SOIL CHEMISTRY

Tillage Management Alters Surface Soil Organic Matter Composition: A Pyrolysis Mass Spectroscopy Study

Steven Sleutel*
Mohammed Abdul Kader
Dep. of Soil Management and Soil Care
Ghent Univ.
Coupure Links 653
9000 Gent
Belgium

Peter Leinweber
Institute for Land Use, Univ. of Rostock
Justus von Liebig Weg 6
18059 Rostock
Germany

Karoline D'Haene
Stefaan De Neve
Dep. of Soil Management and Soil Care
Ghent Univ.
Coupure Links 653
9000 Gent
Belgium

We examined the effects of 10 yr of reduced tillage (RT) management, compared with conventional tillage (CT), on the chemical composition of soil organic matter (SOM) using a combination of physical fractionation and pyrolysis–field ionization mass spectroscopy (Py–FIMS). Surface soil samples (0–10 cm), which were collected from two adjacent CT and RT managed fields, were separated into particulate organic matter (POM) fractions and sand, silt, and clay size separates. Higher amounts of C (77%) and N (64%) were observed in all size separates of the RT soil and, in particular, the proportion of intramicroaggregate POM was two times as large as in the CT soil. The Py–FIMS spectra showed that larger proportions of free fatty acids, sterols, and N-containing compounds were present in the RT soil's sand fraction than in the CT soil. Similar observations were also made for both free and intramicroaggregate POM fractions. In the silt and clay size fractions, no substantial differences in SOM composition were measured between tillage treatments, except that the RT soil's clay fraction had a higher proportion of N-containing compounds and a lower proportion of alkylaromatics. Ratios of carbohydrate hexose to pentose subunits and of lignin monomers + phenols to lignin dimers confirmed a less decomposed character of the SOM in the silt and clay size fractions of the RT soil. These observations can be logically linked to the reduced mixing of crop residues and manure in the soil profile and higher surface soil microbial biomass under RT.

Abbreviations: CT, conventional tillage; fPOM, free particulate organic matter; iPOM, intramicroaggregate particulate organic matter; LF, light fraction; NMR, nuclear magnetic resonance; OC, organic carbon; OM, organic matter; Py-FIMS, pyrolysis–field ionization mass spectroscopy; RT, reduced tillage; SOC, soil organic carbon; SOM, soil organic matter; TII, total ion intensity.

Tillage influences soil properties by causing physical disruption of the soil structure, thereby altering the soil water and O_2 content (Curci et al., 1997). Furthermore, the intensity of tillage determines the stability and turnover of soil aggregates and thus the presence of organic matter (OM) within or between aggregates. The accessibility of organic substrates for microbial activity, and hence their susceptibility to decomposition, depends on this spatial location of a substrate in the soil matrix because: (i) there is reduced access for the microorganisms and reduced diffusion of their enzymes into the intraaggregate space; and (ii) aerobic decomposition is restricted due to a reduced diffusion of O_2. Consequently, there exists a very intimate link between tillage, soil aggregation, and SOM turnover. Indeed, a decreased macroaggregate turnover under RT compared with CT was found to lead to a stabilization of POM in stable microaggregates (Six et al., 2000). Higher C mineralization following crushing of microaggregates (Mikha and Rice, 2004; Bossuyt et al., 2002) corroborates the theory that more otherwise mineralizable, i.e., labile, SOM is stored as occluded POM in less disturbed soils (Elliot, 1986). This occluded POM may account for an important part (10–60%) of the total gain in SOM in no-till soils compared with CT (Six et al., 1999). Next to the differences in the relative distribution of OM across OM fractions between RT and CT managed fields, RT management also significantly increases soil microbial activity (e.g., Granatstein et al., 1987) and enzyme activity (e.g., Curci et al., 1997) and favors a more fungi-dominated microbial biomass, particularly in the surface layer (Frey et al., 1999). The microbial activity and community structure, in turn, are directly determinant on SOM turnover because soil biota mediate decomposition and humification processes and indirectly through the role that bacteria and fungi and their mucilages play in aggregate stabilization (Oades, 1984).

Very few studies, however, have looked into differences in the chemical composition of the SOM between CT and RT managed soils that may result from the above reported shifts in microbial activity, in the microbial community's composition, and in the distribution of SOM across different POM fractions after establishment of RT. Using solid-state ^{13}C nuclear magnetic resonance (NMR), Golchin et al. (1994) and Kölbl and Kögel-Knabner (2004) found occluded light fraction (LF) and POM to contain more alkyl C and less O-alkyl C than the free LF and POM. These data suggest that

during the inclusion of occluded OM, there is a selective decomposition of carbohydrates and relative enrichment of more stable OM compounds compared with free OM. Elaborate investigations on this subject will, however, require more detailed analytical chemical methods. In this context, Py–FIMS has been shown to uniquely yield a detailed chemical characterization of SOM combined with data on the thermal stability of individual SOM compounds (Leinweber and Schulten, 1995). The thermal stability of SOM compounds depends strongly on the strengths of the chemical bonds within the organic macromolecules or between organic materials and minerals and thus provides additional important information on SOM quality. As such, based on Py–FIMS analysis, Monreal et al. (1995) found positive correlations between macroaggregation (>250 µm) and the proportions of lignin dimers, sterols, lipids, and alkylaromatics in the >250-µm aggregate fraction.

