Limitations and Potential of Spectral Subtractions in Fourier-Transform Infrared Spectroscopy of Soil Samples

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Soil science research is increasingly applying Fourier transform infrared (FTIR) spectroscopy for analysis of soil organic matter (SOM). However, the compositional complexity of soils and the dominance of the mineral component can limit spectroscopic resolution of SOM and other minor components. The use of spectral subtraction is an established technique for isolating specific components of multicomponent spectra, and as such is a powerful tool to improve and expand the potential of spectroscopy of soil samples. To maximize the utility of spectral subtraction and avoid its misuse, consideration must be given to the general and soil-specific limitations of FTIR spectroscopy and subtractions, as well as specific experimental objectives. This review summarizes the history of subtractions in FTIR spectroscopy of soil samples, identifies and qualifies misconceptions and limitations on its use, and highlights trade-offs, recent developments, and future directions on the potential of spectral subtractions to improve and expand FTIR applications in soil science.

Abbreviations: ATR, attenuated total reflectance; DFT, density functional theory; DRIFT, diffuse reflectance infrared Fourier transform; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared; HS, humic substance; IR, infrared; LTA, low-temperature ashing; MD, molecular dynamics; MID, mid-infrared; MO, molecular orbital; OM, organic matter; PLS, partial least squares; QCC, quantum chemical calculations; SOC, soil organic carbon; SOM, soil organic matter; TG, thermogravimetry.

FOURIER TRANSFORM INFRARED SPECTROSCOPY IN SOILS

Fourier transform infrared spectroscopy is increasingly employed in soil science because of its versatility, rapidity of measurement and relative low cost, and sensitivity to mineral and organic bonds that compose soil minerals and organic matter (OM; e.g., Si-O, C-O, C=C, C-N; Nocita et al., 2015; Parikh et al., 2014a). To improve the sensitivity and expand the utility of FTIR spectroscopy, spectral subtractions can be performed to remove overlapping absorbances (e.g., mineral) and/or enhance absorbance bands of interest (e.g., organic; Smith, 2011), as well as highlight differences among samples due to treatments or across time. The complexity of soils as mixtures of mineral and organic components entails a high potential for spectral subtractions to improve FTIR spectroscopy in these sample types, but also engenders constraints on the validity and interpretation of resulting subtractions. Spectral subtractions further the versatility of FTIR spectroscopy as

Core Ideas

- Spectral subtractions are a tool for improving FTIR spectroscopy.

- Minor components of soil spectra such as SOM can be resolved by subtracting interfering backgrounds (e.g., minerals).

- Limitations to spectral subtractions are specific to both FTIR spectroscopy and soils.

- There is strong potential to improve accuracy and applications of subtractions in soil spectra.

one component of the soil scientist's toolbox for characterizing SOM (e.g., nuclear magnetic resonance spectroscopy, mass spectrometry, Raman spectroscopy).

This review summarizes the history of subtractions in FTIR spectroscopy of soil samples, identifies advantages and disadvantages of subtractions, and highlights trade-offs and future directions for the use of subtractions to improve its application in soil science. The focus is on the application of subtractions to enhance characterization of SOM, because this is the most common use of subtractions in soil samples. We suggest that there exist proper circumstances, objectives, and methods for the use of subtractions in FTIR spectroscopy, which can improve its ability to characterize OM composition and interactions (e.g., organomineral) in soils. For a review of theory and principles of FTIR spectroscopy and its potential applications for soil samples, the reader is referred to Parikh et al. (2014a).

What are Subtractions for?

The purpose of subtractions is to improve detection at a target wavenumber, rather than guaranteeing full subtraction of all potentially interfering absorbances (Hirschfeld, 1987; Joussein et al., 2005; Smith, 2011; Weis and Ewing, 1998). The use of subtractions to improve FTIR characterization of SOM is operational, and like any other operational technique (e.g., chemical fractionations of SOM) is intended to provide a repeatable measure, specifically the relative abundance of functional groups that constitute SOM. Accordingly, subtractions may be more effective for certain ranges of the mid-infrared (MIR) spectrum. For example, Nguyen et al. (1991) noted that subtractions would be most useful to enhance organic bands within 2000 to 1600 cm^{-1}, which Reeves (2012) identifies as a spectral region in which accurate subtractions are possible, even if there are artifacts from an ashed soil background. Silica absorbances (Si-O) in this region subtract accurately, in contrast to absorbances by minerals (e.g., phyllosilicates, metal oxides) in other regions (e.g., 1000–400 cm^{-1}), and particularly silicate Si-O at 1100 to 980 cm^{-1} (Reeves, 2012) due to strong absorbance and inversions (Nguyen et al., 1991). This illustrates an advantage to spectral subtractions over alternative methods such as hydrofluoric acid (HF) demineralization to reduce mineral absorbances in FTIR spectra, because HF is less able to remove sand-size quartz (Almeida et al., 2012).

Spectral subtractions for FTIR data of soil samples is often perceived as perplexing and/or convoluted because of differing perceptions on the objectives of its use. This tends to reflect a gap between how FTIR spectroscopy has been traditionally used for chemically simple samples (e.g., pharmaceuticals), and limitations on its use because of the inherent heterogeneity of soil samples. Furthermore, general limitations to FTIR spectroscopy in soils are often conflated with limitations specific to spectral subtractions. As pointed out by Painter et al. (2012), one of the first to apply FTIR and subtractions to coal samples (Painter et al., 1978, 1980), the limitations of FTIR spectroscopy have remained largely unchanged. Application of FTIR to new con-

texts, in particular more complex samples like soils, can make these inherent limitations increasingly apparent. As a result, Smith (2011) contends that spectroscopists must use a combination of experience and "common sense" to address the limitations presented by its application to new sample types—such as utilizing FTIR spectroscopy for analysis of soil samples in recent decades, including spectral subtractions.

History of Spectral Subtraction in Soil Science

Subtractions have been used to improve FTIR characterization of soil samples since the mid-20th century (Table 1). The most common application of subtractions is to improve characterization of OM in extracts, fractions, and bulk soils. Other uses of subtractions include highlighting binding mechanisms of sorbates like organic molecules, ions, and anthropogenic compounds (e.g., agrochemicals) with minerals and soils. Schnitzer and Desjardins (1965) first used spectral subtractions to remove mineral bands in spectra of peat soils using acid treatments. Coals, like soils, can have significant amounts of silicates, quartz, and carbonates. Painter and coworkers employed subtractions to resolve OM absorbances in coal samples, using both pure minerals and empirically derived mineral backgrounds via ashing (Painter et al., 1978, 1980). Nguyen et al. (1991) and Janik et al. (1998) proposed the necessity of subtractions for soil spectra to resolve organic bands at 2000 to 1600 cm^{-1}, a region of co-absorbance by silicates (Si-O). This work was followed by numerous studies using mineral subtractions to better resolve organic bands in aqueous soil extracts containing minor mineral components (e.g., Chefetz et al., 1998). Mineral backgrounds were obtained by ashing (550°C) of the (lyophilized) extract, followed by subtraction of the original unashed (DOM [dissolved organic matter] + mineral) and ashed (mineral) sample. Beginning in the 2000s, this method was used to characterize relative differences in SOM composition in more mineral dominated samples, namely bulk soils (Fig. 1). Recently, potential artifacts in subtractions using ashed backgrounds have drawn attention. Since ashing can alter mineral structure (e.g., dehydroxylation, dehydration, interlayer and lattice collapse; Frost and Vassallo, 1996), this can produce changes in mineral absorbances, which when used as a background can yield inaccuracies in the resulting subtraction spectrum. As a result, the use of alternative methods such as low-temperature ashing (LTA) and chemical oxidations has increased in the last decade.

Misconceptions of Subtractions in Soil Samples
Non-Quantitative Absorbance

The ability of FTIR spectroscopy to provide quantitative information is limited by IR absorbance data, which is attributable to sample concentration and composition (e.g., overlap of absorbance bands). Because this issue is incorrectly conflated with limitations of subtractions, it merits clarification.

