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Nuclear Magnetic Resonance Analysis of Changes in Dissolved Organic Matter Composition with Successive Layering on Clay Mineral Surfaces

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Abstract: Dissolved organic matter (DOM) chemistry and the potential for organic matter (OM) to self-associate with other OM components are important aspects of understanding the mechanisms of DOM sorption to clay surfaces. To investigate this further, we sorbed DOM isolated from peat humic acid onto either kaolinite, montmorillonite and gibbsite via ten sequential batch equilibration sorption experiments. Dissolved organic carbon (DOC) sorption to all minerals increased consistently, suggesting that sorption occurred via mineral-OM interactions at the beginning of the experiment. After six successive DOM loadings, the concentration of DOC sorbed by kaolinite and gibbsite began to plateau, likely due to the saturation of mineral surface sorption sites. Solution-state nuclear magnetic resonance (NMR) analysis of unbound DOM showed that kaolinite and montmorillonite sorbed aliphatic, protein and lignin components initially and primarily aliphatic and aromatic constituents in later sorption experiments, whereas gibbsite sorbed mostly aliphatic compounds during all DOM loadings. Analysis of the organo-clay complexes using ^{1}H high resolution–magic angle spinning (HR-MAS) NMR confirmed the preferential sorption of aromatic and aliphatic components to all three minerals. Overall, these results suggest that OM-OM interactions may be important mechanisms of DOM sorption to clay mineral surfaces.

Keywords: gibbsite; kaolinite; montmorillonite; organo-mineral interactions

1. Introduction

Soil organic matter (OM) is a critical component of the global carbon cycle [1], but the environmental factors contributing to soil OM stabilization and persistence are difficult to characterize because of its molecular complexity, variation with environmental controls such as climate and vegetation, and interactions with other soil components such as minerals and biota [2]. Among these interactions, organo-mineral associations, which occur via hydrophobic, van der Waals and electrostatic interactions, cation bridging and ligand exchange are believed to protect OM from degradation and contribute to the accumulation and preservation of soil OM [3–6]. Previous organo-mineral interaction studies have demonstrated that mineral properties such as specific surface area (SSA) and cation exchange capacity (CEC) may dictate the type of dissolved organic matter (DOM) components that are sorbed to mineral surfaces [7–11]. For example, iron and aluminum oxides were reported to sorb DOM components rich in phenol, carboxyl and hydroxyl groups through ligand exchange, while aliphatic components accumulated in solution [8,12,13]. Other studies have reported that aliphatic DOM components preferentially sorbed to kaolinite and montmorillonite, possibly through weak interactions

such as van der Waals forces and hydrogen bonding, while aromatic components were sorbed to a lesser extent and primarily by montmorillonite [14–16]. Although previous studies have provided molecular-level insight into sorption processes, most have only examined one DOM loading onto the mineral surface [8–10,12–16]. In soil, a mineral particle may be coated with several layers of OM to form organo-clay complexes [3,16,17], and the mineral sorption capacity for OM may decrease with successive OM loading as reactive surface functional groups become saturated with OM [18]. Currently, it is unclear whether mineral properties such as SSA and CEC continue to control DOM sorption with increasing OM layering [19–22], which may limit the amount of OM that can be sequestered by organo-mineral associations [23].

Several studies have demonstrated that OM-OM interactions may also stabilize and protect OM from degradation in soil [24–26]. For example, protein encapsulation in humic acid prevented hydrolysis of a proteins by strong acid [26], while coating lignin with dodecanoic acid protected the lignin from chemical degradation by $NaClO_2$ [24]. Similarly, Thevenot et al. [25] observed that lignin and aliphatic soil OM components such as plant and microbial lipids were associated in ligno-aliphatic complexes, which may slow lignin degradation in soil. In another study, the removal of *O*-alkyl soil OM components by acid hydrolysis increased the sorption capacity of several soils for three organic contaminants [27]. This suggested that high affinity aliphatic and aromatic sorption domains in soil are buried beneath a surface layer of more polar compounds such as carbohydrates and proteins. Furthermore, the analysis of organo-clay complexes and whole soils using nuclear magnetic resonance (NMR) spectroscopy showed that aromatic signals were not visible when the samples were swollen in water (D_2O) [15,28,29]. This indicated that aromatic components such as lignin were not in contact with the NMR solvent and thus were not present at the solid-aqueous interface. However, aromatic signals were observed when the samples were swollen in DMSO-d_6, a more penetrating solvent that disrupts hydrophobic and hydrogen bonding and can probe OM components beneath the aqueous interface and surface layer of OM. These previous studies provided further evidence that aromatic components are buried deeper within the soil OM microstructure and may be partially protected from degradation by more polar compounds that are present at the soil–aqueous interface [15,28,29].

Kleber et al. [3] proposed a conceptual model of organo-mineral interactions in which OM may be arranged into three zones on a mineral surface. The zones are differentiated based on the proximity of the OM components to the surface and the types of sorption interactions occurring between the components. The contact zone is composed of protein and amphiphilic molecules that are sorbed directly to the mineral surface via several mechanisms including ligand exchange, electrostatic interactions or hydrophobic bonding [30,31]. These amphiphilic compounds may increase the number of reactive functional groups on the mineral surface, known as preconditioning [32], which may facilitate the sorption of additional DOM from solution [3]. The hydrophobic zone consists of a bilayer of amphiphilic molecules such as fatty acids, which forms above the contact zone [33]. Polar functional groups of these amphiphilic components may interact with bound OM, whereas the non-polar domains may associate via hydrophobic or van der Waals interactions [30]. The outermost kinetic zone consists of polar compounds which are weakly bound to the organo-clay complex through interactions such as cation bridging or hydrogen bonding, and these components may readily exchange with the dissolved phase. The zonal model provides detailed insight into the possible structural arrangement of OM on clay surfaces [3]. However, the type of molecular-level OM-OM interactions that occur and the extent to which they may control DOM sorption to organo-clay complexes when reactive functional groups on mineral surfaces are saturated with OM is still unclear. For example, sorbed aliphatic OM components may interact with aliphatic compounds in solution through non-polar associations between polymethylene chains [34] or polar interactions between charged functional groups [35]. These favourable OM-OM interactions may facilitate the sorption and layering of additional OM on the organo-clay complex [3,16].

