



# Application of DRIFTS, $^{13}\text{C}$ NMR, and py-MBMS to Characterize the Effects of Soil Science Oxidation Assays on Soil Organic Matter Composition in a Mollic Xerofluent

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## Abstract

Chemical oxidations are routinely employed in soil science to study soil organic matter (SOM), and their interpretation could be improved by characterizing oxidation effects on SOM composition with spectroscopy. We investigated the effects of routinely employed oxidants on SOM composition in a Mollic Xerofluent representative of intensively managed agricultural soils in the California Central Valley. Soil samples were subjected to oxidation by potassium permanganate ( $\text{KMnO}_4$ ), sodium hypochlorite ( $\text{NaOCl}$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Additionally, non-oxidized and oxidized soils were treated with hydrofluoric acid (HF) to evaluate reduction of the mineral component to improve spectroscopy of oxidation effects. Oxidized non-HF and HF-treated soils were characterized by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS),  $^{13}\text{C}$  cross polarization magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy, and pyrolysis molecular beam mass spectrometry (py-MBMS), and for particle size distribution (PSD) using laser diffractometry (LD). Across the range of soil organic carbon (OC) removed by oxidations (14–72%), aliphatic C–H stretch at  $3000\text{--}2800\text{ cm}^{-1}$  (DRIFTS) decreased with OC removal, and this trend was enhanced by HF treatment due to significant demineralization in this soil (70%). Analysis by NMR spectroscopy was feasible only after HF treatment, and did not reveal trends between OC removal and C functional groups. Pyrolysis-MBMS did not detect differences among oxidations, even after HF treatment of soils. Hydrofluoric acid entailed OC loss (13–39%), and for  $\text{H}_2\text{O}_2$  oxidized soils increased C:N and substantially decreased mean particle size. This study demonstrates the feasibility of using HF to improve characterizations of SOM composition following oxidations as practiced in soil science, in particular for DRIFTS. Since OC removal by oxidants, mineral removal by HF, and the interaction of oxidants and HF observed for this soil may differ for soils with different mineralogies, future work should examine additional soil and land use types to optimize characterizations of oxidation effects on SOM composition.

## Keywords

Diffuse reflectance infrared Fourier transform spectroscopy, DRIFTS, nuclear magnetic resonance spectroscopy, NMR, pyrolysis molecular beam mass spectrometry, py-MBMS, soil organic matter, SOM, oxidation, potassium permanganate,  $\text{KMnO}_4$ , sodium hypochlorite,  $\text{NaOCl}$ , hydrogen peroxide,  $\text{H}_2\text{O}_2$ , hydrofluoric acid, HF, laser diffractometry, LD

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## Introduction

In soil science, chemical oxidation of soil samples is routinely employed in the study of soil organic matter (SOM). Removing a component of SOM by oxidation can provide a standardized method to fractionate SOM. However, chemical oxidations may not necessarily reflect biological oxidations<sup>1</sup> nor consistently isolate SOM by age<sup>2</sup> or across sites,<sup>3</sup>

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and are increasingly considered to be limited as an operational measure of SOM.<sup>4</sup> A potential strategy to improve interpretation of oxidative fractionations is to characterize the composition of SOM removed or retained using spectroscopic methods that provide information on functional groups and molecular classes that constitute SOM.

Oxidants used to study SOM include potassium permanganate (KMnO<sub>4</sub>),<sup>5–7</sup> sodium hypochlorite (NaOCl),<sup>1,3,8–13</sup> and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>13–20</sup> An advantage of using chemical oxidations is that they offer a standardized measure of SOM and, in some systems, can serve as an indicator of soil C stabilization or predictor of nutrient mineralization.<sup>21–23</sup> The assumption that the chemical reactivity of an oxidant reflects in situ lability of the SOM removed or retained can be problematic for the interpretation of oxidative fractionations.<sup>4,15,24</sup> Assessing the structural selectivity of operational oxidations can improve their interpretation by relating the composition of SOM retained and the amount of organic carbon (OC) removed. Given that organomineral associations can exert strong controls on SOM stabilization<sup>25</sup> and their reactivity to oxidants,<sup>2</sup> combining compositional characterizations with particle size analyses can further improve understanding of SOM fractionation. For example, changes in soil particle size distribution (PSD) can be used to identify differences in organomineral associations.<sup>26,27</sup>

However, current methods for characterizing SOM composition are often challenged by interferences caused by the mineral component of soil samples. These methods include diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) for functional group composition, <sup>13</sup>C nuclear magnetic resonance spectroscopy (NMR) for carbon speciation, and pyrolysis molecular beam mass spectrometry (py-MBMS) for compound-specific fragment fingerprints. The mineral component of soils can limit the feasibility and utility of these methods for characterizing SOM composition analyses. For DRIFTS, the dominant mineral component of soil samples produces strong absorbances that can swamp out or overlap with organic bands.<sup>28,29</sup> While the mineral dominance of soil samples does not inherently pose an obstacle to NMR characterization, paramagnetic ions (e.g., Fe<sup>3+</sup>, Mn<sup>5+</sup>) interfere with magnetization to reduce spectral quality (signal intensity, broadness, and chemical shift).<sup>30,31</sup> For py-MBMS, the challenge presented by the mineral component of soil samples is twofold: (1) the mineral matrix can entrap and selectively retain pyrolysis products,<sup>32,33</sup> and (2) metals and clay minerals can catalyze side reactions during pyrolysis that may yield artifact peaks.<sup>34,35</sup>

A common strategy to minimize interference by the mineral component of characterizations of SOM composition is to treat soil samples with dilute hydrofluoric acid (HF) (1–10% v/v). By dissolving a portion of the silicate and oxide minerals and concentrating SOM, HF has been used to improve characterizations. Demineralization of soil

samples using HF is standard for NMR,<sup>36–39</sup> and has been recently used to improve characterization of SOM composition by DRIFTS<sup>40–42</sup> and py-MS.<sup>32,33,43</sup> However, HF treatment of soil samples may affect the composition and amount of SOM by disrupting organomineral associations and via DOC losses from sequential washes, respectively.<sup>3,37,44</sup> Effects of HF treatment on particle size of soil samples has yet to be characterized and may help explain observed C loss as well as identify potential effects on characterization methods sensitive to particle size distributions, including DRIFTS<sup>45–47</sup> and py-MBMS.<sup>32,33</sup>

The objectives of this study were to evaluate the potential of DRIFTS, NMR, and py-MBMS to characterize the effects of commonly employed oxidations on SOM composition and to assess the potential of demineralization with HF to improve characterizations. We evaluated SOM removal and composition in a cultivated soil of key importance in California agriculture following three commonly employed oxidations using KMnO<sub>4</sub>, NaOCl, and H<sub>2</sub>O<sub>2</sub>, with and without subsequent HF treatment. We expected oxidations to differ in their effects on SOM composition and that removal of the mineral component of soils by HF treatment would enhance characterizations of oxidation effects on SOM compositions.