The relationship between infrared absorbance and sample concentration is described by the Beer-Lambert Law:

Table 1. Applications of subtractions in FTIR characterization of soil and mineral samples. †

Subtraction type	Mode	Wn	Sample	Background	Target/objective of subtraction	Reference
		cm⁻¹				
Enhance organic bands	transmission	4000–600	NaOH extract of soil	lyophilized NaOH extract before HCl washes	Effect of acid washes on HA structure	Schnitzer and Desjardins, 1965
	transmission	1800–400	coal	coal after HCl and HF treatment	Mineral spectrum of coal	Painter et al., 1978
	transmission	3800–700	coal	coal samples at difference points along a transect	Relative changes in coal composition along a coal seam transect	Painter et al., 1980
	transmission	4000–800	coal	coal samples at different oxidation temperatures	Changes in coal composition during low-temperature (<100°C) oxidation	Gethner, 1987
	DRIFT	4000–400	DOM from composting sewage	DOM after 550°C for 8 h	Composition of DOM	Chefetz et al., 1998
	DRIFT	4000–500	soil	soil after 350°C	SOM composition	Skjemstad et al., 1998
	transmission	3950–450	water and pyrophosphate extracts of soil	aqueous extracts following hydrogen peroxide oxidation	Composition of water- and pyrophosphate-extractable OM	Ellerbrock et al. 1999
	PAS	4000–400	soil aggregates	aggregate fractions after LTA at 0.1 kPA for 20 h	SOM composition of aggregates	D'Acqui et al., 1999
	transmission	4000–400	soil	soil after 650°C heating for 15 min	SOM composition, to allow fingerprinting of soils for forensics	Cox et al, 2000
	DRIFT	4000–400	soil	soils after 350°C overnight	SOM composition	Rumpel et al., 2001
	transmission	4000–349	soil	peat along a transect	Differences in SOM composition of peat soils along a transect	Chapman et al., 2001
	transmission	4000–400	water and pyrophosphate extracts of soil	aqueous extracts after 550°C for 8 h	Composition of water- and pyrophosphate-extractable OM	Kaiser and Ellerbrock, 2005
	transmission	3900–400	water and pyrophosphate extracts of soil	aqueous extracts after 550°C for 8 h	Composition of water- and pyrophosphate-extractable OM	Kaiser and Ellerbrock, 2005
	DRIFT	4000–400	soil size fractions	soil size fractions after 550°C for 6 h	SOM composition of size fractions	Sarkhot et al. 2007
	DRIFT	4000–1000	soil amended with charcoal	soil before addition of charcoal	Determine effect of soil matrix on charcoal added to soils	Janik et al., 2007
	transmission	4000–400	soils	soils after heating at 250°C for 5 h	Changes in SOM composition during heating to remove water repellency	Simkovic et al., 2008
	DRIFT	4000–580	soil surface litter	paired litter samples at successional sites	Differences in litter composition among successional sites	Filley et al., 2008
	DRIFT	4000–400	NaOH extracts of soil aggregates	NaOH extracts after LTA at 90°C and 0.1 kPA for 24–96 h	Composition of extracts as affected by different LTA methods	Agnelli et al., 2008
	PAS	4000–1400	soils, soil aggregates	soils and soil aggregates after LTA (24 h), and before addition of prions	SOM composition of soils, and sorption of prions to soils and soil aggregates	Pucci et al., 2008
	DRIFT	4000–400	clay fraction from oil sands	clay fraction after LTA and/or H₂O₂ oxidation	Composition of SOM removed by LTA and/or hydrogen peroxide oxidation from clay fractions	Adegoroye et al., 2009
	DRIFT	4000–400	soils	soils after 400°C for 2 h	Prediction of SOC from MIR spectra	McCarty et al., 2010
	DRIFT	4000–500	soils	soils after 350°C for 24 h	SOM composition, PLS prediction of sorption of pentadecane	Clark Ehlers et al., 2010
	DRIFT	4000–400	soil aggregates	soils after 550°C for 3 h	SOM composition	Calderón et al., 2011b
	DRIFT	4000–400	soil	soils after 550°C for 3 h	SOM composition	Calderón et al., 2011a
	ATR	4000–600	lignite	lignite after 650°C for 15 min, and ashed lignin after acid treatment	Characterize lignite OM and its response to acid treatment	Gezici et al., 2012

Table 1. Continued.

Subtraction type	Mode	Wn	Sample	Background	Target/objective of subtraction	Reference
	transmission	4000–400	coal	coal samples from different transects	differences in composition of coal from two seams in relation to flexural strength	West et al., 2012
	DRIFT	4000–400	soils with added organic standards	soils	organic compounds added to soils	Calderón et al., 2013
	DRIFT	4000–1250	soils	soil following HF treatment	remove SOM interference from spectra of soil-diesel mixture	Forrester et al., 2013
			soil, eolian sediments	soils and eolian sediments after 350, 450, or 550°C for 3 h	SOM composition in wind-blown sediments	Padilla et al. 2014
	DRIFT	4000–400	soils (permafrost)	soils after 550°C for 3 h or hypochlorite oxidation	SOM composition along permafrost depth profile	Ernakovich et al., 2015
	DRIFT	4000–400	soils	soils following NaOCl oxidation	characterize SOM, as estimated by NaOCl-oxidizable C	Margenot et al., 2015
	DRIFT	4000–400	soil-biochar mixtures	soil-biochar mixtures at varying incubation times	changes in SOM and biochar following incubation	Calderón et al., 2015
	ATR	4000–400	soils	soils following hypochlorite oxidation	forensic identification of soils by geographic location by SOM fingerprint	Suarez et al., 2015
Determine bonding mechanisms	transmission	1800–1350	BSA adsorbed to montmorillonite	montmorillonite-buffer	allow resolution of organic fingerprint region to observe protein binding	Quiquampoix et al., 1993
	DRIFT	4000–200	allophane with $B(OH)_3$	allophane	boron sorption to allophane, including binding complex	Su and Suarez, 1997
	transmission	1750–1500	α-chymotrypsin adsorbed to montmorillonite	montmorillonite-D_2O	conformation changes in chymotrypsin upon binding montmorillonite	Baron et al., 1999
	transmission	1800–1350	BSA adsorbed to montmorillonite or talc in buffers	montmorillonite-buffer and talc-buffer solutions	pH effects on conformation and hydration of bovine serum albumin binding to montmorillonite and talc	Servagent-Noinville et al., 2000
	transmission	4000–750	$CaCO_3$ dust particles reacted with nitrogen oxides	$CaCO_3$ particles before reaction with nitrogen oxides	reactions of nitrogen oxides to surface of carbonate and mineral dust particles	Grassian, 2002
	transmission	3000–1100	α-chymotrypsin adsorbed to montmorillonite in D_2O	montmorillonite-D_2O	enable quantification of amide I and its deconvolution to determine conformation changes of chymotrypsin on sorption	Noinville et al., 2004
	ATR	4000–400	mineral coatings on ATR crystal exposed to sorbate solutions	pure minerals	P-containing sorbates (e.g., DNA)	Parikh and Chorover, 2006
	ATR	3000–900	hematite, alumina coatings with LPS	hematite, alumina coatings	LPS sorption and conformation at hematite and alumina surfaces	Parikh and Chorover, 2008
	ATR	1300–950	goethite in solution of sulfate with and without copper	goethite in the absence or presence of Cu^{2+}	binding complex of sulfate to goethite in the presence of copper	Beattie et al., 2008
	ATR	1900–1000	rutile with solutions of glutamate and aspartate	rutile-solution	glutamate and aspartate binding mechanisms to rutile	Parikh et al., 2011
	ATR	940–700	goethite, hematite exposed to dimethylarsinic	goethite, hematite in solution	quantify kinetics of dimethylarsinic sorption to hematite and goethite	Tofan-Lazar and Al-Abadleh, 2012
	ATR	1800–800	hematite, goethite coatings exposed to model compounds, bacterial cultures	hematite, goethite coatings in solution	binding interactions of model compounds on metal oxides	Parikh et al., 2014 b
	ATR	1800–1100	goethite in solution with tetracycline	goethite in solution	identify functional groups and surface complexes involved in tetracycline sorption to goethite	Zhao et al., 2014
	ATR	1800–900	malate and succinate adsorbed to lepidocrocite and maghemite	mineral-malate or mineral-succinate samples before photoirradiation treatment	changes in malate and succinate adsorbed to lepidocrocite and maghemite during photoirradiation	Borer and Hug, 2014

Table 1. Continued.

