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Structural characterization of Humic Materials Using ^{13}C Nuclear Magnetic Resonance Techniques: A Comparison of Solution and Solid-State Methods

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Abstract

The analysis of carbon type distribution and chemical structure of natural organic matter (NOM) by ^{13}C NMR spectroscopy is important for understanding its origins and reactivity. While prior work has used solution-state NMR, solid-state NMR has the potential to provide this information with less instrument time and sample processing, while providing an array of advanced filtering techniques. Quantitative and qualitative analyses with ^{13}C solid-state magic angle spinning (MAS) NMR techniques are described for three commercially available samples and one fulvic acid sample isolated from the Rio Grande in New Mexico. This study demonstrates the utility of solid-state ^{13}C NMR for aquatic NOM structural characterization by determining the % carbon type for three standard natural organic materials and comparing these results to the existing solution-state ^{13}C NMR determinations. The solid-state ^{13}C MAS NMR results are used to determine % carbon distribution, estimates of elemental composition (%C, %H, %(O+N)), aromatic fraction (fa), nonprotonated aromatic fraction (faN), an estimate of aromatic cluster size, and ratio of sp^2 to sp^3 carbons. Additionally, Gaussian deconvolution is used for a more detailed analysis of carbon type than frequency band integration techniques. The solid-state ^{13}C NMR results of these analyses indicate the chemical composition of Rio Grande fulvic acid has higher aromatic fraction and nonprotonated aromatic fraction (fa =0.32, faN = 0.21) and a lower sp^2/sp^3 fraction and cluster size (sp^2/sp^3 = 0.66, C = 6) compared to Suwannee River fulvic acid (fa = 0.18, faN = 0.10, sp^2/sp^3 = 0.82, C = 8).

Keywords: NOM; DOM; Fulvic; Aromaticity; Organic matter; ^{13}C MAS NMR

Abbreviations

NOM: Natural Organic Matter; NMR: Nuclear Magnetic Resonance; MAS NMR: Magic Angle Spinning Nuclear Magnetic Resonance; CP: cross polarization; DP: Direct Polarization; DD: Dipolar Dephasing; CSA: Chemical Shift Anisotropy; T1: Longitudinal Spin-Lattice Relaxation Time

Introduction

Natural organic matter (NOM) is a complex and variable mixture of organic molecules that is a ubiquitous component of natural waters. The chemical reactivity of NOM is important in a variety of water treatment issues, including membrane fouling, halocarbon formation, and bacterial growth [1-3]. The reactivity of NOM in the environment is affected by its chemical composition and structure. For example, both the amount of carboxylate functional groups and the fraction of carbon present in aromatic structures have been shown to affect NOM behavior in environmental systems [4-7].

Carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy is a powerful method that can be used to examine the chemical structure of NOM [8-10]. However, previously used solution-state ^{13}C NMR methods require long acquisition times and large amounts of sample, limiting the utility and availability of this potentially powerful method [11]. The intensity of the NMR signal is dependent on the amount of NOM that can be dissolved into approximately 1 mL of solvent [9] and typically range from 50 to 100 mg/mL [11]. Broad resonance lines, low signal to noise, and baseline distortion also make quantification of the solution-state NMR difficult [12]. In addition, while solution-state ^{13}C NMR is a very useful tool in determining the structure of NOM based on the characteristic isotropic chemical-shift ranges of the various carbon types, the chemical shift alone is not always enough to identify or quantify specific functional groups. For instance, aromatic and alkyl carbons both resonate in the range of 90-121 ppm. While filtering experiments based on spin-echo and polarization transfer techniques could be used to distinguish among overlapping carbon types [13], long acquisition times and baseline distortions have limited their use [9].

Solid-state ^{13}C magic angle spinning (MAS) NMR has the potential to overcome these limitations, and has become more widely available in recent years [8,9,14,15] because the relative concentration of carbon contained in the solid NMR samples is much higher than in solution NMR samples, higher signal to noise ratios and reduced experimental times can be obtained. Additionally, the signal acquisition time can be reduced further using cross polarization (CP) NMR techniques [8]. Finally, an echo technique in the direct-polarization (DP) NMR experiment results in spectra requiring limited baseline correction [16].

Due to shorter acquisition times in solid-state ^{13}C MAS NMR, it also becomes practical to use more complex pulse sequences, including spectral and relaxation-based filters to distinguish different carbon species. For example, nonprotonated carbons can be detected through dipolar dephasing (DD) experiments, while a chemical shift anisotropy (CSA) filter is used to distinguish overlapping bands of aromatics and aliphatics [17,18]. The solid-state ^{13}C MAS NMR methods can also provide an estimate of the elemental composition of these NOM materials in a non-destructive manner, and can provide parameters

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relating to the carbon skeletal structure, such as an estimate of average aromatic cluster size. These types of NMR techniques have been used extensively on other organic matter samples such as soils and coals [19-21], and the technique is currently used by only a few groups for NOM isolates from aquatic samples [9,22-24].

