the salts present in solution. However, this method has recently been used to analyze pore waters collected from marine sediments with great success.[27]

We acknowledge that extended analysis times can be costly. Instrument analysis times can be reduced through the use of a larger or more sensitive probe, such as a cryoprobe. Cryoprobes have greater sensitivity than the 5-mm BBI probe used in this study, potentially increasing the signal-to-noise ratio by a factor of 4.[5] This would translate to a decrease in sample analysis time from 49 hr to approximately 3 hr per sample were a cryoprobe used. However, the use of a larger probe would allow for a larger volume of sample to be analyzed thus increasing the concentration-based sensitivity. Both suggestions are viable options for those that have access to these tools that we unfortunately do not. Another way to further shorten NMR time is to advance to higher field strengths on systems fitted with cryoprobes.

5 | MATERIALS AND PROCEDURES

5.1 | Sample description

The stream water DOM used in this study was collected from five headwater tributaries of the Rio Tempisquito, a pristine watershed with little to no anthropogenic influence located in a tropical evergreen forest of the Guanacaste Conservation Area, in northwestern Costa Rica. The five streams ranged in size from smallest to largest included Quebrada Rosa, Quebrada Kathia, Rio Tempisquito, Rio Tempisquito Sur, and the confluence of Rio Tempisquito and Rio Tempisquito Sur.[28] Surface waters were collected into organic-carbon-free (precombusted at 450°C for 8 hr) 1-L borosilicate glass bottles with precleaned (10% w/v sodium persulfate) Teflon-backed silicone septa (Schott) under baseflow conditions in February 2016 during the dry season and returned to the laboratory on ice. Stream waters had a pH range of 7.6–7.9, conductivity range of 94.4–367.7 μS cm^{-1}, and a DOC concentration range of 59.7–163.9 μM; see Table S1. In the laboratory, all samples were filtered through precombusted (450°C for 8 hr) 0.7-μm glass fiber filters (Whatman GF/F), and samples for ^{1}H NMR analysis were further filtered through 0.2-μm PES membrane filters (Millex GP), frozen, and transported frozen to Norfolk, Virginia, for analysis.

5.2 | DOC data analysis

GF/F-filtered samples for DOC analysis were transferred to triplicate precombusted 40-ml borosilicate vials and stored in the refrigerator at 4°C until analysis on site. DOC analyses were performed with UV-promoted, persulfate oxidation and membrane conductometric detection in an analyzer equipped with an autosampler and inorganic carbon removal module (Sievers-900 Portable TOC analyzer).

5.3 | ^{1}H NMR sample preparation

Frozen stream water was thawed at room temperature and prepared for ^{1}H NMR analysis following two different approaches: whole water and freeze-dried. An internal standard of 1-μM 2,2,3,3-*d*(4)-3-(trimethylsilyl) propionic acid sodium salt (Alfa Aesar) was added to all samples prior to analysis. Ultrapure water blanks were prepared and analyzed following the described protocol for each method. All samples had a final volume of 500 μl and composition of 90:10 (v/v) water:deuterium oxide (H_2O:D_2O).

Stream water DOM for whole water analysis was analyzed directly without any further preparation. Aliquots (1 and 10 ml) of the thawed stream water DOM were transferred into precombusted scintillation vials and frozen. Frozen samples were lyophilized at −80°C at 200 mtorr until dry (36 hr). Aliquots of freeze-dried DOM were redissolved in 1-ml ultrapure water. The 1-ml aliquot represents the nonconcentrated freeze-dried DOM, and the 10-ml aliquot represents the concentrated 10:1 freeze-dried DOM. Due to limited amounts of sample, the comparison of whole water DOM to concentrated freeze-dried DOM was performed with aliquots of a sample from Quebrada Kathia, whereas the comparison of whole water DOM to nonconcentrated freeze-dried DOM was performed with aliquots of a sample from Quebrada Rosa.

To show that ^{1}H NMR analysis of whole water DOM using water suppression is semiquantitative, we performed a series of standard additions on the initial stream water. Acetate was observed in each stream analyzed so the standard additions were performed using calcium acetate hydrate, 99%, extra pure (Acros Organics). The volumes of sample, internal standard, and D_2O were kept constant, whereas the volumes of calcium acetate to