Subtraction type	Mode	Wn	Sample	Background	Target/objective of subtraction	Reference
	ATR	940–700	hematite with arsenate and methyl arsenates in solution	hematite-solution	quantifying As-O bond for calculation of As sorption isotherms	Sabur et al., 2015
	ATR	1200–850	ferrihydrite and HA-coated ferrihydrite exposed to phosphate solutions	ferrihydrite and HA-coated ferrihydrite	concentration-dependency of binding complex of phosphate to pure and HA-coated ferrihydrite	Wang et al., 2015
	ATR	1225–950	hematite and goethite in solutions of phosphate and/or sulfate	hematite and goethite in solution	binding complexes of phosphate and sulfate co-adsorption onto goethite and hematite	Hinkle et al., 2015
	ATR	1000–700	boehmite and chromate solution	boehmite, boehmite-chromate system at different pH or concentration	surface speciation of chromate adsorbed to boehmite as a funtion of pH and concentration	Johnston and Chrysochoou, 2015
Remove water interference	ATR	4000–750	alumina with liquid water	water vapor	characterize liquid water sorption to Al_2O_3 surface	Al-Abadleh and Grassian, 2003
	ATR	4000–800	saturated soil paste	liquid water	improve resolution of nitrate N-O vibration for calibrative prediction	Linker et al., 2005
	ATR	4000–800	fungal filaments	liquid water	improve spectra to quantify soil fungi	Linker and Tsror, 2008
	ATR	4000–800	saturated soil paste	liquid water	quantify N isotopes	Kira et al., 2014

† Wn, wavenumber; DRIFT, diffuse reflectance infrared Fourier transform; ATR, attenuated total reflectance; PAS, photoacoustic; BSA, bovine serum albumin; LPS, lipopolysaccharide; HA, humic acid; OM, organic matter; D_2O, deuterium oxide; $CaCO_3$, calcium carbonate; DOM, dissolved organic matter; LTA; low-temperature ashing; DNA, deoxyribonucleic acid; HF, hydrofluoric acid; NaOCl, sodium hypochlorite; H_2O_2, hydrogen peroxide; Al_2O_3, aluminum oxide.
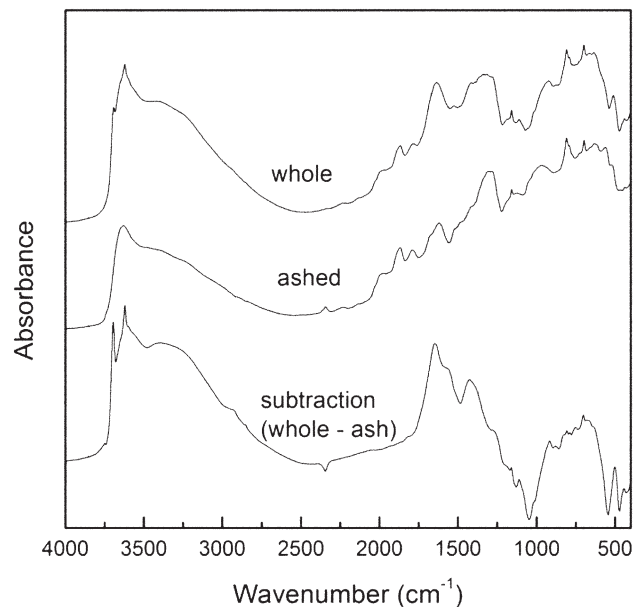

Fig. 1. Illustration of spectral subtraction to enhance SOM bands, using a background spectrum of the same soil following ashing (550°C, 3 h). Spectra were collected by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy of neat (no KBr dilution) soil under agricultural use in northern California (A$_p$ horizon, Mollic Xerofluvent, 22 mg C kg^{-1} soil). A subtraction factor of unity (1.0) was used.

$$A = \mathrm{e}cl \qquad [1]$$

The ability of FTIR spectroscopy to quantify sample components is based on a linear relationship between absorbance (A) at a given wavenumber and concentration (c) of the sample component, assuming constant absorptivity (ε) of the sample component and path length (l). Quantitative FTIR is therefore possible when a target absorbance band can be sufficiently detected to calibrate to a standard curve. For many sample types, subtractions are in fact necessary for quantitative FTIR because they enable isolation of the target absorbance band. The Beer-Lambert Law is thought to be less applicable to reflectance modes than transmission modes, particularly diffuse reflectance modes, the most commonly employed in soils today, because of the unknown path length and scattering effects. However, recent work on quantifying path length of diffuse reflectance in KBr samples has found that the majority (90%) of photons travel ~ 0.1-mm path lengths (Brauns, 2014).

Limitations of spectral subtractions in soils should not be conflated with limitations of FTIR of such sample types, which instead reflect soils' chemical complexity. The complexity of SOM composition is a different issue altogether for the interpretation of organic bands in FTIR subtraction spectra independent of the subtraction technique (Rumpel et al., 2001), and reflects a general challenge to the study of natural OM (Schmidt et al., 2011). Studies on pure or simple organic mixtures in soils have applied subtractions to successfully enable quantification of the target organic bond, demonstrating that subtractions are not a limitation to quantitative FTIR analysis of organics or other minor components of soil samples. Spectral subtractions have been used to quantify specific molecules which would oth-

erwise not be quantifiable, such as nitrate (Linker et al., 2005), and semi-quantitatively detect increases for a variety of organic compounds added to soils (Calderón et al., 2013). In fields like biochemistry, spectral subtractions to remove dominant solvent bands in solutions are requisite for performing spectral deconvolution and quantification (Grdadolnik and Maréchal, 2003).

Potential Subjectivity in Performing Subtractions: The Subtraction Factor

A long-standing criticism of subtractions is that they can be used inadvertently or intentionally to produce false spectral features. However, a distinction must be made between artifacts arising from subtraction, which can be avoided and managed, and the misconception that spectral features can be made to appear even if they are not present in the original spectrum (Smith, 2010). The chief point at which subjectivities can be introduced in subtractions is the subtraction factor. To perform a subtrac-

tion of a sample spectrum and background or reference spectrum, absorbances from the latter are subtracted from those of the former at each wavenumber:

$$S_{sub} = S_{spl} - (S_{ref} \times SF) \; [2]$$

where S_{sub} is the subtraction spectrum, S_{spl} is the sample spectrum, S_{ref} is the reference spectrum, and SF is the subtraction factor.

Since concentrations of constituents in the reference and sample spectrum can be different, the reference spectrum can be scaled by multiplying absorbance of reference absorbances by a subtraction factor (Smith, 1998). Using well-defined, rigorous procedures, which may or may not be computerized, avoids personal biases in judging the subtraction factor (Powell et al., 1986), similar to potential biases in baseline calculations. Depending on the goals of the subtraction, there exist objective criteria and methods for determining an appropriate subtraction factor. For this reason, subtraction factors are perhaps better conceptualized as being 'optimum' for a specific objective, rather than 'correct' (ThermoScientific, 2006). As subtractions gain increasing use in FTIR of soil samples, we recommend that rationale and values for subtraction factors be included in experimental methods.

To illustrate the importance of properly selecting a subtraction factor, and their potential to alter spectral features, consider a series of subtraction spectra calculated using a range of subtraction factors (Fig. 2). As the subtraction factor decreases to 0.1, absorbance of bands representing minerals increase, including aluminol Al-OH at 3618 cm^{-1}, quartz Si-O at 1880 cm^{-1}, and Si-O at 808 cm^{-1}. As the subtraction factor approaches 1.0, subtraction spectra show relative increases in absorbance bands representing organics, such as aliphatic C-H at 3000 to 2800 cm^{-1}, aromatic C=C and amide C-N and N-H at 1558 cm^{-1}, and phenol and carboxyl C-O at 1240 cm^{-1}, but also over-subtraction (inverse peak) of mineral absorbances such as quartz Si-O at 2100 to 1750 cm^{-1}. Since ashing produced a net mass loss of 3.42%, in theory a subtraction factor of ~0.97 should be appropriate. However, nonlinearity of absorbance results in over-subtraction of mineral features at this subtraction factor (Fig. 2).

Subtraction factors can be operationally determined to remove a target absorbance. This method of zeroing target peaks (Smith, 1998) is complemented by restricting the subtraction to a specific range, since the subtraction factor necessary to remove an interfering component may not be appropriate for other regions of the spectrum. For example, biological applications of FTIR commonly entail aqueous samples (e.g., cells, biomolecule solutions) and the necessary subtraction of water can be optimized by restricting the subtraction consideration to a window. The amide I band can be enhanced for protein quantification by restricting water subtraction to 2300 to 1800 cm^{-1} (Rahmelow and Hubner, 1997). Alternatively, a specific water band (e.g., 2515 cm^{-1}) can be used as an internal intensity standard, with the subtraction factor determined with the use of a second-order least-squares fit (Dousseau et al., 1989).
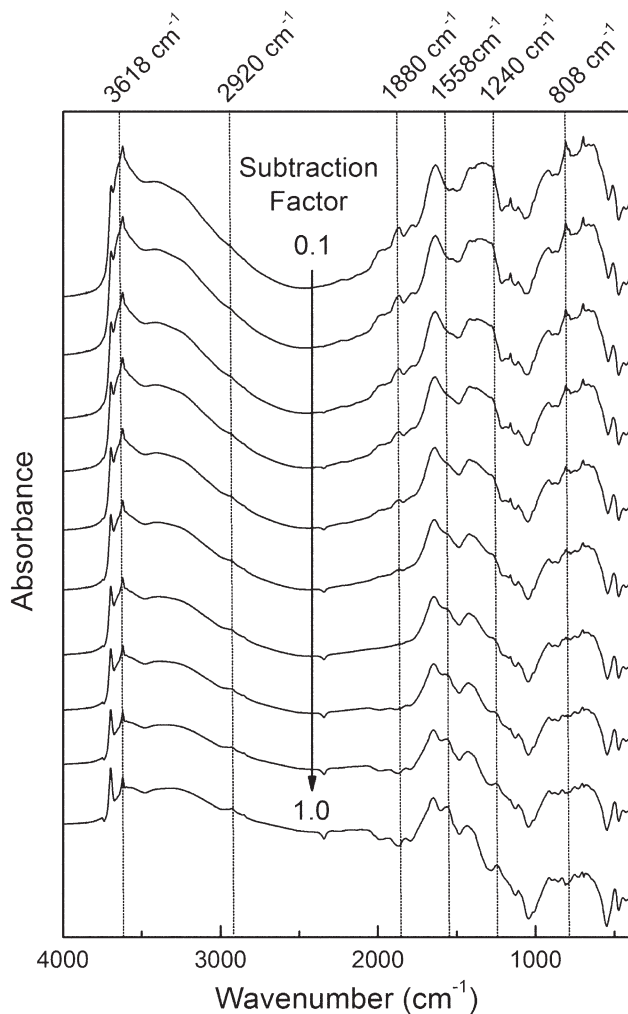