The objective of the present study was to demonstrate the utility of solid-state ^{13}C NMR for aquatic NOM structural characterization by determining the % carbon type for three standard natural organic materials and comparing them to the existing solution ^{13}C NMR results. The solid-state NMR also provides a determination of aromatic (f_a), aliphatic, and nonprotonated aromatic fractions (f_{aN}) of carbon, an estimate of carbon cluster size for aromatic carbon regions (*C*), and the determination of the sp^2/ sp^3 carbon ratio. These solid-state ^{13}C MAS NMR methods are then used to characterize a new NOM sample, a fulvic acid isolated from surface water of the Rio Grande River. The ability of solid state ^{13}C NMR to estimate the elemental composition {%C, %H, and %(O + N)} of aquatic NOM was also evaluated.

Materials and Methods

NOM sample descriptions

Four NOM materials were characterized by solid-state ^{13}C MAS NMR techniques. Three of these are standard samples obtained from the International Humic Substances Society (IHSS): 1) Suwannee River NOM (1R101N), 2) Suwannee River fulvic acid Standard I (1S101F), and 3) Leonardite Humic acid Standard (1S104H). Detailed characterizations of these three standards are available [25,26], including solution-state ^{13}C NMR analysis that was used for comparison in the current study.

The final NOM sample was a Rio Grande River fulvic acid that was collected and isolated for this study according to the methods of [27]. Briefly, the raw surface water (70 L) was collected at Socorro, New Mexico and filtered through a 0.20 μm pore size Whatman Polycap 150 TC filter in the field and stored on ice in acid-washed HDPE carboys. Within 24 hours of collection, the water sample was acidified to pH = 2.0 using ACS grade hydrochloric acid and passed through a glass and PTFE column (Spectrum Laboratories) containing 2 liters of DAX-8 (Supelco) resin. The hydrophobic organic acid fraction of the natural organic matter (referred to as fulvic acid throughout this paper) was then removed from the column using pH 13 NaOH and desalted using proton-saturated AG-MP50 cation exchange resin (BioRad). Once isolated, the sample was lyophilized for storage and analysis using both solution- and solid-state ^{13}C NMR methods.

^{13}C NMR techniques

Solution NMR spectra: The solution-state ^{13}C NMR spectrum of the Rio Grande fulvic acid was obtained on a Bruker DRX 400 instrument at a carbon frequency of 100.5 MHz using an inverse-gated pulse sequence, with 15,360 scan averages, for total experimental time of ~62 hours (limited by the available spectrometer time). A diagram describing the solution-state pulse sequence is presented in Figure 1a. Additional experimental details including NMR sample preparation are provided in the supplemental material.

Solid-state techniques and filtering: All of the solid-state ^{13}C magic angle spinning (MAS) NMR experiments were performed on a Bruker Avance 400 NMR spectrometer at a ^{13}C frequency of 100.6 MHz, using a 4 mm broadband MAS probe spinning at 15 kHz, unless otherwise noted. Recycle delays and the number of scans were chosen in order to maximize the signal to noise in the time available for experiments, ~60 hours/experiment. Diagrams describing all the solid-state pulse sequences used are presented in Figures 1b-e, and include direct polarization (DP), cross-polarization T_1-corrected (CP/T_1), dipolar dephasing (DD), and chemical shift anisotropy (CSA) filtered NMR experiments. Experimental details for each of these different sequences are provided in the supplemental material.

Spectral interpretation

T_1 Corrections and integration methods: The solid-state ^{13}C NMR DP spectra were corrected for T_1 effects utilizing the CP/ T_1 method (supplemental material). The multiplicative T_1 factor was determined from the CP/ T_1 experiment for a 10 ppm region (e.g. 10-20 ppm), and the amplitude of each point within the region in the DP spectrum was then multiplied by that factor. No attempts were made to obtain T_1 correction factors for the individual Gaussian lines in the deconvolutions.

Initially two different integration methods were used to calculate the carbon fraction in these humic materials from the DP or T_1-corrected DP solid-state ^{13}C MAS NMR experiments. In previous solution NMR work a computer integration (CI) method was employed. Each spectrum was segregated into specific frequency bands, numerically integrated over each band, and normalized by the integration of the entire NMR spectrum (e.g. 0–350 ppm) to determine the fraction of carbon present in each band. This CI technique was also used with the solid-state MAS NMR spectra both before and after correction for incomplete relaxation using the CP/T_1 correction factors (supplemental material). Although reasonable, this CI technique ignores the fact that the NMR spectrum results from the spectral overlap for many different carbon environments, whose relative intensity can vary during filtering experiments (see filtering section below). To incorporate different carbon speciation we used the software program DMFIT [28], to deconvolute each ^{13}C MAS NMR spectrum using ~20 Gaussian line shapes (fewer if the signal to noise did not warrant this treatment), summing the integrals of the Gaussians whose center frequencies fell within the frequency band of interest, and normalizing to total carbon signal. As an example, Figure 2 displays the deconvolution of the incompletely relaxed DP solid-state ^{13}C NMR spectrum of the Rio Grande fulvic acid. DMFIT uses a simplex least-squares fitting routine to determine the lines using the chemical shift, amplitude, and line width as parameters. Fewer than 20 lines were used to fit spectra if DMFIT returned negative amplitudes as the best fit result. The Gaussian fit more accurately represents the baseline in each region and allows improved speciation. For some applications, including using multiple filtering techniques, the Gaussian fit allows adjustment of the regions to clearly fit clusters of related resonances.

