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Critical Review

Soil Organic Matter Characterization by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR MS): A Critical Review of Sample Preparation, Analysis, and Data Interpretation

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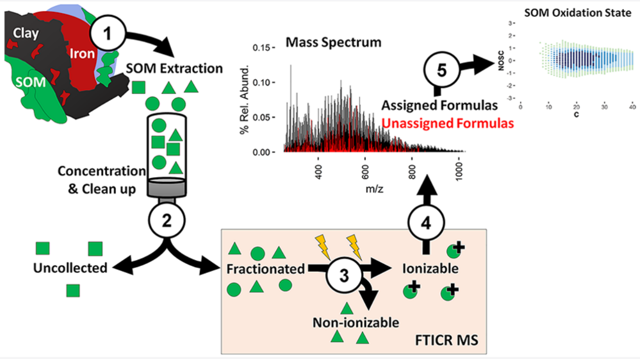

ABSTRACT: The biogeochemical cycling of soil organic matter (SOM) plays a central role in regulating soil health, water quality, carbon storage, and greenhouse gas emissions. Thus, many studies have been conducted to reveal how anthropogenic and climate variables affect carbon sequestration and nutrient cycling. Among the analytical techniques used to better understand the speciation and transformation of SOM, Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) is the only technique that has sufficient mass resolving power to separate and accurately assign elemental compositions to individual SOM molecules. The global increase in the application of FTICR MS to address SOM complexity has highlighted the many challenges and opportunities associated with SOM sample preparation, FTICR MS analysis, and mass spectral interpretation. Here, we provide a critical review of recent strategies for SOM characterization by FTICR MS with emphasis on SOM sample collection, preparation, analysis, and data interpretation. Data processing and visualization methods are presented with suggested workflows that detail the considerations needed for the application of molecular information derived from FTICR MS. Finally, we highlight current research gaps, biases, and future directions needed to improve our understanding of organic matter chemistry and cycling within terrestrial ecosystems.

KEYWORDS: *Dissolved organic carbon (DOC), mineral-associated organic matter, sorptive fractionation, metabolomics, microbial carbon metabolism, organic nitrogen, organo-metal complexation, carbon sequestration*

■ INTRODUCTION TO FTICR MS FOR ENVIRONMENTAL SCIENTISTS

Soil organic matter (SOM) is the accumulated, decaying debris of biota in the soil. It is both the largest active reservoir of terrestrial organic carbon[1] and fuel for microbial activity and plant nutrient production, "giving life" to soil through its decay.[2] SOM affects soil structure, moisture retention, ion exchange capacity, and other properties, and it can be studied from many diverse perspectives, from soil health to contaminant transport. Most recently, urgent attention has been given to its relationship to atmospheric CO_2 concentrations and climate change.[3,4]

The sources of SOM are vast and various, including extracellular compounds likeroot exudates, enzymes, polymeric substances,[5,6] decomposition products from above-ground plant litter, root litter, microbial necromass,[7−10] and abiotic reaction byproducts. The succession of biotic and abiotic reactions and the disparate conditions across both spatial and temporal scales[11,12] create mixtures with chemical diversity that is far more complex than the original biological source materials.[13,14] Recent observations also show that SOM is heterogeneous at high spatial resolution[15,16] and that physical separations (i.e., water extractable, particulate, and mineral-associated organic matter) reflect the formation, persistence, and function of SOM in the environment better than operational fractions produced by solubilities under different pH conditions (i.e., humic acids, fulvic acids, and humins).[17,18] A wide range of complementary analytical techniques are required to study such a complex and dynamic mixture when it is heterogeneously distributed among different physical environments at nearly every measurable scale (i.e., nanometer to kilometer).[19]

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The introduction of Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) analysis established a means to reliably detect and resolve individual SOM molecules when studying its composition, distribution, or transformation. FTICR MS achieves sufficient mass resolving power over a wide mass range (from a hundred to several thousand daltons) to identify a *m*/*z* difference of a single electron, where *m* is ion mass in daltons (Da) and *z* is the ion charge.[20] This resolution is capable of separating the closely spaced *m*/*z* peaks that are found in a typical SOM mass spectra (Figure 1).

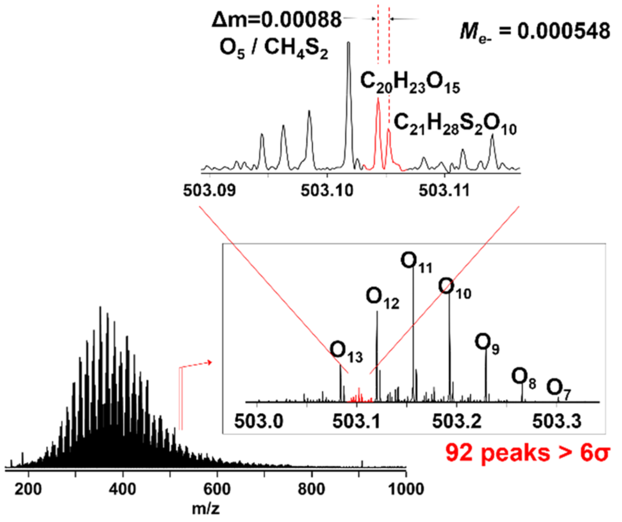

Figure 1. FTICR mass spectra of a SOM extract displaying the resolution needed to differentiate unique peaks. The peaks ($C_{20}H_{23}O_{15}$ and $C_{21}H_{28}S_2O_{10}$) differ by 0.00088 *m*/*z*, requiring a resolution of ∼600 000 (resolution = $m/\Delta m$) to separate and assign. The mass of an electron (M_e^-) is provided as reference. Unpublished data from the National Magnetic Field Lab.

However, biases that originate from sample preparation and the chosen analysis conditions make full characterization of any SOM sample difficult, whatever the mass resolving power of the FTICR MS. Due to the diversity of molecular components that comprise SOM, any extraction method will only target a specific fraction and must be tailored to a specific research question to collect the desired fraction most effectively. Ionization efficiencies vary greatly between molecules of different chemical composition and sample matrices, which complicates the quantitative comparison of SOM mass spectra. Furthermore, once mass spectra are collected, data analysis requires making assumptions to assign reasonable molecular formula to masses, grouping features by calculated indices, and creating visualizations that reflect chemical differences. Robust interpretation relies on critical assessments of the uncertainties introduced during each of these steps.

Importantly, FTICR MS is not, by itself, able to differentiate between isomers, determine molecular structures, or identify functional groups, meaning that FTICR mass spectra are still simplified representations of SOM.[21] FTICR MS provides a qualitative view into a subset of SOM compositional space. However, these data can be further examined using LC-MS and other complementary approaches (section 2 of the Supporting Information) to identify and characterize specific isomers of interest and contextualize relationships between peaks and samples.

There has been significant progress in overcoming the challenges of organic matter analysis using FTICR MS,[22−24] and the user base of FTICR MS analyses is growing rapidly as techniques for SOM analysis become more standardized. Herein, we aim to provide a comprehensive resource to multidisciplinary researchers interested in applying FTICR MS to obtain molecular level insight into SOM chemistry. Workflows for FTICR MS based SOM analysis will be presented from an experimental design perspective, and potential biases from sample extraction, ionization, and formula assignment methods will be highlighted. Data visualization and exploration methods will also be discussed, with suggested usage to avoid misinterpretations. Finally, techniques complementary to FTICR MS for SOM analysis will be presented with their specific strengths to support more robust conclusions about SOM composition, microbial decomposition pathways, and other relevant areas of study (Figure 2).

