

# Pyrolysis-molecular beam mass spectrometry to characterize soil organic matter composition in chemically isolated fractions from differing land uses

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**Abstract** Today's questions concerning the role of soil organic matter (SOM) in soil fertility, ecosystem functioning and global change can only be addressed through knowledge of the controls on SOM stabilization and their interactions. Pyrolysis molecular beam mass spectrometry (py-MBMS) provides a powerful and rapid means of assessing the biochemical composition of SOM. However, characterization of SOM composition alone is insufficient to predict its dynamic behavior. Chemical fractionation is frequently used to isolate more homogeneous SOM components, but the composition of fractions is frequently unknown. We characterized biochemical SOM composition in two previously studied soils from the USA, under contrasting land uses: cultivated agriculture and native vegetation. Bulk soils, as well as chemically isolated SOM

fractions (humic acid, humin and non-acid hydrolysable), were analyzed using py-MBMS. Principal components analysis (PCA) showed distinct differences in the SOM composition of isolated fractions. Py-MBMS spectra and PCA loadings were dominated by low molecular weight fragments associated with peptides and other N-containing compounds. The py-MBMS spectra were similar for native whole-soil samples under different vegetation, while cultivation increased heterogeneity. An approach based on previously published data on marker signals also suggests the importance of peptides in distinguishing samples. While the approach described here represents significant progress in the characterization of changing SOM composition, a truly quantitative analysis will only be achieved using multiple internal standards and by correcting for inorganic interference during py-MBMS analysis. Overall, we have provided proof of principle that py-MBMS can be a powerful tool to understand the controls on SOM dynamics, and further method development is underway.

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## Introduction

Understanding the role of soil organic matter (SOM) has become especially important in the interpretation

of ecosystem functioning, soil fertility and global change. In global change, understanding the dynamics of SOM is vital from the standpoint of predicting the effects of future global change on C storage in soils, as well as assessing the possibility of increasing soil C sequestration to offset atmospheric CO<sub>2</sub> increases. It is generally accepted that SOM dynamics are controlled by an interaction of molecular structure (biochemical complexity and recalcitrance), physical protection in aggregate formation, and interaction with soil minerals such as silt and clay surfaces (Baldoek and Skjemstad 2000; Sollins et al. 1996; von Lützow et al. 2006). All three mechanisms occur simultaneously in both space and time, and are difficult to separate. However, von Lützow et al. (2006) suggest that aggregation influences primarily materials that have low mean residence times in soils such as polysaccharides and proteins, while protection by mineral association protects SOM for the longer-term.

The organic matter of soils has been described as a related continuum of a broad range of substances that occur in somewhat consistent proportions in many soils of the world (Horwath 2007). Recent advances in spectroscopic analyses have provided the means to analyze the biochemical composition of SOM without its extraction or isolation from the mineral matrix (Clapp et al. 2005; Hatcher et al. 2001; Kögel-Knabner 2000). Two techniques increasingly used for SOM characterization are <sup>13</sup>C-NMR and analytical pyrolysis. These techniques are sometimes used in combination (e.g., Mao et al. 2007), because <sup>13</sup>C-NMR provides information on different functional groups of C in SOM while analytical pyrolysis yields data at the molecular scale (Schnitzer 2001). Pyrolysis gas chromatography mass spectrometry (py-GC/MS, Saiz-Jimenez 1994) involves the separation of pyrolysis products into single components in the chromatographic column, with mass spectral data obtained for each component. Pyrolysis field ionization mass spectrometry (py-FIMS, Schnitzer and Schulten 1995) does not involve separation of pyrolysis products, but uses soft ionization to produce predominantly molecular ions of the pyrolysis products. The GC/MS and FIMS methods are generally not suited to rapid analysis because of the retention times of the GC column, and because, in FIMS, each sample must be inserted into the ionization chamber and analyzed individually. Pyrolysis molecular beam mass spectrometry (py-MBMS) was originally devel-

oped for the analysis of biofuels, and has recently been applied to the study of SOM (Hoover et al. 2002; Magrini et al. 2002; Magrini et al. 2007). It is a more rapid and robust method, because volatile pyrolysis products are generated at atmospheric pressure and immediately swept into the mass spectrometer by a supersonic jet expansion through an orifice that cools the pyrolysate to minimize condensation reactions. Using an autosampler, py-MBMS allows several hundred samples to be run per day and the method of sample introduction to the spectrometer preserves high molecular weight products that may be lost when other methods are used (Evans and Milne 1987).

There is growing consensus that characterization of SOM biochemical composition alone is insufficient to explain or predict its dynamic behavior. Schoning and Kögel-Knabner (2006) have suggested that long-term stabilization of SOM is mainly controlled by the existence of various methods of protection offered by the soil matrix and soil minerals, not by the chemical structure of SOM itself. Though few models incorporate these protection mechanisms, Van Veen and Paul (1981) used the concept of physical protection of the different SOM pools in their model of SOM dynamics. One must work with well characterized soils that have been studied with a number of tracer and fractionation techniques to answer the important question concerning the interacting role of the various stabilization mechanisms. Chemical fractionation is frequently used to isolate SOM components that are more homogeneous in terms of biochemical composition and dynamics. However, the biochemical composition of these fractions, particularly fractions termed “resistant”, is frequently not known.

This study involves the analysis of SOM composition by py-MBMS of two previously studied soils, under contrasting land uses: cultivated agriculture and native vegetation. The <sup>13</sup>C and <sup>14</sup>C dynamics of these soils both in the field and during incubation have shown a great range of mean residence times both between and within isolated fractions (Follett et al. 2007; Haile-Mariam et al. 2008). The objective was to characterize the chemical composition of whole-soils from different land uses, as well as chemically isolated SOM fractions from these same soils, using py-MBMS. The combination of well studied long-term sites with isotopic tracer signals,

and biological, physical and chemical fractionation and characterization is considered the best approach to understanding SOM stabilization (Paul et al. 2006).

## Materials and methods

### Soil and site descriptions

Soils were sampled between 2002 and 2004 from two long-term agroecosystem experiments and adjacent “native” soils. At Akron, Colorado (40°09' N, 103°09' W), samples were collected from conventional till wheat-fallow treatments and adjacent native grassland soil. Soils at the site are classified as Aridic Paleustoll according to US Soil Taxonomy, and are in the loam to clay-loam textural class with 27% clay dominated by smectites. Soils from the Akron, CO, site developed under a mean annual temperature of 11°C, with annual rainfall of 424 mm on well drained topography. The site has been under cultivation since 1907, and the current management treatments were initiated in 1967. At Hoytville, Ohio (41°00' N, 84°00' W), samples were collected from conventional-till corn-soybean rotations and adjacent native forest. These soils are classified as Mollic Ochraqualf and are in the silty-clay textural class with 42% clay dominated by illites. The native soil at Hoytville, OH, was formed under a mean annual temperature of 9.2°C and a mean annual precipitation of 845 mm in a poorly drained location. The native deciduous swamp forest consists of mixed oak, hickory, ash, cottonwood and soft maple. Long-term tillage and crop rotation experiments were initiated at this site in 1963 on previously cultivated soil.

At each site, multiple surface cores (0–20 cm) were taken from each experimental field replicate ( $n = 4$ ) and separated into 0–10 cm and 10–20 cm depth increments in the field. Results from only the 0–10 cm samples are reported here as these were expected to show the greatest differences. Soils were packaged to remain cool and uncompacted during transport to the laboratory. In the laboratory, rocks, surface litter and root materials were removed while soil clods were broken by hand and passed through an 8-mm sieve. Soil cores were then composited by field replicate, air-dried, passed through a 2-mm sieve, and stored at room temperature until further analysis.

