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Progressing towards more quantitative analytical pyrolysis of soil organic matter using molecular beam mass spectroscopy of whole soils and added standards

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ABSTRACT

Soil organic matter (SOM) is extremely complex. It is composed of hundreds of different organic substances and it has been difficult to quantify these diverse substances in a dynamic- ecosystem functioning standpoint.Analytical pyrolysis has been used to compare chemical differences between soils, but its ability to measure the absolute amount of a specific compound in the soil is still in question.Our objective was to assess whether utilizing pyrolysis-molecular beam mass spectroscopy (py-MBMS) to define the signature of known reference compounds (adenine, indole, palmitic acid, etc.) and biological samples (chitin, fungi, cellulose, etc.) separately and when added to whole soils it was possible to make py-MBMS more quantitative.Reference compounds, spanning a wide variety of compound categories, and biological samples, expected to be present in SOM, were added to three soils from Colorado, Ohio, and Massachusetts that have varying total C, % clay, and clay type.Py-MBMS, a rapid analysis technique originally developed to analyze complex biomolecules, flash pyrolyzes soil organic matter to form products that are often considered characteristic of the original molecular structure. Samples were pyrolyzed at 550 °C by py-MBMS.All samples were weighed and %C and %N determined both before and after pyrolysis to evaluate mass loss, C loss, and N loss for the samples.An average relationship of $r^2 = 0.76$ ($P = 0.005$) was found for the amount of cellulose added to soil at 25, 50, and 100% of soil C relative to the ion intensity of select mass/charge of the compound.There was a relationship of $r^2 = 0.93$ ($P < 0.001$) for the amount of indole added to soil at 25, 50, and 100% of soil C and the ion intensity of the associated mass variables (mass/charge).Comparing spectra of pure compounds with the spectra of the compounds added to soil and isolated clay showed that interference could occur based on soil type and compound with the Massachusetts soil with high C (55.8 g C kg^{-1}) and low % clay (5.4%) having the least interference and the Colorado soil with low C (14.6 g C kg^{-1}) and a moderate smectite clay content of 14% having the greatest soil interference.Due to soil interference from clay type and content and varying optimum temperatures of pyrolysis for different compounds it is unlikely that analytical pyrolysis can be quantitative for all types of compounds.Select compound categories such as carbohydrates have the potential to be quantified in soil with analytical pyrolysis due to the fact that they: 1) almost fully pyrolyzed, 2) were represented by a limited number of m/z, and 3) had a strong relationship with the amount added and the total ion intensity produced.The three different soils utilized in this study had similar proportions of C pyrolyzed in the whole soil (54–57%) despite differences in %C and %clay between the soils. Mid-infrared spectroscopic analyses of the soil before and after pyrolysis showed that pyrolysis resulted in reductions in the 3400, 2930–2870, 1660 and 1430 cm^{-1} bands.These bands are primarily representative of O—H and N—H bonds, C—H stretch, and δ (CH_2) in polysaccharides/lipid and are associated with mineralizable SOM.The incorporation of standards into routine analytical pyrolysis allowed us to assess the quantitative potential of py-MBMS along with the effect of the mineral matrix, which we believe is applicable to all forms of analytical pyrolysis.

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1. Introduction

Soil organic matter (SOM) consists of a complex array of substances that vary in molecular weight, structural complexity, mean residence times and interactions with the mineral soil matrix. It is challenging to describe and quantify the full diversity of these substances. Thus, they are often aggregated into classes of compounds with similar properties. Most physical and chemical methods used to determine different SOM components are time intensive and produce only operationally-defined information that may not always represent meaningful identities. The application of new techniques has revealed that the classic operationally-defined 'lignin' fraction determined by acid digestion (Klason lignin) contains an abundance of non-lignin compounds (Preston et al., 2009). The methods used to fractionate and characterize SOM have a critical influence on conceptual paradigms of SOM formation, decomposition, and functionality. As new techniques have emerged, they have required us to revisit and revise paradigms such as the contribution of humic substances (Kelleher and Simpson, 2006), the recalcitrance of lignin (Preston et al., 2009), the contribution of microbial constituents to SOM formation (Kelleher and Simpson, 2006; Miltner et al., 2012), and the role of the soil matrix to soil formation and dynamics (Grandy et al., 2009).

Analytical pyrolysis (py) has been widely applied to structural studies of synthetic and biologic macromolecules. The transfer of thermal energy to the polymeric network or macromolecule causes physical cleavage of the chemical bonds and yields pyrolysis products that can be related back to the original structures. When coupled with gas chromatographic mass spectroscopic (GC/MS) detection, it has often been used in a variety of studies to characterize SOM and has proven very useful in providing insight into a wide range of questions relating to soil. Pyrolysis-gas chromatography-mass spectroscopy (Py-GC/MS) separates volatile pyrolysis species on a GC column before MS analysis. It is a powerful technique that requires selecting a column designed for specific analyte classes (hydrocarbons, oxygenates, aromatics) and it is possible that not all pyrolyzates are separated on a given column. Py-GC/MS has been used to characterize peat (Calvert et al., 1989), humic acids (Saiz-Jimenez and Deleeuw, 1986; González-Pérez et al., 2011), burned soils (De la Rosa et al., 2008), soil fractions (Buurman and Roscoe, 2011), allophanic soils (Buurman et al., 2007) and SOM from different ecosystems (Vancampenhout et al., 2009). Field ionization mass spectroscopy (py-FIMS) in which mg quantities of sample are directly introduced into the ion source of the mass spectrometer with no prior separation, has revealed differences in SOM between tillage treatments (Sleutel et al., 2007), the soil rhizosphere (Gillespie et al., 2009), soil fractions (Schnitzer and Schulten, 1992; Leinweber and Schulten, 1995), seasonal variations (Leinweber et al., 1994), and SOM formation over time (Schulten et al., 1992). A related approach to py-FIMS couples analytical pyrolysis with molecular beam mass spectroscopy (py-MBMS), which has the advantage of high sample throughput and rapid measurement of high molecular weight product signals compared to GC/MS methods (Evans and Milne, 1987). Unlike GC/MS data, pyrolysis mass spectra are very complex and representative of all species in the pyrolyzate. Multivariate data analysis (pattern recognition) is generally used to process the large spectral data sets and identify trends to discover the underlying chemical changes that may not be obvious by comparison of such complex mass spectra (Evans and Milne, 1987). Magrini et al. (2002) and Hoover et al. (2002) used py-MBMS to distinguish differences between SOM based on depth, site, and revegetation. It has also been used to distinguish between native and cultivated soils and soil fractions (Plante et al., 2009; Haddix et al., 2011) and relate pyrolysis characteristics to SOM content, particulate organic matter C, mineral C, and soil microbial biomass C in native prairie soils (Magrini et al., 2007).

Analytical pyrolysis provides a wealth of compound identification, but is usually considered semi-quantitative. One reason for this is that different compounds have different pyrolytic responses and some compounds when pyrolyzed are released as secondary by-products that cannot be ascribed to their original compounds (Schulten, 1996), but the use of reference compounds can aide in understanding this issue and help us to determine if certain types of compounds can be quantified. A second reason for this method being considered semi-quantitative is that not all SOM is pyrolyzed due to different optimal pyrolysis temperatures for different compounds (Saiz-Jimenez, 1994) along with the modification of organic matter during the heating process (Miltner and Zech, 1997) possibly producing thermally stable char, which needs to be considered when evaluating quantification. Also the method of detection can limit the quantitative ability. Py-GC/MS has the advantage of more thorough compound identification but only pyrolysis products that are GC separable are detected (Saiz-Jimenez, 1994; Dignac et al., 2006). Py-FIMS, and py-MBMS are considered more quantitative due to their ability to detect a larger suite of compounds that are directly introduced into the mass spectrometer (Derenne and Quenea, 2015). If whole soil are being pyrolyzed the mineral matrix can interfere with the pyrolysis process causing problems with quantification. This interference can be caused by clays, Fe and Al oxides, and carbonates (Faure et al., 2006a; Faure et al., 2006b; Spaccini et al., 2013). While in some cases clays can increase cracking activity (produce less complex pyrolyzates) and make it difficult to associate pyrolysis products back to their original compounds, useable pyrolyzates are still generated for analysis (Magrini et al., 2007). Extraction techniques that target specific organic components have been used with analytical pyrolysis to minimize mineral interference. Pretreatments such as hydrofluoric acid (HF) (Zegouagh et al., 2004; Rumpel et al., 2009; Spaccini et al., 2013; Suárez-Abelenda et al., 2015) or humic extraction utilizing NaOH (Saiz-Jimenez and Deleeuw, 1986; Schulten, 1996; Plante et al., 2009; González-Pérez et al., 2011) can reduce interference from soil constituents and isolate organic matter, but these methods can also cause issues with quantification. HF can cause C losses from 7 to 30% during treatment of surface soils and forest horizons (Skjemstad et al., 1994; Mathers et al., 2002; Rumpel et al., 2006) and losses up to 80–92% in subsurface soils (Dai and Johnson, 1999; Rumpel et al., 2002). HF pretreatments can also cause biases due to chemical alteration of the SOM (Dai and Johnson, 1999; Rumpel et al., 2006; Sleutel et al., 2009). Extraction by NaOH isolates up to 80% of organic matter (Stevenson, 1994), but there is some debate about the prevalence of humics in soil (Kelleher and Simpson, 2006) and if they are the product of the extracting media (Kleber and Johnson, 2010; Lehmann and Kleber, 2015).