The objective of this study is to understand how OM-OM interactions may control both the quantity and composition of DOM that sorbs to organo-clay complexes. To investigate this,

we examined sorptive interactions between DOM derived from peat humic acid and three clay minerals (kaolinite, montmorillonite and gibbsite) with contrasting SSA and CEC [18,36]. Organo-clay complexes were constructed through the repeated loading of DOM onto the minerals over ten sequential 72 h batch equilibration sorption experiments. After each DOM loading, the concentration of dissolved organic carbon (DOC) sorbed to each organo-clay complex was determined. In addition, solution-state ^{1}H NMR was used to compare the composition of the DOM solution before sorption and the unbound DOM following sorption to minerals. Furthermore, components present in the outermost layers of OM sorbed to the organo-clay complexes were characterized at selected intervals using high resolution-magic angle spinning (HR-MAS) NMR. This NMR technique permits the analysis of OM components in solid, solution and gel phases of a sample using a single NMR probe, and has been used previously to study soil OM chemistry at interfaces [14,15,28,37]. We hypothesize that the quantity and composition of OM sorbed to the initial uncoated clay mineral surfaces will be controlled primarily by mineral properties such as SSA and CEC. We further hypothesize that as the clay mineral surfaces become coated in more layers of OM, OM-OM interactions will begin to dictate the composition and quantity of OM that sorbs to the organo-clay complexes.

2. Materials and Methods

2.1. Clay Mineral and DOM Samples

Kaolinite (KGa-1b) and montmorillonite (STx-1b) were purchased from the Clay Minerals Society's Source Clays Repository (Chantilly, VA, USA), while gibbsite (synthetic) was obtained from Ward's Science (VWR, Radnor, PA, USA). The N_2 Brunauer-Emmett-Teller (BET) SSA and CEC values of the minerals are listed in Table 1. SSA and CEC data for the kaolinite and montmorillonite source clays have been reported previously [36]. The N_2 BET SSA of gibbsite was measured at 77 K using a TriStar 3000 gas adsorption analyzer (Micromeritics, Norcross, GA, USA), while the CEC of gibbsite was measured using a Ba^{2+} displacement method [38]. Prior to sorption experiments, clay samples were mixed with 0.01 M $Ca(NO_3)_2$ (Fisher Scientific, Waltham, MA, USA) for one hour on a reciprocal shaker (model 6010, Eberbach Corp., Ann Arbor, MI, USA) to replace exchangeable cations with Ca^{2+}. The samples were then centrifuged at 2700 *g* (Sorvall model RC-5B, DuPont Instruments, Wilmington, DE, USA), the supernatants were decanted and the procedure was repeated twice more, after which the clays were rinsed with deionized water to remove excess salts and then freeze-dried.

Pahokee Peat soil was purchased from the International Humic Substances Society (St. Paul, MN, USA) and peat humic acid was isolated as described previously [15,39]. The peat humic acid was re-dissolved in 0.01 M $Ca(NO_3)_2$ (Fisher Scientific, Pittsburgh, PA, USA), the pH was adjusted to 10 with 0.1 M NaOH (Fisher Scientific) and the solution was stirred for one hour. The solution pH was then re-adjusted to pH = 7 with 0.1 M HNO_3 (BDH, Radnor, PA, USA) and stirred for 1 h, after which the mixture was filtered through a Durapore 0.22 µm polyvinylidene fluoride membrane filter (EMD Millipore, Billerica, MA, USA). The filtrate was then freeze-dried to isolate the neutral pH-soluble fraction of the peat humic acid, which was used for DOM sorption experiments.

Table 1. N_2 Brunauer–Emmett–Teller (BET) specific surface area (SSA) and cation exchange capacity (CEC) values of the clay mineral samples.

Mineral	Specific Surface Area (m^2/g)	Cation Exchange Capacity ($cmol_c$/kg)
Kaolinite [1]	10.05	2.0
Montmorillonite [1]	83.79	84.4
Gibbsite [2]	1.41	0.1

[1] Data from Van Olphen and Fripiat [36]. [2] Values were measured in this study.

2.2. Preparation of Organo-Mineral Complexes

A DOM solution was prepared by reconstituting 150 mg of the freeze-dried peat humic acid in 0.01 M $Ca(NO_3)_2$ (Fisher Scientific), resulting in a solution containing 35 mg/L of DOC. The three clay minerals (150 mg ± 0.01 mg) were separately weighed into 50 mL polytetrafluoroethylene centrifuge tubes, 40 mL of the DOM solution was added, and the mixtures were shaken in the dark for 72 h on a reciprocal shaker (Eberbach). Preliminary sorption experiments determined that sorptive equilibrium was reached before this time, and no DOC degradation or DOC sorption to the vessel walls was detected during the equilibration period (data not shown). After shaking, the mixtures were centrifuged at 600× *g* (model HN-S, International Equipment Company, Needham Heights, MA, USA) and the supernatant was carefully withdrawn using a Pasteur pipette. Successive DOM sorption experiments were conducted by adding fresh DOM solution (40 mL ± 0.1 mL; 35 mg/L DOC) to the organo-clay complexes and repeating the batch equilibration sorption procedure ten times for each clay mineral. All sorption experiments were conducted at room temperature (21 °C).

After each DOM loading, an aliquot of the supernatant which contained unbound DOM was analyzed in triplicate using a total organic carbon analyzer (model TOC-VCSH, Shimadzu Corp., Kyoto, Japan) to quantify DOC (measured as non-purgeable organic carbon). DOC concentrations in the initial DOM solution and the solutions containing unbound DOM after sorptive fractionation were compared to calculate the percentage and amount of DOC sorbed by the minerals or organo-clay complexes during each sequential DOM loading experiment. In preparation for solution-state ^{1}H NMR analysis, an aliquot (35 mL) of the initial DOM solution and the remaining unbound DOM after each sorption step were passed through an ion exchange resin (Amberlite IRA-200C, Alfa Aesar, Ward Hill, MA, USA) to remove salts, freeze-dried and stored under vacuum with P_2O_5 (Sigma-Aldrich, St. Louis, MO, USA) to remove residual water. In preparation for HR-MAS NMR analysis, a kaolinite, montmorillonite and gibbsite organo-clay complex was isolated after each DOM loading, rinsed with deionized water to remove salts and unbound DOM, freeze-dried and stored under vacuum with P_2O_5 (Sigma-Aldrich, St. Louis, MO, USA) to remove residual water.