■ SAMPLING AND EXTRACTION OF SOIL ORGANIC MATTER

SOM sampling methodology depends primarily on the scientific focus of the study and which fraction of SOM will provide the most relevant information. The heterogeneity of SOM, soil surfaces, and microenvironments make a single extraction procedure for all SOM features impossible.[25,26] SOM has been historically isolated into fractions operationally

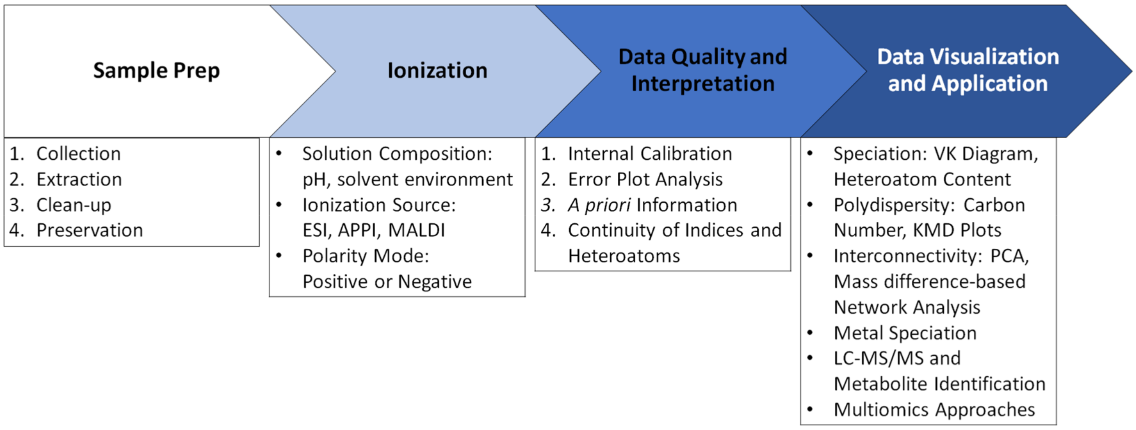

Figure 2. Workflow for analysis involving FTICR MS to provide meaningful conclusions about a set of samples.

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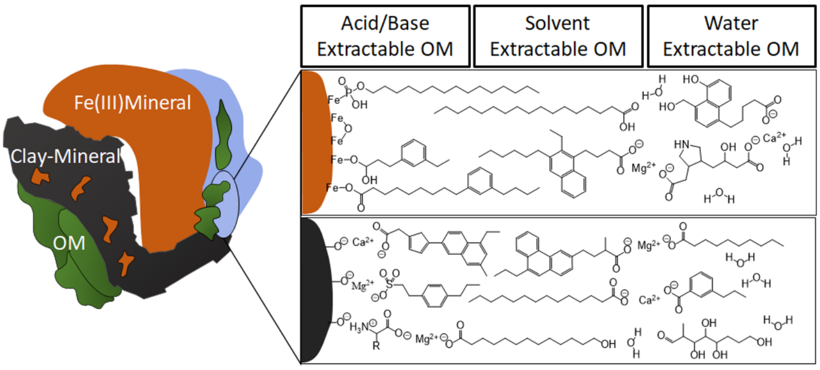

Figure 3. Model soil particle depicting an Fe(III) mineral (brown) and clay (black) surface exhibiting various possible interactions with model SOM. The variety of possible interactions will create fractions with varying extractability based on the extraction solution (e.g., acid/base, organic solvent, and water), mineral surface, and intermolecular forces.

defined by their solubility at different pH.[27] While this method continues to be used,[28,29] other fractions of interest can be collected by targeting specific SOM sorption mechanisms. SOM is bound to soil minerals and other organic matter via different mechanisms, from weak electrostatic, hydrophobic, and other nonspecific interactions to strong polar covalent bonds produced by ligand exchange with carboxylic and phenolic functional groups[25,30] (Figure 3). The most commonly used extraction methods include water, alkaline, mineral dissolution, and organic extractions, and each method has been shown to extract different fractions of SOM (Table S2). Extracted fractions can be related and even complement each other; however they cannot be assumed to be "complete" or truly representative of total SOM as they, by design, only target specific operational fractions defined by the targeted stabilization mechanism.[31] This review outlines the major extraction methods and the key shortcomings of each that must be considered when collecting samples for FTICR MS.

Water-Based Extraction. For approximately two decades, water extraction has been used as a collection method for SOM that is released to the soil solutions when equilibrium conditions change.[32] The resulting fraction, referred to as water-extractable SOM (WEOM),[33,34] is closely related to dissolved organic matter (DOM), which is defined operationally by filtration (i.e., passing through 0.4−0.6 μm pore sizes)[35] of water-extractable or aquatic organic matter.[36,37] These terms describe complex natural organic matter (NOM) and have been shown to be highly related through biomolecular origin and degree of decomposition.[37−40] Therefore, the following discussion on preparation, ionization, and analysis can be similarly applied.

WEOM is considered the most dynamic and bioavailable fraction of SOM and therefore its analysis is highly applicable to a variety of studies.[41−45] The importance and broad application of water extractable SOM are due to its roles in aqueous photochemistry, nutrient cycling, ion distribution, and pH buffering, in addition to acting as an energy and carbon source for microbes.[46−49] Water extraction is performed with ultrapure water or salt solutions such as potassium sulfate (or potassium chloride), which increases ionic strength, generally improving SOM yield.[32,50−53] Additional alteration of extraction conditions, such as increasing temperature, pressure, or time, have failed to produce significant improvement over leaching or batch extractions at room temperature.[45] Overall, water extractions are easily performed but are generally unable to extract SOM adsorbed to mineral surfaces, hydrophobic molecules, or physically protected SOM, which are important when considering carbon sequestration and mineral−organic interactions.[34,50]

Alkaline Extraction. Alkaline extraction of SOM was first described by Achard in 1786 and has remained relatively unchanged to the current day.[54−57] This extraction is widely used due to its high organic carbon yield usually achieved through the use of 0.1 M NaOH. Alkaline extractions are expected to contain a more diverse range of SOM molecules than WEOM, including molecules that are not hydrophilic under the normal range of *in situ* equilibrium conditions. Alkaline extractions function by deprotonation of acidic functional groups present on SOM, greatly increasing SOM polarity and aqueous solubility.[58] This process's effectiveness relies on the extracting solution having a higher pH than the acidity (pK_a) of the targeted functional groups (Table S1, Figure S1) and the abundance and distribution of those functional groups in the SOM.[50,58] The potential for base-catalyzed hydrolysis of SOM collected by alkaline extractions has been debated,[58,59] but many of the extracted features do appear to be identical to other methods.[60−64] Importantly, any extraction that shifts the *in situ* conditions of the SOM, including alkaline extractions, may produce artifacts or side effects that alter "native" SOM composition, so considering the character and extent of such artifacts is essential to any extraction method.[38,58,59,65] Subject to these considerations, alkaline extractions reach SOM that is inaccessible to water extractions and provide a more comprehensive view of SOM composition.[59]

Selective Mineral Dissolution. Study of the role of mineral protection in SOM stability has increased considerably in recent years as mineral−organic associations are thought to increase protection from microbial degradation processes.[38,66] For example, iron oxide minerals are effective sorbents for organic matter through interactions that include co-precipitation and ligand exchange under more acidic conditions.[67−69] To study these mineral−organic associations directly, mineral dissolving solutions are used to selectively extract SOM associated with those minerals.[67] These extractions function by either reduction, chelation, or dispersion of a mineral phase, resulting in the release of SOM stabilized by that mineral.[34,44,70−72] Example mineral phases include short-range order (SRO) Fe(III) oxides, crystalline Fe(III) oxides, aluminum oxides, and aluminosilicate clays, among others. Selection of appropriate extraction solutions has been extensively studied in SOM−mineral or mineral cycling studies.[34,50,67,73−75] Not as well characterized is the potential for SOM alteration by extraction conditions or interactions between the DOM and dissolved minerals.[75−77] After dissolution, new SOM−mineral interactions can be inhibited by inclusion of an appropriate buffering or stabilizing agent, such as citrate for Fe^{2+}, that can complex metal ions and prevent re-adsorption or aggregation of extracted SOM.[76,78,79] All mineral extraction will require a cleanup process to remove the extracting chemicals and stabilizing agents that can affect ionization, discussed below. Finally, mineral extractions have the potential to extract compounds that overlap with water extractions and should be noted when comparing extraction yields and spectra.[51,75,80]

Organic Solvent Extraction. Soil organic matter is known to contain hydrophobic domains,[63] and laboratory studies have indicated that nonselective sorption of DOM contributes substantially to the overall presence of mineral−organic associations.[81] To study these interactions, it is useful to have an extraction method that targets more hydrophobic domains.

