From Spill to Sequestration: The Molecular Journey of Contamination via Comprehensive Multiphase NMR

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1 **FOR SUBMISSION TO ENVIRONMENTAL SCIENCE AND TECHNOLOGY**

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3 **From Spill to Sequestration: The Molecular Journey of**

4 **Contamination via Comprehensive Multiphase NMR**

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ABSTRACT (200/200)

Comprehensive Multiphase NMR is a novel NMR technique that permits all components (solutions, gels, and solids) to be studied in unaltered natural samples. In this study a wide range of CMP-NMR interaction and editing-based experiments are combined to follow contaminants (pentafluorophenol (PFP) and perfluorooctanoic acid (PFOA)) from the solution state (after a spill) through the gel-state and finally into the true solid-state (sequestered) in an intact water-swollen soil. Kinetics experiments monitoring each phase illustrate PFOA rapidly transfers from solution to the solid phase while for PFP the process is slower with longer residence times in the solution and gel phase. Interaction-based experiments reveal that PFOA enters the soil via its hydrophobic tails and selectively binds to soil microbial protein. PFP sorption shows less specificity exhibiting interactions with a range of gel and solid soil components with a preference towards aromatics (mainly lignin). The results indicate that in addition to more traditional measurements such as K_{oc} , other factors including the influence of the contaminant on the soil-water interface, specific biological interactions, soil composition (content of lignin, protein, etc.) and physical accessibility/swellability of soil organic components will likely be central to better explaining and predicting the true behavior of contaminants in soil.

INTRODUCTION

Cost estimates for the clean-up of contaminated sites in the European Union are around 6 billion Euros (€) annually.¹ A fundamental gap in our scientific knowledge as to how and why chemicals become sequestered in the environment hampers the development of optimal remediation strategies. This has led to a situation where ~70% of soils from contaminated sites in Germany are being stored in vast waste disposal facilities until better clean-up technologies can be developed.² In addition, there are important debates over the molecular fate of perfluorinated chemicals (PFCs) which are now ubiquitous in humans and the environment.³ Some scientists hypothesize the partitioning of PFCs into lipid membranes is the cause of PFC persistence in human blood whereas other groups argue the irreversible binding to human serum albumin is responsible.⁴ These examples demonstrate the need for novel technologies that can provide an unprecedented molecular-handle on interactions from dynamic to permanent, and across the full spectrum of states including liquid to solid in intact samples.

One major factor that impedes our understanding of contaminant-soil interactions in whole samples is the lack of analytical methods that can provide comprehensive and detailed molecular-level information on unaltered samples in their native state. Soils are very challenging to study as they are highly heterogeneous and contain a range of components in different phases. For example, organic matter dissolved in soil pores behaves as a solution, swollen organic matter is more “gel-like”, whereas organic matter sorbed to mineral surfaces is closer to a true solid.

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for environmental research and has traditionally been used to characterize soil and soil extracts.⁵ More recently, NMR has shown great promise in elucidating molecular interactions between soil components or between contaminants and soils or soil extracts in solution, gel, and solid phases.⁵ However, traditional NMR technologies, namely solution-state and solid-state NMR, focus on analysis in a single physical state.⁶ In 1996 an NMR technology termed High Resolution Magic Angle Spinning (HR-MAS) was introduced and allowed for swollen samples to be studied intact.⁷ Unfortunately, HR-MAS probes cannot handle the high power radio frequency pulses which are required for high power decoupling or cross polarization, both necessities for the study of solid components. In the case of a spill, a hydrophobic contaminant may enter the soil as a solution. As it interacts with the soil organic matter it may take on properties of a gel before being sequestered into hydrophobic pockets with more solid-like character. To fully elucidate the process of chemical sequestration it is important to be able to investigate and differentiate the molecular interactions from all phases.

Comprehensive Multiphase NMR (CMP-NMR), introduced in 2012, is a novel technology which allows for the analysis of all phases in intact unaltered samples.⁸ CMP-NMR incorporates a lock and full susceptibility matching (for ideal solution state line-width), magic angle spinning and pulsed field gradients (required to study the gel-state), and high power circuitry (to generate intense B_1 fields required for solid state analysis). In combination these features allow all components to be studied and differentiated in soil analysis in its whole natural swollen state thus maintaining the chemical and physical properties responsible for contaminant sorption in the environment.