Fig. 2. Comparison of subtractions performed across a range of subtraction factors (1.0 to 0.1, at 0.1 intervals) for diffuse reflectance infrared Fourier transform (DRIFT) spectrum of 550°C ashed soil from the original soil (Fig. 1). At lower subtraction factors, bands representing minerals increase (3618, 1880, 808 cm^{-1}), and certain bands representing organics decrease (2920, 2985 [not marked], 1558, 1240 cm^{-1}).

In the example illustrated in Fig. 2, zeroing of quartz Si-O bands 2000 to 1800 cm^{-1} suggests a subtraction factor in the range of 0.6 to 0.8 to be more appropriate for removing dominant mineral bands than the a priori subtraction factor of 0.97 based on mass difference between the two spectra (i.e., SOM removed). The reason a priori subtraction factors may need to be adjusted is that interfering bands may not be fully removed if these express nonlinear absorbances, which is typical for mineral bands such as Si-O stretch at 1100 to 950 cm^{-1} (Reeves, 2012). Variability between subtracted spectra can also necessitate adjusting the subtraction factor (Max and Chapados, 1998; Wang et al., 2003), and can be avoided by collecting spectra under the same conditions (e.g., temperature, pH). Normalizing and baseline correcting spectra before subtractions can also ensure spectral consistency (Szafarska et al., 2009). However, variability can also result from component interactions and temperature-dependent effects which are more difficult, and often not possible, to experimentally control (Grdadolnik, 2003).

Spectral software accompanying modern FTIR spectrometers facilitates subtractions. However, the use of softwares' default subtraction factor merits caution because the criteria used may not match or be appropriate for the subtraction goals. Given varying objectives of subtractions, instrument softwares are meant to be interactive (ThermoScientific, 2006) and require some degree of user judgment (ThermoScientific, 2013). For example, the software used for the subtraction in Fig. 1 and 2 suggested a subtraction factor of 0.76 (OMNIC, Thermo Fisher Scientific), based on linearization of baseline and removal of prominent peaks in the original spectrum. The resulting subtraction provides a closer match with the operational zeroing of Si-O peaks (Fig. 2).

The 'subtractive similarity method' involves comparison of subtraction spectrum with a spectrum of the standard and can provide criteria by adjusting the subtraction factor to match areas, such as amide absorbances 1700 to 1480 cm^{-1} in aqueous protein samples (Wang et al., 2003). Early studies employing FTIR to quantify lignin in wood samples performed subtractions overlaying the reference cotton linter spectrum with the wood pulp spectrum to match baselines of the aliphatic C-H band at 3000 to 2800 cm^{-1} (Berben et al., 1987).

Mathematical approaches for selecting subtraction factors have been developed for OM samples but have yet to be applied to more complex samples such as soils. These are based on iterative subtractions verified by a measure of subtraction accuracy, including fitting of original and target spectrum, and derivative-based measures. Iterative linear least square fitting can be used to scale subtractions of known components from spectra of OM mixtures by focusing on regions in which known component spectra fit well with the mixture spectrum (Gillette and Koenig, 1984). Alternatively, matching absorbances between mixture and component spectra is not necessary to perform accurate subtractions. The subtraction factor can be empirically determined as the value at which the total intensity of the first derivative of the resulting subtraction is minimized (i.e., derivative minimi-

zation). A subtraction that accurately removes a spectral component will result in a decrease in the total intensity of the first derivative. Iterative subtraction followed by calculating the first derivative can therefore be used to identify the proper subtraction factor for removing the component (Banerjee and Li, 1991). This technique has been successfully applied to quantify biomolecule concentrations in complex OM mixtures, such as lignin in wood pulp (Friese and Banerjee, 1992).

Limitations of Spectral Subtractions for Soil Samples

Artifacts of subtraction can reflect sample type (inconsistent absorbance by soil components), the accuracy of the background used in the subtraction, and instrumentation (wavenumber scale error). The use of sample sets expressing similar soil properties like texture and mineralogy can keep certain artifacts consistent across samples, allowing relative comparison of subtraction spectra among samples. For example, ashing will produce the same subtraction artifacts for a given mineralogy, and particle-size effects on nonlinear absorbance and consequent incomplete subtractions will be consistent for soils of the same texture.

Particle-Size Effects

Particle-size effects on scattering of infrared light can have pronounced effects on absorbance spectra, with potentially significant consequences for subtractions. Variation in infrared scattering by different particle sizes means that most soils express heterogeneity in particle-based scattering (i.e., non-uniform diffuse reflectance). This has been proposed to explain less accurate predictions of soil C across texturally diverse sample sets and among particle-size fractions using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy (Cozzolino and Morón, 2006). Heterogeneity in scattering behavior, a major component of the DRIFT mode, may cause incomplete subtractions due to nonlinear absorbance of mineral components (Dahm and Dahm, 2001), as well as texture-dependent inconsistencies in subtractions among samples. For example, particle-size dependent scattering of infrared light resulted in incomplete subtraction of minerals from spectra of ethyl methylphosphonate added to soil (Samuels et al., 2006).

Interactions among Sample Constituents

Mineral and organic components in soils exhibit interactions that may affect subtractions. For example, sequential addition of pure and mixed organic standards like cellulose and bovine serum albumin to soils resulted in nonlinear absorbance increases (Calderón et al., 2013), potentially reflecting interactions with mineral particles. By influencing the extent of exposure of infrared active bonds for detection, and potentially inducing shifts in particle-size distribution, such interactions may account for incomplete subtractions of mineral bands and/or subtraction recovery of organic bands.

Instrumentation

Anomalies of subtraction can reflect instrumentation issues, though these are largely a problem for removal of dominant water absorbances (Table 1). Unmatched wavenumber scales of subtracted spectra may compromise subtractions. Wavenumber scale error can result from instrumentation; including beam divergence, mirror misalignment, and mirror drive instability (Griffiths and De Haseth, 2007). Instability in the wavenumber scale of the reference laser is a common and inherent (i.e., non soil-specific) source of potential error in FTIR spectral subtractions (Weis and Ewing, 1998). The instability of wavenumber scale reflects fluctuations in the laser wavenumber of ± 0.03 cm^{-1} due to thermal expansion and contraction of the reference HeNe laser compartment. This is a magnitude sufficient to introduce errors into the wavenumber scale of spectra, and typically manifests as dissonance from offset subtraction scales. Amelioration of wavenumber instability is described further by Weis and Ewing (1998).

Methods of Soil Organic Matter Removal to Obtain Mineral Backgrounds

Ashing to Obtain Mineral Backgrounds

Two general approaches for removing mineral absorbances are (i) subtracting a spectrum of a mineral standard(s), and (ii) empirically obtaining a mineral spectrum by removing organics. To enhance organics bands in FTIR spectra of coal (lignite), Painter et al. (1981) used a background spectrum of pure kaolinite weighed by its composition as determined independently by X-ray diffraction (XRD). The second, empirical method is the focus of this review, as spectral subtractions for enhancing SOM largely involve backgrounds obtained from the same soil sample (Table 1). This approach involves subtracting an ideally mineral-only spectrum from the original soil spectrum (mineral + SOM), resulting in a putatively SOM-only spectrum (Fig. 1).