Organic solvents can be more efficient extractors than polar solvents depending on the source material and target fraction (e.g., the hydrophobic domain).[82] Tfaily et al. showed that extracted H/C and O/C ratios are influenced by solvent polarity by performing a series of parallel extractions, and in a subsequent study showed that low polar or nonpolar solvents detect a greater number of low O/C peaks in FTICR-MS analysis.[41,83] Furthermore, Mckee and Hatcher[84] demonstrated that pyridine extractions of lake sediment isolate a larger range of mass spectral and spectroscopic features compared to other organic and aqueous extractions. Organic extractions can also induce alterations to the SOM, such as esterification following methanol and acetonitrile extraction, that should be considered before usage and when comparing between samples prepared differently.[85] Extraction with organic solvents also dissolves fewer inorganic salts that inhibit SOM ionization compared with aqueous extractions. This is a potentially large benefit as other extractions methods require cleanup that further bias SOM composition.[41]

Sequential Extraction. Extractants can also be applied sequentially to soils to selectively collect SOM bound at different degrees of stabilization. This can be very beneficial when comparing the quantity and characteristics of SOM stabilized by the different mechanisms mentioned above. Lopez-Sangil and Rovira[50] demonstrated this with seven extracting solutions on four soils of varying pH and organic carbon content. Each extractant collected a fraction of SOM, but the majority of SOM was collected with sodium tetraborate or sodium pyrophosphate, indicating that cation bridging or chelating interactions predominated, respectively. Tfaily et al.[41] also showed that sequential extractions with water followed by different organic solvents resulted in an overall increase in peak counts by 2−4-fold. As above, it is possible that extractants overlap in the SOM collected; therefore each extraction should be performed several times to ensure that as much of the target fraction is extracted as possible before continuing with the next extractant. The additional extracts collected will increase preparation time; however a sequential extraction protocol can be tailored to specific research questions based on the mineral−organic associations of interest.

Sample Preservation and Cleanup (for FTICR-MS Analysis). Once a soil sample has been collected, it is important to perform an extraction as soon as possible or store the sample to minimize biological degradation of SOM.[86] If extraction soon after sampling is impossible, it is recommended that soil samples be treated as similarly as possible to maintain their comparability, as no storage method can perfectly preserve the *in situ* conditions.[59,78] Before extraction, soil sample storage by freezing or drying can introduce bias from aggregation of the soil or SOM,[78,87,88] which can alter the structure of the soil matrix, reducing exposure to or contact with the extracting chemicals,[78,87,88] and has shown variable carbon yield[78,88] when compared to "wet" or *in situ* samples.

After extraction, long-term storage of SOM extracts is also preferably avoided to minimize postextraction alteration. SOM stability in the extract should be accounted for, but this is easily addressed by sterile filtration, or immediate processing to prepare the samples for FTICR MS. When the extract is aqueous, freezing can provide long-term SOM stability if the solution cannot be sterile filtered, but SOM aggregation can occur, so analysis before long-term storage is ideal.[89] The use of acidification or biocides to sterilize sample extracts for storage is also not recommended as there is evidence for alteration of SOM properties.[90,91]

After extraction, SOM often requires concentration and salt removal to make it suitable for FTICR MS analysis. This process is most commonly performed by solid phase extraction (SPE), which utilizes the difference in polarity between small, highly polar inorganic salts and the less polar SOM for separation.[92,93] As SPE cartridges use a variety of nonpolar packing material and acidic functional groups are common, retention of the SOM is assisted by adjusting the extract to around pH 2, resulting in protonation of acidic functional groups, decrease in polarity, and improvement of stationary phase binding; however acidification can cause compositional changes in the SOM.[90,94] Additionally, amino functional groups and N-heterocycles can be protonated at low pH, increasing their polarity and preference for cation exchange sorbents.[95]

There are a wide variety of SPE cartridges that retain targeted or broad molecular features and must be selected based on the design of the experiment.[96] The recovery of SOM from this method is highly variable and depends greatly on the cartridge being used, the origin of the SOM, and the elution procedure,[95,97,98] with recoveries varying between 20% and 90%.[93,99] Addressing the source of variable yields is challenging as there is often no reliable way to identify whether the retention or elution were incomplete.[31,93,100]

Alternatives to SPE include ultrafiltration (UF), electrodialysis (ED), and reverse osmosis (RO). Techniques using membranes (i.e., UF and RO) are susceptible to fouling;[101−103] however coupling these techniques with SPE can increase carbon recoveries.[104,105] When coupling SPE with RO or ED methods, Green et al.[98] reported near 100% carbon recoveries for marine DOM samples. It is unclear how the higher recovery would affect FTICR MS spectra, and the RO/ED setup costs at least twice as much to perform than SPE.[98] While the combination of methods is still subject to artifacts from procedures in both methods, the improved recovery warrants evaluation for similar performance in extracted SOM.

IONIZATION TECHNIQUES AND MATRIX EFFECTS

Characterization of SOM by any mass spectral technique requires the conversion of nonvolatile analytes from solution to gas phase ions by ionization. All soft ionization techniques are

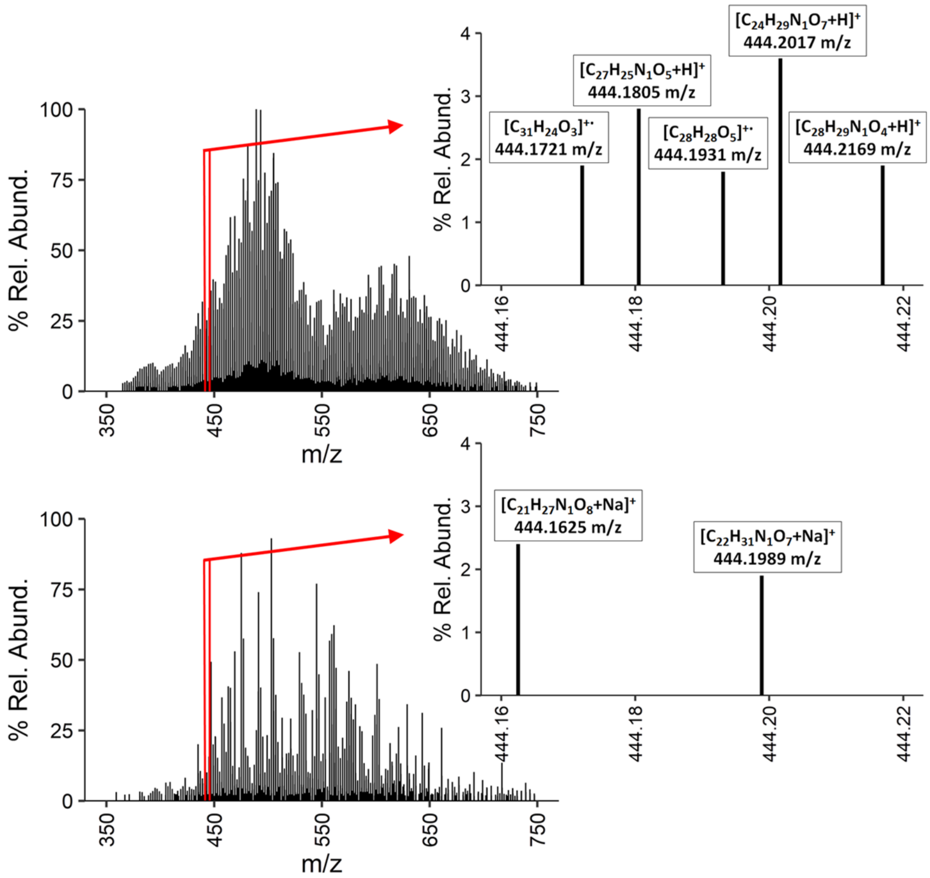

Figure 4. FTICR mass spectra of DOM from Lake Drummond (Great Dismal Swamp National Forest, VA, USA). Samples were prepared using C_{18} solid phase extraction, and spectra were obtained with atmospheric pressure ionization sources: (+)APPI (top) and (+)ESI (bottom). Spectra display peaks assigned with <1 ppm mass error, where insets indicate molecular formulas unique to each ionization source assigned over 444.16–444.22 *m*/*z*. Produced with data obtained from Hockaday et al.[107]

selective, and since OM contains a wide range of polyfunctional species across a wide molecular weight range and range of ionization potentials, ionization efficiency is determined based on the analyte composition. Different ionization mechanisms preferentially ionize certain components of OM (i.e., functional groups), enhancing their signal and suppressing the signal from other ions, since ionization efficiency depends on acidity or basicity, hydrophobicity, molecular weight, and degree of conjugation. Thus, experimental design begins with a consideration of the selectivity of each ionization technique including the potential need for using multiple techniques for a better understanding of the formulas and features present.[106−108]