In this study, the potential of CMP-NMR to study both kinetic transfer and molecular interactions during contaminant sequestration is demonstrated. Specifically, the fate of perfluorooctanoic acid (PFOA) and pentafluorophenol (PFP) in an urban soil is tracked. Kinetic transfer between the liquid, gel and solid states is monitored and the binding orientation of the contaminant as well as the soil materials (protein, lignin etc.) responsible for sequestration are identified for each phase. PFOA is commonly used in high concentration as an anti-foaming agent in the applications of agrochemicals.⁹ It is ubiquitous in the environment and has been found in humans and animals.^{3, 10, 11, 12} PFOA has also shown to bind strongly with soil.^{13, 14, 15} Halogenated phenols like PFP have been detected in environmental systems because of their use as wood preservatives, pesticides, and herbicides.¹⁶ Perfluorinated contaminants are studied here due to their increasing environmental importance and distribution.³ In addition, the ¹⁹F nucleus is very helpful as it provides a sensitive NMR nucleus abundant in the contaminant (but not in the soil matrix) which is important for some of the experiments described here. For other contaminants that do not contain fluorine, ²H labelled analogues could theoretically be used and while less sensitive should permit the study of practically any organic contaminant within a complex matrix.

SAMPLES AND METHODS

Soil and contaminant information

The soil sample used in all experiments was obtained from Hampstead Park in Dublin, Ireland. Hampstead Park soil is a Grey Brown Podzol collected from the A_h horizon with an organic carbon content of 6.85% ± 0.42% C, 0.59% ± 0.04% N, 4.4433% ± 0.4742% Fe, 0.0598% ± 0.0098% Mn, retrieved from an open public area located within Albert College Park. This soil has a pH of 7.6. PFP (99%) was obtained from SynQuest Laboratories (Alachua, FL, USA). PFOA (96%) was obtained from Sigma Aldrich (Oakville, ON, Canada).

Sample preparation

All sorption experiments were performed using 30 mg of soil in 4mm zirconium NMR rotors. For kinetics experiments, the sample was run immediately after preparation and experiments were recorded as quickly as possible. The delay time from contaminant addition to the first scan in the spectrometer was roughly two minutes. For PFP preparation, 51 µl of D₂O (Cambridge Isotope Laboratories, Tewksbury, MA, USA) was added to 30 mg of pre-weighed soil in a 4 mm zirconium NMR rotor and mixed well using a stainless steel mixing rod, selected such that the displaced liquid is minimal and all contents remain within the rotor permitting stirring without loss. 9 µl of PFP was added after the water was added to simulate PFP entering a hydrated soil. PFOA (7.5mg) were dissolved in 60µL of D₂O and the pD was adjusted to 7.0 using NaOD and a glass electrode. The deuterium ion effect was accounted for by adjusting to 7.4 on the pH scale.¹⁷ The 60µL solution was added into 30 mg of soil in a 4 mm NMR rotor and

lightly mixed. The rotor was then sealed and capped as quickly as possible to begin the kinetics experiments.

For the saturation transfer difference (STD) and cross polarization (CP) studies, sample preparation was exactly the same as the kinetics experiments with the addition of an equilibration time. Samples were prepared and stored at room temperature for 1 month before STD and CP experiments were performed. This was to ensure equilibrium conditions to better simulate the environment and to allow for the maximum amount of contaminant to sorb.

All experiments used in this manuscript are relatively low in sensitivity as they specifically target the interactions between the contaminants and the soils and only a finite number of contaminants will be interacting with any given binding site at any time.¹⁸ Many of the experiments used here have been adapted from medical research to study drug binding in tissue.^{19, 20, 21} The experiments such as CP and STD selectively detect only the sub-fraction interacting with the receptor (soil) and it is recommended the ligand (contaminant) is always added in excess.²⁰ For this reason the quantities used here are not reflective of what may be found in the greater environment but more representative of spill conditions or agricultural application. In the case of PFOA the high concentration of PFOA had little effect on the aqueous-soil interface indicated by the ¹H NMR spectrum of the soil (see supporting Figure S1). Conversely, the PFP did act like a solvent swelling the soil itself indicated by a changing ¹H profile upon addition. This shows that the interactions may alter and may not necessarily be the same as trace concentrations. The implications of this are discussed in the main text. However, it also demonstrates that CMP-NMR, with the ability to study the contaminants, interfaces, mechanisms, etc. is an important tool to identify which chemicals impact the soil