### Chemical fractionations

#### *Soil humus fractionation*

Samples from Colorado and Ohio were fractionated to isolate classic humic fractions using 0.1 M NaOH–0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  (Anderson et al. 1974; Anderson and Schoenau 1993; Kononova and Bel'chikova 1961). Twenty-five grams of sample were pretreated with 250 ml of 0.5 M HCl in a 500 ml polycarbonate centrifuge bottle for 1 h to remove floating plant material and inorganic C. The suspension was centrifuged at  $10,000\times g$  for 15 min, re-suspended in 250 ml of deionized water, centrifuged again, and the supernatant discarded to remove excess acid. Two hundred and fifty ml of fresh 0.1 M NaOH–0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  was added to the centrifuge bottle. The bottle headspace was swept with  $\text{N}_2$  gas and capped, then placed on a reciprocal shaker for 18 h. After shaking, the suspension was centrifuged at  $10,000\times g$  for 15 min, and the supernatant was transferred to another centrifuge bottle for further separation. The pellet (humin) was transferred to an aluminum pan and oven-dried at 60°C, ground to  $<100\ \mu\text{m}$ , and stored for further analyses. Approximately 10 ml of 6 M HCl was added to the second centrifuge bottle to reach pH 1.5 and precipitate the humic acids. The suspension was then centrifuged at  $10,000\times g$  for 15 min to separate the humic and fulvic acids. Approximately 40–50 ml of the supernatant (fulvic acid, FA) was set aside in glass tubes for further analyses. The remaining supernatant was discarded, and the pellet (humic acid, HA) was transferred to a 100 ml glass jar, freeze-dried, ground to  $<100\ \mu\text{m}$ , and stored for further analyses.

#### *Acid hydrolysis*

Soil samples were subjected to acid hydrolysis to isolate resistant pools of C and N using the method described in Paul et al. (1997a) and Plante et al. (2006). Briefly,  $\sim 0.5$  g of sample was refluxed at 95°C for 16 h in 25 ml of 6 M HCl. After refluxing, the suspension was washed with de-ionized water and filtered through a glass-fiber filter. The residue was oven-dried at 60°C and subsequently analyzed for organic C and total N content. Hydrolysability of samples is expressed as the percentage of non-hydrolysable C or N (%NHC or %NHN), and was calculated as a simple ratio without accounting for mass

loss of the sample during hydrolysis or incomplete recovery during filtration. The residues and their associated organic matter are subsequently referred to as the non-acid hydrolysable (NAH) fraction.

#### Organic C, $\delta^{13}\text{C}$ and total N analyses

Total C and N analyses were performed on bulk soil samples and each isolated fraction using an automated CHN analyzer (LECO CHN-1000, Leco Corp., St. Joseph, MI). Results of initial acid additions to soil samples to test for the presence of soil carbonates indicated that carbonates were not present, and thus total C concentrations were equated to organic C concentrations. Soil and fraction samples were also analysed for stable C isotope ratios ( $\delta^{13}\text{C}$ ) using a Carlo Erba NA 1500 CN analyzer (Carlo Erba, Milan, Italy) coupled with a Micromass VG isochrome-EA mass spectrometer (Micromass UK Ltd., Manchester, UK).

#### Pyrolysis-molecular beam mass spectrometry

Whole soil samples and isolated fractions were analyzed by py-MBMS to characterize their chemical compositions. Details of the analytical method and instrumentation are provided in Magrini et al. (2002) and Hoover et al. (2002), and are described in brief here. Two aliquots ( $\sim 0.1$  g) from each field replicate sample were weighed in quartz boats and pyrolyzed in a reactor consisting of a quartz tube (2.5 cm inside diameter) with helium flowing through at  $5\text{ l min}^{-1}$ . The reactor was electrically heated and maintained at  $500^\circ\text{C}$ , and was oriented so that the sampling orifice of the MBMS was inside the end of the quartz reactor. The molecular beam system used an Extrel<sup>TM</sup> Model TQMS C50 mass spectrometer for both pyrolysis and combustion vapor analysis. Total pyrolysis time was 5 min, and the residence time of the pyrolysis vapors in the reactor pyrolysis zone was estimated to be  $\sim 75$  ms, which is short enough to minimize secondary cracking reactions in the quartz reactor. Mass spectral data from  $m/z$  20 to 450 were acquired on a Teknivent Vector 2<sup>TM</sup> data acquisition system using 22 eV electron impact ionization and programmed storage in a personal computer. Repetitive scans (typically one 300 amu scan  $\text{s}^{-1}$ ) were recorded during the evolution of a pyrolysis wave from each soil sample. The mass spectrum of the pyrolysis vapor provides a rapid, semi-quantitative representation of

the molecular fragments from the original molecules (Evans and Milne 1987; Milne and Soltys 1983). Overall, 64 samples were generated by the fractionation procedure (2 sites  $\times$  2 treatments  $\times$  4 field replicates  $\times$  4 fractions). Two sub-samples from each of these 64 were analyzed by py-MBMS, resulting in an anticipated 128 total spectra. However, there was insufficient mass of HA samples for multiple analyses (leaving only 25 HA spectra), and some additional spectra were excluded from further analysis due to data quality issues, leaving 31 WS, 31 humin and 28 NAH spectra, for a total of 115 spectra.

#### Statistical analyses

The resultant pyrolysis mass spectra are complex and data rich. Multivariate analysis can be used to reduce the data by finding correlated masses that can be expressed by new variables and to identify trends in compositional chemistry that may not be obvious by visual comparison of such complex mass spectra. Signal intensities from individual spectra ( $m/z$  20–450) were normalized to 100% total ion intensity (TII, the sum of the intensity for each  $m/z$ ), which corrected for differences in sample size and organic C content. Reduced data sets ( $m/z$  57–450) from the complete mass spectral range were used in the multivariate analyses to omit the small mass units typical of water,  $\text{CO}_2$  and other volatiles that dominate the spectra but are not particularly informative about the original chemical composition. Principal components analyses (PCA) were performed using the Unscrambler v.8.0 software package (CAMO Process AS, Oslo, Norway) to compare the similarity of SOM composition between samples, using full cross-validation and ten principal components to build the model.

#### Comparisons of SOM chemical compositions

Description of the chemical composition of a sample by py-MBMS can only be achieved using knowledge of how to assign the individual mass signals. Shulten, Schnitzer and colleagues (Hempfling and Schulten 1990; Schnitzer 1991; Schnitzer and Schulten 1992; Schulten 1993; Schulten and Leinweber 1993; Sorge et al. 1993) have previously identified marker signals for several classes of compounds (Table 1). While relative intensities of each of the marker signals may differ between py-FIMS and py-MBMS, we assume

**Table 1** Typical marker signals for selected classes of compounds detected using Py-MS of samples (adapted from Schulten 1996)