Quantification of aromaticity and nonprotonated carbon fraction : Several different useful parameters related to aromaticity were evaluated using the solid-state ^{13}C MAS NMR spectra. NMR is a direct and widely accepted method for measuring carbon fraction in NOM [5],[8,],[29]. However, most reported NMR estimates of the fraction of carbon in aromatic moieties (fa) have probably suffered from an interference with the signals of alkyl O–C–O, which overlap the aromatic carbon in the 90-121 ppm range, thus either including some alkyl groups or excluding some aromatic groups in the determination. Also, some NMR reports of fa determine the carbon fraction using 1H-13C cross-polarization (CP) NMR techniques which are known to be non-quantitative for aromatic structures [8,14,18]. In this work, we obtained the integral for the aromatic carbons from the T_1 corrected DP spectra in the ranges 90-165 ppm, and removed the alkyl percentage; we refer to this aromatic fraction as fa′. The alkyl percentage was calculated by taking the ratio of anomerics from

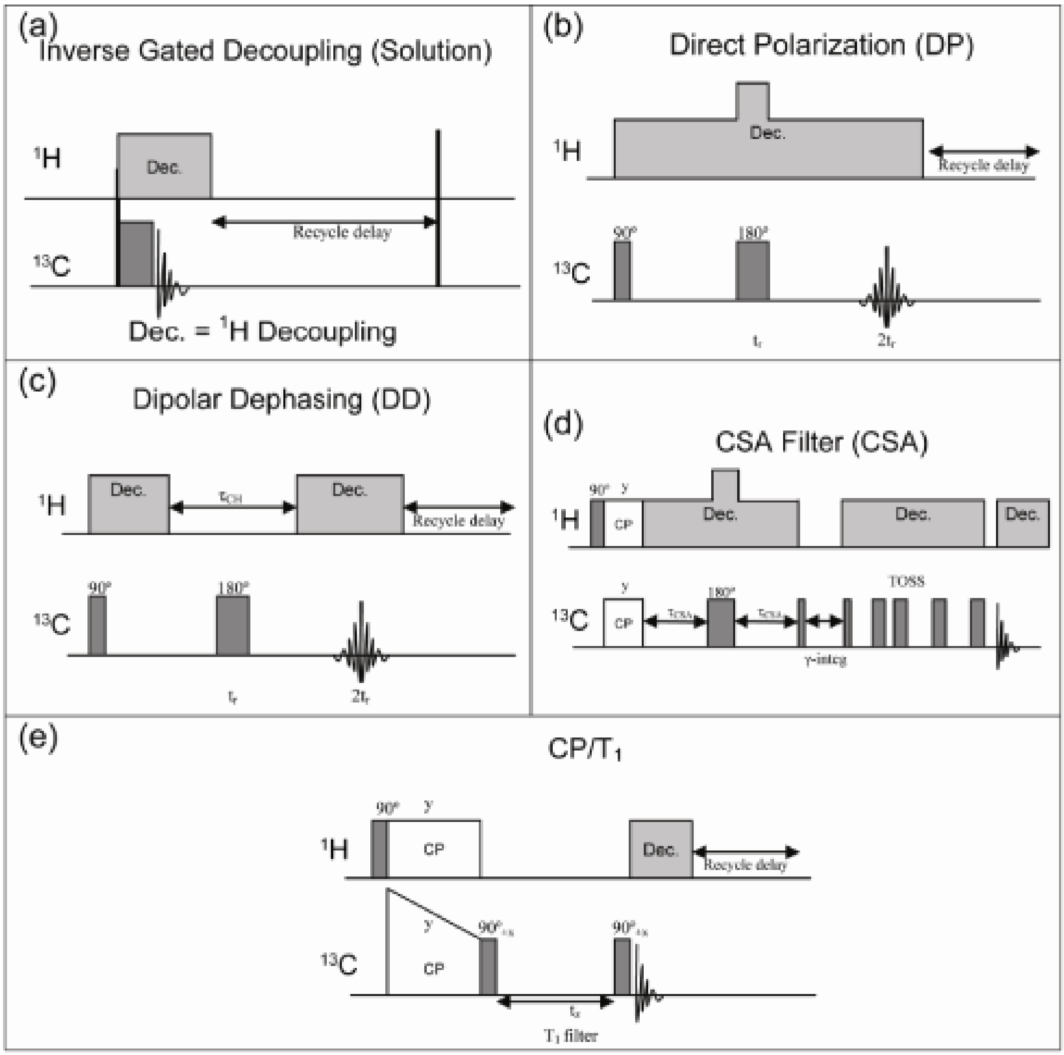

Figure 1: NMR pulse sequences used in this experiment. a) Inverse-gated decoupling, b) Direct Polarization (DP) Rotor Synchronized Hahn Echo, c) Dipolar Dephasing (DD) d) Chemical Shift Anisotropy (CSA) Filter, e) Cross Polarization T_1 determination (CP/ T_1). Details about NMR parameters can be found in the supplemental material.

90-121 ppm and the carbohydrates from 60-90 ppm from the CSA filtered spectrum and multiplying sp^3-hybridized O-C carbon (60-90 ppm) from the DP spectrum. We did not need to take into account the spinning sidebands because they were very small (< 1%) for the spinning speed and magnetic field strength used for data acquisition [18].