structure/interface itself fundamental to truly understanding sorption. Considering that agrochemicals are rarely applied alone and are often mixed with soil surfactants it is probable that co-solvent effects are of environmental relevance and could be assessed using CMP-NMR.

NMR Spectroscopy

All NMR experiments were performed on a 500 MHz Bruker Avance III Spectrometer using a prototype MAS 4 mm ^1H - ^{19}F - ^{13}C - ^2H CMP-NMR probe fitted with an actively shielded magic angle gradient. All samples were spun at a rate of 6666 Hz and all experiments were performed at 288K. Full experimental details of the NMR experiments are provided in the supporting information Section S.1.

Kinetic Curves

All kinetics curves were run in triplicate. Absolute signal integration was used to calculate solution and gel experiments and the quantities of each were based on the contribution of each sub-phase to the total ^{19}F signal. The solid signal was hard to quantify directly as the CP process is not fully quantitative. As such only relative change is reported for the solid components. Estimates of the amount in the solid phase are possible through difference which is discussed more in the main text. Integrals were taken using the multi-integrate tool in AMIX (Bruker BioSpin, Rheinstetten, Germany), and relative integrals were taken using the first time point as the reference spectrum.

166 *Epitope Maps*

167 Epitope maps were calculated using relative signal integration through the multi-
168 integrate tool in AMIX. Heteronuclear saturation transfer difference (HSTD) and reverse HSTD
169 (RHSTD) epitope maps were created by comparing a saturation experiment to a standard
170 experiment without saturation and intensities were normalized to the signal that had the
171 greatest positive change in the difference spectrum (i.e. received most saturation) is denoted
172 by the 100%. ^1H - ^{19}F CP epitope maps were derived through comparison to the ^{19}F spectrum of
173 the solid compound.

RESULTS AND DISCUSSION

Spectral Editing of the Sub-Phases within the Soil

To isolate individual phases using CMP-NMR spectroscopy a combination of diffusion filters were employed along with CP as a filter for true solids. These experiments have all been described before by Courtier-Murias et al.⁸ and are only briefly described here.

Freely diffusing soluble species can be selected through an inverse diffusion experiment (IDE). The IDE is created by the subtraction of the diffusion edited spectrum (diffusion gradient on, contains larger components with restricted diffusion) from a reference spectrum (diffusion gradient off, contains a profile of all components) leaving just the molecules that are free to diffuse (i.e. solubilized).

Molecules with restricted diffusion can be emphasized using diffusion editing (DE). The gradient encodes the spatial position of signals at the start of the experiment and then decodes them at the end.²² The signal from molecules that diffuse and move positions are not refocused and are attenuated. Essentially the DE spectrum contains molecules that are bound and/or have restricted diffusion. To appear in diffusion editing the molecules must not move physical position, but have enough solvent induced bond dynamics to survive the relatively long delays in diffusion editing (see below for discussion of relaxation). As such, the molecules selected here in diffusion editing are going to be strongly biased towards those sorbed at the solid-water interface, which fulfills both of these conditions.

Due to the relatively long delays required for diffusion editing some molecules with fast relaxation are not observed. These signals can be recovered in a complimentary experiment

195 termed Relaxation recovery Arising from Diffusion Editing (RADE). The experiment recovers the
196 components that otherwise may be missed due to their fast relaxation if diffusion editing is
197 used alone.⁸ In conventional RADE, ^1H is normally used for detection and true solids are not
198 detected due to ^1H - ^1H dipole broadening.⁸ However, ^{19}F - ^{19}F dipole couplings are generally
199 weaker making perfluorinated compounds easy to detect using single pulse experiments.²³
200 Therefore, the ^{19}F RADE will include contributions from rigid-gels, semi-solids and some true
201 solids.