Previous work has found that 5–12% of the soil mass is lost during pyrolysis (Sorge et al., 1993a; Sleutel et al., 2007) and that 47–99% of total C and N pyrolyzes in soil and soil fractions (Leinweber and Schulten, 1995; Schulten and Leinweber, 1999). This wide range of values is dependent on pyrolysis temperature, %C, and soil type. Extensive *m/z* score identification lists, which do not differ greatly with the type of instrumentation, have been published for reference compounds (Buurman and Roscoe, 2011; Schulten, 1996).

The use of complementary analysis such as XANES and NMR in conjunction with analytical pyrolysis has shown similarities in composition between compounds identified utilizing different methods (Kaal et al., 2007; Gillespie et al., 2009; Leinweber et al., 2010). We need to determine the relationships between the mass spectroscopic signals from reference compounds expected to be present in SOM and the total ion intensity produced to better measure the quantity as well as the type of SOM constituents. It is also necessary to establish the mass loss and amount of C and N pyrolyzed in different soils as this will aid in our quantification ability. Clays have an important effect on the amount of soil organic matter present in soil. Additionally, the impact of soils with varying properties such as clay content, %C, Fe and Al oxides, etc. on the type and amounts of pyrolysis products needs to be established when pyrolyzing whole soil samples. Our approach in this study is to add compounds representative of the building blocks most commonly found in SOM with the expectation that we can relate select *m/z* from these standards back to actual amounts in the soil. Along with this we

will measure mass loss with pyrolysis and the effect of the mineral matrix to evaluate if we can improve quantification of analytical pyrolysis.

A recent review of analytical pyrolysis-based instrumental techniques characterized py-MBMS as a quantitative analysis (Derenne and Quenea, 2015) though a need to further investigate the quantitative degree of py-MBMS is warranted.The objective of this study was to investigate the degree of quantification achievable with py-MBMS in order to quantify SOM compounds within the soil matrix along with determining the effect of the soil matrix in different soils.Specifically, we aimed to answer the questions:1) Can we associate specific mass spectral data reported as *m/z* scores to a specific reference compound or compound category? 2) Is there a strong relationship between select *m/z* and the amount of an added compound in a sample? 3) What is the degree of mineral interference for various compounds and soil types? 4) What compounds are and are not pyrolyzed?The answers to these questions begin to lay a foundation for the use of py-MBMS, and other analytical pyrolysis techniques, as a quantitative SOM analysis tool.

2. Material and methods

2.1. Sample sites

We utilized three soils that we have previously studied (Haddix et al., 2011; Plante et al., 2009) with varying C, N, pH, and clay content and type (Table 1). The Akron soil with 14.6 g C kg^{-1} and a moderate smectite clay content of 14% was collected from USDA-ARS Central Great Plains Research Station (40°09′N, 103°08′W) near Akron, Colorado. The Akron soil is an Aridic Paleustoll (Halvorson et al., 1997) and was collected to a depth of 0–20 cm in native grassland with a mix of C_3 and C_4 grasses. There were three separate sampling pits within the grassland area, each treated as a separate replicate.

The Hoytville soil with 24.8 g C kg^{-1} and high clay content (35.9%), comprised of illite, was from the Ohio Agricultural Research Development Center near Hoytville, Ohio (41° 00′N, 84° 00′W). This soil is a Mollic Ochraqualfs that has been under continuous corn since 1962 after the removal of the deciduous forest and installation of tile drainage. The site is in no-till and has an extensive proportion of high mean residence time soil organic matter associated with the clay fraction (Paul et al., 2001; Haile-Mariam et al., 2008). This site has three plot replicates and multiple composited cores were taken from each plot to a depth of 0–20 cm. The clay fraction used in this study was fractionated from the Hoytville soil by shaking the soil with 0.5% sodium hexametaphosphate and glass beads for 18 h and then centrifuging the dispersed sample at 200 g for 2 min 56 s after which the clay supernatant was aspirated off and dried at 60 °C.

The Waltham soil was collected from the Boston Area Climate Experiment (BACE) in Waltham, Massachusetts (40°23′N, 71°13′W). This has a higher C content (55.8 g C kg^{-1}) but only 5.4% clay. The site is now a grassland on an abandoned agricultural field, which was originally deciduous forest. The soil is a Mesic Typic Dystrudept (Haven series) (Suseela et al., 2012). This site has three plot replicates and multiple, composited cores were taken from each plot to a depth of 0–5 cm.

2.2. Sample preparation and compound standards

Soils were prepared by removing large plant materials, sieved to 2 mm, oven dried at 60 °C, and finely ground prior to analysis. Total C and N content was determined with a LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI, USA). The absence of carbonates was confirmed using a fizz test, so total C in the soils was determined to be organic C. The pH of soils was determined using 1:1 soil to water slurry solution (Thomas, 1996).

We utilized pure reference compounds that were readily available through manufacturers and biological samples that were purchased through manufacturers or isolated from biological materials (Table 2). The reference compounds spanned a variety of compound categories utilized in other studies (Schulten, 1996; Hempfling and Schulten, 1990) that we are using as model compounds to represent some of the hundreds of compounds that are common in SOM. The biological samples were chosen to represent actual biological components we would expect to find in SOM. The reference and biological samples were run on the py-MBMS by themselves and mixed with soil. The reference compounds and biological samples were added to each soil in an amount to increase the total C content of the sample by 50%. Fifty percent of soil C was chosen to make sure the added compound had a detectable signal, but was not greater than the soil SOM signal. Cellulose and indole were added to the soil in three different concentrations (standard addition) to increase the soil C content by 25, 50, and 100%. Cellulose was also added to an isolated, clay-sized fraction from the Hoytville soil to determine clay interference. All solid standards added to dry soil were mixed with each soil using a mortar and pestle. The bacteria sample (*Escherichia coli*) was in a dilute liquid growth broth and was added to the soil drop-wise just prior to analysis. The original spectra for the reference samples shown in Table 2 are shown in Supplemental Fig. 1.

2.3. Instrument analysis

Samples were analyzed using py-MBMS (Magrini et al., 2002; Hoover et al., 2002). 100–200 mg of soil and 5–20 mg samples of standard were run in duplicate or triplicate replicates. All soils and standards were oven dried at 60 °C prior to analysis except indole and palmitic acid which were placed in a desiccator for 24 h prior to analysis, due to their low melting point. Samples were weighed in quartz boats and pyrolyzed until the total ion intensity returned to background levels, which was approximately 3 min, in a reactor consisting of a quartz tube (2.5 cm inside diameter) with helium flowing through at 5 L min^{-1} heated and maintained at 550 °C. The quartz reactor was connected to the sampling orifice of the molecular beam mass spectrometer (MBMS). The system utilized an Extrel TM model TQMS C50 for analysis of the pyrolysis vapors. Residence time of the vapors was short enough to minimize secondary reactions in the quartz reactor (Evans and Milne, 1987; Plante et al., 2009). Mass spectral data from *m/z* 20 to 625 were acquired on a Teknivent Vector 2TM data acquisition system using 22 eV electron impact ionization and programmed storage in a personal computer. Repetitive scans (one 480 amu scan s^{-1}) were recorded during the evolution of a pyrolysis wave from each soil sample and then averaged across all scans. For all spectra, a blank spectra signal was subtracted prior to any data analysis. Compound category summaries were calculated by using published compound categories and

Table 1
Soil characteristics of samples used for py-MBMS standards (average ± 1 standard error, $n = 3$).

Site	Abb.	Treatment	Mineralogical clay	pH	Depth (cm)	C (g kg^{-1})	N (g kg^{-1})	%Clay
Akron, CO	AK	Native grassland	Smectite	6.7	0–20	14.6 ± 1.1	1.73 ± 0.05	14.3 ± 0.7
Hoytville, OH	HYT	No-till corn	Illite	6.1	0–20	24.8 ± 0.4	2.76 ± 0.02	35.9 ± 0.6
Waltham, MA	WAL	Old field	Smectite/illite	4.6	0–5	55.8 ± 4.1	4.68 ± 0.27	5.4 ± 0.4

Abb.: name abbreviation.

Table 2
Major peaks (mass/charge) associated with various C standards and biological samples.

Material	Abb.	Compound category	Molecular weight	Primary peak (*m/z*)	% Total ion intensity	Secondary peak (*m/z*)	% Total ion intensity
Standards							
Adenine	Ad	Prot	135.1	135	42.1	136	28.3
Alanine	Al	Prot	89.1	44	34.3	90	9.2
Arginine	Ar	Prot	174.2	69	2.8	134	2.6
Asparagine	As	Prot	132.1	123	9.4	97	6.5
Caffeic acid	Cf	Alk Arom	180.2	110	11.2	136	10.4
Cellobiose	Clo	Carb	342.3	60	8.4	73	7.1
Ergosterol	Er	Sterols	396.7	396	18.2	397	6.0
Glucosamine	Gs	Prot	179.2	36	5.4	160	2.1
Glucuronic acid	Gr	Carb	194.1	86	8.4	57	7.9
Glycine	Gy	Prot	75.1	114	12.8	30	9.1
Guanine	Gu	Prot	151.1	151	44.3	152	10.2
Indole	In	Heterocyclic N	117.2	117	40.4	90	24.0
Methionine	Me	Prot	149.2	149	8.7	104	6.7
Palmitic acid	Pa	Lipids	256.4	256	12.0	257	9.9
Ribose	Rb	Carb	150.1	73	3.8	57	3.6
Tannic acid	Ta	Alk Arom	1701.2	126	20.2	170	13.4
Vanillin	Va	Ph&LM	152.2	152	35.5	151	16.4
Xylose	Xy	Carb	150.1	73	3.9	96	3.3
Biological samples							
Bacteria	Ba			112	1.5	152	1.4
Bovine protein	BP	Prot		61	11.6	43	5.4
Casein	Ca	Prot		44	3.7	41	2.1
Cellulose	Cl	Carb		60	11.9	73	8.3
Chitin	Ct			43	4.2	84	3.1
Chlorophyll	Ch			497	2.5	123	2.0
Egg protein	EP	Prot		154	1.6	138	1.1
Lignin poplar	LP	Lig Dimers		154	3.8	167	3.5
Morel	Mo			110	1.7	280	1.6
Pectin	Pe			126	4.7	97	2.8
Shiitake	Sh			43	2.7	44	1.8
Urease	Ur	Prot		154	1.4	84	1.2

Abb.: name abbreviation.
Alk Arom: alkyl aromatic; Carb: carbohydrates; Lipids: lipids, alkanes, alkenes, fatty acids; Prot: proteins, peptides, amino acids, nucleic acids; Ph&LM: phenols and lignin monomers.

associated *m/z* data (Schulten et al., 1986; Hempfling and Schulten, 1990; Schulten, 1996; Magrini et al., 2007; Sykes et al., 2008; Gillespie et al., 2009) along with validation from our standards. For the few instances when one *m/z* was associated with more than one compound category, the ion intensity of that *m/z* was split equally between the two categories.