Methods of obtaining the mineral sample have employed ashing, which is successful at removing organics but can entail changes in mineral absorbance bands due to thermal alteration and manifest as artifacts in subtraction spectra. Reeves identified thermal sensitivity of minerals as a potential compromise on the utility of spectral subtractions using ashed backgrounds, especially at higher temperatures typically employed (e.g., 550°C; Table 1; Reeves, 2010, 2012). Mineral alterations during ashing are specific to mineral type and temperature, including interlayer collapse, dehydration, decarboxylation, and surface hydroxyl cross-linking and dehydroxylation, and have been corroborated by XRD, differential scanning calorimetry (DSC), thermogravimetry (TG), and FTIR (Frost and Vassallo, 1996; Prasad et al., 2006; Suitch, 1986). Such alterations will manifest as peak losses and formations, and peak shifts and broadening in the ashed spectrum used as a background, in particular in regions of overlap with organic bands such as 1600 to 1000 cm^{-1} (Reeves, 2012). Ashing can also produce infrared active combustion products (e.g., ash, char, amorphous metal oxides) or exposure of mineral surfaces previously covered by OM. Subtraction artifacts reflecting mineral alteration during ashing have been identified for bentonite (Yang, 2014), and for organic (permafrost) soils (Ernakovich et al., 2015) and mineral soils (Margenot et al., 2015; Suarez et al., 2015). For example, following ashing at 550°C, Margenot et al. (2015) observed loss of Si-OH at 3700 to 3600 cm^{-1} and Al-OH at 909 cm^{-1} in DRIFT spectra, and in attenuated total reflectance (ATR) FTIR spectra, a +12 cm^{-1} shift in phyllosilicate lattice Si-O-Si/Al at 983 cm^{-1}. Similarly, following ashing at 650°C, Suarez et al. (2015) identified peak losses from dehydration and dehydroxylation as the main sources of subtraction artifacts, limiting forensic discrimination among associated soil map units by ATR-FTIR.

In general, lower temperatures are ideal to avoid mineral artifacts, but these may come at a cost of less efficient SOM removal, restricting enhancement of organics by subtraction to less than the total SOM pool. Loss of OM may also be selective at lower temperatures. For example, lower temperature (350 vs. 450–550°C) preferentially removed soil C relative to soil N and did not highlight absorbances corresponding to amide C=O, aromatic C=C, and phenol and carboxyl C-O at 1600 to 1400 cm^{-1} (Padilla et al., 2014). On the other hand, certain organic bands can be emphasized even at low temperatures (350°C, 3–24 h), including 3325, 2927, 1640, and 1100 cm^{-1}. Identifying mineral artifacts favors a compromise of greater SOM resolution with higher temperature ashing (Kaiser et al., 2007; Padilla et al., 2014). To minimize mineral artifacts, knowledge of soil sample's mineralogy can be used a priori to determine a maximum ashing temperature. Another strategy is to constrain soil mineralogy of sample sets to maintain subtraction artifacts of ashing constant across samples, allowing emphasis of relative differences in organic bands.

Chemical Oxidation

Reeves (2012) hypothesized that chemical oxidations could provide an alternative to ashing for supplying mineral backgrounds. Potential limitations of oxidations include lower SOM removal as compared with ashing and mineral alteration, in particular dissolution of metal oxides. Two recent studies suggest oxidations have strong potential to minimize artifacts of subtractions relative to ashing while removing high amounts of SOM. Ernakovich et al. (2015) and Margenot et al. (2015) evaluated sodium hypochlorite oxidation as an alternative SOM removal to ashing for performing subtractions. Hypochlorite oxidation removed high amounts of organic C in alluvial (85%; Margenot et al., 2015) and permafrost (79–92%; Ernakovich et al., 2015) soils, comparable to ashing (95%) but without spectral artifacts resulting from thermal alteration of minerals. This is consistent with evidence from previous studies demonstrating that hypochlorite removes high amounts of SOM without dissolving mineral oxides, in contrast to oxidants like hydrogen peroxide and sodium hyposulfite (Adegoroye et al., 2009; Mikutta et al., 2005; Siregar et al., 2005; von Lützow et al., 2007).

Low-Temperature Ashing

Low-temperature ashing (LTA) has been proposed as a method for removing SOM with minimal alteration of the mineral component (Marcoen and Delecour, 1976; Sullivan and Koppi, 1987). Low-temperature ashing entails oxidation of OM by oxygen plasma (130 mL O_2 min^{-1}) under low pressure (0.1 kPa) and temperature (<100°C; Adegoroye et al., 2009; Agnelli et al., 2008; D'Acqui et al., 1999). Similar to some chemical oxidations, for LTA the advantage of more accurate subtractions due to minimal mineral alteration comes at a cost of incomplete SOM removal. For example, LTA of the clay-fraction of oil sands did not alter siderite, in contrast to H_2O_2, but it removed less total C (84 vs. 97%; Adegoroye et al., 2009). Removal of C from soil aggregates ranges from 30% (NaOH-extractable C; Agnelli et al., 2008), 40 to 46% (total C; D'Acqui et al., 1999), and 35 to 70% (total C) depending on the duration of ashing (5–24 h; Pucci et al., 2008). Subtraction spectra indicated a high proportion of aliphatic C-H (2980–2930 cm^{-1}) and amide C=O (1650 cm^{-1}) and C-N (1542 cm^{-1}) absorbances relative to aromatic C=C and C-H in aggregate OM removed by LTA (D'Acqui et al., 1999; Pucci et al., 2008). Aggregates from uncultivated and cultivated soils showed differences in LTA-oxidizable OM, including relative enrichment in aliphatic and amide absorbances in uncultivated soils and relative enrichment in carboxylate and phenol C-O (1244 cm^{-1}) in cultivated soils (Pucci et al., 2008).

Incomplete SOM removal may be an advantage for probing SOM composition in relation to physical stabilization because LTA involves stepwise removal of SOM layers, which has been compared with "the peeling of an onion skin," (Pucci et al., 2008). This offers a way to operationally fractionate and characterize SOM. For example, Pucci et al. (2008) coupled LTA with photoacoustic (PAS) FTIR to characterize SOM composition by sequential oxidation of OM with depth in aggregates.

Applications of Spectral Subtractions for Soil Samples
Soil Organic Matter Composition

Subtractions have been used to emphasize organic absorbances in a number of soil samples, including bulk and physical fractions like aggregates, enabling characterization of relative functional group composition of SOM. This is useful for a number of purposes, including (i) complementing additional structural characterizations (e.g., nuclear magnetic resonance spectroscopy, mass spectrometry), (ii) providing a measure of SOM in addition to mass balance analyses such as total soil organic C, (iii) relating SOM composition to specific soil measurements, like labile C fractions, microbial biomass, and soil enzyme potential activities, and (iv) fingerprinting SOM composition for forensic purposes (Table 1).

Subtractions may be less important for improving FTIR predictions of SOM measurements (e.g., TOC, aggregate C). For example, Kamau-Rewe et al. (2011) found no improvement of soil C prediction by DRIFT (4000–400 cm^{-1}) using subtraction spectra calculated with ashed mineral backgrounds (550°C) vs. original soil spectra. The similarity of regression slopes for soil ($m = 0.928$)

vs. subtraction ($m = 0.925$) spectra suggested prediction models using soil spectra already accounted for mineralogical variation among the $n = 87$ Alfisols from eight tropical countries. Notably, subtraction spectra calculated with mineral backgrounds ashed at lower temperatures weakened predictions of soil C (for 350°C, $R^2 = 0.82$; for 450°C, $R^2 = 0.88$) relative to bulk and 550°C subtraction spectra ($R^2 = 0.93$). On the other hand, subtraction spectra obtained by ashing (350°C) have been used to successfully predict sorption of pentadecane to soils using partial least squares (PLS) analysis (Clark Ehlers et al., 2010). The potential of non-ashing subtractions to improve calibrative prediction of SOM fractions, from total C to labile C, has yet to be explored.

Binding Mechanisms

Changes in intensity of bands corresponding to specific bonds of sorbed and unsorbed molecules can be used to provide quantitative information on binding, to the extent of enabling sorption isotherms to be constructed and surface complexes (e.g., bidentate, monodentate) determined (e.g., Elzinga and Sparks, 2007; Parikh et al., 2011; Sabur et al., 2015; Tofan-Lazar and Al-Abadleh, 2012). For example, subtraction can be used to identify phosphate binding by iron oxides in a weathered soil following a phosphate spike (Fig. 3). Phosphate retention by iron oxides is evident in the subtraction spectrum as strong absorbance of Fe-O-P at 1012 cm^{-1} (Guan et al., 2005), and net phosphate enrichment is apparent from by P-O bands such as PO_2^- at 1039 cm^{-1}
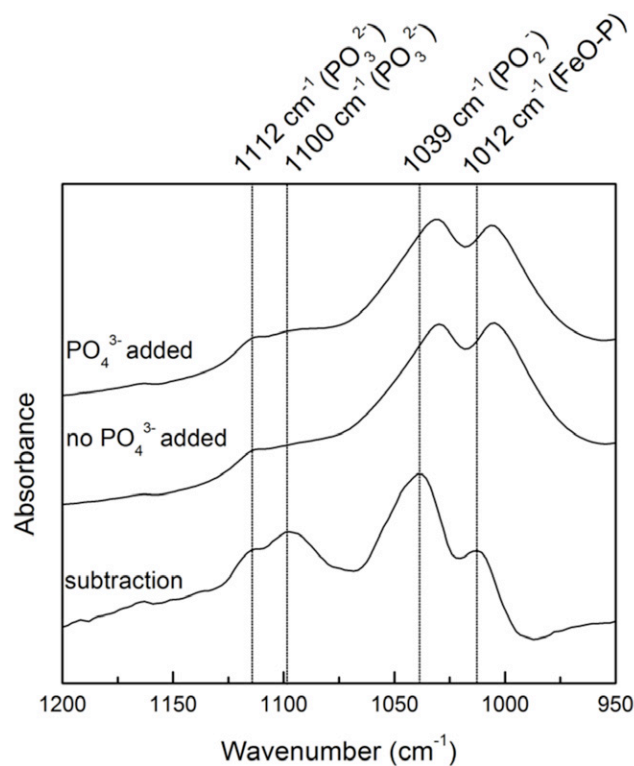
Fig. 3. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of a weathered soil (Typic Kandiudox) before (top) and after (middle) a phosphate spike and the resulting subtraction (bottom). The subtraction spectrum reveals changes in absorbance bands reflecting phosphate binding to minerals (Fe-O-P at 1012 cm^{-1}) and increased phosphate content (1112, 1100, 1039 cm^{-1}).