202 A simple way to select the true solid components is to use cross-polarization. In a
203 completely dry sample, CP-MAS is very efficient as it relies on permanent dipoles which
204 dominate in solid structures. However, upon swelling, water introduces local dynamics which
205 modulate the dipolar interactions in turn reducing CP-MAS efficiency. In previous work, it has
206 been shown that no CP-MAS signal is observed for dissolved species while mobile gels are
207 strongly attenuated.⁸ In this study either the dipole between the soil and the contaminant (^1H -
208 ^{19}F) or within the contaminant itself (^{19}F - ^{13}C) can be used to monitor the solid phase. In both
209 cases, CP is most efficient if the dipole is not modulated which occurs in the true solid phase. In
210 this study, to demonstrate the applicability of both ^1H - ^{19}F and ^{19}F - ^{13}C CP, the ^{19}F - ^{13}C is
211 monitored here for the purposes of kinetics and later in the paper the more informative ^1H - ^{19}F
212 dipole is exploited in regard to binding mechanism in the solid-phase.

213 Figure 1 illustrates the decomposition of the spectra into the individual phases. PFP
214 forms three distinct chemical shift bands corresponding to free (1), bound (2), and solid (3) best
215 seen for the fluorine in the ortho position. The IDE clearly highlights the free form (Figure 1b),
216 while the DE highlights the form with restricted diffusion (1c). The RADE identifies a range of

material from gel through solids (Figure 1d). CP (Figure 1e) selects only the broadest components consistent with the PFP that has been sequestered into the soil to form a true solid-like phase. The use of spectral editing filters makes the assignment of the various bound states easier and less ambiguous. Unfortunately, for PFOA there are no chemical shift perturbations on binding but estimates of contributions from each phase can be performed by comparing the intensities of signal in each sub spectrum relative to the total (Figure 1a).

The results indicate that PFP interacts to a greater extent with soil than PFOA. Only ~9% of the total ^{19}F signal is from molecules in the dissolved state with ~88% sequestered into the rigid-gel, semi-solid, solid-state (Figure 1d). Conversely over half of PFOA remains in the true dissolved state with 41% showing some signs of sequestration. The predicted K_{oc} (ACD labs Phys. Chem. Suite 2012) at pH 7 are 36 for PFP and 3 for PFOA which are in line with a strong interaction for PFP. Interestingly the soil partition coefficient (K_{oc}) at pH 5.5 for PFP is predicted to be 578 while PFOA remains relatively unchanged. Therefore if lower pH micro environments exist within the soil these could show a strong preference towards PFP. The smaller size and roughly spherical shape of PFP may also aid in penetration into the soil. In addition, changes were noted in the ^1H spectrum upon the addition of PFP. PFP acts like a solvent and is capable of swelling and penetrating a wider range of soil components (Figure S1). This behaviour is interesting, easily observable by NMR, but not easily predictable based on the physical-chemical properties of the molecule alone.

Unfortunately the absolute percent in the true solid phase cannot be accurately determined as CP cannot be quantified easily with respect to the total ^{19}F signal. In the case of PFP, as the solid components can be seen in the total spectrum, the contribution in Figure 1a

can be used as a rough guide. It appears that a considerable fraction (~50% based on estimations from Figure 1a) is sequestered into the solid phase. Unfortunately, accurate estimates are not possible for PFOA as the solid components cannot be discerned in Figure 1a. However, there is ~3 times the signal-to-noise in the PFP spectrum than PFOA (Figure 1e) which suggest PFP sequesters ~3 times more than PFOA. The reason behind this is not completely clear but can be elucidated using other NMR techniques demonstrated in the rest of this manuscript starting with individual phase kinetics.

Kinetics Between Phases

Kinetics studies are very useful to explain sorption properties including adsorption mechanisms, surface properties of the adsorbent, and degree of affinity for the adsorbent.²⁴ Most commonly the concentration of sorbent left in the solution phase is used to calculate the amount sorbed by difference. CMP-NMR is highly complementary as it can follow not just the free contaminant but also as it penetrates into the gel and becomes sequestered into the solid phase.