Electrospray Ionization. Electrospray ionization (ESI) is the most[109] routinely applied ionization technique for WEOM components. Ionization is achieved by infusing the analyte (e.g., OM) solution through a positively or negatively charged capillary that generates a fine mist of droplets. Solvent removal, often assisted by heating, yields charged molecular ions. This process is one of the softest methods of ionization and during OM analysis is often assumed to produce intact analyte ions, although some analytes may still undergo in-source fragmentation, particularly at high spray voltages.[110−112]

Ion formation by ESI depends largely on the pK_a of the analyte as well as the ionization polarity (e.g., negative or positive mode). In negative mode, ions are generally formed by deprotonation or formation of adducts with anions such as Cl^-. Negative ionization efficiency generally correlates inversely with pK_a and the extent of charge delocalization, such as in conjugated and aromatic chemical structures.[113] Common acidic functional groups in SOM such as carboxylic acids and phenolic groups tend to form negative ions. In positive mode, ions form by protonation or adduct formation with cations such as Na^+, K^+, NH_4^+, or metal ions. Many functional groups in SOM such as amines, alcohols, and carbonyls can be protonated to form cations, and their ionization efficiency is largely correlated with basicity (conjugate acid pK_a).[114] Thus, positive and negative polarity modes may detect distinct components from a SOM mixture.

Analyte hydrophobicity and molecular size influence ESI efficiency. Hydrophobicity often correlates with higher ionization efficiencies, particularly in aqueous solutions, due to enhanced affinity for the surface of droplets where molecules have a greater probability of being desolvated and charged.[115] ESI sensitivity is often also increased when the analyte is already ionized in solution (i.e., acidic conditions for basic analytes and basic conditions for acidic analytes),[116] although specific pH effects vary depending on analyte chemistry.[117,118]

Solvent composition influences the relative solubility and droplet surface affinity for polar and nonpolar components and therefore ionization efficiency.[115,116] Novotny et al.[119] demonstrates that different solvent compositions, including neat solvents, efficiently ionize specific fractions and influence the representativeness of a sample spectra. Solvent mixtures commonly used for ESI include water, methanol, and acetonitrile and can improve ionization efficiency of target molecules[115] and reproducibility of mass spectra[120] by altering the proportion of organic solvent.

Additives, contaminants, salts, and metals can also enhance or suppress ionization by ESI (i.e., introduce matrix effects) and can also dilute feature signals across multiple adducts, discussed further below. Additives can extend the range of compositional features collected[121,122] and enhance the ionization of specific molecular features.[123] In contrast, there are numerous contaminants that can be introduced accidentally during sample collection and preparation that will suppress SOM ionization. This is especially true for surfactants, which preferentially ionize due to their amphiphilic nature and ionizable head groups (Figure S2).[81] While the bulk SOM characteristics and most ionizable species will likely still be observable, ionization suppression will limit the number of other peaks that can be detected and assigned near the limit of detection. This can dramatically influence heteroatomic assignments and peak count overall; therefore, it is crucial that materials used for sampling, filtration, and processing prior to collection and sample analysis are tested.

The use of micro- or nano-ESI reduces ion suppression from salts and other species and improves the ionization efficiency of compounds with less surface affinity, because droplet sizes are reduced and desolvation is enhanced compared to electrospray at higher flow rates.[124] However, high proportions of surfactants or other contaminants can still influence the number of SOM peaks that can be detected and assigned because it remains desirable to limit the number of trapped ions during SOM analysis to prevent adverse effects from space charging[125] (discussed below).

Ionization Techniques for Nonpolar and Water-Insoluble Soil Organic Matter. While ESI remains the most common ionization technique used for OM analysis, atmospheric pressure photoionization (APPI) offers different ionization mechanisms and selectivity. Using APPI extends the analytical window of FTICR MS to less polar analytes, evidenced by direct comparisons with ESI that display ionization of formulas with lower oxygen content and higher carbon unsaturation.[106,107,126] This will affect heteroatom abundance and peak assignments (Figure 4), as less polar nitrogen-containing compounds can be preferentially ionized using positive ion APPI,[127] yet sulfur atoms presumed in polar functional groups are diminished.[106] For positive ion APPI, radical cation ($M^{+\bullet}$) and protonated $[M + H]^{+\bullet}$ species are formed based on ionization energies of the analyte with respect to the dopant. For negative APPI, analyte ions differ based on electron affinity and include $M^{-\bullet}$, $[M - H]^-$, $[M - X + O]^-$, and $[M + A]^-$ where A is an anion (typically a halogen) and X is H, Cl, or NO_2. Developments and mechanistic discussions of APPI have recently been reviewed.[128]

The main benefit of APPI over ESI is the simultaneous formation of gas phase ions from both polar and nonpolar compounds. Other benefits include less suppression from salt and solvent effects as charge acquisition occurs differently than in ESI.[129] Comparisons of these methods for lipid analysis suggest that APPI is far more sensitive than ESI and is particularly useful for studies focusing on condensed aromatics and hydrocarbons within NOM.[130] However, the additional ionization mechanisms also increase the number of peaks in APPI compared to ESI,[107] increasing the complexity of the mass spectrum and requiring even higher mass resolving power to separate the increased number of isobaric species. The amount of sample required for APPI is also generally higher as injection flow rates can be orders of magnitude larger than in ESI.[106,107,126]

Solid phase samples can also be ionized through desorption techniques, including desorption atmospheric pressure photoionization (DAPPI), laser desorption ionization (LDI), and matrix-assisted laser desorption ionization (MALDI). These techniques allow ordinarily insoluble SOM components to be analyzed directly, such as with the application of DAPPI to pyrolyzed carbon.[131,132] Similar to APPI, LDI and MALDI have been shown to compliment ESI-FTICR MS by ionizing more conjugated and aromatic DOM constituents.[133,134] Mechanisms and substrate selection for LDI and MALDI are reviewed by Zenobi et al. and Dreisewerd.[135,136]

Importantly, while using multiple techniques will provide a larger window into the OM compositions present, it is not feasible to use every technique nor can the use of every technique unequivocally ionize every organic residue present in injected samples. Technique and polarity mode should therefore be chosen based on *a priori* knowledge of the sample and target fraction, as ionization parameters can greatly influence sample representation. Additionally, the intensity and m/z distribution are sensitive to instrumental parameters, meaning FTICR MS is not well suited for resolving controversies regarding the size distribution of SOM.

■ ANALYSIS AND INTERPRETATION OF SOIL ORGANIC MATTER USING FTICR MS

When analyzing natural organic matter (NOM), FTICR MS typically detects and resolves many thousands of peaks in the m/z range from 150 to 1000 m/z. Molecular formula assignment involves calibrating the mass spectra, computing formulas that fit each mass within the applicable mass error window, and choosing an appropriate assignment when there are conflicts using all available information (e.g., elemental content and chemical or structural relationships with other formulas).[137] Uncertainty in assignments rises dramatically with mass and the number of elements[138] as the number of possible formulas increases. While automated assignment procedures often select the formula with lowest mass error or smallest number of nonoxygen heteroatoms (i.e., atoms in the ring of a cyclic compound other than carbon or hydrogen atoms), these approaches can sometimes yield incorrect results.[139] Accordingly, various methods, including m/z vs error[140] and DBE minus O plots,[139] can be used to further evaluate dubious or conflicting formula assignments.[141] Following formula assignment, several assumptions will be present in data visualizations and should be carefully considered to avoid misinterpretations. Additional methods, both online and offline to FTICR MS, will be discussed that can provide additional insights into SOM composition, microbial decomposition pathways, and other relevant areas of study.