## Materials and Methods

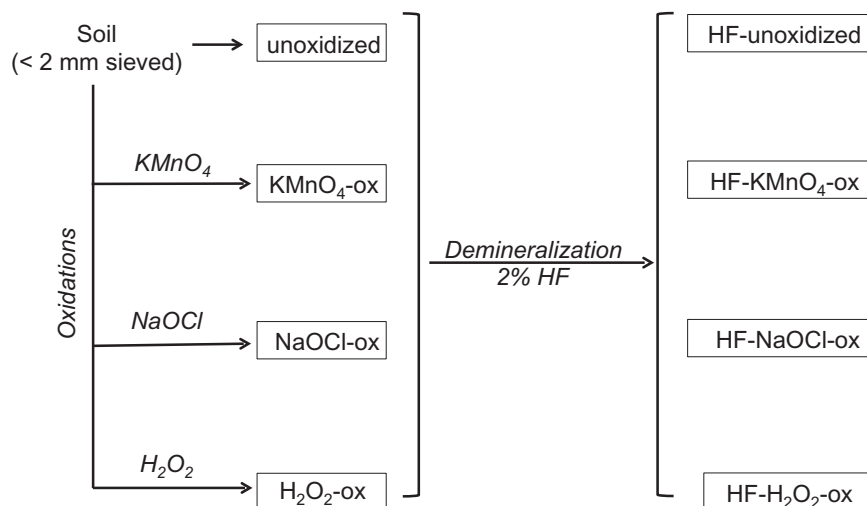
### Soil Properties

A soil of high significance to agriculture in California was used to evaluate oxidation and HF effects on SOM composition across a suite of characterization methods. The Yolo series (mixed, super-active, non-acid, thermic Mollic Xerofluvent) is highly productive for irrigation agriculture (Class I)<sup>48</sup> with an estimated extent of  $5.4 \times 10^4$  ha.<sup>49</sup> This soil type represents a family of alluvial soils used for intensive (e.g., fertilization, irrigation) annual and perennial agriculture in the Central Valley. The Yolo series is developed on fine-loamy alluvium from sedimentary deposits of mixed mineralogy,<sup>50</sup> predominantly smectite, kaolinite, mica, quartz, and montmorillonite.<sup>51</sup>

A Yolo series soil was sampled from a field in Yolo County under annual vegetable cultivation (A<sub>p</sub> horizon, 0–15 cm) as a composite (n = 6). The composite sample was sieved (<2 mm) and air-dried prior to analyses. The soil has 21.1 mg organic carbon (OC) g<sup>-1</sup> soil, pH 6.8 (1:2.5 soil:water), and silt loam texture (11% clay, 48% silt, 41% sand).

### Oxidation and Hydrofluoric Acid Treatments

Soil samples were subjected to oxidation and HF treatments as outlined in Figure 1. Three oxidations commonly employed in soil science were investigated, using KMnO<sub>4</sub>,



**Figure 1.** Schematic of chemical treatments (oxidations and demineralization) and analyses used for SOM characterization. Three oxidative treatments were performed on a Yolo series soil from an A<sub>p</sub> horizon under agricultural use (21.1 mg organic carbon g<sup>-1</sup> soil), and demineralization was performed on the unoxidized and three oxidized soils to furnish a total of eight treatments.

NaOCl, and H<sub>2</sub>O<sub>2</sub>. Oxidations were performed on separate soil samples according to standard assay conditions in soil science, in order to evaluate effects on SOM composition by oxidations as practiced. Hydrofluoric acid treatment was performed after oxidation treatments because it is meant to improve characterization of the composition of SOM following oxidations.

Oxidation by KMnO<sub>4</sub> was performed according to the method for permanganate-oxidizable C (POXC)<sup>52</sup> as modified by Culman et al.<sup>21</sup> Briefly, soil was oxidized using 0.02 mol L<sup>-1</sup> KMnO<sub>4</sub> (pH 7.2, 1:1.25 g soil:mL) with 2 min shaking followed by 10 min incubation at room temperature. A stream of distilled water was then used to remove unreacted permanganate. Hypochlorite oxidation was adapted from the method proposed by Anderson.<sup>53</sup> Soils were incubated with NaOCl (6% wt, pH 9.5, 1:5 g soil:mL) at 70 °C for 20 min. Solutions were centrifuged (15 min, 1081 RCF) and the supernatant discarded. This was repeated twice for a total of three oxidation treatments. Soils were subject to oxidation by H<sub>2</sub>O<sub>2</sub> (10% wt, 1:10 g soil:mL) by shaking (180 rpm) for 20 min at room temperature. Solutions were centrifuged (15 min, 1081 RCF) and the supernatant discarded. This was repeated twice for a total of three oxidation treatments. For each oxidation treatment, soils were washed twice with distilled water and air-dried (25 °C).

Hydrofluoric acid was used to demineralize soils according to Rumpel et al.<sup>30</sup> Unoxidized and oxidized soils (n = 4 treatments) were subjected to repeated washes of HF (2% v/v) (Fig. 1), using three replicate samples (1:10 g soil:mL). Suspensions were shaken for 2 h at room temperature, centrifuged (15 min, 1081 RCF), and the supernatant removed, for a total of five washes. Following HF treatment, samples were washed with distilled water,

centrifuged, and the supernatant discarded, for a total of five washes, then air-dried (25 °C).

Following oxidation and HF treatments (Figure 1), OC and total nitrogen (N) were determined by combustion element analysis (Costech Analytical Technologies, Inc., Valencia, CA, USA). Due to limiting analytic costs, treatment replicates were combined for NMR and py-MBMS characterizations. One-way analysis of variance (ANOVA) and Tukey's studentized range test were used to evaluate effects of oxidation and HF treatments on soil OC and C:N using PROC GLM in SAS v9.4 (SAS Institute, Cary, NC, USA).

### Particle Size Distribution

In contrast to traditional methods of particle size analysis, which provide only three size classes that are operationally defined (i.e., clay, silt, sand), PSD analysis measures particle size across a more resolved scale to enable greater sensitivity to particle size changes,<sup>54</sup> including changes following chemical oxidations.<sup>55,56</sup> Particle size distribution of samples (n = 8) were determined by laser diffractometry across 2000–0.045 μm using a LS-230 (Beckman Coulter Inc., Brea, CA, USA) with a 750 nm laser beam as described by Eshel et al.<sup>57</sup> Briefly, 0.20 g of soil were dispersed in 10 mL of 50 g L<sup>-1</sup> sodium hexametaphosphate (18 h) and transferred to a fluid module (1.7 L distilled water). Samples were sonicated for 1 min before analyses and three replicates of each sample were subjected to four consecutive 1 min runs at a pump speed of 10 L min<sup>-1</sup> with 1 min sonication between runs to prevent re-aggregation. Particle size distributions were calculated as the percent of total particle volume. Difference curves were calculated to isolate the effect of oxidations (e.g., NaOCl-ox – unox), oxidation + HF (e.g., HF-NaOCl-ox – HF-unox), and HF (e.g., HF-unox – unox) on PSD.