In this study, multiple phase kinetics were obtained in a whole soil through the use of CMP-NMR⁸ and CPMG-SS²⁵ techniques, and absolute quantification was performed in a similar method as was done in Figure 1. Figure 2a plots the kinetics in the solution and gel for PFP and PFOA. The solution phase kinetics curve for PFP had a small and steady decline while the solution state curve for PFOA had a rapid decline and reached equilibrium quickly. In the case of PFOA it is possible that the soil becomes saturated and cannot take on any more contaminant.

On the other hand, the solvent like properties of PFP may aid the soil swelling allowing more molecules to penetrate.

The kinetic curve for the gel-phase PFP and PFOA (as determined by diffusion editing) both declined continuously. Essentially these are molecules that have some bond dynamics and have relatively long relaxation, but show no physical movement and will therefore be dominated by molecules bound at the soil-water interface. This signal starts at ~4-5% indicating a rapid initial sorption at the interface (before the first NMR experiment) followed by a slow decline caused by slow transfer across the interface into the more rigid soil interior. For both contaminants only a small fraction (<5%) of the contaminant was bound to the interface at any given time.

Finally, the solid state (Figure 2b), represents the fraction that has essentially become fully sequestered and taken on properties of a solid. Such soil domains are likely hydrophobic and removed from the water which itself would induce local dynamics in turn attenuating CP signal. While it is possible from Figure 1 (see discussion above) that there is ~3 times the amount of PFP in the solid phase when compared to PFOA, unfortunately direct quantification from CP is not possible. As such Figure 2 simply shows the relative change over time. For PFOA this is considerable, rising ~80% after the 2 hour window. This is correlated with a reduction in the solution fraction and demonstrates that the contaminant is crossing the soil interface and becomes sequestered into the hydrophobic interior. Interestingly the signal after the first point (10mins) is already significant (relative change 1.0) and demonstrates that more is sequestered in the first 10mins than over the next 2 hours (relative change 1.8 after 2 hours). In turn, this demonstrates that initially the soil can rapidly take up large quantities of contaminant and this

contaminant becomes rapidly sequestered into the solid phase. A similar trend with PFP is observed but the increase in the solid fraction and the corresponding decrease in the solution phase are less pronounced. In addition, while the solid fraction of PFP has mainly plateaued a very slight rise may still be visible after 2 hours. When considered alongside the continued decrease in the solution and gel fractions (Figure 1a,b) the data suggest the kinetics for PFP have yet to reach full equilibrium. This is likely in part due to the solvent effects of PFP which further swell the soil overtime permitting further sorption. While kinetics provides a deeper understanding of the sorption process it provides little direct evidence of the molecular-level phenomena behind the process. To address this, additional CMP-NMR experiments can be utilized to identify what soil components bind the contaminant as-well as the binding orientation of the contaminant itself.

Molecular Orientation of the Contaminant

The molecular orientation of the contaminant in both the dynamic- and solid- phase are discriminated. As both approaches use different NMR techniques the dynamic interactions will be discussed first.

Orientation in the Dynamic Fraction

A technique called heteronuclear saturation transfer difference (HSTD) was employed. As ^1H atoms from the soil are saturated, the saturation perturbs through spatial dipoles and

is transferred to the ^{19}F atoms that are in closest spatial proximity to the soil.^{4, 26, 27, 28} The ^{19}F groups in the contaminant closest to the soil receive the most saturation, which can be quantified and converted into an epitope map. Each group is normalized to the strongest binding position (denoted 100%) and interactions at other positions are given relative to this. To suppress signals from solid signals, a short CPMG filter was determined empirically and was included prior to acquisition. Only components that show interaction with soil will be detected in HSTD while free components will be completely subtracted. Hence, the HSTD result can be thought of as the average binding orientation of the dynamic fraction. This will include molecules bound at the interface as well as more dynamic species partitioning with the soil.