2.3. NMR Spectroscopy Experiments

^{1}H NMR experiments are sensitive enough to detect small changes in DOM composition [10]. The freeze-dried DOM samples were re-constituted in 50 µL of dimethyl sulfoxide(DMSO)-d_6 (99.9% D, Cambridge Isotope Laboratories, Tewksbury, MA, USA) and transferred to a 1.7 mm NMR tube. Solution-state ^{1}H NMR spectra were recorded using a Bruker BioSpin Avance III 500 MHz NMR spectrometer equipped with a 1.7 mm ^{1}H-^{13}C-^{15}N microprobe fitted with an actively shielded z-gradient operating at 298 K and using TopSpin version 3.1 software (Bruker BioSpin, Rheinstetten, Germany). One-dimensional ^{1}H NMR experiments were performed using 1K scans, a recycle delay of 5 × T_1 and 16K time domain points. DOM samples were further characterized using diffusion-edited (DE) ^{1}H NMR spectroscopy. This technique attenuates signals from small molecules which diffuse quickly in solution, providing insight into the sorption behavior of more rigid, macromolecular DOM components and aggregates in the presence of the three clay minerals [40]. DE ^{1}H NMR experiments were performed using a bipolar pulse longitudinal encode-decode sequence [41] with 4K scans, 16K time domain points, a diffusion time of 200 ms, a recycle delay of 2 s and 2.5 ms, 53.5 gauss/cm gradient pulses. The spectra were processed using a zero-filling factor of two and the spectra were apodized by multiplication with exponential functions corresponding to 1 Hz and 10 Hz line broadening in the transformed spectrum, respectively for ^{1}H and DE ^{1}H NMR spectra. The ^{1}H and DE ^{1}H NMR spectra were integrated into seven regions using AMIX software (version 3.9.15, Bruker BioSpin) based on previously published spectral assignments for protons in specific chemical environments [40,42–44]: aliphatic polymethylene and methyl groups (0.6–1.3 ppm); *N*- and *O*-substituted aliphatic (1.3–2.9 ppm); *O*-alkyl (2.9–4.1 ppm); α-proton of peptides (4.1–4.8 ppm); anomeric proton of carbohydrates (4.8–5.2 ppm); aromatic and phenolic (6.2–7.8 ppm); and amide (7.8–8.4 ppm). The ^{1}H NMR signal within each region was expressed as a proportion of the total

^{1}H signal across the seven regions [45], and the percent change in NMR signal of each type of OM component between the initial DOM and the unbound DOM samples after sorption to minerals was calculated.

Organo-clay complexes after one, five and 10 DOM loadings were analyzed using HR-MAS NMR to follow progressive changes in organo-clay surface chemistry with repeated DOM layering. Clay samples were also analyzed using ^{1}H HR-MAS NMR in a preliminary experiment before sorption to test for any OM that may interfere with detecting clay-sorbed DOM. Freeze-dried organo-clay complex (30 mg) was placed in a 4 mm NMR rotor and swollen with 60 µL DMSO-d_6 (99.9% D, Cambridge Isotope Laboratories). DMSO-d_6 can disrupt hydrophobic and hydrogen bonding and penetrate deeper into the OM microstructure, providing insight into the structure of OM components beneath the outermost layer of OM [14,15]. The rotor was fitted with a Kel-F insert and sealed with a Kel-F cap (Bruker BioSpin). ^{1}H NMR spectra were recorded using a Bruker BioSpin Avance III 500 MHz NMR spectrometer equipped with a 4 mm ^{1}H-^{13}C-^{2}H comprehensive multiphase (CMP) NMR probe fitted with an actively shielded magic-angle gradient [46]. All HR-MAS NMR experiments were conducted at a rotor spinning speed of 6666 Hz with presaturation water suppression. ^{1}H NMR spectra were collected using the Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence [47,48] with 512 scans, a recycle delay of 5 $\times$ T_1 and 8K time domain points. The spectra were processed using a zero-filling factor of two and the ^{1}H NMR spectra were apodized by multiplication with exponential functions corresponding to 2 Hz and 10 Hz line broadening in the transformed spectrum, respectively. In addition, we attempted to collect solid-state ^{13}C NMR spectra of the organo-clay complexes using the CMP NMR probe [46,47], but we were unable to achieve adequate signal-to-noise due to the low organic carbon content of the organo-clay complexes (data not shown).

3. Results

3.1. Dissolved Organic Matter Sorption to Clay Minerals

Figure 1 shows the cumulative concentration of DOC sorbed by each mineral (in milligrams of DOC per gram of clay) during each successive DOM loading experiment. Montmorillonite sorbed a greater concentration of DOC than kaolinite and gibbsite during all ten loading steps. This is consistent with the higher SSA of montmorillonite compared to the other two minerals (Table 1), and with previous studies which suggested that DOC sorption to minerals increases with increasing mineral SSA [18,21,22,48]. Kaolinite and gibbsite initially sorbed a similar concentration of DOC, but from loading five onward gibbsite sorbed a higher concentration of DOC than kaolinite. Given that the SSA for gibbsite is lower than kaolinite (Table 1), this observation suggests that mineral–DOM interactions are not limited by SSA alone. The cumulative concentration of DOC sorbed by each mineral increased consistently over the first five DOM loadings. During these initial loadings, DOM likely sorbed to the organo-clay complexes through primarily direct mineral–OM interactions [5,6]. Although the concentration of DOC sorbed continued to increase with progressive DOM loading, less DOC sorbed to the organo-clay complexes during the last four sorption steps compared to the initial and intermediate loadings, especially for kaolinite and gibbsite (Figure 1). This trend implies that sorption sites on kaolinite and gibbsite surfaces may have approached saturation after several successive DOM loadings. As a result of this mineral saturation, the DOM sorbed to the organo-clay complexes during the later loadings may have sorbed via a combination of mineral–OM and OM–OM interactions [3,24,25], rather than just direct mineral-OM associations as likely occurred in the initial loadings. These OM-OM interactions may be an important factor governing the quantity of DOC sorbed by minerals with low SSA since these mineral surfaces require less DOC to become saturated than minerals with high SSA, such as montmorillonite. Furthermore, it is important to note that we did not explicitly investigate the desorption of DOC from the organo-clay complexes during the DOM loading experiments. However, the DOC measurements before and after sorption showed a net

increase in DOC concentration with each sorption step (Figure 1), suggesting that the amount of DOC sorbed was greater than any potential DOC that desorbed from the organo-clay complexes.