## Characterization of Soil Organic Matter Composition

### *Diffuse Reflectance Infrared Fourier Transform Spectroscopy.*

Soils from all oxidation and demineralization treatment combinations ( $n=8$ ) were analyzed by DRIFTS according to standard practices in soil science. Spectra were collected on finely ground ( $<200\ \mu\text{m}$ ) soil samples loaded into an aluminum well and corrected against a solid aluminum blank in ambient air as the background using a Nicolet 6700 spectrometer (Thermo Scientific) with a DTGS detector and using a diffuse reflectance accessory (Pike AutoDIFF, Pike Technologies). Soil samples were analyzed neat and with KBr dilution (1%). The use of neat samples is widespread in soil analysis<sup>28,58–61</sup> due to its advantage of minimal sample preparation and quantitative use of spectra with chemometrics, which is not necessarily improved by the use of halide salt dilution.<sup>62–64</sup> Additionally, specular reflectance that minimizes silicate peaks in spectra of neat soils can be considered an advantage when assessment of SOM composition is the objective.<sup>45</sup> Spectra were calculated as the mean of 256 scans across  $4000\text{--}500\ \text{cm}^{-1}$  at  $4\ \text{cm}^{-1}$  resolution. Absorbance spectra were expressed as  $\log(I/\text{reflectance})$  by the software OMINIC (Thermo Scientific).<sup>45,65,66</sup> Absorbance at each wavenumber was normalized as a percent of total absorbance across  $4000\text{--}500\ \text{cm}^{-1}$ . For each sample, replicate spectra ( $n=3$ ) were collected on different subsamples.

Effects of oxidation and HF treatments on SOM composition characterized by DRIFTS were evaluated with the humification index (HI), a measure of OM lability calculated as the ratio of absorbance intensity at  $1650\ \text{cm}^{-1}$  with contributions from ketone, quinone, and/or amide  $\text{C}=\text{O}$  and from aromatic  $\text{C}=\text{C}$ , to aliphatic  $\text{C}\text{--}\text{H}$  at  $2930\ \text{cm}^{-1}$ .<sup>41,67–69</sup> Oxidation treatment effects on HI were evaluated separately by HF treatment using one-way ANOVA and Tukey's studentized range test using PROC GLM in SAS v9.4. The effect of HF treatment on HI was evaluated by Wilcoxon's test and pairwise multiple comparisons using PROC NPAR1WAY.

<sup>13</sup>C Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy. Solid-state <sup>13</sup>C NMR was performed at the UC Davis NMR Facility using a Bruker Avance 500 MHz NMR spectrometer (11.75 Tesla). Paramagnetic interference in non-demineralized samples prevented spectra acquisition, so NMR spectra were only collected for HF-treated samples ( $n=4$ ). Spectra were collected using approximately 200 mg of soil sample packed in a 4-mm probe at a <sup>13</sup>C resonance frequency of 125.75 MHz spun at 15 kHz. Scan number was adjusted to maximize signal-to-noise (S/N) and consequently varied by OC content of samples: 94 424 scans for unoxidized (27.5 h), 80 012 scans for  $\text{KMnO}_4\text{-ox}$  (23 h), 95 914 scans for  $\text{NaOCl-ox}$  (28 h), 138 861 scans for  $\text{H}_2\text{O}_2\text{-ox}$  (40.5 h). A contact time of 2 ms and a recycle delay time of 1 s were used for all spectra. Free induction decay (FID) was recorded by the spectrometer and Fourier transformed to

yield a NMR spectrum. The spectra were normalized to total intensity across  $200\text{--}0\ \text{ppm}$ .

Nuclear magnetic resonance spectra were used to assess oxidation effects on SOM composition in three ways: (1) relative C speciation was estimated by integrating intensity for five regions: alkyl C ( $0\text{--}45\ \text{ppm}$ ), O-substituted alkyl C ( $45\text{--}110\ \text{ppm}$ ), C-substituted aryl C ( $110\text{--}140\ \text{ppm}$ ), O-substituted aryl C ( $140\text{--}160\ \text{ppm}$ ), and carbonyl C ( $160\text{--}200\ \text{ppm}$ );<sup>70</sup> (2) the ratio of O-alkyl to C-alkyl was calculated as a measure of OM decomposability;<sup>71</sup> and (3) interpreting resonance peaks previously identified as SOM components in soil samples.<sup>72–74</sup>

*Pyrolysis Molecular Beam Mass Spectrometry.* Pyrolysis molecular beam mass spectrometry was used to identify compound classes in pyrolyzed SOM from soil samples from all treatments ( $n=8$ ). Soil samples were analyzed as described by Magrini et al.<sup>75</sup> Briefly, 20 mg of soil sample was heated at  $550\ ^\circ\text{C}$  under helium flow to generate pyrolysis products sampled directly in real time for spectral collection across  $10\text{--}500\ m/z$  by a molecular beam mass spectrometer. Soils from all treatments were analyzed in duplicate, as well as a soil reference material ( $50\ \text{mg OC g}^{-1}$ ). This technique has the advantage of reducing pyrolysis production condensation, which is critical to avoid pyrolysis artifacts in compositionally complex samples like soils,<sup>75</sup> and the molecular beam sampling ensures that representative products are detected.<sup>76–78</sup> To enable comparison of SOM pyrolysis products, spectra were processed by normalizing intensity of each peak to total intensity, mean-centering peaks, and scaling intensity of peaks by standard deviation.<sup>79</sup>

Multivariate statistical analysis of py-MBMS spectra was used to determine distinguishing features of SOM, such as the proportion derived from carbohydrates, lignin, fatty acids, sterols, phenolics, and N-containing compounds, and identify potential patterns in molecular fragments.<sup>75,77</sup> Unscrambler (CAMO Software AS, Oslo, Norway) was used to explore py-MBMS data using multivariate approaches that enable interpretation of complex fragment patterns in OM-rich samples.<sup>75,80</sup> These included principal component analysis (PCA) to identify potential groupings of oxidation and HF treatments by detected fragments, as well as projection to latent structures (PLS) using SPSS (IBM Corp.) for discriminant classification, and discriminant function analysis (DFA) and multivariate component resolution (MCR) using Interactive Self Modeling Multivariate Analysis (ISMA).<sup>81</sup>

## Results

### *Chemical Treatments*

Oxidation treatments removed soil OC in the order  $\text{KMnO}_4 < \text{NaOCl} < \text{H}_2\text{O}_2$ , with nearly two-thirds of OC removed by  $\text{H}_2\text{O}_2$ , but did not significantly alter C:N

(Table 1). Treatment of soils with HF reduced total mass by 66–72%, enriching OC content of samples inversely with initial OC (Table 2). Losses of OC from HF treatment (13–39%) varied independently from initial OC and differed significantly among oxidation treatments, with greatest loss for unoxidized and H<sub>2</sub>O<sub>2</sub>-ox soil. HF treatment increased C:N following H<sub>2</sub>O<sub>2</sub> oxidation by ~2.5-fold relative to other oxidation treatments and the unoxidized soil.