Figure 3a illustrates the results of the epitope maps calculated for PFP and PFOA for the dynamic phase. The ^{19}F position with the strongest interaction is normalized to 100% and other positions were reported relative to this. PFP displayed non-specific binding since each ^{19}F atom is fairly close to 100%. However, there is a very slight preference towards the OH indicated by the strongest interaction of the ^{19}F nuclei closest to the OH group. This is logical since the aqueous-soil interface has been shown to be relatively polar,²⁹ and with a pKa of 5.5 the OH may be protonated in more acid microenvironments and could be more likely to associate with, rather than repel, the soil organic matter (itself mainly anionic). Conversely with a pKa of 3.8,³⁰ PFOA will be mainly negatively charged and largely repel organic matter. Indeed this is reflected in its epitope map which demonstrates a bias towards the hydrophobic tail end as the point of interaction. This is consistent with previous work that illustrates that ^{19}F rich functional groups play a key role for contaminant sorption to soil.^{4, 26, 28}

324

325 *Binding Orientation in Solid Fraction*

326 The solid phase epitope map was derived from ^1H - ^{19}F CP. In this case the ^1H
327 magnetization is passed from the soil, which acted as a source of ^1H , to the contaminant (^{19}F)
328 via rigid dipoles exclusive to the contaminant in the solid phase. The inherent signal from ^{19}F is
329 suppressed using phase cycling and the only way for signal to arise in the experiments is from
330 magnetization transferred from the soil protons. As such, the signal in the resulting spectrum is
331 proportional to the interaction with the soil from which an epitope map can be constructed. To
332 further support this, CP ^1H - ^{19}F build up curves were recorded for each ^{19}F position. From these
333 curves, the heteronuclear ^1H - ^{19}F dipole couplings can be extracted. Essentially both build up
334 curves and conventional ^1H - ^{19}F CP produce the same binding orientations. For the sake of
335 brevity a more involved analysis of the buildup curves is presented in the supporting
336 information (supporting section S.4).

337 Figure 3b shows the binding orientation for the molecules in the solid-state. PFP in the
338 gel state showed little orientation preference (Figure 3a), however in the solid-phase there is a
339 clear bias towards the most hydrophobic segment of the molecule (Figure 3b). This confirms
340 that hydrophobicity is the overall driving factor for sequestration of PFP within soil. The
341 reduced interaction at the OH strongly suggests the final sequestered site is removed from the
342 water interface. The solid interactions of PFOA also support this finding. However, extracting
343 information along the chain is challenging due to spectral overlap and low S/N, and therefore
344 only an average for the CF_2 portion of the chain can be reported. It is clear that in the solid state

the interaction further favors the hydrophobic end of the molecule and that the COOH group has little influence. This is consistent with previous binding studies that determine the halogenated functionalities in organic contaminants show the strongest preference for both soil and extracted soil organic matter.^{28, 31} Combined kinetics and molecular orientation studies paint detailed molecular pictures as to how and why contaminants become sequestered in soil. However, information regarding the exact soil components that bind the contaminants is missing.

Elucidating the Soil Components Responsible for Binding

Soil Components Involved in Dynamic Interactions

To elucidate which sub-components of the soil bind the contaminant, the reverse of HSTD and CP experiments described above are employed. Reverse HSTD (RHSTD) is used to identify dynamic interactions. In this case the contaminant (^{19}F) is saturated with magnetization which is passed onto the soil ^1H for detection. ^1H - ^1H dipoles in the macromolecular soil components propagate the saturation such that the equilibrium magnetization is perturbed through the entire macromolecular network. A difference approach is used for detection and in simple terms all components that are not interacting with the contaminant cancel (due to a 180° phase shift of the receiver every other scan) leaving only signals from soil components interacting with the contaminant in the final spectrum. In the case of ^1H detection, true solids are attenuated because they have wide spectral profiles. Therefore, the RHSTD biases interaction with dynamic soil components at the interface, in exchange, or swollen sites.

The spectral profile of the dynamic components interacting with PFP is relatively intense and presents a wide range of soil components (lignin, lipids, protein, and carbohydrates) and is shown in Figure 4a. This is in line with PFP having solvent like properties that can indiscriminately swell all components in soil organic matter to some extent. Conversely, very little if any signal is observed for PFOA even if the experiment is run for many days. This is interesting as previous studies have shown with extracted soil organic matter in solution RHSTD spectra can be obtained and PFOA shows a preference towards protein.²⁷ This suggests the protein fraction in extracted matter (known to be lysed from microbial cells)³² is not available at the soil interface in whole samples, and reiterates the importance of studying samples in their unaltered state. As well, this demonstrates the importance of physical accessibility in the sorption process.