Compound class	<i>m/z</i>
Carbohydrates	60, 72, 82, 84, 96, 98, 110, 112, 114, 126, 132, 144, 162
Peptides	57, 70, 73, 74, 75, 84, 87, 91, 97, 99, 115, 120, 129, 135
Phenols and lignin monomers	94, 108, 110, 122, 124, 138, 140, 150, 152, 154, 164, 166, 168, 178, 180, 182, 194, 196, 208, 210, 212
Lignin dimers	246, 260, 270, 272, 274, 284, 286, 296, 298, 300, 310, 312, 314, 316, 326, 328, 330, 340, 342, 356
Lipids, alkanes, alkenes, fatty acids and n-alkyl esters	202, 216, 230, 244, 256, 258, 270, 272, 284, 286, 298, 300, 312, 314, 326, 328, 340, 342, 354, 368, 380, 382, 394, 396, 408, 410, 422, 424, 438, 452, 466, 480, 494
Alkyl aromatics	92, 106, 120, 134, 142, 148, 156, 162, 170, 176, 184, 190, 192, 198, 204, 206, 218, 220, 232, 234, 246, 260, 274, 288, 302, 316, 330, 344, 358, 372, 386
Heterocyclic N-containing compounds	59, 67, 79, 81, 95, 103, 109, 111, 123, 125, 137, 139, 153, 161, 167, 181, 183, 195, 203, 233, 245, 255, 257, 271, 285, 333, 359, 363, 393
Sterols	372, 386, 388, 390, 393, 394, 396, 398, 400, 402, 408, 410, 412, 414, 416, 426, 430

that the identification of compounds by their *m/z* should be the same in py-MBMS compared to py-FIMS. Additional PCA were performed on individual sub-sets of mass spectra variables representing each of the identified classes of compounds to identify which compound classes differed the most between samples. For instance, PCA was performed to compare similarities and differences in the carbohydrate-associated signals of samples. Semi-quantitative SOM compositions were also calculated for each sample using the ion intensity for each identified class of compounds as well as the volatile *m/z* 20–56 class. Differences in SOM composition of each individual class of compounds between land uses and chemically isolated fractions were compared using analysis of variance in JMP v6.0.3 (SAS Institute, Cary, NC). For each test, the statistical model was SITE  $\times$  TREATMENT(SITE)  $\times$  FRACTION where site was a random variable, and treatment and fraction were fixed variables. Differences between least squared means were tested using Tukey HSD, and considered significant at  $\alpha = 0.05$ .

## Results

### Characteristics of bulk soils and chemical fractions

The two soils chosen for this study are from well characterized long-term plots (Paul et al. 1997b) on medium textured soils that have received a great deal

of previous study. The growth of a deciduous, swamp forest consisting of mixed oak, hickory, ash, cottonwood and soft maple at the Hoytville site produced an organic carbon concentration of 7.47% (Table 2) in the surface mineral soil. Tile drainage beginning in the early twentieth century, and management under a corn-soybean rotation decreased the soil C concentration to 1.76%. The change in the  $^{13}\text{C}$  content from  $-26.2\%$  in the forest to  $-23.9\%$  in the corn-soybean rotation indicated that 21% of the SOM of the cultivated site was corn-derived. The contribution of soybeans can not be calculated. At Akron, CO, growth of a native mixture of  $\text{C}_3$  and  $\text{C}_4$  native grasses resulted in a soil C concentration of 2.18%, which decreased to 0.93% under wheat-fallow plow tillage management. The native Akron soil collected for this study had a  $^{13}\text{C}$  content of  $-19.88\%$  (Table 2). This indicates that 56% of the organic C was derived from cool season ( $\text{C}_3$ ) grasses and 46% from warm season ( $\text{C}_4$ ) grasses. The similarity of the  $^{13}\text{C}$  signature of the cultivated soil, which was significantly reduced in C content, indicates that most of the C in the cultivated soil was derived from the original native vegetation. This is corroborated by the carbon dates discussed below.

Humic acid fractionation of the Hoytville soil produced a low-ash fraction containing 35% C for both the cultivated and forested sites, compared to 27% C found in the grassland Akron humic acid samples. In both soils, the  $^{13}\text{C}$  signal of the humic acid fraction was similar to the whole soil (Table 2). Acid hydrolysis is used to remove carbohydrates and proteins,

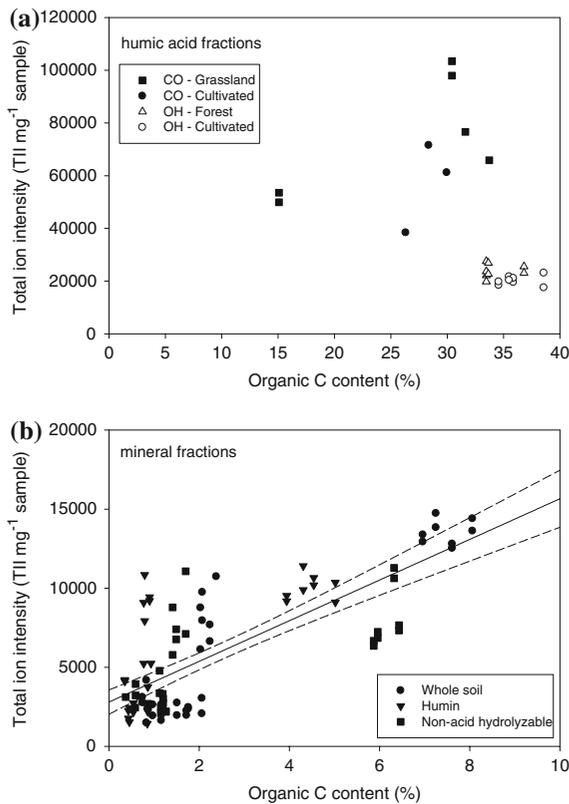
**Table 2** Organic carbon content and  $\delta^{13}\text{C}$  signature of whole soil and isolated fraction samples (mean  $\pm$  standard error)

Site	Land use treatment	Fraction	Organic carbon content (%)	Total nitrogen content (%)	$\delta^{13}\text{C}$ (‰)
Hoytville, OH	Forest	Whole soil	7.47 $\pm$ 0.24	0.61 $\pm$ 0.02	-26.20 $\pm$ 0.11
		Humic acid	34.35 $\pm$ 0.82	2.72 $\pm$ 0.11	-26.72 $\pm$ 0.12
		Humin	4.45 $\pm$ 0.23	0.43 $\pm$ 0.01	-25.87 $\pm$ 0.13
		Non-acid hydrolyzable	6.15 $\pm$ 0.14	0.19 $\pm$ 0.01	-27.28 $\pm$ 0.09
	Cultivated	Whole soil	1.76 $\pm$ 0.11	0.21 $\pm$ 0.01	-23.93 $\pm$ 0.16
		Humic acid	36.13 $\pm$ 0.86	3.33 $\pm$ 0.12	-24.59 $\pm$ 0.12
		Humin	0.86 $\pm$ 0.01	0.17 $\pm$ 0.005	-23.26 $\pm$ 0.17
		Non-acid hydrolyzable	1.20 $\pm$ 0.02	0.06 $\pm$ 0.009	-25.80 $\pm$ 0.22
Akron, CO	Grassland	Whole soil	2.18 $\pm$ 0.08	0.20 $\pm$ 0.006	-19.88 $\pm$ 0.55
		Humic acid	27.71 $\pm$ 4.27	2.44 $\pm$ 0.40	-19.54 $\pm$ 0.47
		Humin	0.85 $\pm$ 0.04	0.14 $\pm$ 0.003	-19.58 $\pm$ 0.40
		Non-acid hydrolyzable	1.43 $\pm$ 0.12	0.01 $\pm$ 0.004	-22.08 $\pm$ 0.40
	Cultivated	Whole soil	0.93 $\pm$ 0.09	0.10 $\pm$ 0.008	-20.07 $\pm$ 0.26
		Humic acid	27.29 $\pm$ 1.19	2.40 $\pm$ 0.07	-19.25 $\pm$ 0.27
		Humin	0.44 $\pm$ 0.04	0.06 $\pm$ 0.002	-19.62 $\pm$ 0.49
		Non-acid hydrolyzable	0.51 $\pm$ 0.06	0.02 $\pm$ 0.002	-22.29 $\pm$ 0.22