### Changes in Particle Size Distribution

Oxidation and HF treatments caused shifts in PSD (Figure 2) and greatest changes resulted from H<sub>2</sub>O<sub>2</sub>, HF treatment, and the combination of these (Table S1, Supplemental Material). Oxidations showed small but similar shifts in particle size (Figure 2a) that corresponded to sharp changes within the medium sand fraction (450 μm and 310 μm) at the expense of silt and clay fractions (50–2 μm) (Table S1). The magnitude of these PSD shifts was greatest for H<sub>2</sub>O<sub>2</sub>, which uniquely produced an increase in the fine sand fraction (130 μm).

Hydrofluoric acid treatment of oxidized soils increased the proportion of silt- and very fine sand-sized particles

(peaking at 55 μm) (Figure 2b). For the H<sub>2</sub>O<sub>2</sub>-ox soil only, the increase in silt and very fine sand fractions following HF treatment was concurrent with a decrease in fine sand and medium sand fractions (peaking at 256 μm). In contrast, KMnO<sub>4</sub>-ox and NaOCl-ox soils exhibited a similar PSD (Figure 2a), with a relative increase in fine sand particles (450 μm) and relative decrease in very fine sand particles (340–110 μm).

Difference PSD curves calculated to isolate the effect of HF treatment demonstrated opposite trends in particle size shifts for H<sub>2</sub>O<sub>2</sub>-ox relative to unoxidized, KMnO<sub>4</sub>-ox, and NaOCl-ox soils (Figure 2c). For the H<sub>2</sub>O<sub>2</sub>-ox soil, HF treatment yielded decreases in sand fractions (650–120 μm) but not for unoxidized, KMnO<sub>4</sub>-ox, and NaOCl-ox soils. Specific to H<sub>2</sub>O<sub>2</sub> oxidation, HF treatment decreased the proportion of the sand-sized fraction (e.g., sharp decreases at 175 μm and 450 μm) and concurrently increased the silt and very fine sand fractions (15–92 μm).

### Characterization of Soil Organic Matter Composition

#### Diffuse Reflectance Infrared Fourier Transform Spectroscopy.

Diffuse reflectance infrared Fourier transform spectra showed differences in organic bands among oxidation treatments (Figure 3a) that were accentuated by HF treatment (Figure 3b). Depending on HF treatment, organic bands were more resolved in DRIFT spectra collected on neat (Figure 3) or KBr diluted soil samples (Figure S1). Increasing OC removal by oxidations corresponded to decreasing absorbance intensity of aliphatic C–H stretch (3000–2800 cm<sup>-1</sup>), which was enhanced by HF treatment (Figure 3b). The humification index (HI; 1650 cm<sup>-1</sup>:2930 cm<sup>-1</sup>) was not significantly affected by oxidation treatments ( $P = 0.58$ ), but following HF treatment the index reflected increasing soil OC removal by oxidations ( $P < 0.0001$ ), indicating lower decomposability of the SOM remaining with progressively harsher oxidation (Table 3).

Hydrofluoric acid treatment enabled additional differences in organic functional groups by oxidation treatments

**Table 1.** Carbon removal and change in C:N following oxidation treatments of a surface horizon (A<sub>p</sub>) of a Mollic Xerofluent under agricultural use.

Sample	OC (mg g <sup>-1</sup> soil)	C:N	OC removal
Unoxidized soil	21.1 ± 0.03 <sup>a</sup>	10.5 ± 0.1 <sup>a</sup>	–
KMnO <sub>4</sub> -ox	18.2 ± 0.0 <sup>b</sup>	10.0 ± 0.1 <sup>a</sup>	13.9 ± 0.8 <sup>c</sup>
NaOCl-ox	12.4 ± 0.0 <sup>c</sup>	10.5 ± 0.1 <sup>a</sup>	41.4 ± 0.7 <sup>b</sup>
H <sub>2</sub> O <sub>2</sub> -ox	6.0 ± 0.0 <sup>d</sup>	10.4 ± 0.2 <sup>a</sup>	71.8 ± 0.1 <sup>a</sup>
P-value	<0.0001	0.085	<0.0001

The letters a–d indicate significant mean differences ( $p < 0.05$ ).

OC, organic carbon; N, nitrogen; HF, hydrofluoric acid; KMnO<sub>4</sub>, potassium permanganate; NaOCl, sodium hypochlorite; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide.

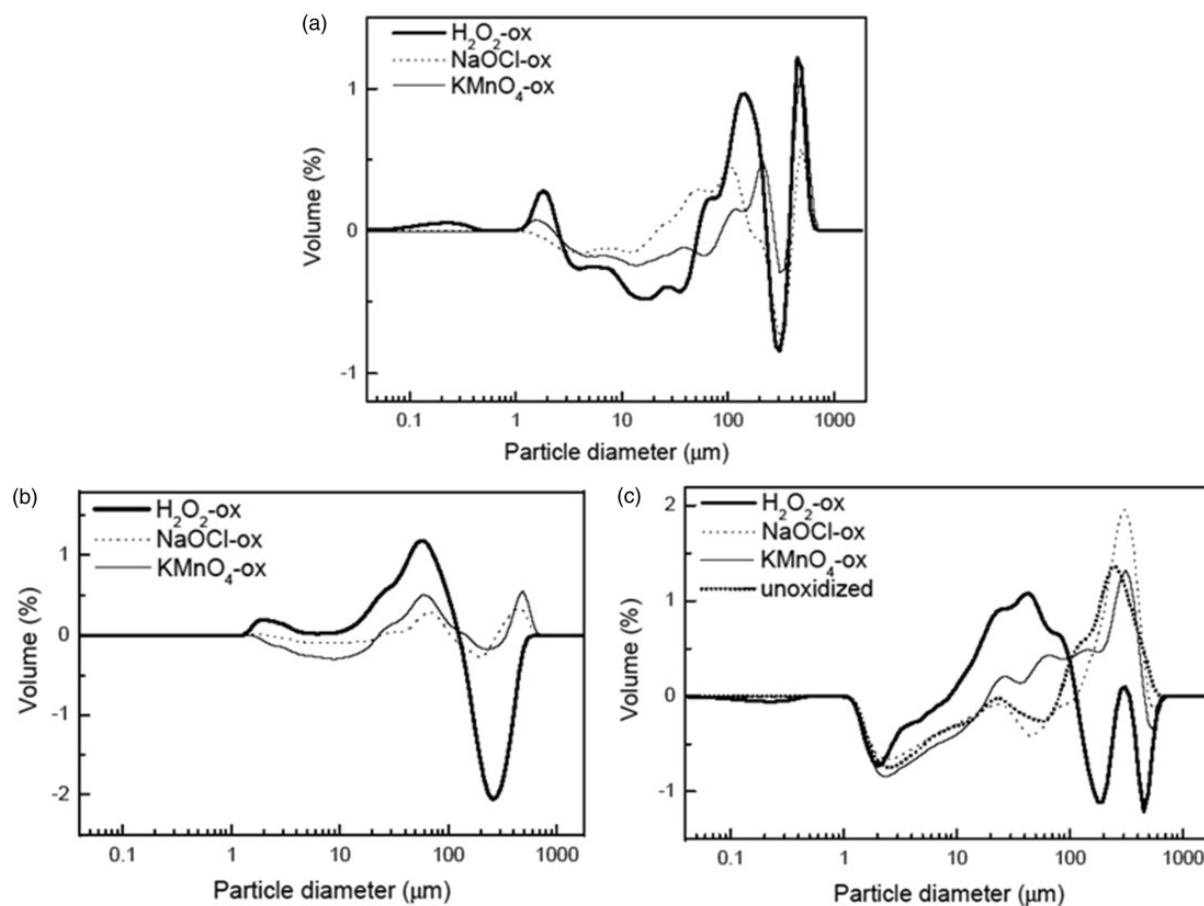
**Table 2.** Mass loss, organic carbon (OC) content, loss and enrichment, and change in C:N following demineralization (2% HF) of a surface soil previously subjected to chemical oxidations. The soil is from a surface horizon (A<sub>p</sub>) of a Mollic Xerofluent under agricultural use.