With the exception of the cellulose and indole regressions, signals from individual samples were standardized to 100% total ion intensity (TII), which corrects for differences in sample size and C content. The amount of mass loss during pyrolysis was determined by weighing the sample before and after pyrolysis.The C content of soil and pyrolyzed residues was determined using a Carlo Erba NA 1500 Elemental Analyzer (Carlo Erba, Milan, Italy).To determine the amount of added compound pyrolyzed in soil it was assumed that the same amount of soil C was pyrolyzed with and without compound addition. Thus, the additional C pyrolyzed was assumed to be from the added compound.

Pyrolyzed and whole (not pyrolyzed) samples from Akron and Hoytville soils were scanned neat on the mid-infrared (MIR) range of 4000 to 400 cm^{-1} on a Digilab FTS 7000 (Agilent Technologies, Walnut Creek, CA) with a Peltier-cooled DTGS detector, KBr beam splitter, and KBr background. The samples were scanned in diffuse reflectance mode, and resolution was set at 4 cm^{-1}, with 64 co-added scans. Prior calibration of the instrument was done using many of the same standards utilized in this study (Calderón et al., 2013). The SUBTRACT.AB application of GRAMS/AI version 9.1 software (Thermo Fisher, Woburn, MA) was used to perform the spectral subtractions of whole minus pyrolyzed soil spectra. This subtraction uses the algorithm described by Banerjee and Li (1991). The resulting spectrum equals the whole soil spectrum minus the pyrolyzed soil spectrum multiplied by a subtraction factor. We used the default factor and tolerance values calculated by the software.

2.4. Statistical analysis

Individual spectra were visually inspected for outliers and then analytical replicates from py-MBMS were averaged for each sample and all statistical analysis was done on the three field replicates for each soil. Comparisons between reference compounds and biological standards mass spectral data were done using non-metric multidimensional scaling (NMS) for *m/z* 57–625 (PC Ord version 6.0 MjM Software, Gleneden Beach, OR). NMS does not assume that the data is normal or that there are linear relationships among variables (McCune and Grace, 2002). For NMS the Sørensen (Bray-Curtis) coefficient was used for calculating distance measures with a random seed and 50 runs with real data. Comparisons between pure compound spectrum to soil + compound were done by subtracting the standardized spectrum of the soil from the standardized soil + compound spectrum. The positive portion of the difference spectra (*m/z* scores associated with the pure compound) was then re-standardized to 100% and the ion intensity of each *m/z* from the pure compound was compared to the re-standardized compound spectra when added to soil. When cellulose and indole where added to the soil in different amounts, comparisons were made between ion intensity of peaks isolated using NMS for indole that was *m/z* 89, 90, 117, and 118 (Fig. 1) and for cellulose that was *m/z* 60, 73, 89, 144 (Fig. 2). Statistical analysis of amount C pyrolyzed between the soils was done using the ANOVA procedure in SAS (v9.3) by soil.

3. Results

3.1. Reference compounds and biological samples

For this study we utilized a wide range of compounds that spanned many general compound categories found in whole soils. We had two

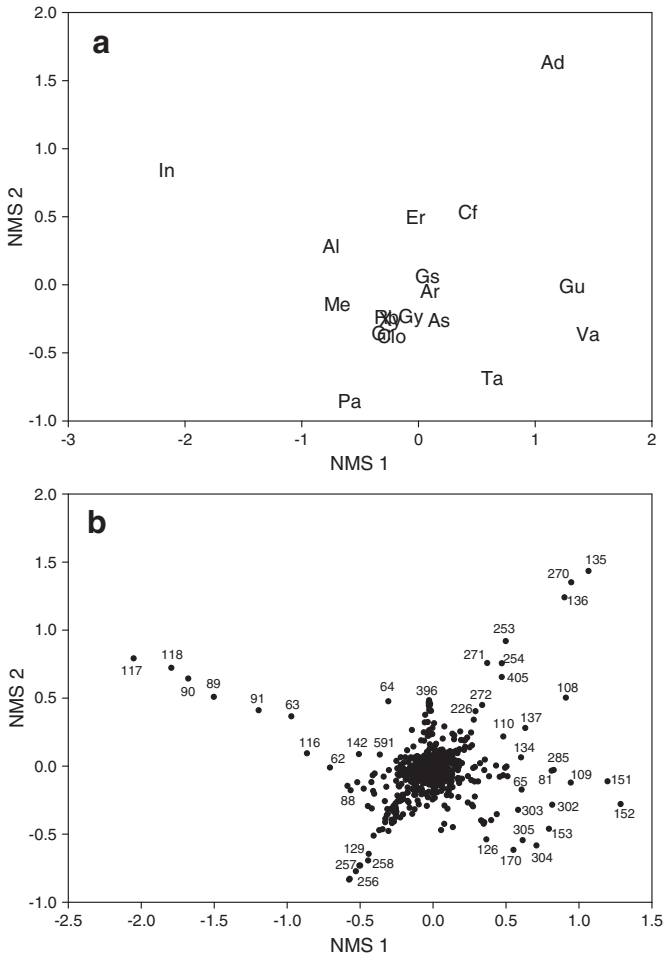

Fig. 1. Non-metric multidimentional scaling (NMS) of py-MBMS data of reference compounds for *m/z* 57–625 of scores (a) and loadings (b). Compound abbreviations are found in Table 2. Values are averages of two or three replicates.

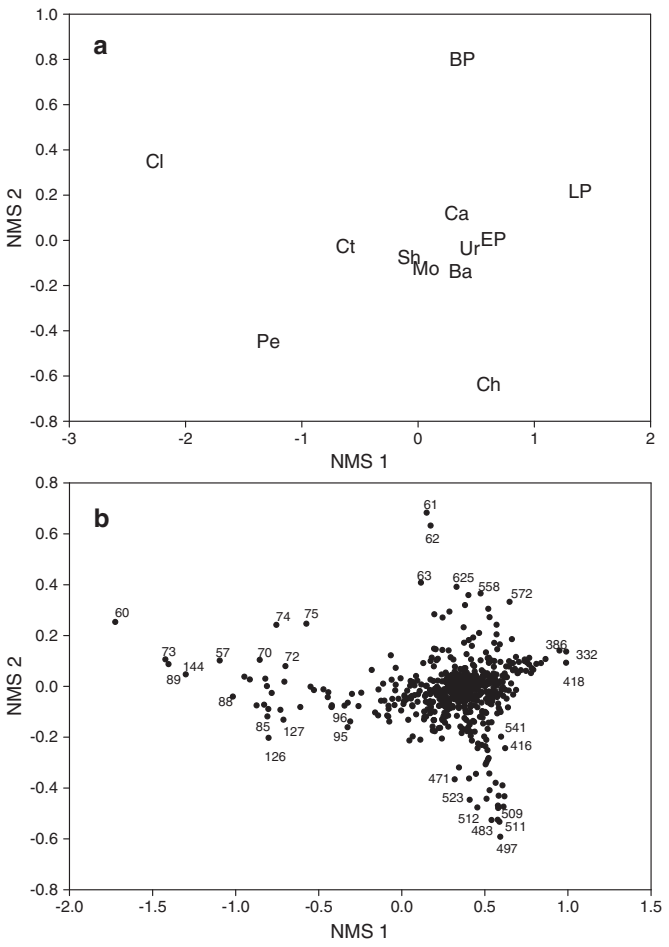

Fig. 2. Non-metric multidimentional scaling (NMS) of py-MBMS data of biological samples for *m/z* 57–625 of scores (a) and loadings (b). Sample abbreviations are found in Table 2. Values are averages of two or three replicates.