and PO_3^- at 1112 and 1100 cm^{-1}. These peaks are not visible in the spiked soil spectrum due to strong absorbance overlap by silicate mineral Si-O at 1032 and 1005 cm^{-1}.

Resolving Chemical Oxidations and Extractions

Soil organic matter measurements that remove OM such as chemical oxidations can be structurally resolved by FTIR subtractions by using the oxidized soil as the background spectrum. This allows characterization of the composition of SOM removed by the oxidant. By providing a measure of SOM quality to the oxidized fraction beyond mass balance, this application of subtractions offers structural resolution of largely operational chemical fractionations, potentially improving their application to fractionate SOM by residence time (Jagadamma et al., 2010). For example, comparison of oxidants that remove different amounts of SOM may be able to identify relationships between composition and reactivity to a given oxidant. Subtractions fol-
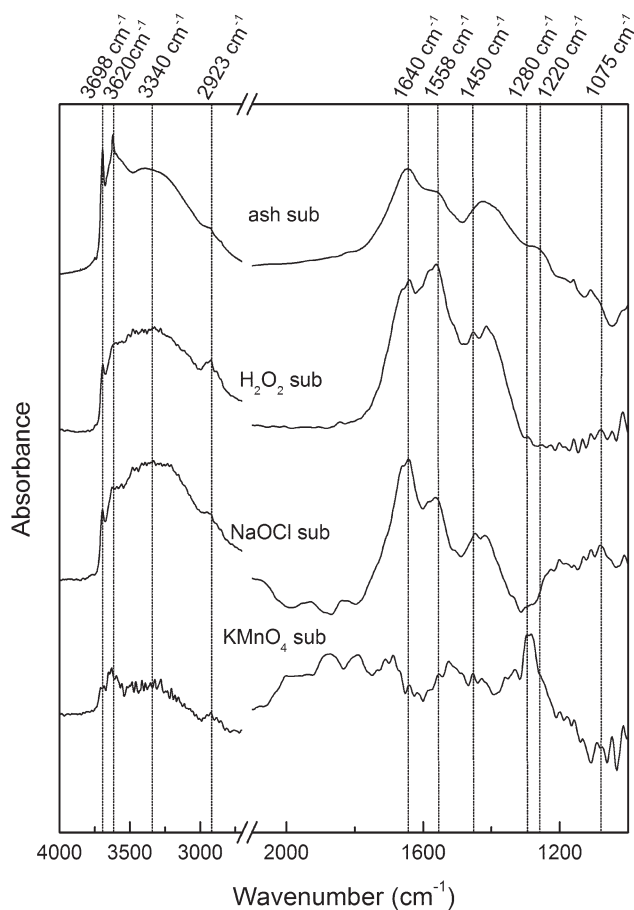

Fig. 4. Diffuse reflectance infrared Fourier transform (DRIFT) subtraction spectra obtained by different methods, including ashing (550°C, 3 h; 95% C loss) and chemical oxidations of hydrogen peroxide (H₂O₂; 72% C loss); sodium hypochlorite (NaOCl; 42% C loss), and potassium permanganate (KMnO₄; 14% C loss). Subtraction spectra demonstrate oxidant selectivity in SOM removal, and as compared with the ashed subtraction spectrum, including absorbances representing aromatic C=C, ketone C=O, and/or amide C=O (1640 cm⁻¹), and aromatic C=C and/or amide C-N and N-H (1558 cm⁻¹), phenol C-O (1280, 1220 cm⁻¹), and polysaccharide C-O (1075 cm⁻¹). The original soil is an A$_p$ horizon (22 mg C kg⁻¹ soil) of Mollic Xerofluvent under agricultural use in northern California.

lowing three commonly employed oxidations suggest oxidant-specific selectivity in SOM removal (Fig. 4). Potassium permanganate (KMnO$_4$) is postulated to oxidize an active or labile fraction (Culman et al., 2012), whereas sodium hypochlorite (NaOCl) and hydrogen peroxide (H$_2$O$_2$) are often used to isolate older pools despite conflicting results (Lutfalla et al., 2014; Zimmermann et al., 2007). Comparison of subtraction spectra indicates selectivity among oxidants in SOM removal and as compared with the ashed subtraction spectrum. Hydrogen peroxide preferentially removed aromatic C=C, ketone C=O, and/or amide C=O at 1640 cm^{-1}, in contrast to KMnO$_4$ and NaOCl. Potassium permanganate generally did not show strong selectivity except for phenol and carboxyl C-O at 1280 cm^{-1}, while H$_2$O$_2$ and NaOCl both showed loss of aromatic C=C and/or amide C-N at 1558 cm^{-1}. Increased noise is also evident for subtractions performed with decreasing C removal, especially in regions with dominant absorbance bands for minerals and mineral-associated moisture at 3800 to 3400 and 1100 to 600 cm^{-1}.

To enhance the effect of oxidations on organic bands, original and oxidized soils were demineralized by HF washes (2% v/v) before collection of spectra, and subtractions were re-calculated (Fig. 5). Demineralized subtractions show differences among oxidants for the same types of organic functional groups as non-demineralized subtractions (Fig. 4), but relative differences among oxidants that were less clear or not present in subtraction spectra without demineralization are now evident. For example, carboxyl C=O (1725 cm^{-1}), aromatic C=C (1650, 1554 cm^{-1}), amide C=O (1650 cm^{-1}), and aliphatic C-H (1450 cm^{-1}) differ most among oxidants. However, there are previously unobserved differences in relative selectivity among oxidants, such as the loss of phenol and carboxyl C-O at 1220 cm^{-1} and polysaccharide C-O at 1075 cm^{-1}, though these features could also be subtraction artifacts from Si-O inversions (Nguyen, et al., 1991, Parikh et al., 2014a). The subtraction spectrum representing SOM oxidized by KMnO$_4$ is less noisy following HF treatment, demonstrating an advantage of HF demineralization to improve subtractions of small mass differences (See *Application of Hydrofluoric Acid to Explore Subtractions* below).

Tracking Processes in Soil Organic Matter

Subtracting spectra of a sample across time points can be used to monitor SOM processes such as litter decomposition and composting. In biochemistry, such 'reaction-induced' or 'time-resolved' subtraction spectra are used to resolve chemical changes during reactions (Mäntele, 1993). For soil incubations and other assays that involve mass balance measures of SOM lability (e.g., C mineralization), subtractions can help identify organic bands reflecting biochemical and biological lability of SOM. This is an important step toward identifying functional significance of specific organic bands (Calderón et al., 2015, 2011b), and more broadly is a concrete step toward using chemical measures to complement in situ study of SOM lability (Schmidt et al., 2011). For example, Calderón et al. (2015) used subtractions to identify changes in SOM following maize stover or maize bio-

char addition over a 48-wk incubation. Relative to the time-zero spectrum, greatest absorbance decreases were found for organic bands representing O-H and N-H at 3400 cm^{-1}, aliphatic C-H at 2925 and 2850 cm^{-1}, carbonyl C=O at 1737 cm^{-1}, and amide C=O but potentially aromatic C=C at 1656 cm^{-1}, suggesting their utility as markers of SOM lability. The typically small mass loss of soil samples from SOM mineralization experienced in incubations may entail noisy subtractions, as is the case for subtractions with oxidants that remove low amounts of OM like KMnO$_4$ (Fig. 4). Subtractions have also been used with bulk soils samples under different management treatments to highlight compositional changes in SOM concurrent with its accrual or loss (Calderón et al., 2011a).