leaving a residue with older, more recalcitrant SOM (Schnitzer and Preston 1983). This was reflected in the more negative  $\delta^{13}\text{C}$  signal of the non-hydrolysable C of all samples (Table 2), consistent with a greater content of lignin, which is depleted by 3–5‰ (Benner et al. 1987) and long chain alkyls that can be depleted by as much as 8‰ (De Niro and Epstein 1978). Previous carbon dating of the cultivated Hoytville soil from a nearby continuous corn rotation resulted in a MRT of  $920 \pm 53$  years, whereas the non-hydrolysable fraction had a MRT of  $1770 \pm 45$  years (Paul et al. 2001). Paul et al. (1997a) previously reported  $^{14}\text{C}$  ages of  $193 \pm 118$  years before present for total SOM from native surface soils from Akron, CO, and  $1994 \pm 134$  years BP for non-hydrolysable residue. Cultivated soils from the same site in Akron, CO dated to  $1296 \pm 262$  years BP with an age of  $3326 \pm 616$  years BP for the non-hydrolysable residue (Paul et al. 1997a). Acid hydrolysis conducted in this study employed a temperature of  $95^\circ\text{C}$ , while the studies for carbon dating of the Akron soil reported in Paul et al. (1997a) and Follett et al. (2007) used  $116^\circ\text{C}$ . In separate trials, the higher temperature has been shown to remove 23% more material (data not shown), which should result in different SOM compositions in the non-hydrolysable fraction.

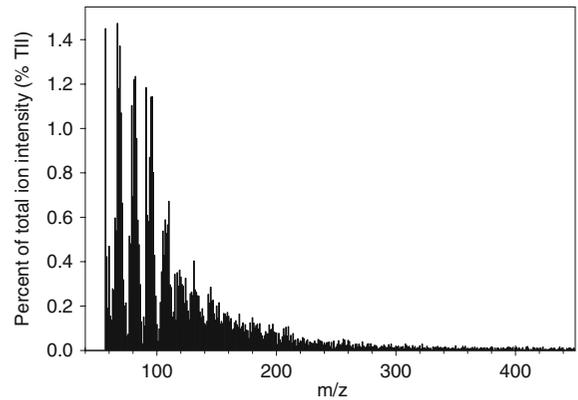
#### Results of pyrolysis molecular beam mass spectrometry

Previous studies have demonstrated that TII in py-MS analyses is proportional to sample organic matter content (Magrini et al. 2002; Schulten et al. 1990; Sorge et al. 1993). However, Schulten et al. (1995) found that in spite of similar organic C contents, the TII for a cultivated soil was only one-sixth of that of the paired native grassland soil. For all sites and fractions combined, we found a statistically significant correlation between TII for py-MBMS and sample organic C content ( $r^2 = 0.453$ ,  $P < 0.001$ ) that was dominated by the large separation between humic acid fractions and the mineral-dominated fractions (Fig. 1). Taken separately, humic acid samples showed no correlation between TII and organic C content (Fig. 1a), while the mineral-dominated fractions (including whole-soil, humin and non-acid hydrolysable) showed a strong correlation (Fig. 1b,  $r^2 = 0.587$ ,  $P < 0.001$ ). The regression results emphasized the need for normalization of the spectral intensities to make fair comparisons between sites or fractions, and thus all ion intensity data were normalized to %TII before subsequent multivariate analyses.

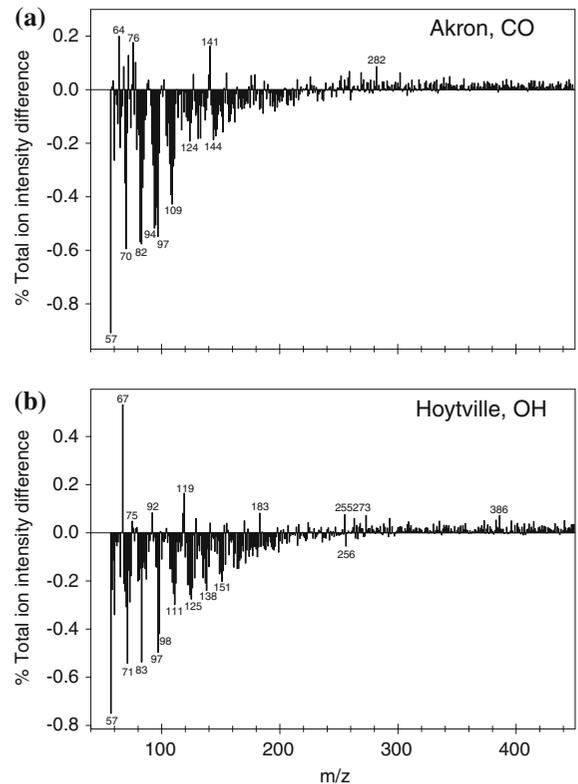


**Fig. 1** Relationship between total ion intensity (TII  $\text{mg}^{-1}$  sample) from the pyrolysis molecular beam mass spectrometry analysis and sample organic C concentration of (a) humic acid fractions, and (b) mineral fractions including whole-soil, humin and non-acid hydrolysable fractions. Dashed lines represent 95% confidence interval on regression

The mass spectrum of the whole-soil native vegetative samples from Akron, CO (Fig. 2) is typical of those observed for other whole-soil samples, with clusters of large peaks at  $m/z$  67–69, 77–82, 91–95 and 105–109. Plots showing the difference in ion intensity between the two land use treatments show major differences (Fig. 3). The difference spectrum from Akron indicates less intensity in the cultivated samples than in the native samples (i.e., negative values in Fig. 3a) at low  $m/z$ , except for small relative increases for  $m/z$  64, 68, 72, 76, 78, 141. Cultivated samples had generally larger intensities for  $m/z > 250$  than did native samples (i.e., positive peaks in the difference spectrum). Similar patterns are observed in the samples from Hoytville (Fig. 3b), with generally less intensity in the cultivated samples for  $m/z < 250$  and larger intensity above that  $m/z$ . A large relative increase is noted for  $m/z$  67, and smaller increases for

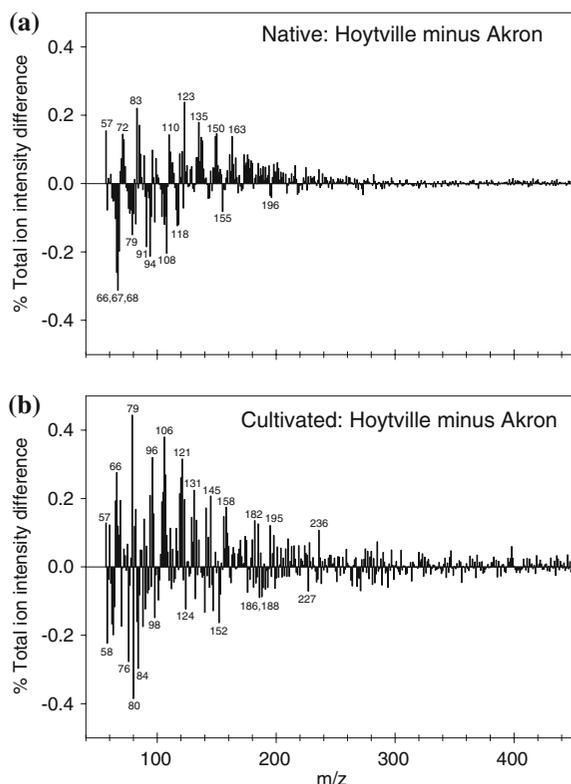


**Fig. 2** Normalized mass spectrum for spectral range  $m/z = 57$ –450 from the pyrolysis molecular beam mass spectrometry analysis of the native grassland whole-soil sample from Akron, CO. Spectrum represents mean values of two aliquots from each of four field replicates



**Fig. 3** Difference spectra of cultivated minus native whole-soil samples of the normalized mass spectra for spectral range  $m/z = 57$ –450 for (a) Akron, CO, and (b) Hoytville, OH