compounds in the alkylaromatic category, caffeic acid and tannic acid, with 34% of the total ion intensity (TII) being associated with the primary and secondary peaks for tannic acid and 22% for caffeic acid (Table 2).Five compounds comprised the carbohydrate category with the most complex being cellulose (Fig. 3a).Cellulose had the greatest percent of TII from the two dominant peaks of the five compounds totaling 20%.The two sugars, ribose and xylose, behaving similarly and had the lowest percent TII at 7% each.Cellulose, a crystalline glucose polymer, and cellobiose, a soluble glucose dimer, had similar spectra with *m/z* 60 (levoglucosan) and *m/z* 73 (C5, C6 sugars) having similar total ion intensity for their dominant peaks (Table 2).Multiple peaks were associated with all or many of the compounds in the carbohydrate category with all five compounds having *m/z* 73 in the top 5 peaks and all but xylose having *m/z* 57 in the top 5 peaks.Indole, a heterocyclic N compound, had 64% of its TII associated with its two dominant peaks (amu) and the primary peak corresponded with its molecular weight of 117 (Fig. 3b).The 12 compounds comprising the proteins, peptides, amino acids, and nucleic acids category had TII associated with the two dominant peaks that varied from 3 to 70% (Table 2).The pure amino acids, alanine, glycine, and methionine, and nucleobases, adenine and guanine, resulted in clear definable spectra whereas the more complex compounds like the proteins- bovine serum, casein, and egg, and the enzyme urease had complex spectra with many *m/z* scores associated with very little TII or uninformative spectra where many of the major *m/z* scores are associated with *m/z*<56 (Supplemental Fig. 1).We did not see similar *m/z* scores or percent TII between the three proteins with bovine serum protein having 17% of TII associated with the two dominant peaks, although only *m/z* 61 is diagnostic for identification, and the egg protein was associated with higher weight *m/z* scores, but little TII associated with any single *m/z*.Casein provided the least useful information with the most abundant *m/z* scores being low weight and low TII associated with any one *m/z*.There were seven compounds where the primary peak corresponded to the molecular weight and these compounds fell into five different compound categories (Table 2).

The biological samples tended to have fairly complex spectra with numerous *m/z* products and a low percent of the total ion intensity attributed to primary and secondary peaks, with the exception of bovine protein and cellulose (Fig. 3a) (Table 2). For example, the morel mushroom had 1.7% of the total ion intensity (TII) associated with *m/z* 110 which has been identified as a furaldehyde (Van Smeerdijk and Boon, 1987; Hempfling and Schulten, 1990) or a dihydroxybenzene (Van Smeerdijk and Boon, 1987). The furans can have a microbial origin and large amounts of furans tend to be produced with pyrolysis of fungal biomass (Gutiérrez et al., 1995), but *m/z* 110 can also be associated with compounds of a non-microbial origin. The second peak, *m/z* 280, represented 1.6% of the TII, which has been identified as a C20:1 alkene (Gillespie et al., 2009; Hempfling and Schulten, 1990). Along with these *m/z* scores, there were a total of 49 *m/z* scores that represented at least 0.5% of TII for the morel mushroom (Fig. 3c). 2-and 3-furaldehyde and 5 methyl 2-furaldehyde can be pyrolysis products of microbial organic matter (Buurman et al., 2007) and so we would expect *m/z* 110 and 97 to be high in the bacteria sample and fungal samples. We did find *m/z* 110 to be a major peak in the morel mushroom spectrum, but not in the shiitake mushroom or bacteria samples (Table 2). In the shitake

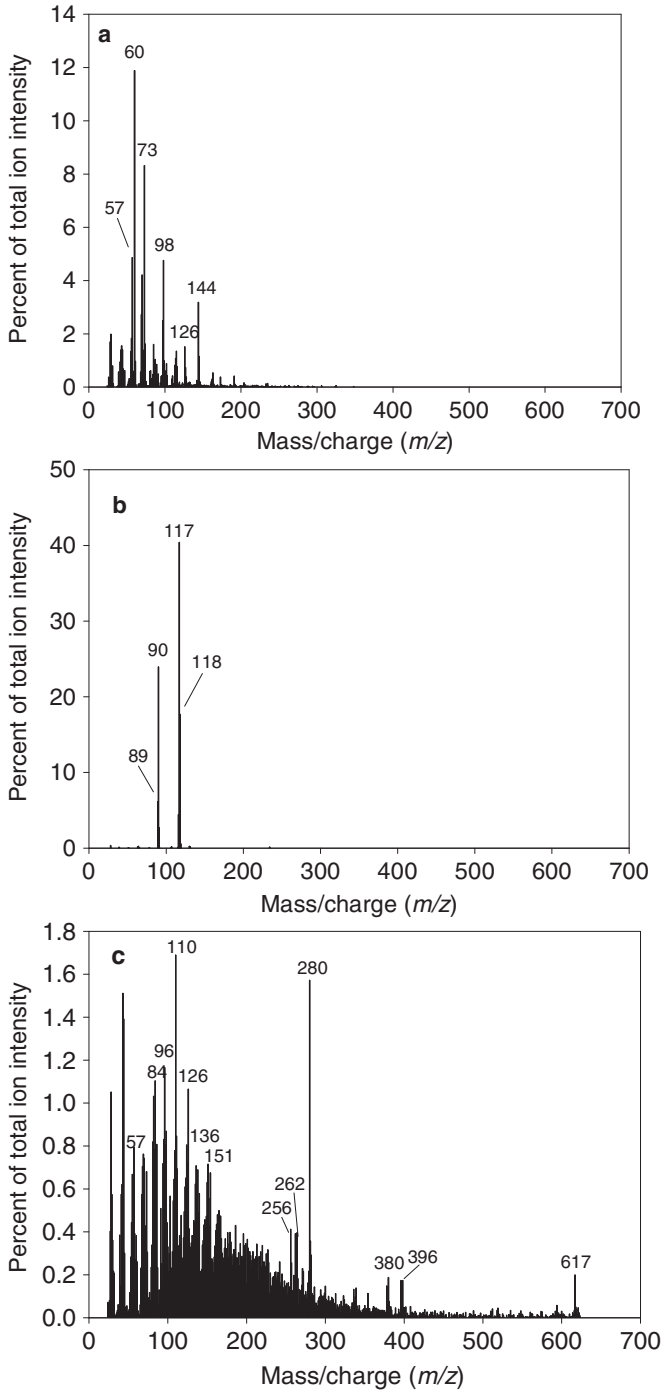

Fig. 3. Py-MBMS spectra in percent of total ion intensity for *m/z* 20–625 for cellulose (a), indole (b), and morel mushroom (c).

the two *m/z* scores combined represent 1.9% of the total ion intensity and in the bacteria these two peaks combined contribute 1.5% to the total ion intensity.

We utilized non-metric multidimensional scaling (NMS) to isolate unique *m/z* associated with the different reference compounds and biological samples.The NMS for the reference compounds had a two dimensional solution recommended and stabilized after 40 iterations with a final stress of 9.9 and the two dimensions accounted for 72% of the variance (Fig. 1).Indole and adenine separated out from the other compounds (Fig. 1a) and *m/z* scores 89, 90, 117, and 118 being associated with indole and comprising 88% of the TII and *m/z* scores 135, 136, and 270 being associated with adenine and comprising 78% of the TII (Fig. 1b). The carbohydrates clustered together (cellobiose (Clo), glucuronic acid (Gr), ribose (Rb), xylose (Xy)), but were clustered next to many of the compounds from the proteins, peptides, amino acids, and nucleic acids category (arginine (Ar), asparagine (As), glucosamine (Gs), glycine (Gy)) so NMS does not separate out any distinct *m/z* for these compounds (Fig. 1a).Guanine and vanillin cluster together due to the same *m/z* (151 and 152) being associated with both compounds, but they are from different compound categories.Both palmitic acid and tannic acid separate from the cluster of compounds with distinct *m/z* being associated with each.

A two dimensional solution was recommended for the NMS analysis of the biological samples with a final stress of 4.5 after 61 iterations and the two dimensions explaining 75% of the variance (Fig. 2).The NMS for the biological samples shows separation of cellulose, bovine protein, and lignin (Fig. 2a).There were four *m/z* associated with cellulose (60, 73, 89, and 144) comprising 24% of the TII (Fig. 2b).The *m/z* scores 61 and 62 had the greatest association with the bovine protein and account for 12% of the TII in that compound.The *m/z* scores 332, 386, and 418 appear to have the greatest association with the isolated poplar lignin, but those *m/z* scores only account for 2% of the TII in lignin. Chlorophyll also separated out and was associated with multiple high molecular weight *m/z* and the microbial samples clustered together (Bacteria (Ba), Morel (Mo), Shiitake (Sh)).