The fraction of nonprotonated aromatic carbon (faN) was also determined and is given in Table 3. The faN was calculated using integrals from the dipolar dephasing (DD) experiment, and the direct polarization experiment (DP), using the technique described by [18]. In essence, this experiment determines the fraction of nonprotonated carbon in the region 90-162 ppm by accounting for the nonprotonated alkyl carbon identified in the DD NMR experiment. Due to the difference in the number of scans taken for the DP and DD spectra, all integrations were normalized to the number of scans. The ratio of nonprotonated to protonated aromatic carbons, faN / fa, increases towards 1 with increasing size of aromatic clusters, and can be used to estimate the average size of aromatic clusters in the humic material (see Elemental Analysis).

Ratio of sp^2/sp^3-carbon environments: The ratio of aromatic to aliphatic (sp^2/ sp^3) carbon allows comparison of the experimental data to model compounds and allows classification of the NOM sample. The ratio was calculated using equation 1. This allows consistency with prior literature data although some sp^2 carbon environments identified through the filtering experiments are included in the sp3 carbon environment range.

$$\frac{sp^2}{sp^3} = \frac{DParea(108-220PPm)}{DParea(0-108PPm)} \qquad (1)$$

Unlike previous parameters describing humic materials [20], this ratio was determined nearly assumption free and can be used to validate various models for different types of natural organic material. It should be noted that the models for aquatic humic materials will likely be different than those for coals, soils, or plant extracted material.

Estimation of aromatic cluster size: Solum showed that it is possible to estimate the number of carbons in aromatic clusters by using the mole fraction of aromatic bridgehead carbons [21]. The

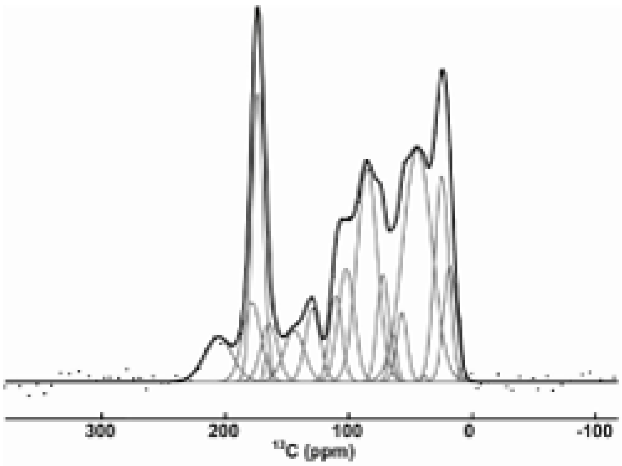

Figure 2: Deconvolution of the incompletely relaxed direct polarization (DP) solid-state ^{13}C MAS NMR spectrum of Rio Grande fulvic acid. This spectrum was decomposed into 19 Gaussian lines, shown in gray. The black line is the model spectrum while the dotted line is the experimental spectrum.

nonprotonated aromatic carbons (faN) can be subdivided into three groups or fractions, the phenolic or phenolic esters (142-162 ppm), alkylated aromatic carbon (135-142 ppm), and remaining bridgehead carbons, fb, (90-135 ppm). The mole fraction of aromatic bridgehead carbons, X_b, is calculated as the ratio of bridgehead carbons to the aromatic fraction corrected for alkyl carbons, fa′ and is given by the equation

$$x_b = \frac{f_b}{f_a^{'}}.$$

Using the relationship of X_b to the structure of polycondensed aromatic hydrocarbons, Solum arrived at an empirical function that relates X_b to the number of carbon atoms per aromatic cluster (C) shown here as equation 3,

$$X_b = \frac{1-\tanh\left(C-C_0\right)}{2}\left(\frac{1}{2}-\frac{3}{C}\right)+\frac{\left(1+\tanh\left(\frac{C-C_0}{m}\right)\right)}{2}\left(1-\sqrt{\tfrac{6}{C}}\right)$$

where C_0 and mare shifting and scaling parameters best fit by C_0=19.57 and m=4.15 [21].

Elemental analysis: Using the procedure outlined by [20], the elemental composition of each of the NOM samples was estimated from the T_1 corrected NMR spectral intensities. In this procedure, the percentage of spectrum found in each functional group was multiplied by the atomic weight for C, H and O+N and divided by average molecular mass of the functional groups in each chemical shift region (See Table 2 for elemental composition of functional groups). The O and N contents were calculated together with a weight of 16 because the O and N functional groups tend to overlap in the NMR spectra, the percentage of N is low in humic and fulvic acids, and the molecular weight of the two elements are close. We compared this data to that provided by IHSS for the Suwannee River NOM, Suwannee River fulvic acid, and Leonardite (Table 4).

Results and Discussion

Carbon fractions

The solution-state ^{13}C NMR spectra for the Rio Grande fulvic acid is shown in Figure 3.

For the IHSS samples (Leonardite humic acid, Suwannee River NOM, and Suwannee River fulvic acid), the solution ^{13}C NMR spectra have previously been reported and are available at the IHSS website [25]. Figure 4 shows the DP solid-state ^{13}C MAS NMR spectra for both the IHSS and Rio Grande fulvic acid samples. Figure 5 demonstrates the effect of the filtering sequences on the Rio Grande fulvic acid. From these NMR spectra, the various carbon fraction and ratios described above were determined.