HF-treated sample	Mass loss (%)	OC (mg g <sup>-1</sup> soil)	C:N	Mean OC enrichment (%)	Mean OC loss (%)
Unoxidized	71.9 ± 2.8 <sup>a</sup>	45.8 ± 0.3 <sup>a</sup>	12.6 ± 0.2 <sup>b,c</sup>	117 ± 2 <sup>b</sup>	38.9 ± 6.6 <sup>a</sup>
KMnO <sub>4</sub> -ox	71.7 ± 3.0 <sup>a</sup>	42.2 ± 0.6 <sup>b</sup>	12.4 ± 0.1 <sup>c</sup>	132 ± 3 <sup>a,b</sup>	34.4 ± 6.1 <sup>a</sup>
NaOCl-ox	66.3 ± 0.1 <sup>a</sup>	31.9 ± 0.5 <sup>c</sup>	13.0 ± 0.1 <sup>b</sup>	158 ± 4 <sup>a</sup>	13.0 ± 1.2 <sup>b</sup>
H <sub>2</sub> O <sub>2</sub> -ox	72.0 ± 0.9 <sup>a</sup>	15.0 ± 0.7 <sup>d</sup>	32.2 ± 0.3 <sup>a</sup>	153 ± 11 <sup>a</sup>	29.4 ± 1.7 <sup>a</sup>
P value	0.240	<0.0001	0.0003	0.006	0.019

The letters a–d indicate significant mean differences ( $p < 0.05$ ).

N, nitrogen; HF, hydrofluoric acid; KMnO<sub>4</sub>, potassium permanganate; NaOCl, sodium hypochlorite; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide.



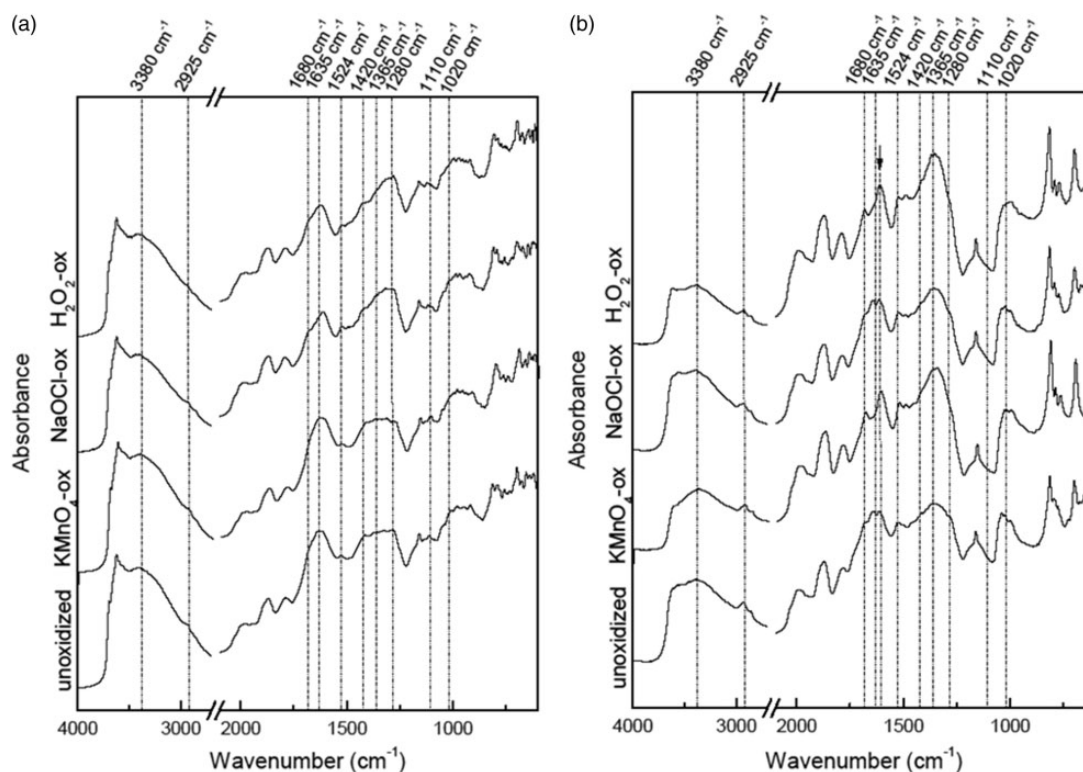


**Figure 2.** Particle size distribution (volume %) difference curves demonstrating effects of oxidation and HF treatments. Differences in PSD (a) among oxidized soils relative to the unoxidized soil, calculated by subtracting the PSD of the unoxidized soil from the PSD of the oxidized soil, (b) among oxidized treatments following HF treatment relative to the unoxidized soil, calculated by subtracting the PSD of the unoxidized, HF-treated soil from the PSD of the oxidized, HF-treated soil, and (c) the effect of HF treatment on changes in particle size distribution among oxidation treatments, calculated by subtracting PSD of non-HF-treated samples from the PSD of HF-treated samples.

to be identified by DRIFTS. For example, in the region corresponding to aromatic C=C, ketone and quinone C=O, and amide C=O and C=N (1680–1520 cm<sup>-1</sup>), HF treatment increased sharpness of peaks at 1680 cm<sup>-1</sup> and 1635 cm<sup>-1</sup> in unoxidized and KMnO<sub>4</sub>-ox soil, and revealed a peak loss at 1635 cm<sup>-1</sup> from NaOCl and H<sub>2</sub>O<sub>2</sub> oxidation and the presence of a peak at 1615 cm<sup>-1</sup> across oxidation treatments. Significant demineralization by HF for this soil (70% mineral removal; Table 2) corresponded to a decrease in absorbance of mineral moieties such as phyllosilicate OH (3700–3620 cm<sup>-1</sup>), but increased absorbance of recalcitrant mineral components such as quartz Si–O (2000–1780, 1159 cm<sup>-1</sup>). Definitive assignment of organic bands 1800–1000 cm<sup>-1</sup> is challenged by artifacts arising from specular reflection. The presence of less strongly absorbing mineral Si–O bands 2000–1780 cm<sup>-1</sup> in spectra indicates the likely contribution of specular reflection for the more strongly absorbing Si–O stretch 1150–950 cm<sup>-1</sup>, even after HF treatment.