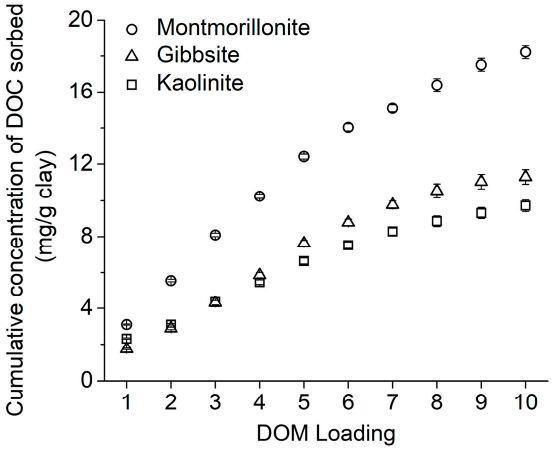

Figure 1. The cumulative concentration of dissolved organic carbon (DOC) sorbed by kaolinite, montmorillonite and gibbsite organo-clay complexes during ten successive dissolved organic matter (DOM) loading experiments. Error bars represent standard error (n = 3).

3.2. NMR Characterization of Unsorbed DOM and Organo-Clay Complexes

The initial DOM solution and the unbound DOM samples following sorption to minerals contained several types of OM components that have been identified previously in DOM using solution-state NMR spectroscopy [15]. Representative ^{1}H and DE ^{1}H NMR spectra of the initial DOM solution (before sorption) with specific OM components labelled are shown in Figure 2a,b, respectively. In addition, Supplementary Materials Figures S1–S3 and S4–S6 show the ^{1}H and DE ^{1}H NMR spectra, respectively, of the thirty unbound DOM samples following sorption to kaolinite, montmorillonite or gibbsite. Signals in the aliphatic region include protons of CH_2 groups in DOM constituents with polymethylene chains, such as cutin, suberin, lipids and waxes (1.25 ppm), while protons of CH_3 groups in aliphatic lipids, lignin and proteins (0.85 ppm) also resonate in this region [40,42]. *N*- and *O*-substituted aliphatic resonances arise from protons in CH_2 groups near polar functional groups, such as those β or γ to carboxylic acid groups in lipids or in amino acid sidechains [45]. Signals within this region also contain contributions from protons of N-acetyl groups in biopolymers such as peptidoglycan and chitin as well as protons in microbial-derived, short-chain carboxylic acids which are products of OM degradation [44]. The *O*-alkyl region contains signals from protons in DOM constituents such as carbohydrates, with lesser contributions from protons in proteins and methoxy groups of lignin. Signals from anomeric protons in carbohydrates were also observed. Distinct signals for protein-derived DOM components include the α-proton and amide proton resonances [42]. Aromatic and phenolic resonances originate from protons in aromatic amino acids such as phenylalanine and tyrosine as well as from lignin-derived compounds [43,45].

To complement the solution-state NMR analysis of DOM samples, ^{1}H HR-MAS NMR was used to characterize components present in the outermost layers of OM sorbed to the organo-clay complexes, which are mobile and in contact with the two different NMR solvents [29]. Figure 2c shows a representative ^{1}H HR-MAS NMR spectrum of a montmorillonite organo-clay complex after five DOM loadings (swollen in DMSO-d_6) in which specific OM components that are sorbed to the clay mineral are labelled. The most prominent signals in the ^{1}H HR-MAS NMR spectra were the aliphatic CH_2 (1.25 ppm) and CH_3 (0.85 ppm) groups as well as lower intensity aromatic resonances (6–9 ppm). The HR-MAS NMR spectra of the organo-clay complexes swollen in DMSO-d_6 also contained a residual water resonance (3.3 ppm) which partly obscured the *O*-alkyl and α-proton regions and prevented

an assessment of their sorption behaviour. This water is likely bound to the clays and was not removed by freeze-drying and vacuum desiccation.

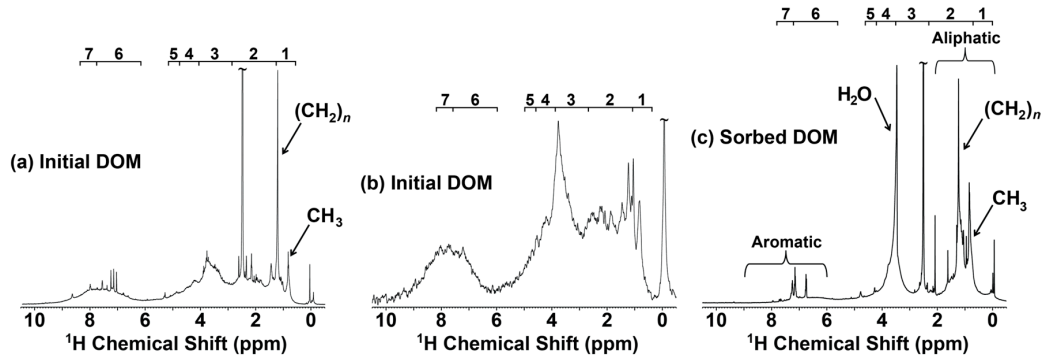

Figure 2. Representative solution-state (**a**) ^{1}H and (**b**) diffusion-edited ^{1}H nuclear magnetic resonance (NMR) spectra of the initial dissolved organic matter (DOM) which was used in the successive DOM sorption experiments. (**c**) Representative ^{1}H high resolution-magic angle spinning (HR-MAS) NMR spectrum of DOM sorbed to a montmorillonite organo-clay complex with specific OM components labelled. Braces indicate the ^{1}H chemical shift ranges of DOM components in the following seven chemical environments: (1) aliphatic polymethylene and methyl groups (0.6–1.3 ppm); (2) *N*- and *O*-substituted aliphatic (1.3–2.9 ppm); (3) *O*-alkyl (2.9–4.1 ppm); (4) α-proton of peptides (4.1–4.8 ppm); (5) anomeric proton of carbohydrates (4.8–5.2 ppm); (6) aromatic and phenolic (6.2–7.8 ppm); and (7) amide (7.8–8.4 ppm). The peak at 2.50 ppm arises from the solvent (DMSO-d_6).