With a particular focus on OM in free and microaggregate-associated POM, however, none of these very few studies have yet addressed the effects of RT management on the whole SOM composition. In a review of the literature, Schulten and Leinweber (2000) found not only sand but also silt size fractions to be enriched in relatively undecomposed OM under SOM-conserving management. Clay fractions, in turn, are unequivocally richer in OM from the soil biomass and its metabolites, which are represented by N-containing compounds and carbohydrates (Leinweber and Schulten, 1995). A larger input of fresh plant material and a larger soil microbial activity in the surface layer of RT soils could therefore possibly alter SOM composition in silt and clay fractions under RT as well. Detailed investigations on this matter are at present absent, which obstructs our understanding of processes involved in tillage-induced changes in SOM content and quality.

Accordingly, the underlying aim of this study was to further elucidate the relationship between tillage management and SOM quality. Specific objectives were: (i) to verify the hypothesized accumulation of biochemically labile OM in the surface layer brought about by a shift in management to RT by assessing the distribution of SOM across individual soil size fractions and POM fractions; and (ii) to examine the effects of RT on the chemical composition of SOM in sand, silt, and clay size separates as well as in the whole soil and intramicroaggregate particulate organic matter (iPOM) and free particulate organic matter (fPOM) fractions compared with CT.

Based on the above considerations, we used physical fractionation and Py–FIMS to study SOM and SOM fractions in the surface soil of two adjacent RT and CT fields to address these objectives.

MATERIALS AND METHODS
Site Description and Sampling

The soil samples were collected from two adjacent farm fields located in Villers-le-Bouillet (Belgium) under RT (for the past 10 yr) and CT (soil type: Alfisol, silt). The RT field has a slightly higher clay percentage than the CT field. The annual mean temperature is 9.8°C and the mean annual rainfall 780 mm. A three-year crop rotation of winter barley (*Hordeum vulgare* L.)–sugarbeet (*Beta vulgaris* L.) or potato (*Solanum tuberosum* L.)–winter wheat (*Triticum aestivum* L.) was established 25 yr ago. White mustard (*Sinapis alba* L.) and winter oat (*Avena sativa* L.) were sown as green manures

after harvest of winter barley and potato, respectively. An average dose of cattle farmyard manure of 25 Mg ha^{-1} was applied right before sowing of the green manure on both RT and CT fields. Moldboard plowing (20–25 cm) was performed annually in the CT treatment. Tillage operations for the RT treatment consisted of chopping and superficial incorporation of harvest residues before seedbed preparation and sowing of sugarbeet or potato and of direct drilling of winter wheat. Harvest of beet or potato also constituted an additional heavy soil disturbance every 2 or 3 yr in both RT and CT fields. The exact cropping history of these fields is unknown but arable cereal–root crop based rotations have been practiced for at least 50 yr. Three plots of 150 m^2 (10 by 15 m), each spaced 10 m apart, were sampled in the RT and CT fields in March 2005 by boring five times per subplot with an auger (0–10, 10–20, 20–30, 30–40, and 40–60 cm). Soil samples were air dried at room temperature. The main physicochemical properties of the different soil layers were determined per subplot (Table 1). For each depth layer, the subplot soil samples of the replicate plots were then bulked for subsequent texture analysis, physical fractionation, and mass spectroscopic analyses. Microbial biomass organic C (OC) was measured with a chloroform fumigation extraction using a 0.1 M KCl extractant (1:2 soil / extractant w/v) (Voroney et al., 1993). The OC in the extracts was analyzed with a total organic C analyzer (TOC-V CPN, Shimadzu Corp., Kyoto, Japan). An extraction efficiency K_{EC} value of 0.25 was used, as suggested by Voroney et al. (1993).