<sup>13</sup>C Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy. Across oxidation treatments, NMR spectra identified a similar signal intensity from alkyl C, which represented the most common C species, and least similarities for aryl and carbonyl C (Figure 4). Oxidations generally decreased alkyl C and increased O-alkyl C, but total alkyl signal (alkyl C + O-alkyl C) was highly similar across oxidation treatments (67.0–67.7% of total intensity), and the O-alkyl:alkyl C ratio did not reflect oxidative removal of OC (unoxidized, 1.24; KMnO<sub>4</sub>, 2.00; NaOCl, 1.29; H<sub>2</sub>O<sub>2</sub>, 2.09). Hydrogen peroxide and KMnO<sub>4</sub> produced greatest changes in C speciation, such as increased O-aryl C (+22%) and decreased carbonyl C (–37%) following H<sub>2</sub>O<sub>2</sub> oxidation (Table 4). Least changes in C speciation resulted from NaOCl oxidation.

Differences in specific NMR peaks indicate selective removal and preservation of compounds by oxidations. For example, H<sub>2</sub>O<sub>2</sub> removed the peak at 173 ppm present in other treatments, which corresponds to amide and



**Figure 3.** Diffuse reflectance infrared Fourier transform spectra of an agricultural soil subjected to different chemical oxidations routinely employed in soil science: (a) unoxidized soil and three oxidation treatment soils; and (b) HF-treated unoxidized soil and three oxidation treatment soils. Spectra were collected on neat samples. Bands representing organic matter functionalities include O–H and N–H at  $3380\text{ cm}^{-1}$ , aliphatic C–H at  $2925\text{ cm}^{-1}$ , carbonyl C=O at  $1680\text{ cm}^{-1}$ , amide C=O and/or aromatic C=C at  $1635\text{ cm}^{-1}$  and  $1524\text{ cm}^{-1}$ , aliphatic C–H at  $1420$  and  $1365\text{ cm}^{-1}$ , phenol and carboxyl C–O at  $1280\text{ cm}^{-1}$ , and polysaccharide C–O at  $1110\text{ cm}^{-1}$  and  $1020\text{ cm}^{-1}$ .

**Table 3.** Effect of oxidations and HF treatment on the humification index of the  $A_p$  horizon of a Mollic Xerofluvent determined using DRIFTS.

Oxidation	HF treatment	Humification index ( $1650\text{ cm}^{-1} : 2930\text{ cm}^{-1}$ )
Unoxidized	No	$1.062 \pm 0.012^a$
$\text{KMnO}_4$	No	$1.053 \pm 0.012^a$
$\text{NaOCl}$	No	$1.082 \pm 0.015^a$
$\text{H}_2\text{O}_2$	No	$1.067 \pm 0.018^a$
Unoxidized	Yes	$1.348 \pm 0.003^b$
$\text{KMnO}_4$	Yes	$1.370 \pm 0.003^c$
$\text{NaOCl}$	Yes	$1.365 \pm 0.001^c$
$\text{H}_2\text{O}_2$	Yes	$1.391 \pm 0.002^a$

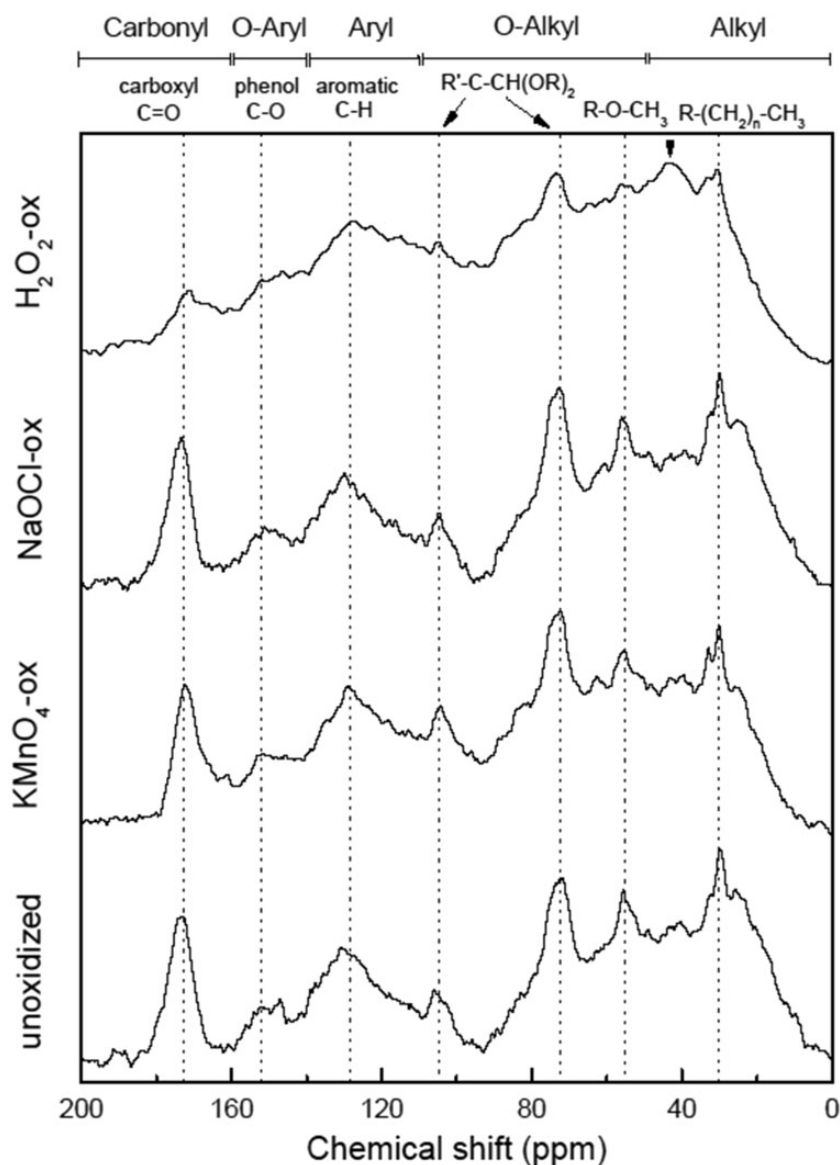
The letters a–c indicate significant mean differences ( $p < 0.05$ ).

HF, hydrofluoric acid;  $\text{KMnO}_4$ , potassium permanganate;  $\text{NaOCl}$ , sodium hypochlorite;  $\text{H}_2\text{O}_2$ , hydrogen peroxide.

carboxyl C=O<sup>73</sup> and can be attributed to lignin.<sup>13</sup> Additional chemical shifts at 56, 130, and 150 ppm associated with lignin<sup>73,82</sup> were similarly present following oxidation with  $\text{KMnO}_4$  and  $\text{NaOCl}$  but not with  $\text{H}_2\text{O}_2$ , which

produced a peak at 40 ppm corresponding to unsubstituted alkyl C.<sup>83</sup> The peak at 75 ppm can be assigned to hexose ring<sup>84</sup> (i.e., carbohydrates) and mid-chain methylene C, analogous to the DRIFTS aliphatic C–H stretch, is observable at 20–50 ppm with a distinct peak at 30 ppm for polymethylene.