If all the data presented so far are considered, it can be seen that very little PFOA is in the gel-state (Figure 1) with the majority either not interacting at all (~60%), or in the semi-solid/solid-phase (~40%, Figure 1d). As such, it seems that PFOA exhibits a more specific targeted interaction. If these locations are available it will bind and become sequestered as a solid, but if not PFOA will stay mainly in the solution state without swelling the soil interface (see supporting section S.2). As mentioned above ^1H - ^1H dipoles lead to spectral components for solids many kHz wide. The lack of signal in the RHSTD suggests the components that PFOA interacts with have a strong solid-like character.

Soil Components Involved in Solid Interactions

The solid phase components that are responsible for sequestration of contaminants are identified through ^{19}F - ^1H CP. In this case the ^{19}F magnetization is passed from the contaminant to the soil (^1H) via rigid dipoles found in the solid phase. The result is a spectrum of the soil components that bind the components in the solid phase (Figure 4b).

PFP demonstrates a markedly different spectrum than for the dynamic fraction. This spectrum is dominated by a broad aromatic resonance consistent with lignin in soil. This is in line with previous work that has shown perfluoroaromatics binding to lignin components in isolated organic matter and soil.^{27, 33} It has been previously shown that lignin components are buried under the soil-water interface in a domain where water cannot penetrate soil organic matter.²⁹ The mechanism of PFP interaction could be the following. First PFP indiscriminately swells soils such that lignin components are uncovered and once exposed shows a strong preference towards these sub-components. Previous work identifies that the overlap of the electrostatic potential density profiles between the prefluoroaromatic contaminants and the aromatic sub-units of lignin are responsible for the strong interaction in extracted organic matter.²⁶ Ultimately, this does not rule out an interaction with other aromatic species in soil organic matter including tannin, suberin, or black carbon.

PFOA displays a strong preference towards the aliphatic components of soil. The apex of the soil component peak is centered around 0.7ppm for methyl ^1H from proteins rather than 1.3ppm which would be expected for long chain aliphatics. Indeed previous work on soil organic matter has shown that these components are from soil protein mainly derived from microbes.³² Furthermore extensive NMR based work in human blood and soil organic matter have shown that PFOA specifically binds to albumin proteins.^{4, 27} Albumins are by design fatty acid transport

proteins and PFOA is essentially a perfluorinated fatty acid and therefore binds to the same sites, specifically Sudlow's site I, but irreversibly.⁴ In whole soil, it appears that the fate of PFOA is the same as in extracted organic matter and that PFOA binds to the protein fraction. It is not clear whether the protein fraction is within living microbial biomass or necromass. If PFOA is within living microbial cells, then the microbial community could have a much more direct and profound role on contaminant sequestration, fate and transformation than previously anticipated. However, given the high concentrations of contaminant used here, microbial cells in these experiments may be inactive because of the toxicity of PFOA. It has been previously reported that in eukaryotic and bacterial cells, a relatively low concentration (milli-molar) of PFOA is required to inhibit bioactivity.³⁴ Further considerations as to the general applicability of CMP-NMR are discussed in supporting section S5.

This manuscript, using PFP and PFOA as examples, has demonstrated that CMP-NMR is a versatile technique to better understand and monitor contaminant sequestration at the molecular-level. PFP shows more general partitioning behavior, but its ability to act as a solvent and change the properties of the soil-water interface itself is hard to predict without NMR data. The results indicate that the impact of the contaminant itself on the soil-water interface (especially if a liquid) is an important factor to consider in predicting contaminant sorption and could vary with concentration (i.e. the more contaminant the more the soil properties themselves are altered) and may vary from soil to soil. Conversely, PFOA which is found to selectively bind to soil microbial protein is of particular significance. The EPA reports that in its ionic form PFOA can readily migrate from soil to ground water and be transported long distances.^{35, 36} The data here both support and contrast this. When binding sites are not