Mass Calibration. Molecular formula assignment relies on mass error windows, so mass calibration is a critical part of the formula assignment process.[142] External calibration cannot provide better mass accuracy than a few parts per million because the number of ions in the ICR cell varies from sample to sample.[143] As a result, complex organic mixture FTICR mass spectra benefit from internal calibration, wherein the ion cyclotron resonance frequency (f) is converted to ion mass-to-charge ratio (m/z) by fitting experimental data spanning the entire detected m/z range (e.g., multiple homologous series) to

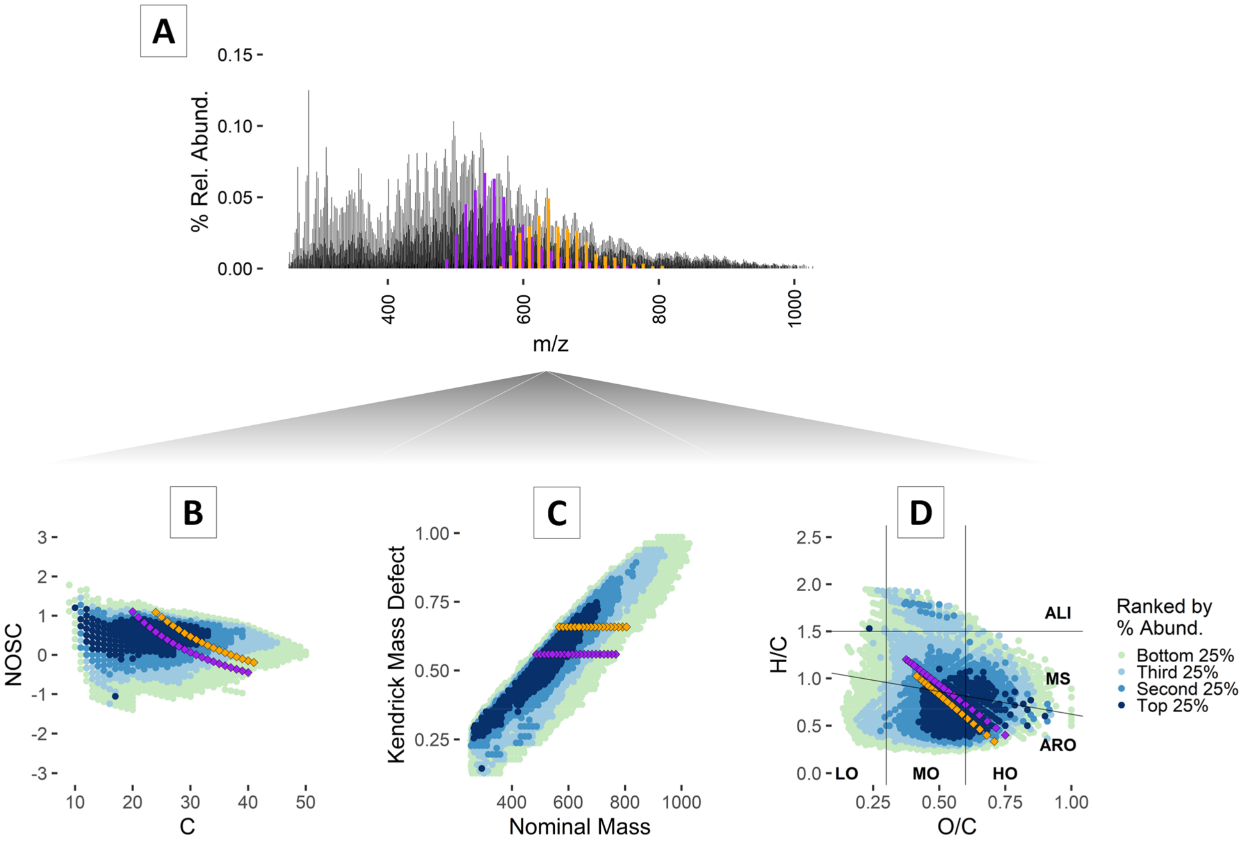

Figure 5. 21T FTICR mass spectrum of Pahokee Peat extracted with pyrophosphate with formula assignment conducted using PetroOrg software[153] to assign 15506 molecular formulas. Two series are highlighted (orange and purple) that represent CH_2 Kendrick mass defect (KMD) series (0.55857 and 0.65806) and are present in each of the plots to illustrate their appearance and patterns through different visualizations: (A) mass spectra displaying semigaussian abundance distributions, (B) nominal oxidation state carbon (NOSC),[163] (C) CH_2 KMD series over the entire mass spectrum, and (D) van Krevelen diagram in which sections plot regions indicating oxygen content (LO = low-oxygen, MO = mid-oxygen, HO = high-oxygen content) and degree of saturation (ARO = aromatic,[162] MS = midsaturated, and ALI = aliphatic[182]). Formulas are colored based on abundance quartiles. Unpublished data from the Borch Lab.

the relation $m/z = A/f + B/f_c^2$ to yield root-mean-square mass error as low as ∼10−200 ppb.[144]

Savory et al. developed a calibration protocol in which the spectrum is divided into dozens of adjoining segments, with separate calibrations applied to each, to eliminate systematic error with respect to m/z, and introduced a third calibration term to minimize systematic error with respect to ion abundance.[140] In addition, Dittmar et al. recently reported improved mass accuracy by averaging mass spectral data from independent environmental samples and increased mass precision through peak alignment during post-detection data processing.[145]

The minimum resolution requirements to adequately analyze a sample composition will change depending on signal-to-noise (S/N) thresholds, the dynamic range of the instrument measurements, digital resolution, and isotopic fine structure.[146] Ideally, the instrument and resolving power should be considered based on *a priori* information as additional constraints, such as inclusion or exclusion of specific heteroatoms, which can substantially change the minimum resolving power required.[22] Increasing magnetic field strength will increase mass resolution, dynamic range, and peak noncoalescence.[20,147] Increasing the time-domain acquisition period will also increase resolution; however if sample signal magnitude is low (i.e., sample ions are very low in abundance), instrument noise can also erroneously be assigned.[20] Increasing ion accumulation times can increase sensitivity; however high ion densities in the ICR cell can produce space charge effects that coalesce peaks or alter ion frequencies, adversely affecting both formula assignment and mass accuracy.[20,125,148,149] The limited ion capacity during FTICR MS measurement also means that intensity and m/z distributions are sensitive to instrumental parameters. Molecular weight distributions are better determined in low resolution MS systems like linear ion trap and time-of-flight that also capture lower molecular weights (<200 m/z) efficiently.[150,151] Thresholds for S/N will also strongly affect peak detection and reproducibility. Discussions by Sleighter et al.[152] indicate how S/N affects minimum common peak assignments and describe methods to evaluate meaningful differences between samples.

Automated Molecular Formula Assignment. There are a range of tools available for automated molecular formula assignment, including in-house programs (PetroOrg/EnviroOrg at the National High Magnetic Field Laboratory),[153] open-source programs (Formularity, UltraMassExplorer, and ICBM-OCEAN), and publicly available R or Matlab scripts (MFassignR, CIA, and TRFu).[137,154,155] The Compound Identification Algorithm (CIA) developed by Kujawinski et al. computes elemental compositions for low molecular weight compounds (<500 m/z), where the potential for conflicting solutions is minimal,[156] and uses a "formula extension" approach to connect the assigned formulas with higher molecular weight compounds via established chemical and functional group relationships (e.g., CH_2, $CH_4 - O$, H_2, CO_2 and NH). The existence of these relationships has also been separately established by mass difference and network analyses, discussed in section 3 of the Supporting Information.[157,158] Indeed, some version of formula extension is used in almost every formula assignment algorithm. The CIA algorithm has been incorporated into the freely available Formularity software platform, which includes an associated database and formula filters based on the "Seven Golden Rules".[159] The CIA component is restricted to C, H, N, O, S, and P, but a separate isotopic pattern algorithm (IPA) is available to identify other elements, particularly when they present a strong isotopic signature (e.g., Br, Cl, or Hg).[160]

ICBM-OCEAN is a server-based tool that integrates published and novel approaches for standardized processing of ultrahigh resolution mass spectrometry data from complex molecular mixtures.[161] Importantly, ICBM-OCEAN incorporates diagnostic and validation tools for each step in data processing, including noise and systematic error reduction and spectra recalibration and alignment, and it has been tested and applied to both FTICR MS and Orbitrap MS for more than 1000 NOM samples.