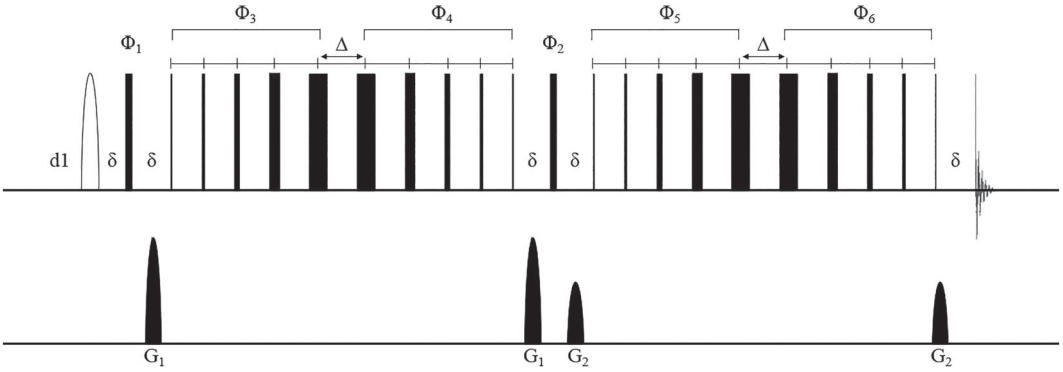

FIGURE 5 Depiction of the modified water-suppression pulse sequence, PEW5shapepr. The unfilled shape at the beginning of the sequence represents a soft pulse that has been modified from the original sequence. The filled rectangles represent hard pulses. G represents gradient pulses. Φ represents the following phase cycles: $\Phi_1 = x, -x$; $\Phi_2 = y$; $\Phi_3 = x, x, y, y, -x, -x, -y, -y$; $\Phi_4 = -x, -x, -y, -y, x, x, y, y$; $\Phi_5 = x, x, x, x, x, x, x, x, y, y, y, y, y, y, y, y, -x, -x, -x, -x, -x, -x, -x, -x, -y, -y, -y, -y, -y, -y, -y, -y$; $\Phi_6 = -x, -x, -x, -x, -x, -x, -x, -x, -y, -y, -y, -y, -y, -y, -y, -y, x, x, x, x, x, x, x, x, y, y, y, y, y, y, y, y$. Time delays are represented by δ and Δ. Full pulse sequence and values are detailed in the Supporting Information

ultrapure water were varied giving a final volume of 500 μl. Each standard addition spectrum was compared with the CH_2 peak at 1.24 ppm.

5.4 | ^{1}H NMR data acquisition and analysis

Solution state, ^{1}H NMR spectra were obtained on a 400-MHz Bruker AVANCE III equipped with a 5-mm broadband inverse probe housed in the COSMIC facility at Old Dominion University. All samples were analyzed at room temperature using a modified water-suppression pulse sequence (PEW5shaperpr), see Figure 5, with a 1.57 s acquisition time and a total of 7.22 s relaxation delay (d1 + 2 s delay built in for p20-shaped pulse). The appropriate value for d1 was chosen following measurements of spin-lattice relaxation times using the inversion recovery method. ^{1}H NMR spectra were acquired with 20,000 scans for whole water DOM and nonconcentrated freeze-dried DOM, 2,000 scans for the concentrated 10:1 freeze-dried DOM, and 600 scans for the standard additions. Spectra for the whole water process blank were acquired for 2,000 scans instead of 20,000. If any contaminant peaks were present in the whole water process blank, they would have been observed within the first 1,000 scans. The free-induction decay signal was digitized with 16 K of data and processed with 3 Hz of apodization. Duplicates of two separate stream water samples were analyzed yielding identical spectra and minimal error (standard error ± 0.79%) in the relative peak area of integrated regions. Spectra were processed and integrated using Bruker TopSpin software version 4.0.6. The integral curves for all spectra can be found in Figures S8 and S9.

To ensure that there was no sample degradation during the long analysis time (49 hr), a time lapse experiment was conducted on whole water DOM collected from Quebrada Rosa. Spectra were collected every 1,000 scans for a total of 20,000 scans over a 49 hr period using the same instrumental parameters described above. The 20 spectra were integrated across each region and showed little to no change over time; see Figure S10. These results indicate that the whole water DOM does not degrade during analysis at room temperature.

5.5 | Process blank assessment

Process blanks were prepared using ultrapure water following the same procedures as the stream water. ^{1}H NMR spectra of the whole water process blank revealed no contribution from compounds other than the internal standard (Figure S6). A few broad peaks were detected between 0.6 and 4.2 ppm at low intensity in the duplicate freeze-dried process blank indicating a low level of contamination obtained during the freeze-drying process; these peaks accounted for roughly 3% of the total signal acquired from the freeze-dried DOM (Figure S7). Peak area integrations of the freeze-dried DOM were blank subtracted to account for the signal contribution from the process blank.

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REFERENCES

[1] a) L. A. Kaplan, T. N. Wiegner, J. D. Newbold, P. H. Ostrom, H. Gandhi, *Freshwater Biol.* **2008**, *53*, 855. b) C. J. Volk, C. B. Volk, L. A. Kaplan, *Limnol. Oceanogr.* **1997**, *42*, 39.