Particle Size Separation

Bulked (three field replicates) whole-soil samples of the 0- to 10-cm depth layer were separated into three particle size fractions: clay (<2 µm), silt (2–63 µm), and sand (63–2000 µm). For this purpose, soil samples were dispersed in a two-step procedure according to Amelung (1997) and Thiele-Bruhn et al. (2004) using an ultrasonic vibrator (Sonics Vibracell 600 with Sonotrode CV 26, Sonics & Materials Inc., Newton, CT). A 30-g sample was weighed into a beaker and 500 mL of deionized H$_2$O was added. To preserve particulate organic matter from disruption, the soil slurry was first sonicated at 60 J mL^{-1} while the temperature was kept constant at 20°C. The sand fraction was separated by wet sieving through a 63-µm mesh screen. Deionized H$_2$O was added to the remaining soil suspension, giving a soil/solution ratio of 1:20, and it was additionally sonicated at 440 J mL^{-1}. The energy input was calibrated from the heating of H$_2$O in a Dewar vessel, as described by Thiele-Bruhn et al. (2004).

Table 1. Soil texture, bulk density (ρ_b), pH in KCl, organic C (OC) and N content, C/N ratio, and microbial organic C (OC$_{mic}$) content at different depths in the soil profile of the reduced tillage (RT) and conventional tillage (CT) fields at Villers-le-Bouillet.

Depth	Soil texture			ρ_b	pH$_{KCl}$	OC	N	C/N	OC$_{mic}$
	>50 µm	2–50 µm	<2 µm						
cm	———%———			g cm^{-3}		g C kg^{-1}	g N kg^{-1}		mg C kg^{-1}
					RT				
0–10	7.9	72.2	19.8	1.47	6.5	16.1	1.55	10.4	135
10–20	7.4	71.8	20.8	1.47	6.6	9.3	1.13	8.3	23
20–30	5.7	68.8	25.6	1.53	6.4	5.0	0.74	6.7	33
30–40	7.4	67.5	25.1	1.53	6.1	5.6	0.85	6.6	41
40–60	6.4	66.6	27.0	1.53	–	1.8	–	–	–
					CT				
0–10	9.4	74.6	16.0	1.51	5.8	9.4	0.97	9.7	57
10–20	9	75.7	15.3	1.51	5.7	8.9	0.94	9.5	28
20–30	7.5	75.7	16.8	1.56	5.8	6.8	0.79	8.6	24
30–40	8	74.3	17.6	1.56	5.8	6.4	0.77	8.2	28
40–60	6.5	69.3	24.2	1.56	–	2.8	–	–	–

The resulting soil suspension was separated into the clay and silt particle size fractions by gravity sedimentation in water following Stoke's law. Sedimentation was repeated up to 10 times, and about 4 L of suspension containing the clay fraction was collected. A 1-L subsample of the clay suspension was centrifuged at 16,900 × g. Clay and silt fractions were dried at 50°C after decanting the clear supernatant. Particle size fractionations were performed in duplicate.

Particulate Organic Matter Fractionation

Soil organic matter in the bulked (three field replicates) surface soil samples (0–10 cm) was physically fractionated into OM associated with the clay + silt particles, OM residing in stable microaggregates (53–250 μm), and the free POM based on the "microaggregate isolation" methodology proposed by Six et al. (2000). The applied methodology, described in detail in Sleutel et al. (2006), results in the sequential isolation of (i) water-stable microaggregates from coarse fPOM (>250 μm) and silt- and clay-associated OM (<53 μm) by means of wet sieving, (ii) density separation of fine fPOM (53–250 μm) from iPOM and intramicroaggregate silt- and clay-associated OM (<53 μm), and (iii) isolation of iPOM (53–250 μm) from the latter
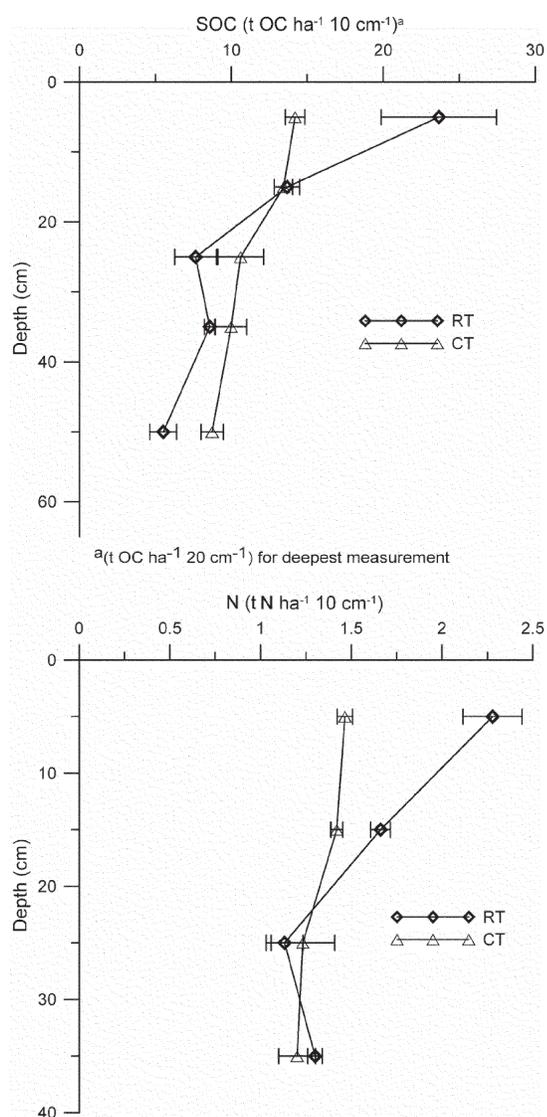

Fig. 1. Depth distribution of the average soil organic C (SOC) and N stocks in the reduced tillage (RT) and the conventional tillage (CT) fields at Villers-le-Bouillet, Belgium; error bars represent corresponding standard deviations.