Supplementary Materials Figures S7 and S8 show the proportion of ^{1}H NMR signal arising from the seven major types of OM components that were identified in the ^{1}H and DE ^{1}H NMR spectra, respectively, of the initial DOM and the thirty unbound DOM samples following sorption to kaolinite, montmorillonite or gibbsite. To better visualize compositional differences between the DOM before sorption and the unbound DOM components after batch equilibration, the percent change in the NMR signal area for each of the seven components following each DOM loading are shown in Figure 3 and Supplementary Materials Figure S9 for the ^{1}H and DE ^{1}H NMR spectra, respectively. A positive percent change indicates sorption of a DOM component to the organo-clay complex, whereas a negative percent change indicates that a specific component accumulated in solution and was not sorbed (e.g., unbound). To provide further evidence of DOM sorption onto clay minerals, Figure 4 shows the ^{1}H HR-MAS NMR spectra of OM components sorbed to the kaolinite, montmorillonite and gibbsite organo-clay complexes (swollen in DMSO-d_6) after one, five and 10 DOM loadings. The HR-MAS NMR signal intensity of the sorbed OM increased between loadings one and five, in agreement with the DOC results in which the concentration of DOC sorbed increased consistently during the first five DOM loadings (Figure 1). From loadings five to 10, the HR-MAS NMR signal intensity increased marginally for gibbsite and small increases were observed for kaolinite and montmorillonite (Figure 4). This is consistent with the sorbed concentration DOC data where a lower concentration of DOC was found to sorb to the organo-clay complexes during the higher loadings compared to the initial loadings. Therefore, the HR-MAS NMR results further supports that the clay mineral surfaces became saturated with DOC during the later sequential DOM loading steps and their capacity to sorb more carbon was reduced.

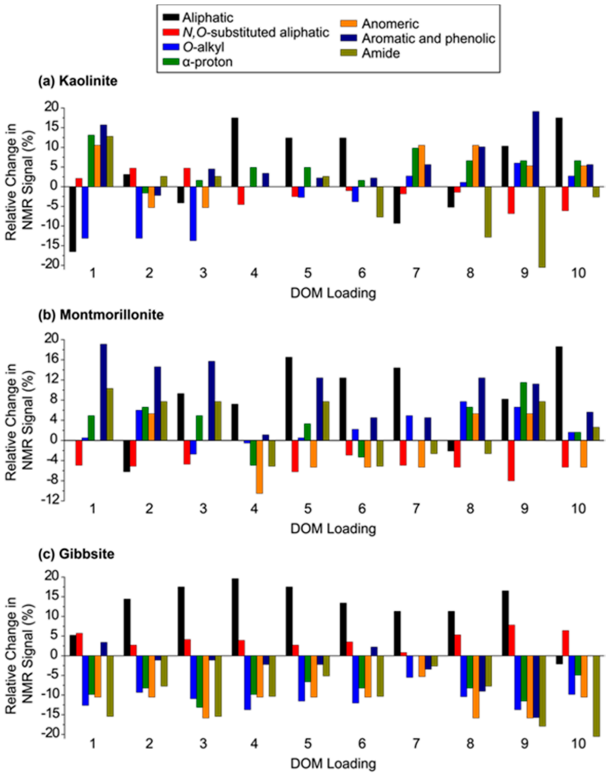

Figure 3. Percent change in integrated solution-state ^{1}H nuclear magnetic resonance (NMR) signal area of seven classes of dissolved organic matter (DOM) components between the initial DOM and unbound DOM samples following sorptive fractionation by (**a**) kaolinite, (**b**) montmorillonite and (**c**) gibbsite over ten successive DOM loading experiments. A positive percent change indicates sorption of a component to the organo-clay complex, whereas a negative percent change indicates that a component accumulated in solution and was not sorbed.

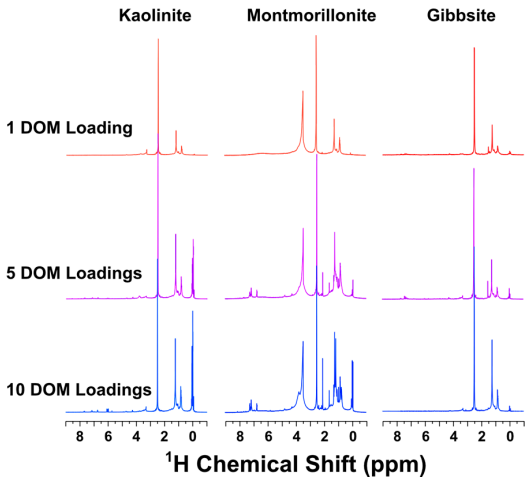

Figure 4. ^{1}H high resolution-magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectra of dissolved organic matter (DOM) sorbed to kaolinite, montmorillonite and gibbsite organo-clay complexes after one, five and 10 successive DOM loading experiments. The compounds visible are bound to the organo-clay complex but are mobile and in contact with the NMR solvent. The peak at 2.50 ppm arises from the solvent (DMSO-d_6).

3.3. Sorption of Specific DOM Components to Kaolinite

The ^{1}H NMR integration results showed that kaolinite sorbed a mixture of *N*- and *O*-substituted aliphatic, protein and aromatic components during the first DOM loading (Figure 3a). The DE ^{1}H NMR results revealed that kaolinite initially sorbed proteins as well as other macromolecular aliphatic DOM constituents such as waxes, cutin and suberin (Supplementary Materials Figure S9a). HR-MAS NMR analysis of the initial kaolinite organo-clay complex showed resonances for CH_2 and CH_3 groups, further indicating the sorption of aliphatic components or proteins (Figure 4). These results agree with previous studies that have observed sorption of aliphatic- and protein-derived DOM components to uncoated kaolinite surfaces [7,14,15,49,50]. The initial sorption of proteins to kaolinite is also consistent with the proposed zonal model of organo-mineral interactions [3]. In the contact zone of this model, proteins are proposed to sorb to the surface of phyllosilicate minerals through polar interactions, including electrostatic, cation bridging or ligand exchange associations, as well as more non-polar interactions such as hydrophobic and van der Waals bonding [51,52]. In addition, Masoom et al. [28] reported that protein components of soil OM were not visible when a soil was swollen in D_2O, but could be observed using DMSO-d_6, a solvent which disrupts hydrogen bonding and hydrophobic interactions and penetrates beneath the outermost layers of OM [29]. This suggested that proteins are buried deeper within the soil OM microstructure and may be sorbed to the mineral surface as observed in the present study. Sorption of aromatic and phenolic components during the first DOM loading further implies protein sorption, but may also signify lignin sorption to kaolinite in the contact zone. Similarly, this result agrees with previous studies which reported that lignin domains of soil OM were buried beneath the soil–aqueous interface where they may closely associate with the clay mineral surface [15,28].