Table 1 shows the % carbon fraction of the solid-state study compared to the % carbon values published by IHSS. The CI and Gaussian integration of the different spectral regions are also detailed in Table 1. For the Leonardite humic acid and Suwannee River NOM spectra which were fully relaxed (short spin lattice relaxation times, T_1 as determined by using the CP/ T_1 filter), comparison of the non- T_1-corrected CI and Gaussian % carbon fractions gave values within 6% of each other in all ranges for all samples. For the Leonardite humic acid and Suwannee River NOM, the Gaussian fractions determined from the solid state spectra were within 4% of the reported solution-state values except for the ketone/quinone region and the hetero-aliphatic region for the Leonardite humic acid and the aliphatic region in the Suwannee River NOM. The CI analysis of the solid-state NMR spectra for the ketone/quinone region was consistently lower than the solution ^{13}C results reported by Thorn [13,31]. In general, the ketone/quinone region was a broad, low intensity peak which was difficult to distinguish from the nonlinear baseline found in raw solution-state spectra. The echo sequence used in the solid-state ^{13}C MAS NMR removed the baseline roll typically encountered in solution-state spectra, so our conclusion was that the lower fraction determined in the solid-state NMR experiments was probably more accurate, in agreement with Thorn's conclusions [13]. Similarly, the fraction in the aliphatic region was greatly affected by the choice of baseline in solution-state spectra. The lower fractions determined in the solid-state spectra for the Suwannee River NOM, Suwannee River fulvic acid, and Rio Grande fulvic acid are probably more accurate using the echo sequence. The hetero-aliphatic region of the Leonardite sample was made up of a number of broad, low peaks that have significant area spreading into both the acetal aromatic and aliphatic regions. The Gaussian method considers the entire area of the peak to lie in the hetero-aliphatic region thus increasing that fraction.

The solid-state ^{13}C NMR of both of the Suwannee River and Rio

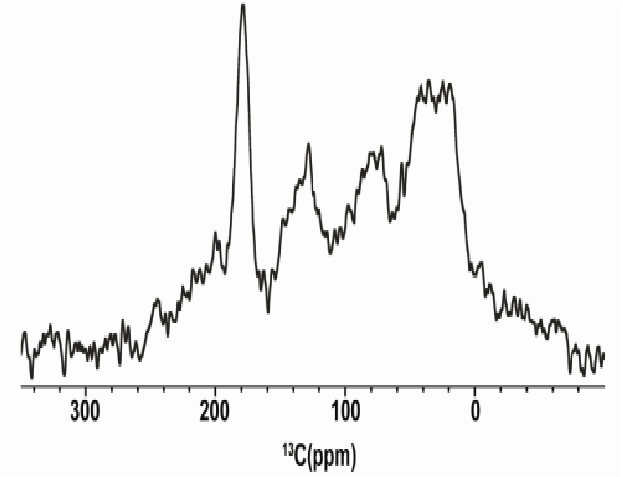

Figure 3: Quantitative ^{13}C solution-state NMR spectra of the Rio Grande fulvic acid, see Solution NMR Spectra for instrumental parameters.

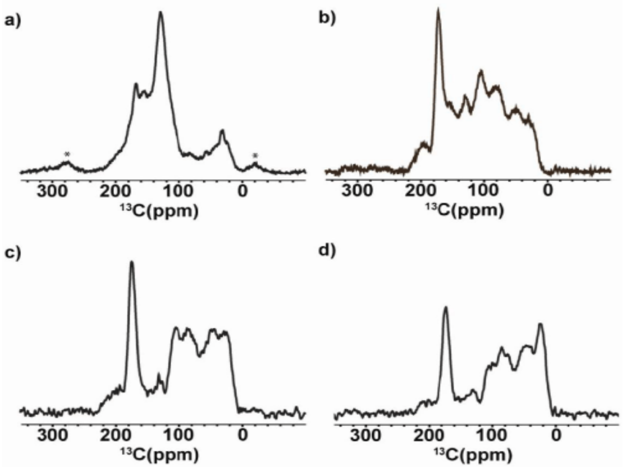

Figure 4: Quantitative direct polarization (DP) ^{13}C solid-state spectra of a) Leonardite, b) Suwannee River NOM, c) Suwannee River fulvic acid, and d) Rio Grande fulvic acid, see Solid-state techniques and filtering and Supplemental Material for instrumental parameters. Asterisks (*) denote spinning sidebands.