After automated formula assignment, equivocal assignments (i.e., peaks above S/N with multiple feasible compositions) will generally remain. Molecular formula assignment for equivocal assignments can be highly subjective; therefore different calculated indices and empirical evidence should be used to increase the reliability of manually distinguished assignments wherever applicable. For instance, the selection of heteroatoms during assignment can dramatically alter the number of equivocal assignments (Figure S3); however prematurely excluding elements can also result in erroneous assignments.[21] Kendrick mass defect analysis can be used to study patterns in OM assignments, discussed below. Double-bond-equivalents-minus-O (DBE-O) plots are also used that compare equivocal and unequivocal assignments using heteroatoms and oxygen content (Figure S4).[138]

Visualization and Data Analysis. Once elemental compositions have been assigned, several parameters can be calculated using the stoichiometry of the assigned molecular formulas. These include rings-plus-double-bonds equivalents (RDBE), aromaticity indices (AI_{mod}),[159,162] and the average nominal carbon oxidation state (NOSC) (Figure 5B).[163] NOSC has been used to examine chlorinated disinfection byproduct formation,[164] water solubility,[165] and the biogeochemical reactivity of SOM.[166] Indeed, NOSC makes it possible to calculate the Gibbs free energy (ΔG) provided by the oxidation of a particular compound (ΔG°_{Cox}) given the available electron acceptors and environmental conditions.[167−169] Thus, NOSC and ΔG°_{Cox} have been used to provide a thermodynamically relevant metric for approximating the energy stored in organic matter or whether microbial oxidation of OM is thermodynamically favorable under a given set of redox conditions.[170]

Kendrick mass defect (KMD) analysis[171] was initially adopted by the organic matter community as the standard means for visualizing complex mass spectra prior to formula assignment.[172−176] Kendrick plots are generated by plotting nominal mass as a function of KMD, most commonly based on a methylene (CH_2) subunit (Figure 5C). In effect, each m/z is normalized by the accurate mass of the subunit (e.g., 14/14.01565 for CH_2 subunits, common to lipid (Figure S1), sugar, and lignin derivatives[177]). Afterward, compounds whose elemental compositions only differ by the number of subunits possess the same KMD value and line up horizontally in the associated KMD plot. KMD analysis on SOM is useful for (i) molecular formula assignment by assisting in assigning molecular formulas at higher m/z ions when there are more formulas that match an exact m/z value, (ii) identifying multiply charged ions, and (iii) multiply charged polymer ions of plant origin in soils.[178−181]

Van Krevelen (VK) diagrams[183] are constructed with assigned formulas using molar ratios of hydrogen-to-carbon (H/C) on the y-axis and oxygen-to-carbon (O/C) on the x-axis (Figure 5D). Points can be colored or a z-axis can be added to provide additional information such as relative abundances, molecular weights, or elemental classes (e.g., CHO vs CHNO). VK plots are widely used to make inferences about predominant reaction pathways (e.g., methylation, hydrogenation, condensation) and to estimate the abundance of major compound categories based on the major biogeochemical components of the source material (e.g., lignin-like, lipid-like, or carbohydrate-like), which have characteristic H/C versus O/C ratios.[184−186] However, the boundaries of the compound classes vary among published studies, and often overlap. Moreover, "lignin-like" components may be derived from nonlignin source materials[187] or even reflect synthetic chemicals if anthropogenic impacts are present (Figure S5), and lignin sources may generate some non-"lignin-like" signatures.[188] Accordingly, while the major compound categories do signify molar ratios similar to well-known biogeochemical classes, they are not reliable indicators of source or structure without additional, complementary information (e.g., on the prevalent reaction pathways;[184] section 2 of the Supporting Information). Recent revisions proposed by Rivas-Ubach et al.[189] improve compound classification in biological samples using C/H/O/N/P stoichiometric ratios but were only tested on different metabolite compositions and omitted "lignin-like" and "condensed hydrocarbon" compounds that could be a major component of soil organic matter.

Regardless of the VK plot limitations, this tool can still provide useful insights into C cycling and changes in soil organic matter composition with perturbation. Users are advised to use O/C and H/C boundaries that are consistent with the chemistry of their sample and carefully interpret their results while acknowledging that these classifications are only putative. Relative abundance values of different compound classes can also be calculated from count values associated with each observed biomolecule group normalized by the total number of C molecules identified (i.e., number-weighted).[190,191] When comparing multiple samples in side-by-side comparisons or combined analyses, it is important to ensure that systematic biases (e.g., ion suppression or differences in SPE extraction efficiency) are minimized or eliminated, particularly because many SOM constituents are detected at signal intensities close to the detection limit. Molecular weight distribution, heteroatom content, constituent presence or absence, and VKD coverage can all be influenced by these low-intensity signals. Past approaches to eliminate systematic bias include extracting the same number of the most intense signals from every sample[192] or the formulas common to every sample[81,193] and then normalizing the extracted signals based on total intensity.

Other visualizations are common in the petroleum and aerosol field for rapid visual comparisons to highlight compositional differences or similarities between samples and can be beneficial to SOM studies.[163,194−196] These include (i) plotting the number of carbons in each formula versus its nominal mass (C versus M), where the molecules are classified into different categories based on their sum of carbon and oxygen atoms (i = C + O), (ii) contoured plots of RDBE vs carbon number[197] for members of a single heteroatom class, (iii) relative abundance histograms for heteroatom classes, and (iv) Kroll diagrams where NOSC is plotted as a function of number of carbons (Figure 5B).[193] Kroll diagrams are especially useful when looking at changes in SOM composition under different redox[198] conditions.

When comparing multiple samples, such as DOM from different sources, principle component analysis (PCA)[193,199] and hierarchical clustering analysis (HCA)[200] can be used to group similar samples. PCA takes advantage of the fact that many variables are correlated (e.g., H/C ratios and double bond equivalents) and produces diagrams that group similar samples and show correlations among variables. In one example, PCA was used with intensity-weighted element number, m/z, H/C, O/C, AI_{mod}, and DBE values to compare the DOM in ten world rivers, associating the intensity-weighted variables with different levels of anthropogenic influence and other watershed characteristics (Figure 6).[199]

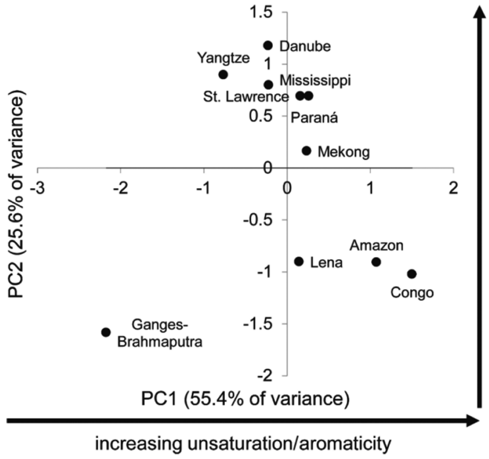

Figure 6. Ten rivers around the world were evaluated using FTICR MS and compared with PCA to identify sample groupings. The largest variance originated from the unsaturation/aromaticity and heteroatom content. Reproduced from Wagner et al.[199]

PCA is particularly useful for visualizing the relative similarities and differences of multiple samples and for identifying the variables that best explain their differences. Importantly, compositional data, such as the %CHO of a DOM sample, are generally not appropriate for PCA.[201] In comparison, HCA uses Bray−Curtis dissimilarity or another distance calculation to perform a series of partitioning calculations that group samples into clusters.[100,202,203] The result is a tree-like dendrogram, where common branches indicate greater similarity.