3.2. Sample quantification

Cellulose and indole were added to the Akron and Hoytville soils in different amounts to determine the relationship between the ion intensity of select *m/z* scores and the amount of compound added.For both cellulose and indole, the *m/z* most associated with each compound using NMS, for indole that was 89, 90, 117, 118 and for cellulose that

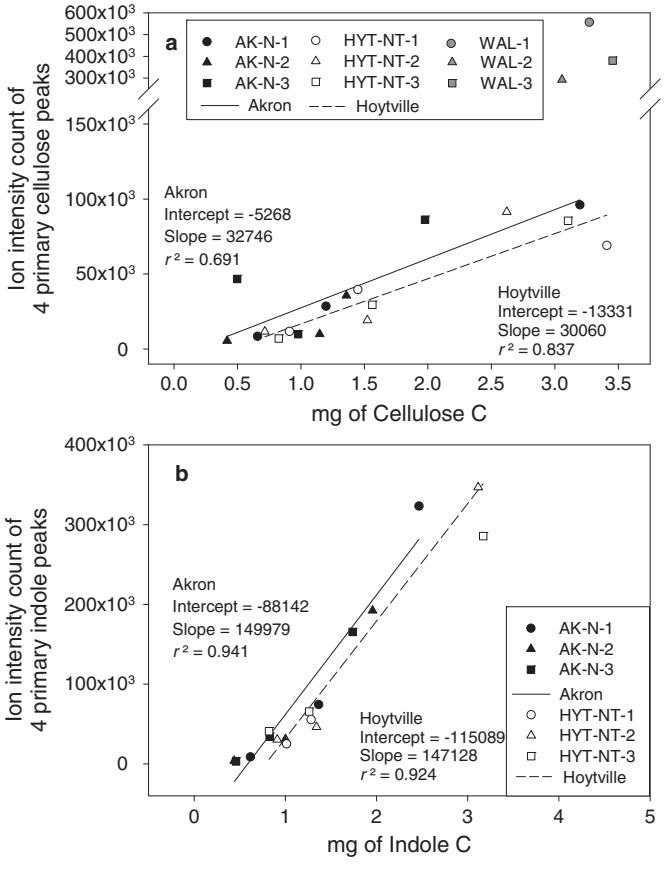

Fig. 4. Regression of amount of compound added in mg C versus ion intensity counts of primary peaks for cellulose (a) and indole (b).

was 60, 73, 89, 144 (Figs. 1 and 2), were summed and the TII of the soil for those peaks subtracted from the soil with added compound.This was then compared with the amount of reference sample C added.There was a relationship of $r^2 = 0.691$ ($P = 0.006$) for cellulose in Akron soil and 0.837 ($P < 0.001$) between the amount of cellulose C added to the Hoytville (Fig. 4a), indole had an $r^2 = 0.941$ ($P < 0.001$) and 0.924 ($P < 0.001$) (Fig. 4b) for Akron and Hoytville respectively.Cellulose was added in only one concentration to the Waltham soil, but the one addition amount was included in Fig. 4a to illustrate the much higher ion intensity count for cellulose that occurred in that low clay soil.Using the regression equation for cellulose to estimate carbohydrate C, we calculate an average 1.7 g C kg^{-1} in the Akron soil representing 11% of the total SOC. The 3.4 g C kg^{-1} calculated carbohydrate C in the Hoytville soil accounts for 14% of the SOC of this sample.

The difference spectra (Fig. 3a versus Fig. 5a, b, and c) show the impact of soil composition on the cellulose pyrolysis spectra. For the difference spectra, all the positive peaks are associated with cellulose and all negative peaks are associated with the soil.Many of the dominant *m/z* scores in the pure compound were also found in the difference spectra (above the line) although at a lower percent TII.The positive portion of the difference diagram for both Hoytville and Waltham showed fairly similar spectra to pure cellulose. However *m/z* 126, a small percentage in pure cellulose, was found in the positive portion in spectra for all soils as well as clay (Fig. 5).Akron also had an addition of *m/z* 110 in the cellulose spectra (Fig. 5a). The clay fraction appears to produce more differences with the reduction of *m/z* 60 (Fig. 5d) showing the influence of both soils and clay on the *m/z* scores.

To understand the effect of different soils on signal interference, we plotted the percent TII in our standard cellulose against the percent TII of the cellulose added to soil.With this method, a slope of one would indicate no soil interference.Akron and Hoytville soils show a reduction of the total ion intensity for cellulose when added to soil, as shown by regression lines below the 1:1 line (Fig. 6a, b).The *m/z* 60, which is the primary pyrolysis peak of cellulose, is still the primary peak in the Akron and Hoytville soil plus cellulose, but *m/z* 73 is no longer the secondary peak.The Waltham soil, in contrast, has little to no soil interference with a regression very similar to the 1:1 line (Fig. 6c) and a slope of 1.20 and a r^2 of 0.947 (Table 3).The isolated clay produced the greatest interference with a regression well below the 1:1 line with a slope of 0.29 and an r^2 of 0.20.None of the dominant peaks found in pure cellulose were dominant peaks in cellulose-clay mixtures (Figs. 5d and 6d).This result as well shows differences in the fragmentation of the pyrolysis products likely due to cracking from the clay.

Recovery equations (coefficient of determinations and slopes) were calculated for the compounds and biological materials added to the soils (Table 3).The relationships varied greatly between compounds with r^2 values from 0.02 to 0.96.Some of the more complex biological materials like chlorophyll and morel mushroom tended to have lower r^2 though the shiitake mushroom did not follow this trend.The Akron and Hoytville soils had very low r^2 for ergosterol.It appears that the soils changed the pyrolysis characteristics of the major fungal lipid ergosterol from an *m/z* 396 to 253 and 254 in Akron and 249 and 253 in Hoytville.This also occurred in the Waltham soil, but to a lesser extent with the dominant peaks of the ergosterol in this soil comprising *m/z* 378 and 253.When these additional peaks were added to *m/z* 396 in ergosterol, the r^2 increased in Akron from 0.02 to 0.37, 0.03 to 0.56 in Hoytville, and 0.44 to 0.82 in Waltham.Although some of the more complex compounds had low r^2 and slopes, adenine, egg protein, guanine, indole, and vanillin had minimal soil interference with slopes similar to one and high r^2 values.Across the different compounds, the Waltham soil tended to have higher r^2 and slopes closer to one, except in the case of urease, but because of the high SOC content more of the standard had been added to the Waltham soil.The Hoytville soil tended to have similar or higher r^2 and slopes than the Akron soil.The proteinaceous compounds that gave poorly defined spectra had coefficients of determination similar to those for other compounds with more defined spectra.

3.3. Pyrolysis of soils

Utilizing previously characterized compound categories (Schulten et al., 1986; Hempfling and Schulten, 1990; Schulten, 1996; Magrini et al., 2007; Sykes et al., 2008; Gillespie et al., 2009) and our standards, we were able to classify into compound categories 58% of the TII of the

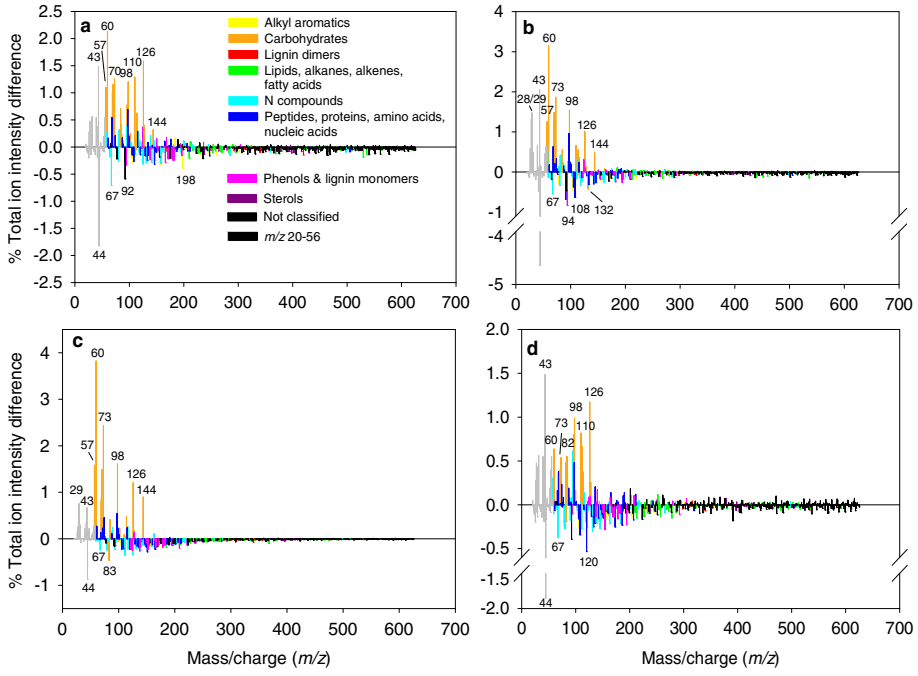

Fig. 5. Relative ion intensity difference from py-MBMS for *m/z* 57–625 between soil with added cellulose minus soil for Akron (a), Hoytville (b), Waltham (c), and Hoytville isolated clay fraction (d). Positive values are associated with cellulose and negative values are associated with soil.

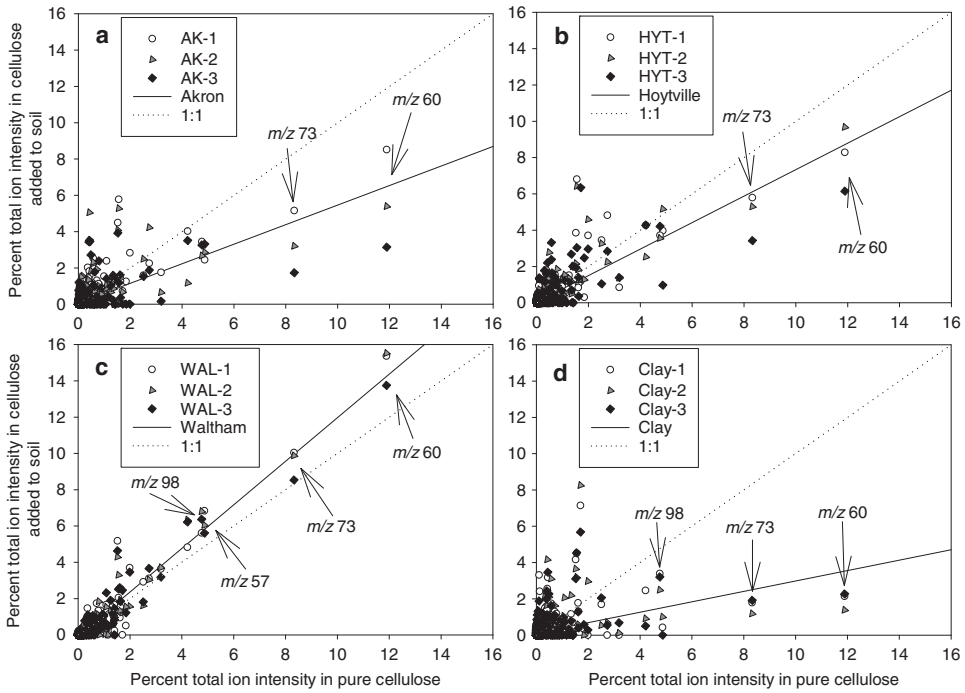

Fig. 6. Relationships between the percent total ion intensity of each *m/z* in pure cellulose and in cellulose added to soil in Akron (a), Hoytville (b), Waltham (c) soils, and Hoytville clay (d).