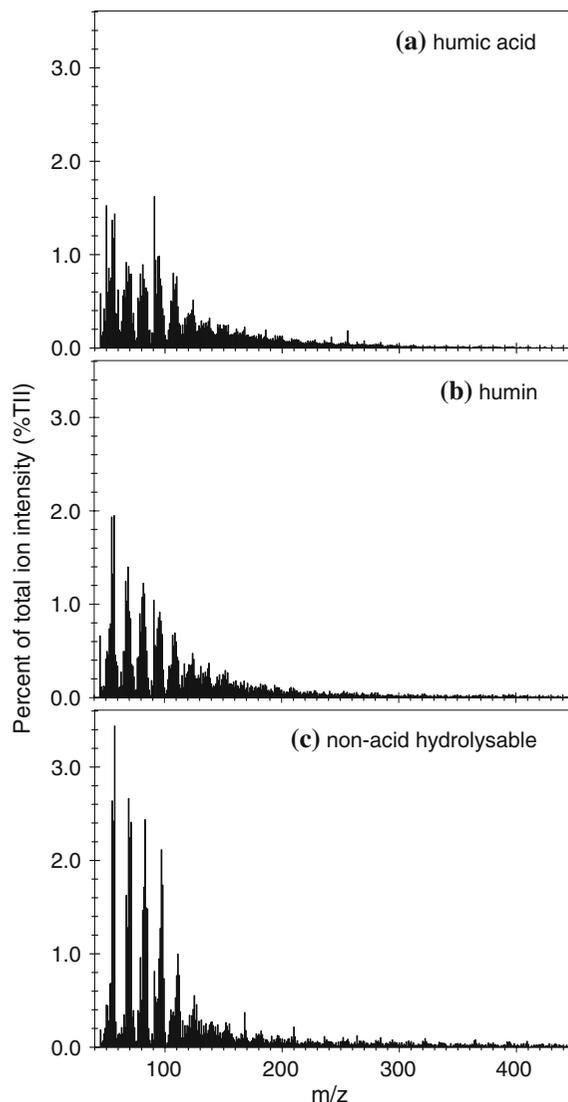
$m/z$  75, 92, 119 and 183. These results indicate the relative preservation of larger molecular weight compounds after long-term cultivation.



**Fig. 4** Difference spectra for spectral range  $m/z = 57$ –450 for (a) native whole-soil (Hoytville, OH minus Akron, CO) and (b) cultivated whole-soil (Hoytville, OH minus Akron, CO)

Figure 4 illustrates spectral differences between soils from the two sites (i.e., native OH minus native CO, and cultivated OH minus cultivated CO). The smaller range in intensity difference under native land use compared to the broader range under cultivated land use suggests that the SOM composition is more similar under native forest and grassland than in the corresponding soils under long-term cultivation. This suggests that cultivation increases the heterogeneity of SOM composition.

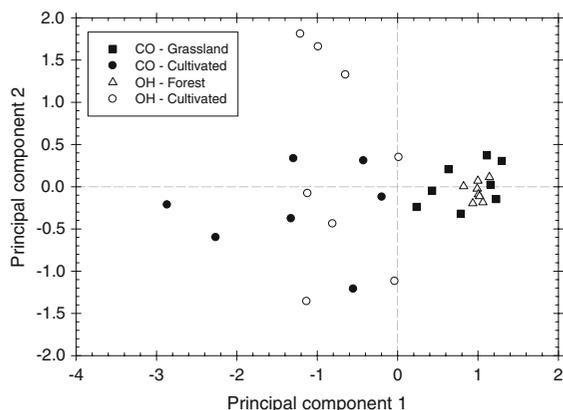
Mass spectra of humic acid (Fig. 5a) and NAH (Fig. 5c) from the native grassland soil at Akron differ visually from the whole-soil samples (Fig. 2), while the spectrum of humin (Fig. 5b) was relatively similar to whole-soil except for less peak intensity overall. These general differences and similarities were also found for the other site and treatment combinations. Spectra from the non-acid hydrolysable samples (Fig. 5c) showed the highest individual peak intensities; suggesting a simplified chemical composition, which is the goal of the hydrolysis procedure.



**Fig. 5** Normalized mass spectra for spectral range  $m/z = 57$ –450 for (a) humic acid, (b) humin, and (c) non-acid hydrolysable fractions isolated from the native grassland soil from Akron, CO

#### Statistical analyses of mass spectra

Visual or simple data analysis by pair-wise comparisons of mass spectra is insufficient for such complex data. Multivariate analysis has proven to be an important tool for pattern recognition in pyrolysis mass spectrometry (e.g., Schulten et al. 1988). Multivariate analysis by PCA can much better illustrate both the similarities and differences between samples, and additionally provide an indication about



**Fig. 6** Principal components analysis scores for spectral range  $m/z = 57\text{--}450$  of whole-soil samples alone. Open symbols are OH samples and closed symbols are CO samples. ●, cultivated samples; △, forest samples; ■, grassland samples

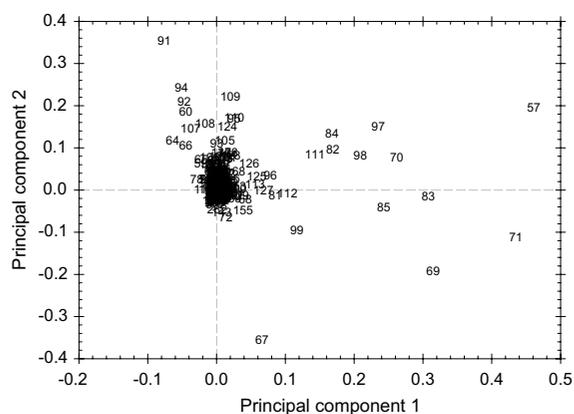
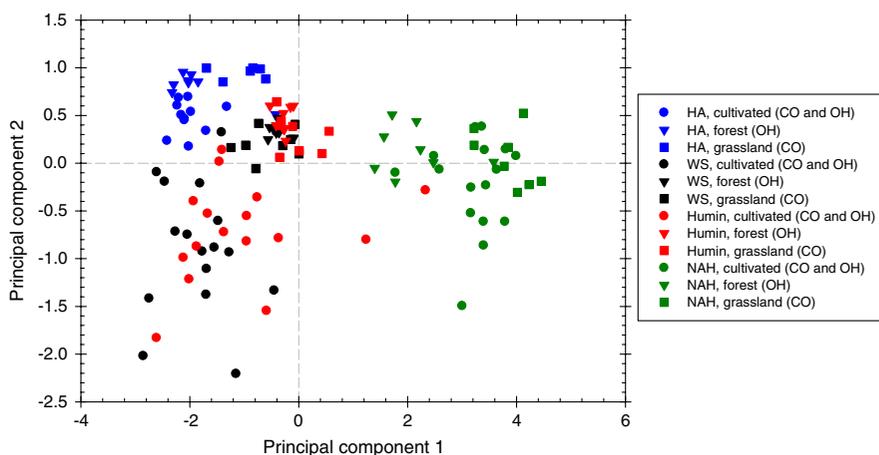
which components of the mass spectra are responsible for the differences. Samples with similar chemical compositions are illustrated in a tight grouping of data points in a PCA score plot, while samples with heterogeneous compositions are more scattered. Examining the SOM composition of whole-soil samples, tight and overlapping groups in Fig. 6 suggest that the native land uses (forest and grassland) were highly homogeneous and similar in composition, supporting the evidence from the difference spectrum in Fig. 4a. The first principal component explained 24% of the variance and the second component explained 13% of the variance (Table 3). After long-term cultivation, the SOM composition of the soils changes significantly (i.e., shift to the left in Fig. 6), and appears to become more chemically heterogeneous (i.e., less grouping). Data for the cultivated Akron soil also show more spread along PC2, and those from the cultivated Hoytville soil are more