After the first DOM loading, the ^{1}H NMR results showed that kaolinite sorbed non-polar aliphatic DOM constituents in most subsequent sorption experiments (Figure 3a). The DE ^{1}H NMR results also indicated the sorption of aliphatic-rich macromolecular DOM to kaolinite during the initial and intermediate DOM loadings (Supplementary Materials Figure S9a). This was further supported by CH_2 and CH_3 resonances of aliphatic OM components that were observed in the HR-MAS NMR spectra of the kaolinite organo-clay complexes (Figure 4). With respect to more polar forms of aliphatic OM, kaolinite sorbed *N*- and *O*-substituted aliphatic compounds only in the first three DOM loadings (Figure 3a). The sorption of both non-polar aliphatic and *N*- and *O*-substituted aliphatic components to kaolinite during the intermediate DOM loadings may indicate the presence of a region of amphiphilic, aliphatic-rich compounds sorbed to the surface-bound OM, in agreement with the zone of hydrophobic interactions proposed by Kleber et al. [3]. Aliphatic components such as lipids and fatty acids may sorb to the bound OM through several mechanisms, including electrostatic, cation bridging or ligand exchange interactions between terminal functional groups of lipids and reactive sites of proteins, lignin or other sorbed OM [30,35]. The non-polar hydrophobic polymethylene chains of these aliphatic components may also associate via OM–OM interactions [34]. Furthermore, the ^{1}H NMR results indicated that aromatic and phenolic DOM components such as lignin or aromatic amino acids sorbed to kaolinite from the third DOM loading onward, albeit to a small extent in most cases (Figure 3a). This is corroborated by aromatic and phenolic resonances that were observed in the HR-MAS NMR spectra of the kaolinite organo-clay complexes after DOM loadings five and 10 (Figure 4 and Supplementary Materials Figure S8). These results suggest that aromatic DOM components may also be present in the hydrophobic zone of the zonal model of organo-mineral interactions [3].

Only low amounts of *O*-alkyl and anomeric components sorbed to kaolinite, and sorption of these constituents was observed only during DOM layering experiments 7–10 (Figure 3a and Supplementary Materials Figure S9a). Signals in these regions arise primarily from carbohydrates, which may have accumulated in the solution during the initial DOM loadings due to their polarity and affinity for the dissolved phase [8,10]. These results agree with previous studies which reported weak sorption of *O*-alkyl constituents to kaolinite [7,14,15]. Sorption of *O*-alkyl compounds to kaolinite in the later DOM loadings may indicate their presence in the kinetic zone of the zonal model of organo-mineral

interactions [3], which is composed of OM components that are weakly and transiently sorbed to the organo-clay complex and may readily desorb into solution. In addition, the α-proton integration results showed that protein constituents sorbed to kaolinite during DOM loadings 7–10 (Figure 3a). Therefore, protein-derived OM may also be an important component of the kinetic zone of the proposed zonal model [3]. However, it should be noted that amide signals arising from proteins did not show the same trend as the α-proton resonances, and thus protein sorption to kaolinite in the later DOM sorption experiments must be interpreted cautiously.

3.4. Sorption of Specific DOM Components to Montmorillonite

The solution-state ^{1}H NMR analysis showed that montmorillonite initially sorbed aromatic and phenolic DOM components such as protein- or lignin-derived compounds (Figure 3b). However, this trend was not observed when the DOM samples were analyzed using DE ^{1}H NMR (Supplementary Materials Figure S9b), suggesting that primarily small aromatic and phenolic molecules sorbed to montmorillonite initially. Positive percent changes in α-proton and amide integration values provided further evidence of protein sorption to montmorillonite during the first DOM loading (Figure 3b). These results suggest that protein- or lignin-derived DOM may be the primary constituents of the contact zone of OM that initially sorbs to exposed montmorillonite surfaces [3]. In addition, greater positive percent changes in aromatic and phenolic integration values for montmorillonite compared to kaolinite (Figure 3a,b) indicated that montmorillonite sorbed more aromatic DOM components than kaolinite initially, in agreement with a previous study [14].

The solution-state ^{1}H NMR results did not indicate any sorption of non-polar aliphatic or *N*- and *O*-substituted aliphatic DOM components to montmorillonite during the first DOM loading (Figure 3b). However, the DE ^{1}H NMR results showed that montmorillonite initially sorbed macromolecular aliphatic constituents such as waxes, cutin and suberin as well as amide components of proteins (Supplementary Materials Figure S9b). Similarly, the ^{1}H HR-MAS NMR spectra of the montmorillonite organo-clay complex after the first DOM loading showed prominent CH_2 and CH_3 resonances (Figure 4), which also indicated the sorption of non-polar aliphatic compounds, lignin or proteins to montmorillonite. As such, montmorillonite likely sorbed primarily large, macromolecular aliphatic components during the initial DOM loading. Collectively, the solution-state and HR-MAS NMR results are consistent with previous studies that have reported the sorption of aliphatic, aromatic and protein-derived DOM components to uncoated montmorillonite surfaces [7,14,15].

After the first DOM loading, the solution-state NMR analysis of DOM (Figure 3b and Supplementary Materials Figure S9b) showed that montmorillonite sorbed non-polar aliphatic DOM components from the third DOM loading onward. This was further supported by the HR-MAS NMR analysis of the montmorillonite organo-clay complexes, which showed prominent CH_2 and CH_3 group signals after DOM loading 5 (Figure 4). As noted previously for kaolinite, these results suggest the formation of a hydrophobic zone of amphiphilic aliphatic compounds above the surface-bound OM as proposed in the zonal model of organo-mineral interactions [3]. However, in contrast to kaolinite, more polar *N*- and *O*-substituted aliphatic compounds did not sorb to montmorillonite during the study. In addition to aliphatic compounds, montmorillonite continued to sorb aromatic and phenolic components during the intermediate and final DOM loadings (Figures 3b and 4), suggesting that DOM constituents such as lignin or aromatic amino acids may also be present in the hydrophobic zone of OM sorbed to the montmorillonite organo-clay complexes [3].

O-alkyl constituents such as carbohydrates were sorbed by montmorillonite to a slight extent and only during DOM loadings 6–10 (Figure 3b). Similarly, the α- and amide proton integration data showed marginal protein sorption to montmorillonite during DOM loadings 8–10 (Figure 3b). As noted for kaolinite, these results may indicate the presence of carbohydrate and protein-derived DOM components in the outermost kinetic zone of OM sorbed to the montmorillonite organo-clay complexes [3]. Furthermore, montmorillonite and kaolinite exhibited comparable integration results

after DOM loading 10, indicating that these minerals sorbed similar types of components during the final DOM sorption experiment (Figure 3a,b).