*Pyrolysis Molecular Beam Mass Spectrometry.* Multivariate statistical analyses did not identify patterns or groupings for oxidation and HF treatments in SOM fragments detected by py-MBMS. Hydrofluoric acid treatment produced a significant increase in py-MBMS signal intensity. Additionally, a representative difference spectrum highlighting HF treatment effects on unoxidized soil (Figure S2) indicated that HF caused a relative increase in the detection of fragment markers representing undecomposed biomass such as carbohydrates ( $m/z$  60, 73, 85, 98, 126) and the monomethoxy signature of lignin ( $m/z$  124, 128, 150, 178), and a relative decrease in fragment markers of more decomposed organic matter such as aromatic hydrocarbons ( $m/z$  78, 92, 106, 120, 134) and N-aromatics like pyrrole ( $m/z$  67) and indole ( $m/z$  117).



**Figure 4.**  $^{13}\text{C}$ -Carbon cross polarization magnetic angle spinning nuclear magnetic resonance spectra of an agricultural soil (unoxidized) and resulting soils following separate treatment by three different oxidations. Ranges of chemical shifts representing five carbon types (alkyl, O-alkyl, aryl, O-aryl, and carbonyl) are indicated, as well as specific chemical shifts associated with lignin and carbohydrates corresponding to polymethylene C (30 ppm), methoxy C, and potentially  $\alpha$ -amino C (56 ppm), saccharide anomeric C (75 ppm) and ring C (110 ppm), aromatic C–H (130 ppm), phenol C–O (156 ppm), and carboxyl C=O (173 ppm). A peak specific to the  $\text{H}_2\text{O}_2$ -ox soil is indicated at 40 ppm. All samples were treated with HF (2% v/v) prior to NMR spectroscopy.

## Discussion

### Quantity versus Quality: Oxidation Effects on Soil Organic Matter

Differences in SOM composition following oxidations support the hypothesized selective removal of SOM by different oxidations commonly employed in soil science. Across the wide range of soil OC removed (14–72%) by the three oxidations investigated, soil C:N did not change,

but changes in SOM composition were detected using DRIFTS. Application of HF treatment enhanced the trend of decreasing aliphatic C–H with increasing OC removal, and enabled NMR, which was able to identify oxidation-specific effects on composition such as preferential removal of lignin by  $\text{H}_2\text{O}_2$ . This underscores the importance of measures of SOM beyond mass balance (e.g., %C removed) to understand oxidative fractionations.

Changes in SOM composition induced by oxidations were not necessarily consistent across characterization



**Table 4.** Relative abundance of C types identified by  $^{13}\text{C}$  CP-MAS NMR spectroscopy for an unoxidized soil and its treatment by different oxidants. All samples were demineralized prior to NMR spectroscopy using 2% HF. The soil is from a surface horizon ( $A_p$ ) of a Mollic Xerofluvent under agricultural use.

Range (ppm)	Assignment	Unoxidized	KMnO <sub>4</sub>	NaOCl	H <sub>2</sub> O <sub>2</sub>
Abundance of C types (%)					
0–45	alkyl C	30.2	22.3	29.5	21.9
45–110	O-alkyl C	37.3	44.7	38.0	45.8
110–140	aryl C	15.6	17.4	15.1	18.1
140–160	O-aryl C	6.2	6.8	6.6	7.6
160–200	carbonyl C	10.6	8.7	10.8	6.6
Change in abundance of C types, relative to untreated soil (%)					
0–45	alkyl C	–	–26.2	–2.5	–27.7
45–110	O-alkyl C	–	+19.8	+1.9	+22.7
110–140	aryl C	–	+11.5	–3.5	+15.9
140–160	O-aryl C	–	+9.1	+6.1	+21.9
160–200	carbonyl C	–	–17.5	+2.0	–37.4

C, carbon; HF, hydrofluoric acid;  $^{13}\text{C}$  CP-MAS NMR,  $^{13}\text{C}$  cross-polarization magnetic angle spinning nuclear magnetic resonance; KMnO<sub>4</sub>, potassium permanganate; NaOCl, sodium hypochlorite; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide.

methods, because methods provide information on different units of SOM (e.g., polar bonds in DRIFTS versus pyrolysis fragments in py-MBMS) and have varying sensitivity to changes in SOM composition.<sup>30</sup> For example, the decrease in aliphatic C–H stretch in DRIFT spectra with increasing removal of soil OC by oxidations was not detectable as an analogous trend in aliphatic C species in NMR spectra (e.g., C-alkyl, O-alkyl, total alkyl, or polymethylene C). This highlights a trade-off between complementarity and corroboration of SOM composition among characterization methods that provide information on different measures of organic matter composition.

### Contextualizing Oxidation and Hydrofluoric Acid Treatments with Particle Size Changes

This study is the first to evaluate particle size effects of oxidation and HF treatments in tandem with spectroscopic characterizations. The PSD changes following oxidations across a range of SOM removal represents a potential physical artifact of oxidations, which should be considered for post-oxidation analyses sensitive to matrix or particle size effects such as DRIFTS.<sup>45–47</sup> Coupling PSD analyses and oxidations across a range of soil and land use types could be used to identify oxidations' preservation or removal of organomineral associations considered to represent more functional pools of SOM.<sup>2,4</sup> The convergence of PSD of soil samples after HF treatment toward smaller sizes (< 110  $\mu\text{m}$  diameter) could improve comparability of particle size-sensitive analyses, and for DRIFTS could reduce scattering effects that commonly occur in samples with > 100  $\mu\text{m}$  diameter particles.<sup>46,85</sup> In addition to concentrating SOM and reducing mineral interference, the increased fineness of

soil sample texture may contribute to improved quality of DRIFT spectra following HF treatment.<sup>40,86</sup>