spread along PC1. Application of this technique to a cultivation chronosequence rather than two end-points (e.g., this study and Schnitzer et al. 2006) may yield important information about the dynamics and evolution of SOM composition following a change in land use. Figure 7 shows the PCA score plot for all soil and fraction samples combined. The first four components explained 49, 5, 4 and 3% of the variance, respectively (Table 3). The plot suggests that the chemical composition of the humic acid and NAH samples differ from each other and from whole-soil and humin samples, but that whole-soil and humin samples have similar compositions. Humic acid fractions appear to have highly homogeneous chemical compositions that differ somewhat between land uses. Whole-soil and humin SOM compositions are also distinguished by land use, but NAH compositions are not well separated by land use. Overall, the results suggest that classic humic acid and acid hydrolysis fractionations separate SOM fractions that are chemically distinct from whole-soil SOM. The humic acid fraction appears to be more chemically homogeneous than the NAH residues. We can infer the underlying chemistry that drives the grouping of samples by looking at the masses that are most important in loading the principal components (Table 3). Figure 8 shows the loadings for the first two components whose scores are plotted in Fig. 7. Each number in the plot represents the loading of that particular  $m/z$ , and a longer vector from the origin represents a stronger influence on the PCA scores. The loadings are dominated by low molecular weight and odd-numbered  $m/z$  fragments (Table 3), which are likely derived from carbohydrate, amino acid and peptide side-chains as discussed in the next section.

**Table 3** Amount of variance explained and signals with loadings  $> |0.15|$  (in order of decreasing loading) for each of the first four principal components in the PCA of pyrolysis molecular beam mass spectra

	PC 1	PC 2	PC 3	PC 4
Whole soil samples alone				
Variance explained	24%	13%	9%	7%
$m/z$	57, 83, 70, 97, 82, 98, 96, 109	71, 81, 91, 72, 58, 95, 70	67, 69, 82, 68, 94, 85, 70, 99	70, 91, 96, 94, 68, 118, 98, 77, 57, 71
Whole-soil and isolated fractions combined				
Variance explained	49%	5%	4%	3%
$m/z$	57, 71, 69, 83, 70, 85, 97, 98, 82, 84, 111	67, 91, 94, 109, 92, 57, 69, 60, 110, 95, 108, 97	57, 69, 96, 67, 70, 97, 68, 95, 82	85, 71, 68, 91, 99, 97, 83, 64

**Fig. 7** Principal components analysis scores for spectral range  $m/z = 57$ –450 of whole-soil and isolated fraction samples combined. Black symbols are whole-soil samples, blue symbols are humic acid samples, red symbols are humin samples and green symbols are non-acid hydrolysable samples. ●, cultivated samples (CO and OH); ▼, forest samples (OH); ■, grassland samples (CO)



**Fig. 8** Principal components analysis loadings for spectral range  $m/z = 57$ –450 of whole-soil and isolated fraction samples combined. Numerical symbols represent individual  $m/z$  values

### Comparisons of chemical compositions

A semi-empirical quantification of SOM composition can be achieved using typical marker signals and the measured ion intensities associated with those signals (Schnitzer et al. 2006; Schulten et al. 1995). Overall, the sum of all identified marker signals represented  $79.4 \pm 4.3\%$  of TII (Table 4), of which  $62.3 \pm 10.6\%$  was associated with the volatile fraction ( $m/z$  20–56) that is dominated by  $\text{CO}_2$  produced during pyrolysis. The next largest classes of identified compounds were peptides ( $7.6 \pm 2.8\%$  of TII) and heterocyclic N-containing compounds ( $7.7 \pm 1.5\%$  of TII), though some of this might also be attributable to ionized or  $^{13}\text{C}$ -containing compounds that also have odd-numbered  $m/z$  (i.e.,  $M + 1$ ). Carbohydrates, phenols and lignin monomers, and alkyl aromatics were also significant

contributors (in decreasing order), while lignin dimers, lipids and sterols each represented  $<1\%$  of TII. Separate principal components analyses of TII-normalized data for each of the compounds classes showed that the soil and isolated fraction samples differed mostly in their carbohydrate and peptide contents (Table 5).

Analyses of variance of calculated contributions to TII also revealed significant differences in composition between fractions. Land use also affected SOM composition. Cultivated samples had significantly greater volatiles (51.5–56.1% of TII) than either forest (46.8%) or grassland (44.4%) (Table 4). Cultivated samples also had significantly less carbohydrates, peptides, heterocyclic N-containing compounds, phenols and lignin monomers, and alkyl aromatics than either forest or grassland samples, but significantly greater concentrations of lignin dimers and sterols. Lipids showed no statistical differences between site and land use combinations.

The use of acid hydrolysis followed by characterization by py-MBMS resulted in an interaction, highlighting the strong effect of the mineral matrix on the results of py-MBMS. Hydrolysis with 6 M HCl is generally used to remove proteinaceous and carbohydrate materials, with the remaining NAH residue having a more aromatic and alkyl chemical composition (e.g., Schnitzer and Preston 1983). We did not observe a depletion of carbohydrates or peptides in the NAH fractions once the signals had been normalized to TII. Our analysis of NAH resulting from hydrolysis at a lower temperature suggests that NAH residues from cultivated samples produced signals on pyrolysis that indicated significantly greater concen-

**Table 4** Proportions (%) of ion intensity attributable to selected classes of compounds (mean,  $n = 4$ )

Site	Land use treatment	Fraction <sup>a</sup>	$m/z$ 20–56	Carbohydrates	Peptides	Phenols	Lignin dimers	Lipids	Alkyl aromatics	HCN cmpnds <sup>b</sup>	Sterols	Not classified
Hoytville, OH	Forest	WS	46.7	6.1	7.6	5.4	0.4	0.7	3.2	8.6	0.2	21.1
		HA	57.0	4.7	5.8	5.1	0.2	0.4	3.5	6.8	0.1	16.5
		Humins	43.5	5.6	7.7	6.3	0.5	0.9	3.5	9.3	0.4	22.2
	Cultivated	NAH	40.0	6.4	10.7	4.9	0.6	0.9	3.3	8.9	0.3	24.0
		WS	58.0	3.8	5.0	3.0	0.6	0.9	2.9	7.2	0.4	18.2
		HA	63.9	3.6	5.6	3.7	0.2	0.4	3.0	5.5	0.1	14.0
Site mean Akron, CO	Grassland	Humins	52.0	4.0	6.6	3.1	0.8	1.0	2.9	7.9	0.5	21.1
		NAH	32.2	7.5	12.1	4.4	0.9	1.4	3.3	9.3	0.6	28.3
		WS	49.2	5.2	7.6	4.5	0.5	0.8	3.2	7.9	0.3	20.7
	Cultivated	WS	50.3	6.1	7.0	4.7	0.4	0.6	3.2	7.9	0.2	19.8
		HA	47.4	5.4	7.4	6.0	0.6	1.0	4.0	7.7	0.1	20.3
		Humins	47.8	6.0	7.6	5.3	0.5	0.8	3.0	8.3	0.3	20.5
Site mean Overall mean	Grassland	NAH	34.0	8.9	12.6	4.9	0.8	1.2	2.9	9.3	0.4	25.1
		WS	62.3	3.5	4.2	2.5	0.6	0.8	2.4	5.8	0.4	17.5
		HA	60.2	4.3	5.6	4.3	0.3	0.6	3.1	6.0	0.1	15.6
	Cultivated	Humins	62.4	2.9	4.6	3.1	0.7	0.9	2.3	5.7	0.4	16.9
		NAH	37.1	7.5	10.7	4.6	0.8	1.3	2.7	8.5	0.4	26.5
		WS	49.9	5.6	7.5	4.4	0.6	0.9	2.9	7.4	0.3	20.5
Overall mean		49.5	5.4	7.6	4.4	0.6	0.9	3.1	7.7	0.3	20.6	