Analysis of NOM-Associated Metals. Mineral associated SOM and DOM are known to bind a range of metals and thus control the biogeochemical cycling, mobility, and bioavailability of both toxic metals (e.g., Hg, As, Cd) and essential mineral elements (e.g., Fe, Mn, Zn).[198,204] FTICR MS is particularly well suited for elucidating the speciation of metals and micronutrients that are complexed by or incorporated into organic molecules that influence their solubility and reactivity. In soils, these elements are often binned into operationally defined fractions based on extraction protocols[205,206] that seek to differentiate water-soluble, mineral-associated, or bioavailable content. However, understanding the origin and dynamics of these elemental pools requires knowledge of their chemical identity. FTICR MS can resolve diagnostic isotopologues and mass defects that are characteristic of many heteroatoms, including trace metals (e.g., Fe,[207−209] Zn, Cu,[210] Ni, Hg[211]) (Figure 7) and halogens (Cl, Br, I[212]), thus providing a means to identify the particular SOM components that play a role in the cycling of these elements.

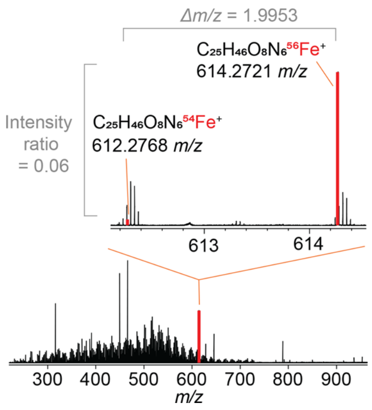

Figure 7. Mass spectra of a water extract of SOM that depicts the isotope pattern for a siderophore−Fe complex, ferrioxamine B. Inset displays the mass and abundance difference indicative of iron complexation. Unpublished data from the Boiteau Lab.

Direct infusion FTICR MS analysis, however, is not well suited to identifying metal-bound molecules in DOM due to the number of peaks that result in unrelated species that mimic mass differences and relative abundances of metals. Furthermore, molecular formula assignments become more ambiguous as heteroatoms are included in the assignment algorithm (Figure S3).[21] Boiteau et al.[207] developed a method for confident detection of metal−organic complexes by comparing direct infusion FTICR MS spectra of samples spiked with both natural and rare isotopes of metals of interest, comparing the ratio of features across samples. This was compared to another method based on separation using online HPLC−FTICR MS and detection of isotopologues with coherent elution profiles. While the isotope exchange method required significantly shorter analysis time, nearly twice as many metal−organic species were measured by HPLC−FTICR MS. This highlights how ion suppression is one of the key challenges in overcoming the detection of metal−organic species in complex mixtures.

Techniques such as liquid chromatography−inductively coupled plasma mass spectrometry (LC-ICPMS) have been used to quantify the various metal species present in DOM samples,[210] and FTICR MS and other high-resolution mass spectrometry techniques have been used to determine the molecular formulas of these compounds. This approach has been used to identify and quantify chelating agents of biological origin in soil samples and the chemical form of heavy metal or halogenated contaminants in terrestrial environments.[211] Thus, FTICR MS combined with metal quantification provides insight into the processes that govern the transport, fate, and ecological effects of nutrients and contaminants in soils by providing unprecedented information on elemental speciation.

Fragmentation, Separation, and Metabolomics. While FTICR MS analysis can attribute chemical formulas to molecules within complex SOM mixtures, identifying specific molecular structures such as metabolites or their transformation products requires pairing FTICR MS with chromatographic and fragmentation analyses. Liquid chromatographic separation prior to offline or online FTICR MS has several benefits. The separation of molecules that must otherwise compete for charge reduces the suppression of poorly ionizing molecules, reducing matrix effects and enabling the detection of a greater number of species.[213−215] In addition, separations can often resolve structural isomers that appear as a single feature with direct infusion FTICR MS (Figure 8).[216] These structural variants may have distinct sources, functional groups, and fates in soils, and thus distinguishing between isomers by LC-FTICR MS could be important to decipher processes that form, preserve, and degrade SOM. The chromatographic separation modes generally selected for these analyses are aimed at separating low molecular weight molecules within the *m*/*z* range of FTICR MS. Reverse phase liquid chromatography is typically applied to organic matter extracts, often mimicking the mode of retention used during solid phase extraction.[207,213,215−219] Hydrophilic interaction chromatography (HILIC), a variant of normal phase chromatography, is also well suited for the separation of polar components of SOM.[220,221] Both separation and ionization are strongly impacted by the choice of mobile phase. Methods commonly employ mass spectrometry grade water, alcohols, and acetonitrile along with volatile pH buffers (e.g., formic acid, acetic acid, ammonium formate, ammonium acetate, or ammonium hydroxide) due to their compatibility with ESI MS. The selectivity of different chromatographic methods for separating DOM has recently been reviewed by Sandron et al.[222]

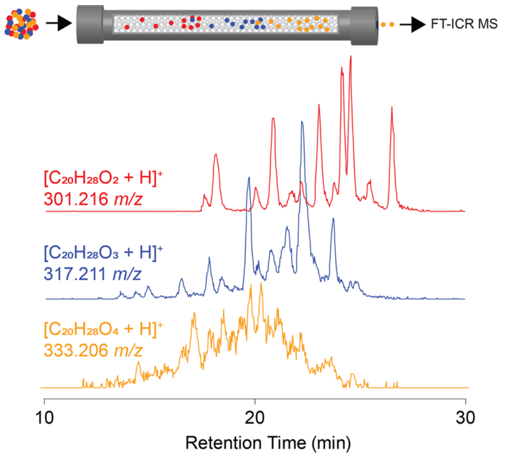

Figure 8. Extracted ion chromatograms from an LC-FTICR MS (21T) of a soil water extract. Mass ranges about each *m*/*z* were ±0.005 Da, where each trace represents the protonated monoisotopic form of the molecular formula indicated. Peaks separated for each formula represent different isomers. Unpublished data from the Boiteau Lab.

Separation also facilitates the acquisition of tandem MS/MS fragmentation spectra for individual compounds by reducing the number of ions that may appear within a single MS isolation window. Fragment analysis has been used to determine the presence of specific functional groups or structures within DOM and other complex mixtures such as carboxylic acids,[215] sulfates,[223] aromatic archipelagos,[224,225] and metal-binding moieties.[210] MS/MS spectra provide a diagnostic molecular fingerprint that can be compared to the fragmentation spectra of known molecules to facilitate structural elucidation. Libraries of MS/MS fragmentation spectra collected with collision-induced dissociation are ever-growing,[226] and developing computational tools for predicting spectra of molecular structures *in silico* is an active field of research.[227−229] Longnecker and Kujawinski[230] coupled LC FTICR MS/MS with *in silico* fragmentation computational tools on *Thalassiosira pseudonana* to study novel intracellular metabolites, finding that they were related through sulfoquinovosyl head groups. Another developing tool is feature-based molecular networking, which links MS/MS spectra by their spectral similarity, permitting the use of precursor masses and structural information from compounds with library matches to help determine the structures of spectrally similar compounds with no library matches.[231,232] Putative identifications using tools such as *in silico* fragmentation or feature-based molecular networking can be confirmed by comparing retention time and MS/MS spectrum of an authentic compound using the same analytical method used for the soil sample.