pyrolysed materials in the Akron soil, 61% in the Hoytville soil, and 78% in the Waltham soil (Fig. 7). These results suggest that the Waltham soil had different SOM constituents or possibly less interference of the mineral constituents as was also seen with the added standards (Table 3). Summing the individual peaks into classes on the basis of our standards and literature show that for the Waltham soil the N compounds and proteins, peptides, amino acids, and nucleic acids categories contributed the most to the TII at 18% each with the next largest category being carbohydrates at 17% (Fig. 7c). Compared to the other two soils, Waltham had more total carbohydrates, proteins, peptides, amino acids, and nucleic acids, as well as phenols and lignin monomers than the Akron and Hoytville soils and less unidentified compounds (Fig. 7). Although the Akron and Hoytville soils have visibly different py-MBMS spectra, the summarized total ion intensities for the compound categories are similar for the two soils (Fig. 7a & b).

3.4. Characterization of pyrolyzed soil samples and standards

Pyrolysis caused 8% mass loss in the Hoytville soil and between 14.9 and 15.8% in the Akron, Waltham, and the Hoytville clay fraction (Table 4). The amount of C pyrolyzed, corrected for mass loss, was 53 to 57% and was not statistically significant between the soils or the clay fraction. Fifty-one to 100% of the reference compounds and biological samples added to soil were pyrolyzed, but there was high variability associated with some of these estimates (Table 5). The amount of sample pyrolyzed differed between the standards, but tended to be similar

Table 3
Correlations between pure material total ion intensity and material spectra added to soil total ion intensity as a means of expressing soil interference ($n = 605$).

Standard/	Akron			Hoytville			Waltham		
Biological samp.	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept
Adenine	0.674	0.953	0.01	0.675	1.160	−0.03			
Bacteria				0.560	0.855	0.02			
Casein	0.372	0.609	0.06	0.397	0.663	0.06			
Cellulose	0.623	0.539	0.08	0.774	0.729	0.04	0.947	1.200	−0.03
Chlorophyll	0.188	0.399	0.10	0.371	0.637	0.06			
Egg protein	0.653	1.043	−0.01	0.627	1.142	−0.02			
Ergosterol	0.020	0.050	0.16	0.028	0.100	0.15	0.444	0.689	0.05
Glucosamine				0.218	0.248	0.12			
Glycine	0.658	0.484	0.09	0.537	0.580	0.07			
Guanine	0.963	0.817	0.03	0.963	0.878	0.02			
Indole	0.749	0.957	0.01	0.741	1.214	−0.04			
Methionine				0.214	0.277	0.12			
Morel	0.353	0.606	0.07	0.551	0.873	0.02			
Palmitic acid	0.551	0.667	0.05	0.631	1.043	−0.01	0.744	1.813	−0.13
Shiitake	0.506	0.730	0.04	0.543	0.965	0.01			
Tannic acid	0.679	0.667	0.06	0.767	0.939	0.01			
Urease	0.550	0.959	0.01	0.659	1.123	−0.02	0.468	0.916	0.01
Vanillin				0.769	1.009	0.00			
Xylose				0.467	0.703	0.05			

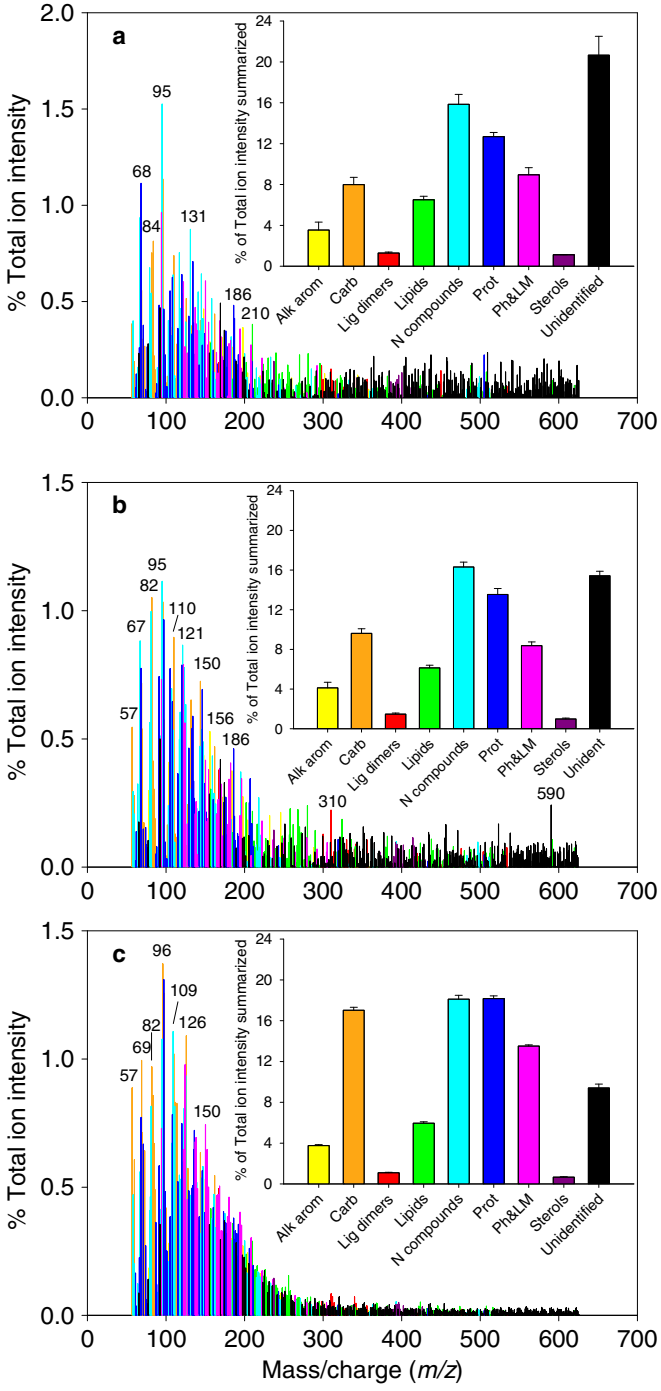

Fig. 7. Py-MBMS spectra in percent of total ion intensity for *m/z* 57–625 for Akron (a), Hoytville (b), and Waltham (c) soils with the percent total ion intensity (average ± 1 standard error, $n = 3$) summarized for each compound category in inset. Alk Arom-alkyl aromatics; Carb-carbohydrates; Lipids (alkanes, alkenes, and fatty acids), Prot-peptides, proteins, nucleic acids, and amino acids; Ph&LM-phenols and lignin monomers.

Table 5
Amount of added material pyrolyzed in each soil (average ± 1 standard error, $n = 3$).

Standard/biological samp.	% of standard pyrolyzed		
	Akron	Hoytville	Waltham
Casein	76.8 ± 8.2	80.7 ± 16.5	
Cellulose	96.2 ± 6.6	95.6 ± 7.6	100 ± 0.0
Glycine	88.5 ± 16.7	92.1 ± 13.7	
Guanine	73.5 ± 23.5	67.5 ± 36.1	
Indole	69.7 ± 11.0	50.7 ± 19.6	
Morel	72.7 ± 25.0	73.7 ± 12.7	
Palmitic acid	94.4 ± 4.9	100 ± 0.0	98.1 ± 3.3
Shiitake	82.2 ± 20.7	82.6 ± 30.1	
Tannic acid	84.1 ± 13.8	71.7 ± 12.0	
Urease	77.3 ± 6.7	85.6 ± 12.3	95.8 ± 7.3

between the soils, except for urease where Akron had 77% of the standard pyrolyzed and Waltham had 96% of the standard pyrolyzed. Cellulose and palmitic acid both pyrolyzed almost completely in all three soils with values ranging from 96 to 100% in cellulose and 94–100% in palmitic acid. Indole pyrolyzed at a much lower percentage, from 51 to 70% (Table 5). These compounds are completely pyrolyzed when analyzed alone and palmitic acid has been correlated with soil microbial biomass (Magrini et al., 2007). Both results suggest that minimal interaction occurs with these compounds during py-MBMS analysis of whole soils.

Characterization of the whole and pyrolyzed Hoytville soil by MIR showed that pyrolysis caused reductions in absorbance at 3400, 2930–2870, 1660, and 1430 cm^{-1} (Fig. 8a). This is consistent with the SOM in the samples losing O—H and N—H bonds (3400), C—H stretch (2930–2870), and δ (CH_2) in polysaccharides/lipid (1430) (Movasaghi et al., 2008). Absorbance at 1660 cm^{-1} has been attributed to amide I-like absorption (C═O/C—N) or aromatic C═C, but it should be noted that other moieties and minerals can complicate this assignment. Absorbance at 1270–1370 cm^{-1}, which encompasses bands for C—O stretch and CH overtones, increased in the pyrolyzed soil relative to the whole soil (Fig. 8a). The Akron spectra of the whole and pyrolyzed soils showed similar changes to the Hoytville soil (Fig. 8b), suggesting that pyrolysis has consistent results across agricultural soils.