Another application of this type of subtraction is to check for effects of soil on amendments. For example, subtraction of a soil spectrum from the spectrum of soil plus added charcoal revealed that the added charcoal interacted with soil components via aromatic functional groups, indicated by broadening of a peak 1607 cm^{-1} to 1581 cm^{-1}, and the appearance of a peak at 1199 cm^{-1} (Janik et al., 2007).

Correction for Non-Sample Interferences

Subtraction of interferences due to water or CO$_2$ are common and often necessary, especially in liquid-solid and gas-solid FTIR experiments in which water absorbance bands dominate spectra (Weis and Ewing, 1998). Removal of water absorption by subtraction has been used to enable quantitative prediction of nitrate from saturated soil pastes (Linker et al., 2005) and characterize organic molecule sorption to minerals, including lipids, proteins, and amino acids (Parikh and Chorover, 2008; Parikh et al., 2014a). In a study of water interaction with aluminum oxide, subtraction of water was necessary to obtain mineral-only spectra despite experimental attempts to remove interfering gaseous moisture (Al-Abadleh and Grassian, 2003). An excellent consideration of factors influencing accuracy in subtractions of water absorbances in spectra of aqueous samples is provided by Rahmelow and Hubner (1997).

Improving Fourier Transform Infrared Assignments

Assignment of IR bands in soil spectra and OM samples is made difficult by overlap of mineral and organic bands, as well as overlap of organic absorbances (Parikh et al., 2014a). Subtractions can improve assignments of organic bands in soils by highlighting effects of treatments known to affect specific organic functionalities. This is analogous to the use of D$_2$O
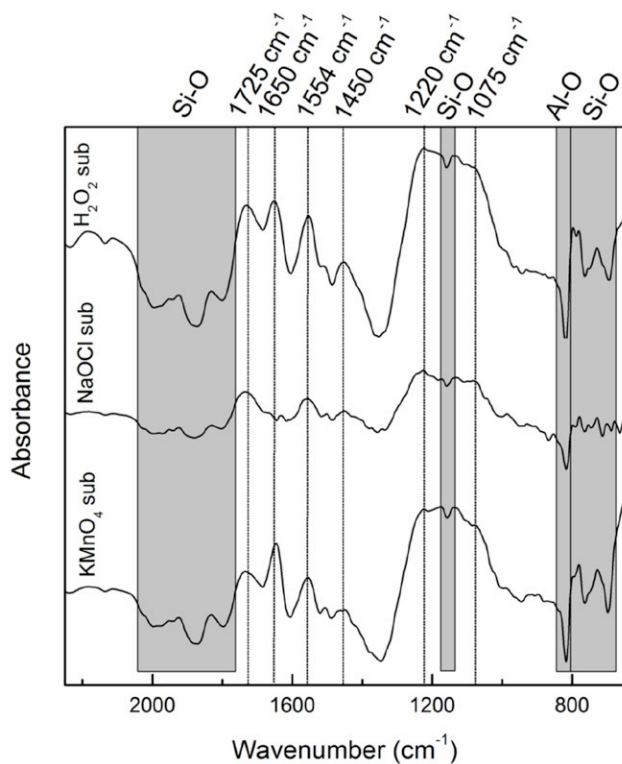
Fig. 5. Diffuse reflectance infrared Fourier transform (DRIFT) spectra obtained by subtracting spectra of soils following chemical oxidations from the original spectrum of a surface soil (22 mg C kg^{-1} soil) of a Mollic Xerofluvent under agricultural use in northern California. Original and oxidized soils were HF-washed before collection of spectra to remove minerals, thereby reducing mineral artifacts of subtraction and enhancing organic bands. Oxidations show strong differences in absorbance of bands representing carboxyl C=O (1725 cm^{-1}), aromatic C=C, amide C=O, and/or ketone C=O (1650 cm^{-1}), aromatic C=C and amide C-N (1554 cm^{-1}), aliphatic C-H (1450 cm^{-1}), and fewer differences for phenol and carboxylate C-O (1220 cm^{-1}) and polysaccharide C-O (1075 cm^{-1}).
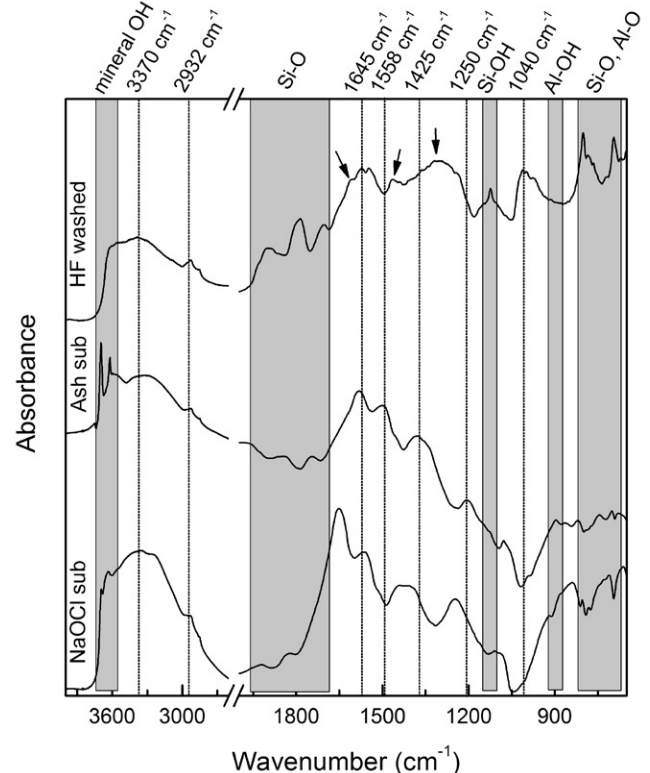
Fig. 6. Diffuse reflectance infrared Fourier transform (DRIFT) spectra of hydrofluoric acid (HF)-washed soil with subtraction spectra obtained by ashing (550°C) or wet chemical oxidation (sodium hypochlorite). Mineral bands are featured in gray boxes and organic bands are indicted by dotted lines. Spectral features present in the HF-washed spectrum but not the subtraction spectra are denoted by arrows. The original soil is the A$_p$ horizon (22 mg C kg^{-1} soil) of Mollic Xerofluvent under agricultural use in northern California.

(deuterium oxide) in protein FTIR to identify exchangeable and surface exposed amide functionalities: treatment of a protein by D_2O leads to D-H isotopic exchange with amide N-H, which manifests as a shift in the amide II band frequency. The effect of washing coal with HCl was identified by subtraction to decrease intensity of carboxylate (COO^-) at 1580 cm^{-1} and concurrently increase carboxylic acid (COOH) at 1710 cm^{-1} (Painter et al., 1981), consistent with protonation from HCl.

Future Directions for Improving Spectral Subtractions in Soil Samples
Method of Background Obtainment

For simple systems, the use of mineral standards as backgrounds ensures high accuracy of subtractions. Mineral backgrounds are less straightforward when a subtraction spectrum of SOM is the goal, as these are empirically derived from the original soil sample by a treatment that removes the SOM. The main challenge is obtainment of mineral backgrounds with minimal artifacts from SOM removal. Alternatives to high-temperature ashing like hydrogen peroxide, sodium hypochlorite, and LTA have been explored (See *Chemical Oxidation* section above, Table 1), but are limited. Expanding study of these and other alternatives, in particular the variety of chemical oxidations used in SOM removal pretreatments for analyses such as XRD and particle size is a logical next step. For example, sodium peroxodisulfate ($Na_2S_2O_8$) has been used with high SOM removal (>90%) in clays of sediments with minimal mineral alteration, determined via a suite of methods including FTIR, XRD, and scanning electron microscopy (SEM; Meier and Menegatti, 1997, Menegatti et al., 1999).

Concurrently, the validity and interpretation of subtractions in general can be improved by accounting for artifacts introduced by the method of SOM removal, including high-temperature ashing and chemical oxidation. Since different minerals will respond differently to ashing, compiling FTIR-detectable artifacts across a range of ashing conditions for soil minerals is a first step toward predicting and accounting for subtraction artifacts from knowledge of soil mineralogy (Reeves, 2012). Trade-offs between SOM removal and mineral alteration for performing subtractions can be leveraged by knowledge of how the latter affect spectra.

Application of Hydrofluoric Acid to Explore Subtractions

By removing minerals, HF has been used to improve FTIR of organics directly on soil samples (Dick et al., 2006; Fontaine et al., 2007; Lima et al., 2009; Poirier et al., 2005; Rumpel et al., 2006; Veum et al., 2014). Conversely, when the HF-treated spectrum is used as the background, subtractions can be used to resolve the mineral component (Forrester et al., 2013).

Hydrofluoric acid treatments offer an alternative for FTIR characterization of SOM that can be used to evaluate SOM characterization performed by subtractions. In theory, a spectrum of HF-treated soil should approximate the subtraction spectrum for

the soil obtained by a background in which the majority or all of the OM was removed. However, there are limitations to HF demineralization, including incomplete and selective removal of minerals. Thus, like subtractions, HF demineralization is a way to enhance organic bands. Additionally, HF carries risk of serious injury or death, and its use and waste disposal require great caution.