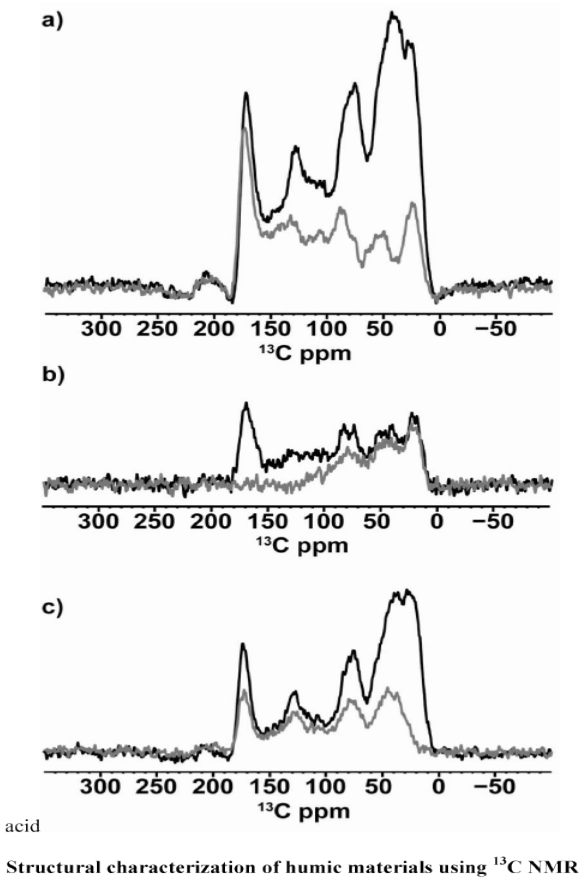

Figure 5: Filtered ^{13}C solid-state spectra of Rio Grande fulvic acid — a) dipolar dephasing (DD) τ_{CH} = 65 μs, b) chemical shift anisotropy (CSA) filter, c) cross polarization T_1 determination (CP/ T_1) t_z = 30 s. The gray traces in (a) and (c) are the filtered spectrum, and the black trace is unfiltered. In figure (b), the gray trace has a τ_{CSA} = 35 μs, while the black trace has a τ_{CSA} = 3 μs. See Supplemental Material and Figure 1 for additional instrumental parameters.

Grande fulvic acid samples were incompletely relaxed after the 30 s recycle delay, requiring a correction for T_1 effects prior to analysis (T_1 column in Table 1 using the CP/ T_1 filter). This was most notable in the carboxyl and aromatic regions. These carbon environments tended to have longer T_1s due to few effective relaxation mechanisms. The T_1 correction not only increased the carbon fraction in those regions, but also lowered the carbon fraction in others, especially the aliphatic region (Table 1). In general, the correspondence between the fractions determined from solid-state and solution-state were improved following this T_1 correction (Table 1).

Quantification of aromaticity and nonprotonated carbon fraction, estimate of aromatic cluster size, sp^2/ sp^3-carbon ratio.

Solid-state ^{13}C MAS NMR along with the DD and CSA filtering techniques were used to determine carbon fraction, and to provide important insights into the NOM chemical structure. The DD experiment (Figure 5a) removed protonated carbon from the spectrum. From the residual signal, it was apparent that there was a non-negligible proportion of complex aliphatic and quaternary carbon. The CSA filter (Figure 5b) suppressed aromatic carbon and specifically selected the alkyl-carbons, which helped identify aromatic carbons in the DP spectrum as described in Quantification of aromaticity and nonprotonated carbon fraction.

As noted above, the percent aromatic carbon has been identified as an indicator of the chemical behavior and structure in humic materials. For example, Traina demonstrated a relationship between this parameter and the UV-light absorbing properties of the NOM [31], Chin showed that aromaticity correlated to both UV-light absorbance and NOM molecular weight [5], A number of studies have shown that the aromatic fraction of NOM have a higher affinity for mineral surfaces [4,32]. Finally, photochemical decomposition of NOM has been shown to disproportionally affect aromatic NOM moieties [7]. Understanding the fraction of carbon contained in these aromatics is an important first step in understanding this material as well as a useful parameter for classifying samples.

The aromatic fraction removing alkyl carbons fa', the nonprotonated aromatic fraction faN, the mole fraction of aromatic bridgehead carbons X_b, and the number of carbon atoms per aromatic cluster C for each of the samples are shown in Table 3.

The fa' was compared with the aromatic fraction (fa) as determined by Thorn for the IHSS using the simple integration method (110-165 ppm) for the solution NMR results and the same method for the solid samples, fa″. For Leonardite and Suwannee River NOM the aromatic fractions fa and fa″ were equivalent, meaning solvent interactions were unimportant in comparing the aromatic fraction between solution and solid-state NMR. In determining fa', some of the alkyl carbon concentration was removed. Additionally the definition uses a slightly different range (90-165 ppm) rather than (110-165 ppm) for fa or fa″; therefore fa' included aromatic rings with two attached oxygen. The fa' and fa″ aromatic fractions were equivalent for Leonardite (fa' = 0.59 vs. fa″ = 0.58), but were significantly different for the Rio Grande fulvic acid (fa' = 0.32 vs. fa″ = 0.18). This result suggested that the Rio Grande fulvic acid had more aromatic singly bonded oxygen than the other IHSS samples. This assumption was also born out in the relatively high faN for the Rio Grande fulvic acid compared to the Suwannee River NOM and fulvic acid samples.

Although Solum used these structural characterization parameters, particularly the number of bridgehead carbons X_b in the determination of cluster size C in coal humic material, Mao used similar models for soil humic materials as well as plant extracted materials [33], and the method should also apply to aquatic organic material. For Rio Grande fulvic acid the calculated fb was less than zero, so we assumed $X_b=0$, and the typical carbon cluster size was six carbons in one aromatic ring.