3.5. Sorption of Specific DOM Components to Gibbsite

Gibbsite sorbed both non-polar aliphatic and more polar *N*- and *O*-substituted aliphatic DOM components during the first DOM loading (Figure 3c and Supplementary Materials Figure S9c). CH_2 and CH_3 group signals in the ^{1}H HR-MAS NMR spectra of the gibbsite organo-clay complex further indicated aliphatic DOM sorption initially (Figure 4). The solution-state ^{1}H and DE ^{1}H NMR integration results (Figure 3c and Supplementary Materials Figure S9c) as well as the HR-MAS NMR analysis (Figure 4) showed that gibbsite continued to sorb both polar and non-polar aliphatic components during all subsequent DOM loadings. In addition, the DE ^{1}H NMR results showed positive percent changes in the amide NMR signal during several DOM loadings, indicating slight sorption of proteins to gibbsite (Supplementary Materials Figure S9c). However, this trend was not observed in the ^{1}H NMR integration results, and sorption of α-protons of proteins to gibbsite was not detected using solution-state ^{1}H or DE ^{1}H NMR (Figure 3c and Supplementary Materials Figure S9c). As such, proteins likely comprised a minor proportion of the OM sorbed by gibbsite over the successive DOM sorption experiments, which consisted of primarily aliphatic compounds. The HR-MAS NMR spectra of the gibbsite organo-clay complexes also showed aromatic resonances after DOM loadings five and 10 (Figure 4). Since the solution-state NMR data showed slight sorption of protein-derived DOM to gibbsite (Supplementary Materials Figure S9c), the aromatic signals observed using HR-MAS NMR likely originated from aromatic amino acids such as phenylalanine and tyrosine [43,45].

As discussed previously, the initial sorption of proteins to kaolinite and montmorillonite may increase the number of available sorption sites on the mineral surface and facilitate the binding of amphiphilic species such as aliphatic lipids in the hydrophobic zone [3]. However, only minor sorption of proteins to gibbsite was detected using solution-state ^{1}H NMR, suggesting that aliphatic compounds may have sorbed to gibbsite via other mechanisms. Iron and aluminum oxide minerals such as gibbsite are believed to sorb DOM primarily via inner-sphere ligand exchange interactions [12,13,53]. Therefore, polar functionalities of aliphatic DOM components, such as hydroxyl and carboxylic acid groups, may have sorbed directly to hydroxyl groups on the gibbsite surface via ligand exchange. Cation bridging between negatively-charged functional groups is another possible sorption interaction, but since the CEC of gibbsite is low (0.1 $cmol_c/kg$), this mechanism may be of minor importance. The continued sorption of both polar and non-polar aliphatic components to gibbsite in the subsequent DOM loadings may have occurred though both ligand exchange with available mineral surface functional groups and OM-OM interactions between aliphatic polymethylene chains [3,34]. The sorption of primarily aliphatic compounds by gibbsite is consistent with previous studies that have reported high concentrations of aliphatic OM in aluminum oxide-rich soils [19,54] and the sorption of *N*- and *O*-substituted aliphatic DOM components to oxide minerals [55]. Using infrared spectroscopy, several earlier studies reported that aluminum oxides selectively sorbed DOM constituents containing hydroxyl and carboxylic acid groups [8,13,56]. Based on our NMR data, these observations may be partly explained by the binding of aliphatic DOM components such as lipids or fatty acids which contain terminal hydroxyl and carboxylic acid groups.

4. Discussion

We found that the concentration of DOC that sorbed to the clay minerals kaolinite, montmorillonite and gibbsite during repeated DOM loadings increased consistently during the initial loading steps and then approached saturation after ten loadings. Similarly, HR-MAS NMR analysis of OM sorbed to the organo-clay complexes showed increased NMR signal intensity for bound OM components after the first five DOM loadings, but little increase in signal intensity between loadings 5–10. Collectively, these results suggest that OM-OM interactions may occur between OM components that are sorbed to organo-clay complexes once the mineral surfaces approach saturation with OM. In particular,

these OM-OM interactions may be more prevalent for organo-clay complexes containing low SSA minerals such as kaolinite and gibbsite since the functional groups available for sorption on the surfaces of these minerals require less DOC to become saturated compared to high SSA minerals such as montmorillonite. However, the concentration of DOC sorbed by all three minerals in the later DOM loadings, where OM-OM interactions were believed to play a more prominent role, was lower compared to the initial DOM loadings where direct mineral-OM interactions are thought to govern DOM sorption [3]. Therefore, OM-OM interactions likely account for the sorption of a lower concentration of DOC to the organo-clay complexes than mineral–OM associations.

The structural characterization of DOM and organo-clay complexes using solution-state ^{1}H NMR and HR-MAS NMR, respectively, showed similarities as well as distinct differences in the composition of DOM sorbed to kaolinite, montmorillonite and gibbsite during the repeated DOM loading experiments. For example, both kaolinite and montmorillonite sorbed aliphatic, protein and lignin components initially, followed by sorption of aliphatic and aromatic constituents during the intermediate DOM loadings and marginal sorption of proteins and *O*-alkyl components in the final sorption experiments. Although kaolinite and montmorillonite have different mineral properties such as SSA and CEC [36], the results showed that these minerals sorbed OM of similar composition, especially after the mineral surfaces were coated with OM. This is consistent with a previous solid-state ^{13}C NMR-based study which reported that two different mixtures of phyllosilicate minerals sorbed DOM of similar composition during repeated DOM sorption experiments [11]. In contrast to kaolinite and montmorillonite, gibbsite sorbed primarily aliphatic compounds during all DOM loading experiments, while proteins were sorbed to a minor extent and no *O*-alkyl component sorption was observed. This indicated that organo-clay complexes containing short-range ordered oxide minerals and phyllosilicate clays sorbed DOM of contrasting composition. However, our results also suggest that similar OM-OM interactions may occur between OM components sorbed to organo-clay complexes containing phyllosilicate or oxide minerals, such as non-polar associations between aliphatic polymethylene chains [34]. These aliphatic-aliphatic interactions may be an important factor governing DOM sorption to organo-clay complexes containing clay minerals with varying SSA and CEC.