Taken together, spectra of SOM obtained by HF versus subtractions provide an opportunity to cross-validate spectral features corresponding to organics and identify artifacts from the subtraction process. Spectra of HF-treated soil with subtraction spectra calculated by ashing (95% C loss) and hypochlorite oxidation (85% C loss) illustrate similarities and differences for organic bands (Fig. 6). Spectral features present in the HF-washed spectrum but not subtraction spectra include amide C=O at 1680 cm^{-1}, aromatic C=C, ketone C=O, and/or amide C=O at 1615 cm^{-1}, aromatic C=C and amide C-N at 1525 cm^{-1}, and aliphatic C-H and/or carboxylate C-O at 1350 cm^{-1}. Mineral peaks removed by HF are present as incompletely subtracted features in subtraction spectra, including phyllosilicate Si-O(H) and Al-O(H), whereas Si-O absorbance of HF-resistant quartz are enriched. Peaks present in both types of spectra and thus likely representing SOM bands include N-H and O-H at 3400 cm^{-1}, aliphatic C-H at 3000 to 2800 cm^{-1}, and aromatic C=C, ketone C=O, and/or amide C=O at 1645 cm^{-1}. Overall, comparison of HF versus subtraction spectral representation of SOM support the ability of subtractions to accurately resolve organics bands in the region 2000 to 1600 cm^{-1} (Nguyen et al., 1991; Reeves, 2012).

Peaks present in subtraction spectra but absent or present as local minima in the HF treatment spectrum may be inversion artifacts from subtraction (Reeves, 2010), including bands 1558, 1425, and 1250 cm^{-1}. However, because HF demineralization typically entails C loss (30% C loss for this soil), inversions in HF spectra may represent HF-labile or soluble organics. For example, 1558 cm^{-1} may be hypothesized to reflect amide C-N and N-H given that HF can selectively remove proteinaceous OM (Schmidt and Gleixner, 2005; Zegouagh et al., 2004), though this absorbance region also corresponds to aromatic C=C. As HF-washed spectra are not entirely mineral-free (e.g., quartz Si-O at 2050–1780 cm^{-1}), in regions of potential overlap (e.g., 1800–1000 cm^{-1}) it is difficult to determine whether peaks present in the HF-washed spectrum but not subtraction spectra are in fact organic bands or mineral bands (e.g., C-O vs. Si-O at 1040 cm^{-1}). Additionally, HF may alter SOM structure (Dai and Johnson, 1999; Gonçalves et al., 2003; Rumpel et al., 2006; Sleutel et al., 2009), which can translate to changes in absorbance bands such as decreased absorbance of carboxylic acid C=O at 1730 to 1710 cm^{-1} and conversion to COO^- at 1600 and 1400 cm^{-1} (Mathers et al., 2002). To date, few comparisons have been made for HF treatment on SOM characterization, though it has been suggested that HF does not necessarily improve FTIR spectra of organics (Poirier et al., 2005). Subtractions may offer a more cost effective and rapid alternative, and additionally avoid the safety precautions and risks surrounding the use and waste disposal of a reagent such as HF.

Internal Standards to Improve Quantification

In FTIR spectroscopy, internal standards are substances with easily identifiable absorbances that can be used to quantify the sample via correlation between absorbances of the internal standard and sample. Internal standards can be part of the sample (endogenous) or from an added substance (exogenous). In both cases, a calibration curve is constructed to relate the standard's absorbance and concentration (Bellamy, 2010; Stuart, 2000). There are numerous potential advantages to exogenous internal standards: 1. Provide an easily quantifiable absorbance, if targeted to a region of minimal sample absorbance; 2. Quantify absorbance(s) across samples, not just relative to absorbances within an individual sample spectrum; 3. Account for variation in sample preparation, instrumentation, and lab conditions (e.g., humidity); and 4. Remove the need to measure path length. Ideally, exogenous standards have a simple spectrum with no bands overlapping sample bands, are stable under experimental conditions of spectral acquisition, do not absorb moisture, and can be readily incorporated into samples (Smith, 2011; Stuart, 2000).

Exogenous standards should be able to improve the accuracy of subtractions (e.g., ensuring complete subtraction, accounting for incomplete subtraction) and quantification of OM absorbances in subtraction spectra. Moreover, endogenous internal standards commonly used for soil samples such as silicate Si-O at 1081 cm^{-1} (Kaiser and Ellerbrock, 2005) and 800 to 795 cm^{-1} (Bernier et al., 2013) are not possible for subtraction spectra representing SOM.

Limited work highlights the potential of exogenous internal standards for quantitative FTIR of SOM, though its utility in subtraction spectra is unknown. Davis et al. (1999) added potassium thiocyanate (KSCN) to humic substance (HS) to quantify HS functional groups among samples using $n_s(C\equiv N)$ absorbance at 2050 cm^{-1} (Kabešová and Gažo, 1980). Potassium thiocyanate was added (1.97 mg mL^{-1}) to HS extracts (10 mg mL^{-1}), and the resulting solution was used to dry films for transmission FTIR. Advantages of KSCN include low cost, solubility in the sample solution, and strong absorbance at 2050 cm^{-1} that does not overlap with the sample absorbances. Davis et al. (1999) further demonstrated that absorbance height and area of KSCN absorbance at 2050 cm^{-1} provided equally accurate quantification of model organics, though the complexity of HS spectra made peak height a more suitable choice. This approach was also successfully applied to quantify glycosidic cross-linking in starch (Wongsagonsup et al., 2014). Additional exogenous standards in OM samples include polyacrylonitrile [$n_s(C\equiv N)$ at 2241 cm^{-1}] to quantify hemoglobin content of hemoglobin-based polymeric nanoparticles (Shan et al., 2010). Since soil spectra generally have minimal absorbance in the region 2700 to 2000 cm^{-1} (Fig. 1), potential exogenous standards include additional compounds containing $C\equiv N$ functionalities.

Modeling-Based Computational Subtractions

The use of computers to model subtractions holds promise for elucidating the organic and mineral spectra of soil samples.

For example, the use of quantum chemical calculations (QCC) could offer a potential strategy to perform spectral subtractions that account for interaction(s) of soil components that are difficult to resolve with linear subtractions (See *Interactions among Sample Constituents*). Quantum chemical calculations employs hybrid molecular orbital/density functional theory (MO/DFT) calculations to model infrared vibration frequencies and thus FTIR spectra), including interactions of organics and minerals (Kubicki et al., 2007). It is well established that FTIR spectra can provide empirical data to test predicted spectra modeled by QCC (e.g., Kubicki et al., 1996, 1997), including organomineral interactions (Omoike et al., 2004; Parikh et al., 2011). Theoretically, it could be possible to calculate subtraction spectra using QCC models verified by experimental spectra. Target subtraction spectra could be used to model QCC calculation of subtraction spectra of increasingly complex organomineral mixtures, beginning with synthetic mineral and OM systems. The hypothetical computer model would likely require additional sample information such as total elemental content (e.g., C, Si, Fe, Al, Ca, Mg) and mineralogy profiles (X-ray diffraction data) to accurately predict discrete organic and mineral spectra. Following computer output, the modeled vibrational frequencies and intensities from the subtraction spectra would be compared with experimental spectra to determine the most accurate QCC model for calculating subtraction spectra. One major hurdle to this approach is that QCC models available do not calculate peak widths and, thus, developments in combining DFT with molecular dynamics (MD) with other models or corrections will be necessary. In addition, current limitations on computing resources and required user input pose obstacles to this approach; however, advances in computer science and software development may allow this approach to be realized in the foreseeable future.

CONCLUSION

Fourier transform infrared spectroscopy is increasingly employed in soil science for its ability to provide low-cost, sensitive measures of functional group composition, in particular for the OM fraction. Spectral subtractions are an integral component of the spectroscopist's toolbox, and in soil science offer a suite of applications that improve or enable characterization of soil components and processes. The complexity of soil samples and the dominance of the mineral component make spectral subtractions an ideal tool for resolving the minority organic component, but also engender soil-specific considerations, which partly depend on the experimental objective. Limitations of FTIR in general and the complexity of SOM should not be conflated with issues specific to spectral subtractions, which in fact allow quantitative applications of FTIR in soil samples and are a necessity under aqueous experimental conditions. Recent work identifies strong potential of alternative techniques to expand the utility of subtractions in soil samples, by furnishing mineral backgrounds, evaluating potential artifacts, and establishing spectral assignments to better link SOM chemistry with in situ dynamics. Future work should further identify sample-specific limitations to better inform trade-

offs involved in the operational use of spectral subtractions to enhance its structural insights to soil samples.

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