### Hydrofluoric Acid Effects on Compositional Characterizations

Hydrofluoric acid treatment enabled NMR analysis and improved DRIFTS detection of oxidation effects on SOM composition, supporting the hypothesis that demineralization with HF can benefit application of these analyses to understand oxidations used in the study of SOM. This reflects significant demineralization (70%) achieved by HF for the investigated Mollic Xerofluvent. Though the increased sensitivity in py-MBMS (greater signal) did not improve identification of oxidation effects on SOM composition, it did improve DRIFTS characterization. For example, though the aliphatic C–H stretch at 3000–2800  $\text{cm}^{-1}$  is considered one of the most mineral overlap-free organic bands in DRIFT spectra of soils,<sup>87</sup> reduction of the mineral component with HF enhanced the trend of decreasing aliphatic C–H absorbance with increasing oxidative removal of OC. By reducing absorbance of overlapping mineral bands, HF treatment also accentuated peaks in the organic fingerprint region (1600–1000  $\text{cm}^{-1}$ ) and revealed additional peaks corresponding to aromatic and amide functional groups at 1680–1520  $\text{cm}^{-1}$ , which was also observed for four soils ranging widely in mineralogy (e.g., Ferralsol, Luvisol).<sup>40</sup> Increased sensitivity of DRIFTS to changes in organic functional groups is consistent with the use of HF to improve spectral subtractions,<sup>86</sup> and indicates strong potential of demineralization to extend the utility of DRIFTS for SOM composition characterizations. Comparison of DRIFT spectra collected on neat and KBr

diluted soil samples confirms suggestions that specular reflection of silicate minerals in the former can enhance resolution of organic bands.<sup>45</sup> On the other hand, greater resolution of the mineral bands in DRIFT spectra of KBr diluted samples more clearly identified changes in the mineral component due to HF treatment. Nonetheless, interpretation of organic bands in spectra collected on soils with and without HF treatment should be performed with caution given specular reflectance by Si–O stretch bands, which can distort spectral features (e.g., inversions) and compromise the quantitative relationship between band intensity and concentration.<sup>29,46,87,88</sup>

Artifacts of HF treatment for this soil, however, may compromise interpretation of oxidation effects on SOM composition. Loss of OC by HF treatment makes it difficult to determine whether changes (DRIFTS) or lack thereof (py-MBMS) in SOM composition reflect oxidations, or fractionation by HF. Loss of OC in the studied Mollic Xerofluvent (13–39%) is within the range reported by others for HF treatment, though high variation within and among soil types are reported, e.g., 12–67% for a Spodosol<sup>36</sup> and 0–85% for an Oxisol.<sup>44</sup> Loss of SOM has been proposed to reflect the number of washes, rather than HF concentration,<sup>39</sup> or initial OC content, mineralogy, and soil texture.<sup>44</sup>

In the studied Mollic Xerofluvent, stoichiometric and compositional changes suggest selectivity in OC loss during HF treatment, which as previous studies have shown can affect characterization using MS and FT-IR, but generally not NMR.<sup>3,30,36,44</sup> Relative enrichment in markers of undecomposed biomass (py-MBMS) as a result of HF treatment are consistent with dissolution of organomineral associations, which can stabilize chemically labile OM.<sup>32,89</sup> These results agree with Rumpel et al.,<sup>30</sup> who found from py-GCMS analyses that non-cellulosic saccharide content tended to increase slightly after HF treatment. Improvement of MS sensitivity to differences in SOM composition by HF treatment has been found to enable discrimination of low OC soils by management.<sup>32</sup> For the low OC soil in this study, HF treatment did not enable py-MBMS to detect differences in SOM composition among oxidations, despite differences identified using DRIFTS and NMR. This may reflect the coarse texture and/or relatively low OC content of the studied agricultural soil, as greatest improvements in MS are observed for high OC (forest O and A horizons) or finely-textured soil samples.<sup>32</sup>

Mass spectrometry characterizations in this and other investigations demonstrate a strong effect of HF on SOM composition,<sup>3,30</sup> which for the studied soil overshadowed effects of oxidations. Demineralization can increase detection of mineral-associated SOM, supported by the observed increase in signal intensity and shift in SOM composition characterized py-MBMS. HF treatment is also used to fractionate mineral-associated OM in soils and sediments (e.g., “HF-resistant OM”).<sup>3,90</sup> The apparent contradiction between these two applications of HF in soil samples is

driven by the practical benefit of enabling NMR of bulk soils, because changes in SOM are typically not detectable at the level of C speciation provided by <sup>13</sup>C NMR.<sup>30,91</sup>

### *Potential Oxidant-Specific Interactions of Oxidations and Hydrofluoric Acid*

Variations in soil particle size distribution implicate organomineral associations in the interaction of HF and H<sub>2</sub>O<sub>2</sub> on OC loss and SOM composition. Hydrogen peroxide can selectively oxidize OM in the silt- and clay-sized (<53 μm) fraction,<sup>92</sup> with increases in clay-sized particles as a result of H<sub>2</sub>O<sub>2</sub> removing clay-associated OM.<sup>93</sup> N-enrichment of clay-associated OM,<sup>94,95</sup> and preferential removal of clay-associated OM by H<sub>2</sub>O<sub>2</sub>, is consistent with changes in C:N and functional groups (DRIFTS) in this and other studies.<sup>15,96</sup>

Disruption of organomineral associations by H<sub>2</sub>O<sub>2</sub> may explain the stronger effect of HF on decreasing particle size and increasing C:N following H<sub>2</sub>O<sub>2</sub> oxidation in this soil. This interpretation is complicated by the potentially strong influence of soil properties on H<sub>2</sub>O<sub>2</sub> oxidation of OM. Hydrogen peroxide reactivity in soils involves three potential pathways that can become individually dominant depending on the amount of metals (e.g., Mn), enzymes (e.g., catalase), and OM content,<sup>97</sup> as well as pH influence on oxidation.<sup>92</sup>

### **Conclusion**

Chemical oxidations are routinely employed in soil science to study SOM and their interpretation could be improved by understanding oxidation effects on SOM composition. Using a Mollic Xerofluvent representative of intensively managed agricultural soils in the California Central Valley, this study demonstrates the potential of applying DRIFTS, NMR, and py-MBMS to identify changes in SOM composition following oxidations using KMnO<sub>4</sub>, NaOCl, and H<sub>2</sub>O<sub>2</sub>. Extending this approach to additional soils and oxidations could be used to identify potential relationships between SOM oxidative removal and composition across factors influencing SOM stability, such as mineralogy and land use. This study also demonstrates the feasibility of using HF to improve characterizations of SOM composition following oxidations as practiced in soil science, in particular for DRIFTS. Across the range of soil OC removed by oxidations (14–72%), aliphatic C–H stretch at 3000–2800 cm<sup>-1</sup> (DRIFTS) decreased with OC removal, and this trend was enhanced by HF treatment due to significant demineralization in this soil (70%). This improves the favorability of DRIFTS as a complement to NMR because HF treatment of soil samples is generally necessary to enable NMR characterization, as for this soil. Despite previous findings of HF improving MS characterization, HF treatment did not improve py-MBMS detection of differences among

oxidations. Hydrofluoric acid treatment entailed OC loss (13–39%), and for H<sub>2</sub>O<sub>2</sub> oxidized soils increased C:N and decreased mean particle size, which could affect spectral analyses and compromises interpretation of SOM composition. Oxidant-HF interactions may differ among soil types due to the significant role of mineralogy in the amount and stability of organomineral complexes. Minimizing mineral interference and OC loss should be assessed across additional soil and land use types to optimize the utility of HF treatment to improve characterizations of oxidation effects on SOM composition.

### Conflict of Interest

The authors report there are no conflicts of interest.

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### Supplemental Material

All supplemental material mentioned in the text, consisting of one table and two figures, is available in the online version of the journal, at <http://journals.sagepub.com/doi/suppl/10.1177/0003702817691776>.

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