4. Discussion

Our objective was to investigate how effectively py-MBMS could be used as a quantitative tool for understanding the chemistry of SOM.There are many aspects involved in achieving a more quantitative analysis, but the four that we focused on were 1) can we associate specific *m/z* scores to a specific compound or compound category? 2) is there a strong relationship between select peaks and the amount of compound in a sample? 3) what is the degree of mineral interference for various compounds and soil types? 4) what compounds are and are not pyrolyzed?

For the first aspect, associating specific *m/z* scores to specific compounds, the type of mass spectrometric analysis used will play a role in this objective. Py-MBMS sacrifices more detailed identification compared to py-GC/MS for the ability to detect a larger suite of compounds. The increased number of peaks in py-MBMS can

Table 4
Amount of mass and C lost with pyrolysis of the soils (average ± 1 standard error, $n = 3$).

Soil	Soil mass pyrolyzed (%)	C before pyrolysis (g kg^{-1})	C remaining after pyrolysis (g kg^{-1})	C pyrolyzed (%)
Akron, CO	14.93 ± 4.07	14.6 ± 1.1	7.2 ± 0.8	56.93 ± 1.37
Hoytville,OH	8.44 ± 1.34	24.8 ± 0.4	12.6 ± 0.7	53.44 ± 3.08
Hoytville Clay	15.78 ± 2.99	40.2 ± 3.2	21.3 ± 3.4	55.77 ± 3.98
Boston, MA	15.19 ± 1.15	55.8 ± 4.1	28.7 ± 3.1	53.70 ± 3.99

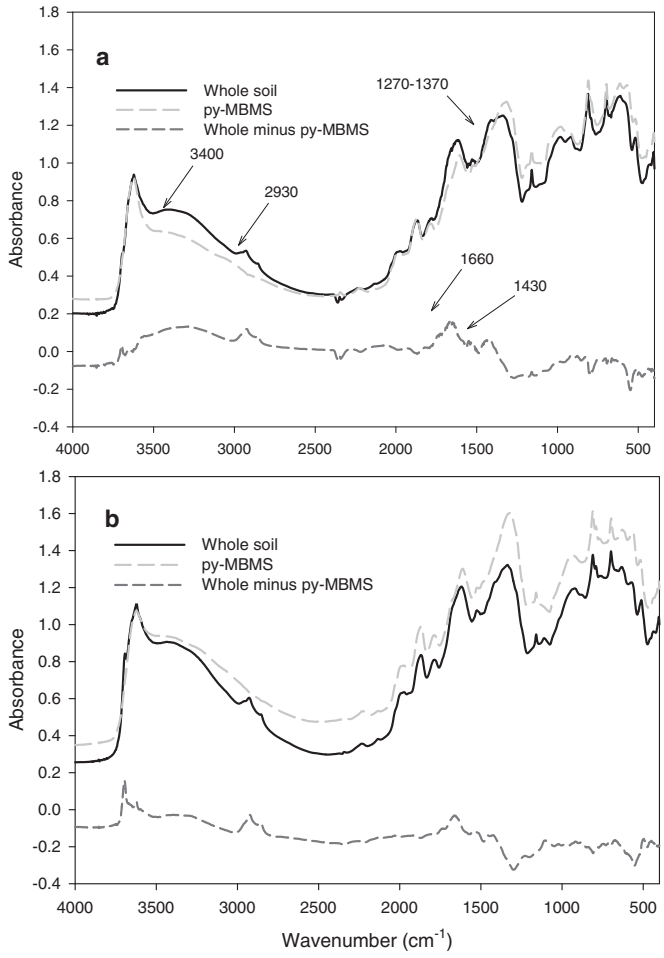

Fig. 8. Mid-infrared diffuse reflectance spectra of whole soil, pyrolyzed (py-MBMS) soil, and subtracted whole minus pyrolyzed soil from the Hoytville (a) and Akron soil (b).

complicate interpretation because multiple compounds can be associated with the same *m/z*. With respect to compound categories, the cyclic N compound, indole, had a large amount of the total ion intensity (TII) associated with a few distinct peaks which allowed it to separate from the other compounds on NMS, making it promising for quantification, however, other compounds in this class need to be investigated. Both alkylaromatic compounds also had a significant amount of total ion intensity associated with only a few peaks, but more compounds need to be assessed to see if this trend continues. The carbohydrate category and cellulose individually are promising for quantitative analysis with many of the same *m/z* (60, 73) being associated with all or most of the compounds compared, a fair amount of TII being associated with a few *m/z* scores, and a clustering of these compounds in NMS, but *m/z* scores from carbohydrates can also be associated with compounds in other categories. The two sugars studied had a lower amount of TII associated with the two largest peaks. Some carbohydrates have been found to pyrolyze at temperatures lower than 550 °C (Syverud et al., 2003) which may be causing the low TII for the two sugars. Although secondary reactions can occur during the pyrolysis of cellulose (Pastorova et al., 1994; Saiz-Jimenez, 1994), we still see a distinct spectra associated with a limited number of *m/z*. We studied a large number of compounds in the protein, peptide, amino acid, nucleic acid category and as an entire category there is a significant amount of variability in the ion traces that make quantification difficult. The ability to accurately quantify proteins in soil would be useful, but with limited informative *m/z* scores being consistently associated with the proteins and low TII associated with *m/z* scores this does not appear feasible. Phenols and alkyl-phenols are signature compounds for proteins, but the same components are found after pyrolysis of lignins, cellulose, or humic substances (Stuczynski et al., 1997), which might be indicative of why there are not distinctive peaks found for the proteins. During protein pyrolysis, secondary reactions can occur and the range of pyrolysis products increases as the variety of building units increases with a large number of unknown compounds (Saiz-Jimenez, 1994), which also contributes to the difficulty in identifying protein pyrolyzates. The amino acids and nucleobases within this larger group did give consistent and promising results for quantification and should be investigated further. Reference compounds like cellulose, indole, palmitic acid, and tannic acid where the majority of the total ion intensity is associated with a limited number of distinct *m/z* scores may be the most useful starting compounds to quantify compounds in soil.

Quantification of bacteria, fungi, and total microbial biomass is useful when trying to understand SOM changes and dynamics. There are pyrolysis products associated with microbial biomass such as 2-and 3-furaldehyde and 5 methyl 2-furaldehyde (Buurman et al., 2007), but complex biological samples like bacteria and the fungi (morel and shiitake) have numerous *m/z* scores and very little of the TII associated with any one *m/z*. The bacteria sample was in a dilute growth broth so that may have also attributed to the numerous peaks as sodium and other ions could crack pyrolysis products. The spectra from microbes are different enough from SOM to indicate that although pyrolysis may not be useful for biomass determinations, it should have some use in the estimation of the role of microbial productions in SOM formation.

Different compounds have optimal pyrolysis temperatures (Saiz-Jimenez, 1994) which makes quantification of a variety of compounds difficult at a single pyrolysis temperature. We utilized 550 °C as a pyrolysis temperature for it tends to be a suitable temperature for biomass pyrolysis (Syverud et al., 2003), but if quantification of a specific compound category is desired modification of pyrolysis temperature should be considered to optimize for that compound. Pyrolysis temperatures of 550–650 °C have been found to be good ranges for pyrolyzing carbohydrates (Syverud et al., 2003) whereas temperatures as high as 770 °C may be necessary to study more resistant macromolecules (Saiz-Jimenez and Deleeuw, 1987).

The second aspect in achieving quantification is understanding the relationship between selected peaks and the amount of compound in a sample. This was only investigated with indole and cellulose as both compounds showed promise with a high percentage of TII associated with a only few distinct *m/z* scores. Both cellulose and indole had robust relationships between the amount of compound added and the ion intensity of the two dominant peaks for both the Akron and Hoytville soils. Both soils had similar, although slightly different, relationships possibly indicative of soil interference (likely clay). Relationships may need to be developed for individual soil types in order to accurately quantify the amount of a specific compound in a soil. The solid relationship between the intensity of selected peaks and amount of compound added to the sample as well as the similar regressions between the soils indicates the usefulness of this approach for quantitative analysis if the complexities involved are taken into consideration. Our estimation of the amount of total carbohydrates in the two soils using the cellulose standard was very similar to the amount of total carbohydrates estimated in our soils using the summation of *m/z* associated with carbohydrates with a 3–4% difference in the values. It is important to note that there may be some differences in residence time and microbial and fungal processing between the pyrolysis of a pure compound like cellulose versus native carbohydrates in soil. A study by Syverud et al. (2003) was able to quantify carbohydrate amounts in chemical pulps utilizing py-GC/MS, supporting the idea of quantification of certain compound categories. Utilizing an approach taken by Sorge et al. (1993b) where 23 amino acid standards were run on py-GC/MS and py-FIMS and then 12 different *m/z* scores were used to quantify α-amino N in soil may also be a useful approach to quantify other well resolved compounds in soil.

The third aspect to consider for more quantitative analysis is the degree of mineral interference for various compounds and soil types. Clay minerals can act as a catalyst during the pyrolysis process and cause the formation of secondary artifacts in pyrolysates (Faure et al., 2006a; Faure et al., 2006b; Spaccini et al., 2013), but the actual amount of interference for quantitative analysis has not been adequately established nor has whether the same breakdown products are always formed. Studies have found that during pyrolysis the clay minerals cause the new formation of aromatic units such as alkylbenzene and polycyclic aromatic hydrocarbons with increasing smectite causing the aliphatic chains to disappear and in parallel a relative enrichment in aromatic structures occurs (Faure et al., 2006a). The effect of clay on pyrolysis transformation can vary between compounds with a wax ester having limited bond breakage in the presence of clay compared to an alkanol and an alkanoic acid (Nierop and van Bergen, 2002). This result could be due to the more stable aliphatic bonds in long chain hydrocarbons compared with the more labile oxygenates.