Finally, while they play a significant role in microbial growth,[233−235] essential metabolites with a molecular weight <200 Da such as acetate, pyruvate, amino acids, glucose, fructose, and succinate are typically either outside the optimum mass range of FTICR MS (i.e., 150−1000 *m*/*z*) or harder to ionize among matrix effects.[96] Gas chromatography mass spectrometry (GC-MS),[236] nuclear magnetic resonance (NMR) spectroscopy,[237,238] and liquid chromatography mass spectrometry (LC-MS)[221] are the preferred instrumentations to identify these critical small metabolites and their structure in SOM. Recently, Swenson et al.[236] used GC-MS to estimate microbial metabolite availability in soils, while Dalcin Martins et al.[238] used ^{1}H NMR to measure acetate and methanol metabolite concentrations using samples from the Prairie Pothole Wetlands in North America. Using LC-MS/MS in positive- and negative-ionization modes, Ladd et al.[221] were able to identify a wide range of compounds (e.g., amino acids, plant/microbial metabolites, sugars, lipids) present in artic soil DOM. Thus, combining multiple analytical techniques can help improve detection and annotation of central and secondary metabolites in SOM important for microbial biogeochemical cycling in soil ecosystems.[169]

Coupling mass spectrometry with microbial techniques (e.g., metaproteomics,[239] community composition profiling[238]) and novel bioinformatics approaches such as correlation networks and metabolite prediction tools[240−243] (Figure S6) can also be useful for determining microbial decomposition pathways of OM and gaining insight into relationships between bacterial communities and SOM composition.[82,240,242,244−246] Using FTICR MS, ^{1}H NMR, and community composition, Dalcin Martins et al.[238] found that extremely high sulfate reduction rates and methane fluxes in Prairie Pothole Wetlands were driven by abundant carbon substrates. Graham et al.[247] also used FTICR MS, gene expression, and community composi-

Table 1. Common Sources of Error or Bias during Sample Preparation and Analysis for FTICR MS with Aspects to Consider and Recommendations

	sources of error or bias	considerations and recommendations
sample collection and preparation	sample uniformity	ensure samples are well mixed when applicable; analyze pooled samples periodically throughout sample batches to monitor instrument stability and sensitivity
	contamination	clean sampling equipment (e.g., combusted glass or medical grade polypropylene) prior to sampling
	extraction methods	using *a priori* knowledge, prepare polar, nonpolar, and mineral-bound extracts separately to avoid biases in ionization
	extraction conditions	extraction and storage conditions (e.g., pH, temperature, volume of extractant, extraction time) should be consistent across sample sets
		artifacts (e.g., byproducts formed during extraction or storage) can be introduced from harsh conditions, so conditions should be noted in following spectra and visualizations
		solvent of choice should be compatible with FTICR MS
data collection and mass spectrometry acquisition modes	ionization method selection	method and mode should target fraction selected during extraction. For bulk SOM, multiple modes provide the largest window into SOM composition
	sample matrix/ instrument variability	quality control strategies and samples (e.g., pooled quality control and standard samples run per analysis session)
		S/N should be carefully considered based on the calibration performed (e.g., not set arbitrarily)
data preprocessing and formula assignment	molecular formula assignment pipeline	heteroatoms included during assignment should be chosen based on *a priori* information
	formula assignment quality	exercise caution and manually assign and remove known artifacts (e.g., solvent clusters, extraction byproducts) and contaminants (e.g., surfactants, solvent contaminants) in data postprocessing
		assignments should be supervised with quality control strategies (e.g., error plots, continuity in calculated indices and heteroatoms)
data visualization	data overinterpretation	data visualization approaches will influence the outcome of the results (e.g., grouping by biomolecular class approximations)
		methods using assigned formulas (e.g., VK diagrams, NOSC vs C) only approximate features and origin; approximations need support from *a priori* information, experimental design, and complementary analyses
		formula assignment should not be assumed to represent bulk SOM; only a subset of SOM residues are measured.
		not all indices are appropriate for use in statistical technique

tion to gain mechanistic insight into hyporheic zone organic matter processing. This multiomics coupled approach revealed a pronounced phenotypic plasticity in the hyporheic zone microbiome with similar microbiome structure, functional potential, and expression across sediments with dissimilar metabolic rates. Diverse nitrogenous metabolites and biochemical transformations as inferred by FTICR MS appeared to be the significant regulatory factor influencing hyporheic zone organic matter processing.

FUTURE DIRECTIONS

The strength of FTICR MS as an analytical tool lies in its unrivaled ability to detect individual organic molecules that make up the tremendously complex mixtures found in soils.[144] Despite this, challenges associated with selectivity and the numerous equivocal peak assignments ultimately limit the processing and comparison of samples between studies. The utilization of complementary techniques that reinforce and constrain FTICR MS data is often needed to fully interpret the large and complex FTICR MS data sets. The use of spectroscopy provides highly complementary data on molecular origin or structure[248] and is discussed in section 2 of the Supporting Information.

Experimental design and sample preparation are critical steps because data are mostly qualitative and poorly reproducible across laboratories even on similar samples and within the same experiment on the same instrument.[120,152,249,250] Thus, it is important to ensure standardized protocols that minimize influences on dependent and independent variables; numerous biases and considerations are summarized in Table 1. Several future developments and needs for the application of FTICR MS to SOM are discussed below.

Unified Sample Preparation. The high chemical diversity of organic compounds in SOM means there is no universal technique for complete and comprehensive extraction, and the resultant analysis and discussion should reflect the limitations of the selected technique to avoid data misrepresentation. Comparisons between studies using different preparations are therefore limited as our understanding of how each preparation alters and isolates the SOM content is still developing.[95,97,98] Characterizing the effects on SOM during preparation would aid in normalizing SOM content against artifacts from preparation, supporting more effective comparisons between data sets. Therefore, a more thorough understanding of the biases introduced during preparation is needed to develop unified sample preparation techniques specific to a target fraction. Standardized protocols for specific sample environments are critical in establishing comparable samples between laboratories and studies.

Automated Internal Calibration. While calibration strategies continue to become more refined in ultrahigh-resolution data, calibration remains dependent on instrument conditions and is subject to user input, which ultimately contributes to the poor reproducibility across laboratories.[152] Developing automated internal calibration strategies would therefore aid in the consistency and removal of bias during the analysis of FTICR MS samples, such as with the implementation of the ICBM-OCEAN[161] protocol; however procedures remain unique to different laboratories. Standardized SOM processing methods are expected to increase sample reproducibility and provide more opportunity for comparison between data sets.

Investigation of Ionization Techniques. Negative ESI remains the most commonly applied ionization polarity and technique for SOM, and other techniques, such as APPI, are capable of extending the range of ionizable features to less polar and aromatic functionalities. These techniques can be coupled to MS/MS systems for fragmentation; however collecting MS/MS spectra free from coisolated precursor fragments has remained a major challenge when fragmenting complex mixtures. Charge acquiring leaving groups such as COO− are abundant during fragmentation of SOM and result in the loss of attributable daughter ions. Structurally informative fragmentation requires the investigation of new solution-based ionization techniques and methods that employ functionality specific ionization. To this end, the development of metal cationization or solvent pH modification methods for specific precursor compounds may provide much higher specificity in the ionizable fraction.

Unified Database Repository. Due to the significant effort required to validate mass spectral features and the large number of features in soil that do not match any fragmentation spectra in databases, comprehensive identification of metabolites in soils remains a major challenge. Therefore, the implementation of a unified database repository using standardized protocols with elemental compositions for SOM data sets worldwide is of great interest for comparisons across temporal and spatial scales in environmental samples. Such repositories could be used to rapidly compare and identify peaks as potential metabolites by linking known and unknown compounds in correlation and network analysis, multiomics techniques, and fragmentation spectra similarity in targeted analysis.[251] These advances would be critical in conducting statistics using larger data sets, identifying stabilization mechanisms and pathways, and informing multiomics approaches in SOM analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c01135.

> Predicted log P and pK_a values for different biomolecules to demonstrate the variety of features in SOM, extraction methods with example conditions and target fractions, comparison of spectra indicating the effect of surfactant contaminants, the number of formula assignments as heteroatoms are included in assignment protocols, DBE vs O and DBE minus O plots to identify confident formula assignments, peaks plotted in van Krevelen plots with no relation to SOM, correlation networks grouped by pairing metabolomics with FTICR MS, discussion and figures on spectroscopic techniques that complement FTICR MS data and can be applied to SOM analysis, discussion and figure detailing the use of mass difference-based network analysis using FTICR MS (PDF)

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Notes

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