Results for Rio Grande fulvic acid

According to our NMR analysis results, the Rio Grande fulvic acid showed a higher proportion of acetal aromatic carbons, had a higher aromatic fraction (fa′=0.32) and a higher nonprotonated aromatic fraction (faN = 0.21) than Suwannee River fulvic acid (fa′=0.18, faN = 0.10). These data also pointed towards more anomeric carbon and saccharide content in the Rio Grande as born out in the lower sp^2/ sp^3 ratio and smaller carbon cluster size ($sp^2/ sp^3 = 0.66$, C = 6 for Rio Grande fulvic acid and $sp^2/ sp^3 = 0.82$, C=8 for Suwannee River fulvic acid). The aromatic cluster size suggested that the Rio Grande fulvic acids were made up of aromatic rings with few to none bridging carbons linked by longer chain structures. The Rio Grande fulvic acid also contained less carboxylate carbon, relative to the Suwannee River samples.

It is important to note that most of the differences in chemical structure between the Rio Grande and Suwannee River samples noted above would not be evident without the use of the combination of solid-state NMR techniques described here. This observation highlights the utility of these methods for aquatic NOM samples. While the use of the CI approach with solution-state ^{13}C NMR analysis is most commonly employed for aquatic NOM samples, solid-state ^{13}C NMR is now available at most research universities. The time savings inherent in this type of NMR and the additional information that it allows the user to collect provides much useful insight into the chemical structure of NOM. We recommend the widespread adoption of solid-state ^{13}C NMR and the filtering and data analysis techniques described here by researchers investigating aquatic NOM samples.

There are few NMR data published for NOM samples from semi-arid lands, such as New Mexico. The Rio Grande fulvic acid does not closely resemble fulvic acids collected from southwestern waters described by Mash [34]. It has a much higher $sp^{2/}$ sp^3 C ratio than their southwestern lake samples, and significantly more carbon present in the 90-110 ppm range. However, the samples collected by Mash and coworkers were taken from impoundments and shown to contain significant carbon contributions from algal sources. At Socorro, New Mexico, the Rio Grande has drained a huge terrestrially-dominated watershed that includes both alpine ecosystems in northern New Mexico and southern Colorado and semiarid, lower elevation ecosystems in central New Mexico.

Elemental analysis

Table 2 shows a detailed assessment of the chemical composition data as determined from the T_1 corrected Gaussian fits of the solid-state spectra. The chemical shift ranges in this table were used to calculate the aromaticity as described in Spectral interpretation section.

Unlike traditional elemental analysis techniques that use combustion analysis, NMR is non-destructive. It has been argued that it can be used as a first estimation of elemental composition for carbon (%C), hydrogen (%H), and the combination of nitrogen and oxygen (%N+O) [20]. The results for both the solid-state 13C NMR analysis and the elemental analysis as determined by the IHSS [25] are shown in Table 4.

The solid-state NMR method tended to underestimate the %C by as much as 21% (relative), in comparison to the elemental analysis result, but reproduced the trends between samples. The solid-state NMR data also overestimated %H and %O+N, compared with the IHSS elemental analysis. Ignoring spinning sidebands in the MAS spectra of the aromatic type carbons would be expected to produce no more than 1% error. One explanation for this error could be a loss of NMR signal from carbon in close proximity to paramagnetic materials, such as iron not removed during the isolation procedure. However, additional errors in this analysis method occur for the low-ash, long T_1 Suwannee River Fulvic Acid sample due to incomplete relaxation (Table 4).

Carbon Type	Chemical Shift Range (ppm)	Leonardite Humic Acid			Suwannee River NOM			Suwannee River Fulvic Acid				Rio Grande Fulvic Acid			
		G†	CI‡	Solution§	G†	CI‡	Solution§	G†	CI‡	Solution§	T_1¶	G†	CI‡	Solution§	T_1¶
Aliphatic	0-60	13	13	14	21	18	27.3	31	31	33	27	40	37	36	26
Hetero-aliphatic	60-90	6	5	1	19	17	15.2	30	18	11	17	20	18	15	19
Acetal/aromatic	90-110	8	7	4	11	14	7.3	6	14	5	12	6	11	7	16
Aromatic	110-165	58	57	58	23	29	22.7	11	14	24	16	14	12	20	18
Carboxyl	165-190	12	14	15	20	17	19.9	16	19	20	24	16	17	14	18
Ketone/ quinine	190-220	3	4	8	6	4	7.6	6	4	7	5	4	4	7	2
All aromatic	90-165	66	63	61	34	43	30	16	28	28	28	20	23	27	34
Error#		3.6	2.7		3.4	5.7		9.6	6.4		5.8	3.9	4.2		6.4

Table 1: Carbon type composition of natural organic material determined by different methods.
† G = Gaussian fit of T_1 uncorrected solid-state spectra.
‡ CI = computer integration of area under curves for the solid-state spectra in regions given in the leftmost column.
§Solution = solution-state spectra integrated in the same manner as for CI as reported by the IHSS (http://www.humicsubstances.org/).
¶ T_1 = CI method applied to the T_1-corrected solid-state spectra.

$$^{\#}\,Error = \sqrt{\frac{1}{N}\sum(\mathrm{G\ (or\ CI)} - \mathrm{Solution})^2}$$