Mineral components have been shown in other studies to be a controlling factor when pyrolyzing carbohydrate containing biomass (Evans and Milne, 1987; Sorge et al., 1993a; Faure et al., 2006a). We utilized cellulose as an example of how the spectrum changes from the pure standard when added to different soils. The TII of the dominant *m/z* scores of cellulose are reduced in the presence of soil and there was an increase in some of the secondary *m/z* scores. The increases in *m/z* 110 and 126 when cellulose was added to soil and clay also occurred in another study utilizing py-MBMS (Evans and Milne, 1987). When we pyrolyzed cellulose with the isolated clay size fraction, the spectrum associated with cellulose was greatly altered. The addition of alkali material is thought to favor the release of furfural instead of levoglucosan and lead to a product state composed of furfural derivatives (Evans and Milne, 1987). The changes in the cellulose spectrum varied between the three soils and the clay isolate with the Waltham soil, which had the highest %C and lowest clay content having minimal soil interference.

Regression analysis was performed for a variety of compounds to better understand the degree of soil interference among compounds. The degree of soil interference varied between the three soils and between the various compounds. Compounds like guanine and indole had high r^2 values and slopes close to one for both Akron and Hoytville, indicating limited soil interference, whereas compounds like chlorophyll and ergosterol had low r^2 values and slopes for all soils. Interestingly tannic acid, which is a fairly complex compound, had a relatively high coefficient of determination. Compounds that have minimal soil interference are good candidates for quantification, such as cellulose, guanine, indole, palmitic acid, and tannic acid. In our three example soils the Waltham soil with high %C and low % clay tended to have the least amount of soil interference. The Akron soil with moderate clay content had the greatest degree of soil interference, so contrary to Faure et al. (2006a) higher clay content doesn't necessary lead to more soil interference and suggests that the clay composition may also contribute to cracking degree. Faure et al. (2006b) found that the clay type played a large role in the degree of aromatization that occurred during the pyrolysis process with Na-smectite having the greatest modification influence. The clay in the Akron soil is smectite and that may be the reason for that soil exhibiting the most soil interference. Although it is important to point out that even with the higher soil interference in Akron there is still a strong relationship between the amount of cellulose and indole added to this soil and the TII associated with the respective peaks. The variation in interference between soils indicates that comparisons of SOM bound to the mineral matrix between soils of vastly different characteristics should be done with caution. Soils having higher interference during pyrolysis have less potential for quantification and may only be considered semi-quantitative. Caution should be taken with these soils and possibly only comparisons within a soil type should be done.

Not all of the organic matter in soil is pyrolyzed and this makes quantification difficult because we need to know what is and is not being pyrolyzed and if different compounds categories have different pyrolysis efficiencies.This issue causes us to consider the first part of our fourth objective: what compounds are pyrolyzed. Organic matter components that are fully pyrolyzed will provide the most accurate quantification.Compounds like cellulose and palmitic acid were essentially completely pyrolyzed, whereas indole which had high r^2s when added to soil had only 51–70% of the standard pyrolyzed. We did not see a systematic difference in the amount of standard pyrolyzed between the soils, although there was high variability in the estimate of amount pyrolyzed, but this may mean that the mineral constituents have limited effect on the amount of organic matter pyrolyzed.Standards added to the soil may not pyrolyze the same as organic matter bound to the mineral matrix, so our results may only give us a potential estimation of what may be occurring in the soil matrix itself.

Sorge et al. (1993a), utilizing py-FIMS on whole soils, measured 4.7% mass loss during pyrolysis and Sleutel et al. (2007) measured 8–12% mass loss during pyrolysis. Our mass loss of 8–15% is similar to the range seen by Sleutel et al. (2007). Other studies using py-MBMS have shown a greater range of mass volatilized during pyrolysis (6% to 25%) (Plante et al., 2009). We found that 53–57% of the soil C was pyrolyzed compared to previous work on litter where 68–78% of C was pyrolyzed using the same pyrolysis conditions (Wallenstein et al., 2013). This amount of %C volatilized is quite similar to the 57% of C volatilized in soil by Leinweber and Schulten (1995) using py-FIMS. Schulten and Leinweber (1999) found that different amounts of C volatilized in different size and density fractions and hypothesized that the non-pyrolyzable fraction was a thermally stable fraction of mineral-bound organic matter. Some of the non-pyrolyzable material may be C bound to the mineral matrix, although not exclusively, since not all plant C is pyrolyzed (Wallenstein et al., 2013). Pyrolysis can modify organic matter during the heating process (Miltner and Zech, 1997) possibly producing char, so the material remaining after pyrolysis may be a combination of inherently and modified thermally stable organic matter. The pyrolysis temperature and degree of heat transfer to the sample greatly determines the types and amounts of compounds pyrolyzed (Saiz-Jimenez, 1994) with pyrolysis temperatures as high as 770 °C utilized to study more resistant macromolecules, such as aliphatic biopolymers (Saiz-Jimenez and Deleeuw, 1987). With that in mind it is important to use caution when comparing pyrolysis amounts from studies that utilized different pyrolysis temperatures, in different mineral matrices.

In this study, 43 to 47% of the soil C was not volatilized by pyrolysis, prompting us to ask the question of what is the C chemistry of the pyrolysis-resistant material undetected by py-MBMS, which is the second part of our fourth objective in this study. For this purpose, we scanned the un-pyrolyzed soils and pyrolyzed soils in MIR. One important assumption is that the organic material remaining with the pyrolyzed soils is made up of the temperature resistant organic matter. We recognize that a portion of the pyrolyzed organics could have been modified by the 550 °C pyrolysis temperature, but with the extremely rapid heating along with the free jet expansion minimizing interactions among the pyrolyzates we believe that modification is minimal. This pyrolysis temperature can be considered a moderate pyrolysis temperature that may not fragment more resistant moieties though it is possible that chemical transformations take place especially with respect to complex carbohydrates. Even so, characterizing the pyrolyzates may yield information this is related to compound recalcitrance. For example, previous studies have shown that higher temperatures are needed in order to properly pyrolyze acid-resistant humic acids (Saiz-Jimenez and Deleeuw, 1987). The spectral changes during py-MBMS are consistent with loss of thermolabile organic functional groups during dehydrogenation and dehydration reactions (Schnitzer and Hoffman, 1965). The MIR shows that N-containing functional groups such as amides and N—H bonds are lost, consistent with the recovery of peptide and N-containing fragments as pyrolysis products. The 1370–1270 cm^{-1} region that showed resistance to pyrolysis treatment

forms a broad peak in agricultural soils (Calderón et al., 2011). While the exact nature of this spectral feature is unknown, it is probably due to a combination of multiple functional groups including COO stretch, COOH stretch of phenolics, amide III, and aromatic C═C. Vanillin, with its highly aromatic character, absorbs at 1295 cm^{-1}, possibly due to aromatic C═C bands (Calderón et al., 2013), which suggests that the material remaining consists in part from thermo-resistant, complex compounds, which are known to require temperatures to fragment and leave the sample (Saiz-Jimenez, 1994). It will be interesting to determine if this spectral region, associated with resistance to pyrolysis, is also related to recalcitrance to microbial decomposition. The possibility that some of these constituents were formed during the pyrolysis procedure also must be considered. It is difficult to know what compounds are not being pyrolyzed and the extent of modification of organic matter during the pyrolysis process. Pyrolysis has been found to modify organic matter similar to oxidative decomposition patterns as observed during litter decomposition (Miltner and Zech, 1997), which makes it difficult to determine if the material remaining after pyrolysis is resistant to pyrolysis or has been transformed by pyrolysis. Regardless of the difficulty, it is useful to try to understand what is not pyrolyzed and the incorporation of multiple analytical tools such as NMR or XANES may assist in answering these questions.

The ability to quantify various organic matter compounds in the soil during the pyrolysis process would be very valuable; especially for SOM within the soil matrix as pretreating the soil to remove minerals is time consuming and can cause losses and modification to the SOM (Dai and Johnson, 1999; Rumpel et al., 2006; Sleutel et al., 2009). When working with whole soils, determining the degree of soil interference during pyrolysis is necessary for quantitative estimates and when making comparisons across soils. Our results indicate the promise of making analytical pyrolysis, specifically py-MBMS, a more quantitative approach. There are compounds like cellulose and total carbohydrates that appear to fully pyrolyze, can be represented by a limited number of characteristic peaks, and have strong relationship with the amount added and the total ion intensity produced. Our results are specific to py-MBMS, but the trends and techniques would be applicable to other analytical pyrolysis methods as well. Although pyrolysis may not fully pyrolyze all organic compounds and soil interference may reduce our ability to identify certain compounds, analytical pyrolysis does show promise in being quantitative for certain compound categories. In addition to using standards, py-MBMS analysis can be greatly enhanced by the inclusion of complementary methods of analyzing SOM molecular structure such as MIR, NMR, or thermogravimetry. Our results point to the need to routinely incorporate reference standards and known biological samples into the analyses. We also encourage broadly sharing data online to facilitate information exchange, so that a more quantitative approach can be routinely used in the analysis of the molecular structure of SOM.

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