<sup>a</sup> WS, whole soil; HA, humic acid; NAH, non-acid hydrolysable residues<sup>b</sup> HCN cmpnds: heterocyclic N-containing compounds

**Table 5** Amount of variance explained by first four principal components during PCA of spectral components associated with specific compounds classes defined by Schulten (1996) for all soil and isolated fraction samples

Compound class	PC 1 (%)	PC 2 (%)	PC 3 (%)	PC 4 (%)
Carbohydrates	60	10	7	7
Peptides	69	9	6	5
Phenols and lignin monomers	32	18	10	7
Lignin dimers	19	10	10	9
Lipids, alkanes, alkenes, fatty acids and n-alkyl esters	17	10	9	8
Alkyl aromatics	36	13	10	7
Heterocyclic N-compounds	29	21	13	9
Sterols	24	20	9	7

**Table 6** Mass loss during pyrolysis (mean  $\pm$  standard deviation) and resulting total ion intensity (mean (coefficient of variance))

Site	Land use treatment	Fraction	Mass loss (%)	Total ion intensity (TII mg <sup>-1</sup> )
Hoytville, OH	Forest	Whole soil	25.4 $\pm$ 4.6	13528 (6)
		Humic acid	48.1 $\pm$ 5.8	23941 (11)
		Humin	21.4 $\pm$ 8.5	10037 (8)
		Non-acid hydrolyzable	20.2 $\pm$ 9.0	8001 (23)
	Cultivated	Whole soil	20.7 $\pm$ 19.6	2360 (16)
		Humic acid	37.8 $\pm$ 3.2	20197 (9)
		Humin	11.7 $\pm$ 10.3	2492 (26)
		Non-acid hydrolyzable	11.3 $\pm$ 6.1	2552 (17)
Akron, CO	Grassland	Whole soil	14.5 $\pm$ 5.0	8233 (20)
		Humic acid	31.2 $\pm$ 4.4	74550 (30)
		Humin	14.8 $\pm$ 10.4	8138 (26)
		Non-acid hydrolyzable	11.4 $\pm$ 7.6	6882 (35)
	Cultivated	Whole soil	6.2 $\pm$ 3.3	2546 (34)
		Humic acid	24.7 $\pm$ 3.7	57033 (30)
		Humin	10.2 $\pm$ 8.9	2648 (38)
		Non-acid hydrolyzable	5.4 $\pm$ 4.9	3243 (19)

trations of carbohydrates (7.6% of TII versus 4.4–4.8%) and peptides (11.6% of TII versus 5.9–6.6%) than whole-soil (Table 4). This we attribute to protection generated by the mineral matrix. It is possible that hydrolysis of complex organic macromolecules solubilized a large amount of low molecular weight carbohydrates and peptides, that were rapidly sorbed to the mineral surfaces. Pyrolysis of the low-C containing cultivated NAH fractions thus resulted in a preferential detection of carbohydrate- and peptide-associated marker signals. A number of studies have also demonstrated that hydrolysis of carbohydrates and peptides is incomplete. Leinweber and Schulten (1998) reported that 28–34% of total ion intensity from py-FIMS analysis of acid hydrolysis residues was associated with low-mass N compounds.

Incomplete hydrolysis is insufficient to explain our differences. Overall, pyrolysis resulted in the volatilization of 19.8  $\pm$  14.0% (mean  $\pm$  standard deviation) of the initial sample mass (Table 6). Humic acid fractions had the greatest mass loss during pyrolysis (37.9  $\pm$  9.3%), followed by whole-soils (17.8  $\pm$  12.7) and humin fractions (13.9  $\pm$  9.7). Non-acid hydrolyzable fractions had the lowest mass loss (12.7  $\pm$  8.5%). These mass losses are attributed primarily to the initial amount of organic matter associated with the fraction that is volatilized during pyrolysis, with minor contributions from hygroscopic water loss and the decomposition of some minerals such as sesquioxides. Normalizing the measured TII by these mass losses, however, suggests a decreasing yield of ion intensity measurable by the mass spectrometer with

decreasing organic matter content. We also found that the NAH residues had significantly less volatile materials ( $m/z$  20–56,  $35.7 \pm 4.5\%$  of TII) compared to bulk soil ( $54.4 \pm 6.8\%$ ), humin ( $51.5 \pm 8.2\%$ ) or humic acid ( $57.3 \pm 6.9\%$ ). Leinweber and Schulten (1998) reported shifts to higher volatilization temperatures (from  $400^\circ\text{C}$  to  $500^\circ\text{C}$ ) in non-hydrolysable residues compared to bulk samples, which they attributed to strong organo-mineral complexation.

## Discussion

Extensive studies on SOM across the globe have shown an overall similarity in the large continuum of compounds ranging from amino acids and carbohydrates, to more complex residues or condensates (Horwath 2007). This is likely due to the fact that SOM production and decomposition are largely microbially-mediated and constrained by their physiological demands and generation of subsequent metabolic products (e.g., Cleveland and Liptzin 2007). An additional constraint may be the nature of the organo-mineral complexation bonds at the mineral surface as proposed by Kleber et al. (2007). The dominance of both labile N-containing compounds (e.g., peptides) and potentially recalcitrant N-containing heterocyclic compounds in our py-MBMS results may be evidence to support their conceptual model. The similarity in whole-soil SOM composition observed through PCA analysis of the py-MBMS results on native wetland forest whole-soil samples from Ohio and grassland soils from Colorado may be surprising at first glance, but is consistent with the extensive microbial alteration this SOM has undergone. The significant alteration in whole-soil SOM composition after cultivation is also not surprising. A combination of  $^{13}\text{C}$ -NMR and analytical pyrolysis techniques have been used to study the changes in SOM composition after long-term cultivation. Schulten and colleagues (Schulten et al. 1990; Schulten et al. 1995) reported decreased molecular diversity after long-term cultivation, and found that py-FIMS ion intensities for carbohydrates, phenols, alkylaromatics and heterocyclic N-compounds were greatly reduced. Cultivation resulted in significant decreases in the contribution of carbohydrates, peptides and phenols to TII (Table 5). The increase in apparent SOM heterogeneity (as

illustrated by reduced grouping in PCA scores, Fig. 7), however, is contrary to the expectation that cultivation might result in a “simpler” SOM composition. A potential explanation for such heterogeneous SOM composition in the cultivated soils is that the PCA analysis did not adequately resolve this complex mixture to be able to discern important differences at high  $m/z$  values. Schnitzer et al. (2006) used three instrumental techniques to compare SOM composition from virgin prairie and similar cultivated soils. Their  $^{13}\text{C}$ -NMR data suggested significant increases in aromaticity of the SOM after long-term cultivation. Their Curie point py-GC/MS data showed that the virgin soil was richer in alkenes than the cultivated soil. Lastly, their py-FIMS data showed that the cultivated soil was enriched in carbohydrates, phenols and lignin monomers, alkyl aromatics and N-containing compounds such as peptides. The native-cultivated difference spectra (Figs. 2b, 3b) and the PCA loadings (Fig. 8 and Table 3) from the current study show that a few peaks were responsible for most of the differences. The  $m/z$  of 57, 60, 64, 66, 69, 70, 71, 82, 83, 84 and 85 were said by Schulten and his colleagues to represent carbohydrate and nitrogenous compounds, and those in the 91–94 range are phenols. This is consistent with previously observed relative increases in N-containing pyrolysis products between permanent pasture and cultivated samples (Nierop et al. 2001), which were attributed to microbial residues. These compound classes contributed significantly to TII in the current study, and thus small changes in these contributions may be magnified compared to proportionally equivalent changes in contributions by lignin components, lipids, alkyl-aromatics or sterols. These larger  $m/z$  materials also show differences but were not evident in the current multivariate analysis. The presence of high  $m/z$  alkanes and the contribution of charcoal need to be further assessed.