[2] S. Kim, L. A. Kaplan, P. G. Hatcher, *Limnol. Oceanogr.* **2006**, *51*, 1054.

[3] a) K. Beck, J. Reuter, E. Perdue, *Geochim. Cosmochim. Acta* **1974**, *38*, 341. b) M. Berggren, H. Laudon, M. Haei, L. Ström, M. Jansson, *ISME J.* **2010**, *4*, 408. c) R. M. Cory, L. A. Kaplan, *Limnol. Oceanogr.* **2012**, *57*, 1347. d) T. Dittmar, A. Stubbins, in *Treatise on Geochemistry*, 2nd ed. (Eds.: B. Birrer, P. Falkowski, K. Freeman), Elsevier, Oxford, UK, **2014**, pp. 125-; e) S. W. Frazier, L. A. Kaplan, P. G. Hatcher, *Environ. Sci. Technol.* **2005**, *39*, 1479. f) P. G. Hatcher, K. J. Dria, S. Kim, S. W. Frazier, *Soil Sci.* **2001**, *166*, 770. g) J. I. Hedges, *Mar. Chem.* **1992**, *39*, 67. h) J. I. Hedges, G. Eglinton, P. G. Hatcher, D. L. Kirchman, C. Arnosti, S. Derenne, R. P. Evershed, I. Kögel-Knabner, J. W. de Leeuw, R. Littke, *Org. Geochem.* **2000**, *31*, 945. i) J. Leenheer, J. Croué, *Environ. Sci. Technol.* **2003**, *37*, 18A. j) W. H. McDowell, G. E. Likens, *Ecol. Monogr.* **1988**, *58*, 177. k) L. J. Tranvik, *Aquatic humic substances, Springer* **1998**, 259.

[4] a) L. I. Aluwihare, D. J. Repeta, R. F. Chen, *Nature* **1997**, *387*, 166. b) D. J. Repeta, T. M. Quan, L. I. Aluwihare, A. Accardi, *Cosmochim. Acta* **2002**, *66*, 955.

[5] A. J. Simpson, D. J. McNally, M. J. Simpson, *Prog. Nucl. Magn. Reson. Spectrosc.* **2011**, *58*, 97.

[6] a) L. I. Aluwihare, D. J. Repeta, *Mar. Ecol. Prog. Ser.* **1999**, *186*, 105. b) T. Grinhut, N. Hertkorn, P. Schmitt-Kopplin, Y. Hadar, Y. Chen, *Environ. Sci. Technol.* **2011**, *45*, 2748. c) N. Hertkorn, R. Benner, M. Frommberger, P. Schmitt-Kopplin, M. Witt, K. Kaiser, A. Kettrup, J. I. Hedges, *Geochim. Cosmochim. Acta* **2006**, *70*, 2990. d) N. Hertkorn, M. Harir, B. P. Koch, B. Michalke, P. Schmitt-Kopplin, *Biogeosciences* **2013**, *10*, 1583. e) A. Simpson, *Soil Sci.* **2001**, *166*, 795. f) E. Tagliavini, F. Moretti, S. Decesari, M. Facchini, S. Fuzzi, W. Maenhaut, *Atmos. Chem. Phys.* **2006**, *6*, 1003.

[7] a) H. A. Abdulla, E. C. Minor, R. F. Dias, P. G. Hatcher, *Cosmochim. Acta* **2010**, *74*, 3815. b) R. L. Sleighter, P. Caricasole, K. M. Richards, T. Hanson, P. G. Hatcher, *Chem. Biol. Technol. Agric.* **2015**, *2*, 9.

[8] A. Simpson, J. Burdon, C. Graham, M. Hayes, N. Spencer, W. Kingery, *Eur. J. Soil Sci.* **2001**, *52*, 495.

[9] R. Benner, J. D. Pakulski, M. McCarthy, J. I. Hedges, P. G. Hatcher, *Science* **1992**, 1561.

[10] a) T. Dittmar, B. Koch, N. Hertkorn, G. Kattner, *Oceanogr. Methods* **2008**, *6*, 230. b) S. Kim, A. J. Simpson, E. B. Kujawinski, M. A. Freitas, P. G. Hatcher, *Org. Geochem.* **2003**, *34*, 1325.

[11] J.-F. Koprivnjak, P. H. Pfromm, E. Ingall, T. A. Vetter, P. Schmitt-Kopplin, N. Hertkorn, M. Frommberger, H. Knicker, E. M. Perdue, *Cosmochim. Acta* **2009**, *73*, 4215.

[12] a) J. E. Kilduff, S. Mattaraj, A. Wigton, M. Kitis, T. Karanfil, *Water Res.* **2004**, *38*, 1026. b) B. Lam, A. J. Simpson, *Analyst* **2008**, *133*, 263. c) J. Peuravuori, *Environ. Sci. Technol.* **2005**, *39*, 5541.