In addition to aliphatic-aliphatic interactions, the sorption of both aliphatic and aromatic components to kaolinite, montmorillonite and gibbsite during the intermediate DOM loadings may indicate the presence of aliphatic-aromatic associations, which have been reported previously [24,25]. For example, Clemente and Simpson [24] found that lignin coated in dodecanoic acid was protected from chemical oxidation by $NaClO_2$, possibly due to strong aliphatic-aromatic interactions which prevented the oxidant from accessing lignin. Moreover, Thevenot et al. [25] reported that close associations develop between lignin and aliphatic OM components over time as lignin is incorporated into soil OM, which may slow lignin degradation in soil. Although the mechanism by which these aliphatic and aromatic components interact is not discernible in the present study, hydrophobic associations between aromatic rings and aliphatic chains, or interactions between charged functional groups may play a role [3,5,25]. Since these aliphatic–aromatic associations may occur in organo-clay complexes containing minerals with contrasting properties such as SSA and CEC, they may be an important mechanism by which DOM sorbs to various soil minerals as noted for aliphatic–aliphatic interactions.

5. Conclusions

The layering of OM and the aliphatic-aliphatic and aliphatic-aromatic interactions [24,25,34] observed may be important factors controlling the structural arrangement of OM components on mineral surfaces that are saturated with OM. As hypothesized, the composition of DOM initially sorbed by clay minerals was dictated by mineral properties such as SSA and CEC, in agreement with previous studies [8,12–15]. However, with successive OM layering on mineral surfaces, OM-OM interactions appeared to play a greater role in controlling the type of OM components that were sorbed than mineral properties, which supports our second hypothesis. For example, kaolinite

and montmorillonite exhibit different SSA and CEC, but these minerals sorbed similar types of DOM components after ten sequential DOM loading experiments. The sorption of DOM with comparable composition suggests that kaolinite and montmorillonite organo-clay complexes may have similar conformational arrangements of OM [57,58], in agreement with the zonal model of organo-mineral interactions [3]. Conversely, minerals with largely contrasting mineral properties such as montmorillonite and gibbsite sorbed different types and proportions of OM constituents and thus may exhibit different OM conformational arrangements. However, the same types of OM-OM interactions may occur between sorbed OM components despite these conformational differences, such as aliphatic-aliphatic and aliphatic-aromatic associations [24,25,34]. These physical and chemical interactions may afford protection from degradation and contribute to the stabilization of OM in conjunction with organo-mineral interactions [3,6,24,25,28,59]. For instance, proteins are considered to be a labile soil OM component [60], and protein sorption to phyllosilicate mineral surfaces and the subsequent layering of non-polar constituents such as lipids on proteins via OM-OM interactions may limit the accessibility of oxidants and contribute to the preservation of proteins in soil [6,61]. Since this study was conducted using one set of experimental conditions, parameters such as pH and dominant cation should be varied to determine whether the relative roles of organo-mineral and OM-OM interactions change under different sorption conditions [14,62,63]. Furthermore, additional clay minerals should be tested to determine whether the trends observed are consistent between minerals with a range of properties such as SSA and CEC [8,64,65].

Supplementary Materials: The following are available online at www.mdpi.com/2571-8789/2/1/8/s1, Figure S1: Solution-state ^{1}H nuclear magnetic resonance (NMR) spectra of unbound dissolved organic matter (DOM) remaining in solution after sorption to kaolinite over ten successive DOM loading experiments. For comparison, all spectra are scaled to the same maximum intensity. The resonance at 2.50 ppm is from the NMR solvent (DMSO-d_6), Figure S2: Solution-state ^{1}H nuclear magnetic resonance (NMR) spectra of unbound dissolved organic matter (DOM) remaining in solution after sorption to montmorillonite over ten successive DOM loading experiments. For comparison, all spectra are scaled to the same maximum intensity. The resonance at 2.50 ppm is from the NMR solvent (DMSO-d_6, Figure S3: Solution-state ^{1}H nuclear magnetic resonance (NMR) spectra of unbound dissolved organic matter (DOM) remaining in solution after sorption to gibbsite over ten successive DOM loading experiments. For comparison, all spectra are scaled to the same maximum intensity. The resonance at 2.50 ppm is from the NMR solvent (DMSO-d_6), Figure S4: Solution-state diffusion-edited ^{1}H nuclear magnetic resonance (NMR) spectra of unbound dissolved organic matter (DOM) remaining in solution after sorption to kaolinite over ten successive DOM loading experiments. For comparison, all spectra are scaled to the same maximum intensity. The NMR solvent is DMSO-d_6, Figure S5: Solution-state diffusion-edited ^{1}H nuclear magnetic resonance (NMR) spectra of unbound dissolved organic matter (DOM) remaining in solution after sorption to montmorillonite over ten successive DOM loading experiments. For comparison, all spectra are scaled to the same maximum intensity. The NMR solvent is DMSO-d_6, Figure S6: Solution-state diffusion-edited ^{1}H nuclear magnetic resonance (NMR) spectra of unbound dissolved organic matter (DOM) remaining in solution after sorption to gibbsite over ten successive DOM loading experiments. For comparison, all spectra are scaled to the same maximum intensity. The NMR solvent is DMSO-d_6, Figure S7: Integration results for seven types of dissolved organic matter (DOM) components in the solution-state ^{1}H nuclear magnetic resonance (NMR) spectra of the initial DOM and the unbound DOM samples following sorption to (a) kaolinite, (b) montmorillonite and (c) gibbsite over ten successive DOM loading experiments. The peak area of each region is expressed as a percentage of the total ^{1}H NMR signal across the seven regions, Figure S8: Integration results for seven types of dissolved organic matter (DOM) components in the solution-state diffusion-edited ^{1}H nuclear magnetic resonance (NMR) spectra of the initial DOM and the unbound DOM samples following sorption to (a) kaolinite, (b) montmorillonite and (c) gibbsite over ten successive DOM loading experiments. The peak area of each region is expressed as a percentage of the total ^{1}H NMR signal across the seven regions. Diffusion-edited ^{1}H NMR experiments provide information about relatively large molecular components within the DOM and complements information obtained from ^{1}H NMR spectra, Figure S9: Percent change in integrated solution-state diffusion-edited ^{1}H nuclear magnetic resonance (NMR) signal area of seven classes of dissolved organic matter (DOM) components between the initial DOM and unbound DOM samples following sorptive fractionation by (a) kaolinite, (b) montmorillonite and (c) gibbsite over ten successive DOM loading experiments. A positive percent change indicates sorption of a component to the organo-clay complex, whereas a negative percent change indicates that a component accumulated in solution and was not sorbed. Diffusion-edited ^{1}H NMR experiments provide information about relatively large molecular components within the DOM and complements information obtained from ^{1}H NMR spectra.

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