431 available PFOA indeed remains in the solution phase. However, if protein binding sites are
432 available it irreversibly binds to these sites to become a solid. Considering that the microbial
433 protein content of soil varies considerably from less than 1% in acid forest soils (which are
434 dominated by fungi) to up to 50% of the carbon in grassland soils (dominated by bacteria),³² it is
435 likely the behavior of PFOA in soils will differ greatly and may in part be proportional to the
436 available protein binding sites. The EPA further report that “PFOA has a high affinity for fatty
437 acid binding protein” and that it can “interfere with fatty acid metabolism and may deregulate
438 metabolism of lipids and lipoproteins”.³⁵ As such, the impact of PFOA binding to microbial
439 protein in soil is not clear but of importance given that PFOA is used in high concentration as an
440 anti-foaming agent in the applications of agrochemicals.⁹ If PFOA does indeed penetrate living
441 cells and impacts some microbial species more than others then the potential to disrupt the
442 microbial diversity in soils exists. In summary the results suggest more traditional approaches
443 that predict contaminant partitioning into soils based on simple parameters such as K_{oc} may be
444 limited. In reality numerous and complex factors including the soil’s chemistry (contents of
445 protein, lignin etc.), physical accessibility of soil components to contaminants and water, soil
446 biology (probability for contaminant to undergo specific biological interactions) and potential of
447 the contaminant itself to alter to soil-water interface likely all play important roles.

SUPPORTING INFORMATION

Detailed methods on instrumental parameters for spectral editing, kinetics, and interactions; Figure S1, illustrating swelling capabilities of PFP vs. PFOA as well as a discussion; Figure S2, spectra used for epitope map calculations; a CP build up curve explanation; Figure S3, CP build up curves; Table S1, calculated $1/T_{HF}$ values; and a discussion on future considerations. The Supporting Information is available free of charge on the ACS Publications website at...

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565 Figure 1: An overview of the spectral editing used in this manuscript. a) A conventional ^{19}F
566 spectrum shows all phases. Using the ortho position as an example, solution (1), bound (2), and
567 sequestered (3) peaks can be discerned whereas this is not the case for PFOA. b) The solution
568 phase is isolated using IDE. c) Diffusion editing illustrates the small number of contaminants
569 that are at the interface of soil and water. d) The RADE accounts for any fast relaxing species
570 lost in the delays of the diffusion experiments and encompasses a range of materials from rigid
571 gels through to true solids. e) ^1H - ^{19}F CP selects for components with rigid dipoles (most solid-
572 like material present). PFP shows much higher signal to noise in the CP experiment indicating
573 more material sequestered into a solid-like environment. f) CP-CPMG-SS was employed to focus
574 the ^{19}F - ^{13}C signals into a single spike to improve sensitivity and decrease experiment time. The
575 single spike experiment permits the total amount in the solid fraction to be monitored with
576 higher temporal resolution for kinetic studies.

Figure 2: The kinetics curves for PFP and PFOA interaction in whole soil for individual phases are plotted. a) Solution state curves show PFOA losing intensity quickly and reaching equilibrium while PFP exhibits a steady decline. Gel phase curves show slow declines for both contaminants while PFP has a stronger decline. b) Solid phase curves show a strong increase for PFOA while a smaller increase exists for PFP. Standard deviations were all smaller than the width of the representative markers and were excluded for figure clarity.

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594

595 Figure 3: Epitope maps for PFP and PFOA in the gel and solid phases in whole soil were
 596 calculated using HSTD and CP. For PFP, there is a discriminant shift in binding mechanism from
 597 the gel to the solid phase where the OH group becomes less important in solid phase
 598 sequestration. Conversely, the CF₃ group in PFOA drives the sorption in both the dynamic and
 599 solid phases.

a Gel Phase Interaction Profile

b Solid Phase Interaction Profile

600

601 Figure 4: To determine which components in soil sequester PFP and PFOA, ^{19}F - ^1H RHSTD was
 602 employed in the gel phase (a) while ^{19}F - ^1H CP is used in the solid phase (b). PFP shows an
 603 indiscriminate profile in the gel phase showing interaction with most soil components. In the
 604 solid phase however, PFP shows a strong preference to towards aromatics (lignin). PFOA shows
 605 few interactions in the gel-state, but a strong preferential interaction towards proteins in the
 606 solid phase.