The NAH fraction has often been found to be the oldest fraction of SOM (e.g., Paul et al. 1997a; Trumbore et al. 1989), and is frequently used to calibrate the most stable pool of SOM in dynamic models (e.g., Falloon and Smith 2000; Trumbore and Zheng 1996), in spite of a lack of extensive chemical characterization of NAH residues reported in the literature. Preston and Schnitzer (1984); Schnitzer and Preston (1983) examined the effects of acid hydrolysis on extracted humic substances by  $^{13}\text{C}$ -nuclear magnetic resonance spectrometry. More recently, Poirier et al.

(2003) used a combination of FTIR,  $^{13}\text{C}$ -NMR and Curie-point py-GC/MS to characterize the NAH fraction isolated from forest and cultivated soils in southwestern France, and found a predominance of aliphatic moieties, melanoidins, black carbon and small amounts of condensed tannins. Their fractionation procedure, however, was significantly more drastic than what is normally employed, and the characterized non-hydrolysable C represented only 6% of the original SOM. Leinweber and Schulten (1998) reported on non-hydrolysable organic N. Our data also confirm Schulten's observations that differences in the amount of mineral matter change the pyrolysis products. While our hydrolysis procedure was conducted at a lower temperature than used previously for carbon dating, a likely explanation for our observations of high amounts of carbohydrates and peptides is that hydrolysis breaks up some of the large macromolecules into smaller units that we are detecting during py-MBMS. In addition to removing many organic materials, hydrolysis may also remove some interfering mineral constituents that otherwise interfere with pyrolysis and detection of organic materials. Hydrolysis also leaves behind some protected amino compounds and carbohydrates that are later pyrolyzed (Leinweber and Schulten 1998; Piper and Posner 1972). This preconditioning has not been previously considered, but is not surprising. A portion of the non-hydrolysable constituents have been shown in other studies to have rapid turnover times. Using their pyrolysis data, Leinweber and Schulten (1998) showed that non-hydrolysable N is biologically active. Unpublished work in our laboratory indicates that the NAH residues contain significant amounts of low molecular weight fatty acids (Drijber, personal communication) that can be decomposed by microorganisms during incubation. It has been previously reported that acid hydrolysis does not release all amino acids from humic materials. Piper and Posner (1972) used alkaline and hydrogen peroxide treatments following acid hydrolysis to release amino acids that are stable to non-oxidative acid hydrolysis, and suggested that N-phenyl amino acids are oxidized to more readily-released quinones. These results help explain the conundrum that while hydrolysis is an excellent way to concentrate old materials in soils it is not as useful an indicator of the resistant fraction as was originally hoped (Helfrich et al. 2007; Plante et al. 2006; Poirier et al. 2006). Follett et al. (2007)

found that NAH comprised 63% of the Akron wheat-fallow cultivated soil C, and had a MRT of 3157 years compared to whole-soil MRT of 1072 years. Incubation for 853 days resulted in a loss of ~35% of whole-soil C as well as the NAH constituents. The MRT of the NAH after incubation increased to 4967 years, indicating that the incubation removed significant portions of the NAH fraction that was initially non-hydrolysable.

Humic acid fractionation has a long history of use and some criticism in soil science (MacCarthy 2001). The separation of a high molecular weight series of colloids by fractionation with weak  $\text{Na}_4\text{P}_2\text{O}_5$ -NaOH in a nitrogen atmosphere were similar to those previously studied by this technique (Clapp et al. 2005). The mass spectra, as well as the PCA analysis, showed the humic acids from both soils have unique characteristics specific to this group of compounds. The humic acids also reflected differences in management and soils. A major argument for including humic acids in a study such as this is that it allows one to use the tremendous wealth of knowledge that exists about these compounds in the interpretation of today's questions concerning SOM dynamics in ecosystem functioning and global change. The humic fractionation does not greatly affect the chemistry of the compounds, but it does allow the fractionation of a relatively homogeneous series of materials that are much more susceptible to pyrolysis without artifact than if conducted on the whole soil. The fact that the humic acids from the forested environment as well as its cultivated counterpart contained much less ash minerals is consistent with the much earlier knowledge about these materials in different soils (Kononova and Bel'chikova 1961). Our results also demonstrated that the humic acid fraction proved to be the most chemically homogenous of the isolated fraction as determined by PCA of py-MBMS spectra.

The fact that the humin remaining after fractionation is not too dissimilar from the whole soil should not be unexpected. It has previously been shown that the residue remaining after extraction of fulvic and humic acids is a mixture of these closely associated with mineral surfaces (Kononova and Bel'chikova 1961), as well as non-extractable plant and animal residues and charcoal. It is most often intermediate in MRT between the younger fulvic and the older humic material (Campbell et al. 1967) and is similar in both  $^{13}\text{C}$  and  $^{14}\text{C}$  to the total soil. Preston et al. (1989) also

showed that with increased de-ashing with HCl/HF, the  $^{13}\text{C}$ -NMR spectra of humin samples became more similar to humic acid. Our py-MBMS analyses further confirmed these observations.

Today's questions concerning the role of SOM in soil fertility, ecosystem functioning and global change can only be addressed through knowledge of the controls on SOM stabilization and their interactions. Pyrolysis molecular beam mass spectrometry provides a powerful and rapid means of assessing the molecular structure (biochemical composition) of SOM. However, it produces an enormous amount of data that is often only indirectly associated with specific source compounds because many of the products of pyrolysis are partial breakdown products of soil constituents and because specific  $m/z$  signals can be attributed to several classes of compounds. We have used the extensive database generated over decades of work by Schulden and his colleagues as our basis for an initial interpretation of the py-MBMS analysis of two soils that have been previously characterized by isotopic tracers and biological, physical and chemical fractionation (Follett et al. 2007; Haile-Mariam et al. 2008; Paul et al. 1997a, 2001). The  $m/z$  values should be directly comparable between analytical methods (py-FIMS vs. py-MBMS) and laboratories, but differences in pyrolysis conditions, and even soils, could produce differences in the relative amount of various pyrolysis products generated and detected, and we therefore use Schulden's database to interpret our results with caution. While the approach described above represents significant progress in the characterization of changing SOM composition, a fully quantitative analysis will only be achieved using multiple internal standards and measurement of interference by inorganic soil constituents. Such standards would be samples spiked with organic compounds representative of a given class that would yield predictable ion intensities at predictable  $m/z$ . Until then, the confounding factors of the matrix effect on pyrolysis yield and total ion intensity and differing SOM compositions will remain difficult to deconvolute. We have demonstrated the principle that py-MBMS can be used to characterize SOM in chemically isolated fractions, but further methodological experiments need to be conducted. We are in the process of utilizing a range of internal standards with more attention to the effects of different types and levels of mineral interferences and better resolution of higher  $m/z$  materials.

This should allow a better quantification of data and verification of peak identities. The large wealth of data obtained and the large throughput of samples in py-MBMS has convinced us it will be a powerful tool to use in combination with biological, chemical, physical and soil surface interactions to understand the controls on the dynamics of SOM

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