Chemical Shift Range (ppm)	Carbon Types	Functional Groups	Elemental Composition	Leonardite Humic Acid	Suwannee River NOM	Suwannee River Fulvic Acid	Rio Grande Fulvic Acid
0-25	Methyl	CH_3	CH_3	3.4	3.3	6	5.9
25-35	Methylene in simple aliphatics	CH_2	CH_2	3.8	3.9	5.9	5.4
35-50	Complex aliphatic	CH_2, CH, C	CH	3.9	6.6	9.8	9
50-60	Methoxy, methyne, quaternary	CH_3O-, CH-NH, CH, C	$CH_{1.5}O_{0.5}$	2	4.6	5.4	6.3
60-96	Saccharide, alcohol, ether	CHOH, CH_2OH,	$CH_{2.5}O$	6.1	20.9	20.1	24.2
96-108	Anomeric, aromatic carbon Neighboring phenolic carbons	O-CH-O, CH, CH_2-OH	CHO	4.3	8.7	7.9	9.3
108-121	Aromatic	CH	CH	9.8	7.2	5.1	5.1
121-145	Aromatic	CH, C	$CH_{0.5}$	32.1	12.5	5.8	8.6
145-162	Phenolic	C-O-, C-OH	$COH_{0.5}$	13.8	8.8	4.1	4.7
162-190	Carboxyl, ester, quinone	COO, COOH	$CO_{1.75}H_{0.5}$	16.3	19.3	25.5	19.2
190-220	Ketone, quinone, aldehyde	C=O, HC=O	$COH_{0.5}$	4	4.1	4.7	1.9

Table 2: Detailed assessment of chemical composition resulting from Gaussian fits after T_1 correction to the solid-state NMR spectra. † †Error is ±0.5.

	Solution	Solid State					
Sample	f_a†	f_a''‡	f_a' §	f_{aN}¶	sp^2/sp^3#	X_b††	C‡‡
Leonardite Humic Acid	0.58	0.58	0.59	0.26	1.37	0.036	7
Suwannee River NOM	0.23	0.23	0.29	0.19	1.08	0.208	10
Suwannee River Fulvic Acid	0.24	0.16	0.18	0.10	0.82	0.122	8
Rio Grande Fulvic Acid	0.20	0.18	0.32	0.21	0.66	0§§	6

Table 3: Analysis of aromaticity using ^{13}C NMR.
† f_a = aromatic fraction (110–165 ppm) of solution-state NMR spectra.
‡ f_a'' = aromatic fraction (110–165 ppm) of the T_1 corrected solid-state NMR spectra.
§ f_a' = aromatic fraction (90-165 ppm) removing overlapping alkyl carbons (90-121 ppm) of the T_1 corrected solid-state NMR spectra.
¶ f_{aN} = nonprotonated aromatic fraction (90-162 ppm) of the T_1 corrected solid-state spectra
# sX_b ɔ^3 = ratio of carbon environments of the T_1 corrected solid-state spectra using equation [1].
†† X_b = mole fraction of bridgehead carbons using equation [2].
‡‡C = estimate of X_b ɔon cluster size using equation [3].
§§ As calculated, X_b <0, the assumed number of bridgehead carbons was zero.

	NMR Analysis				IHSS Elemental Analysis			
	Leonardite	Suwannee River NOM	Suwannee River Fulvic Acid	Rio Grande Fulvic Acid	Leonardite	Suwannee River NOM	Suwannee River Fulvic Acid	Rio Grande Fulvic Acid
%C	54	47	45	47	64	52	53	NA
%(O+N)	42	49	50	48	33	44	45	NA
%H	3.9	4.7	4.9	5.4	3.7	4.2	4.4	NA

Table 4: Comparison of elemental composition calculated from NMR and chemical analysis from IHSS.

We conclude that the solid-state ^{13}C NMR methods are not able to provide a reasonable estimate of %C, %H, and %(O+N) concentration for aquatic samples unless used on low ash samples, correcting for spinning sidebands, and waiting for a well relaxed signal (less than half of the initial value).

Conclusions

- Solid-state ^{13}C NMR pulse sequences helped elucidate the structure of carbon in aquatic humic materials.
- By utilizing solid-state DD and CSA NMR filtering sequences, quantitative measures of carbon fraction, aromatic fraction, nonprotonated aromatic fraction, sp^2/sp^3 carbon environments, and aromatic cluster size were obtained.
- The solid-state NMR techniques were helpful in determining structure of the humic acids by filtering protonated carbon alkyl signals.
- Solid-state NMR techniques gave more reliable carbon fractions in the ketone/quinone (190-220 ppm) range through reduction of baseline distortions.
- Solid-state NMR techniques achieved higher signal-to-noise ratios than solution-state NMR spectra in the same amount of time.
- Solid-state NMR techniques eliminated a processing step and were non-destructive.
- Using a Gaussian line-fitting technique to determine the integrals related the carbon fraction more closely to the physical causes for the NMR spectrum.
- Using these NMR techniques, we characterized the Rio Grande fulvic acid for the first time.

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Supplemental Material

Details about the NMR parameters for solution- and solid-state pulse sequences are available in the supplemental material.

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