[13] K. Mopper, A. Stubbins, J. D. Ritchie, H. M. Bialk, P. G. Hatcher, *Chem. Rev.* **2007**, *107*, 419.

[14] a) H. Masoom, A. Adamo, A. J. Simpson, *Environ. Chem.* **2016**, *13*, 767. b) A. J. Simpson, M. J. Simpson, R. Soong, *Anal. Chem.* **2018**, *90*, 629.

[15] a) A. S. Wozniak, R. U. Shelley, S. D. McElhenie, W. M. Landing, P. G. Hatcher, *Mar. Chem.* **2015**, *173*, 162. b) A. S. Wozniak, R. U. Shelley, R. L. Sleighter, H. A. Abdulla, P. L. Morton, W. M. Landing, P. G. Hatcher, *Mar. Chem.* **2013**, *154*, 24.

[16] a) B. Lam, A. Baer, M. Alaee, B. Lefebvre, A. Moser, A. Williams, A. J. Simpson, *Environ. Sci. Technol.* **2007**, *41*, 8240. b) J. Leenheer, G. Brown, P. MacCarthy, S. Cabaniss, *Environ. Sci. Technol.* **1998**, *32*, 2410.

[17] a) A. Willoughby, A. Wozniak, P. G. Hatcher, *Atmos. Chem. Phys.* **2014**, *14*, 10299. b) A. S. Willoughby, A. S. Wozniak, P. G. Hatcher, *Atmosphere* **2016**, *7*, 79.

[18] a) B. A. Cottrell, M. Gonsior, S. A. Timko, A. J. Simpson, W. J. Cooper, W. van der Veer, *Mar. Chem.* **2014**, *162*, 77. b) B. G. Pautler, G. C. Woods, A. Dubnick, A. J. Simpson, M. J. Sharp, S. J. Fitzsimons, M. J. Simpson, *Environmental Science & Technology* **2012**, *46*, 3753.

[19] J. S. Clemente, E. G. Gregorich, A. J. Simpson, R. Kumar, D. Courtier-Murias, M. J. Simpson, *Environ. Chem.* **2012**, *9*, 97.

[20] B. G. Pautler, A. J. Simpson, M. J. Simpson, L.-H. Tseng, M. Spraul, A. Dubnick, M. J. Sharp, S. J. Fitzsimons, *Environmental Science & Technology* **2011**, *45*, 4710.

[21] R. D. Majumdar, L. Bliumkin, D. Lane, R. Soong, M. Simpson, A. J. Simpson, *Water Res.* **2017**, *120*, 64.

[22] S. Decesari, M. C. Facchini, S. Fuzzi, E. Tagliavini, *J. Geophys. Res. Atmos.* **2000**, *105*, 1481.

[23] a) D. Sinnaeve, *Journal of Magnetic Resonance* **2014**, *245*, 24. b) M. M. Hoffmann, H. S. Sobstyl, V. A. Badali, *Magn. Reson. Chem.* **2009**, *47*, 593.

[24] a) W.-C. Chin, M. V. Orellana, P. Verdugo, *Nature* **1998**, *391*, 568. b) Y.-X. Ding, C.-C. Hung, P. H. Santschi, P. Verdugo, W.-C. Chin, *Atmospheric and Oceanic Sciences* **2009**, *20*, 7. c) P. Verdugo, *Annu. Rev. Mar. Sci.* **2012**, *4*, 375.

[25] a) C. A. Chateauvert, L. F. Lesack, M. L. Bothwell, *Journal of Geophysical Research: Biogeosciences* **2012**, *117*, G04013. b) I. De Vicente, E. Ortega-Retuerta, I. P. Mazuecos, M. L. Pace, J. J. Cole, I. Reche, *Aquatic sciences* **2010**, *72*, 443. c) K. Attermeyer, S. Andersson, N. Catalán, K. Einarsdottir, M. Groeneveld, A. J. Székely, L. J. Tranvik, *Limnol. Oceanogr.* **2019**, *0*.

[26] L. Sigg, R. Behra, in *Met. Ions Biol. Syst., Vol. 44* (Eds.: A. Siegel, H. Siegel, R. K. O. Siegel), Taylor & Francis Group, Boca Raton, FL, **2005**, p. 47.

[27] C. A. Fox, H. A. Abdulla, D. J. Burdige, J. P. Lewicki, T. Komada, *Front. Mar. Sci.* **2018**, *5*, 172.

[28] J. D. Newbold, B. W. Sweeney, J. K. Jackson, L. A. Kaplan, *J. N. Am. Benthol. Soc.* **1995**, *14*, 21.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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