OM fraction. The field-moist soil was gently broken apart by hand and was passed through an 8-mm sieve to break down large macroaggregates. The soil was then dried at 50°C. Three replicate 10-g subsamples were used for the physical fractionation procedure. Subsamples of fractions or entire fractions were analyzed for their total C and N content with a Variomax CNS analyzer (Elementar Analysensysteme, Hanau, Germany).

Pyrolysis–Field Ionization Mass Spectroscopy

Whole soil samples, size separates, and POM fractions were analyzed by means of Py–FIMS. The coarse fPOM and fine fPOM were combined into one fPOM fraction for Py–FIMS analysis. For temperature-resolved Py–FIMS, about 2 to 5 mg of sample material was thermally degraded in the ion source of a modified Finnigan MAT 731 high-performance mass spectrometer (Finnigan MAT, San Jose, CA). The samples were heated under a high vacuum from ambient temperature to 700°C at a heating rate of 10 K per magnetic scan ($\sim$1.7 K s^{-1}) (three replicates). After about 20 min of total registration time, about 60 magnetic scans were recorded for the mass range 16 to 1000 Da (single spectra). The single-scan spectra were integrated to obtain one summed spectrum. In general, the summed spectra of three replicates were averaged to give the final survey spectrum. These survey spectra, in particular the assignment of marker signals to chemical compounds, were interpreted as described by Schulten and Leinweber (1999), modified to include 10 compound classes. These are (i) carbohydrates with pentose and hexose subunits, (ii) phenols and lignin monomers, (iii) lignin dimers, (iv) lipids, alkanes, alkenes, bound fatty acids, and alkylmonoesters, (v) alkylaromatics, (vi) mainly heterocyclic N-containing compounds, (vii) sterols, (viii) peptides, (ix) suberin, and (x) free fatty acids. As well as series of marker signals, the volatilization temperature is also considered for identification. For each of the 60 single scans, the ion intensities of these marker signals were calculated and the average ion intensities for each class of compound were plotted against the pyrolysis temperature, giving characteristic thermograms. All samples were weighed before and after Py–FIMS to normalize ion intensities per milligram of sample. Detailed descriptions of the Py–FIMS methodology (Schulten, 1993) and statistical evaluations of sample weight and residue, volatilized matter, and total ion intensities are given by Sorge et al. (1993).

RESULTS AND DISCUSSION
Soil Organic Carbon, Nitrogen, and Microbial Organic Carbon Depth Profiles

The soil organic C (SOC) and N content decreased with depth in the soil profile under the CT and RT tillage systems (Table 1). Compared with CT, the SOC and N contents in the 0- to 10-cm layer and, to a much lesser extent, in the 10- to 20-cm layer in the RT field were higher, in correspondence with numerous reports in the literature (e.g., Frey et al., 1999; Denef et al., 2004). Slightly smaller SOC concentrations were measured in the 20- to 30- and 30- to 40-cm layers in the RT soil, which is probably due to the lower input of fresh OM in these deeper layers in the absence of plowing. Accounting additionally for small differences in soil bulk density as well, the higher amount of SOC in the 0- to 20-cm layer under RT was partly compensated by a lower SOC stock in the 20- to 60-cm depth layer (Fig. 1). In total, the SOC (2.0 Mg OC ha^{-1} higher in 0–60 cm) and N (0.1 Mg N ha^{-1} higher in 0–40 cm) content in the RT soil were slightly higher compared with the CT soil as a consequence of the reduction in soil disturbance. This difference closely compares with the average accumulation rate of 0.2 Mg OC ha^{-1}

yr^{-1} in the 0- to 30-cm layer in temperate soils under no-till, as calculated by Six et al. (2004). In the RT field, the microbial biomass OC was 2.5 times higher than in the 0- to 10-cm layer of the CT field, but not in the deeper soil layers. Under a temperate climate, higher surface layer microbial biomass OC contents following the adoption of RT have indeed often been observed (Höflich et al., 1999; Kandeler et al., 1999). Changes in SOM stocks are generally accompanied by a redistribution of SOM across individual fractions (Tiessen and Stewart, 1983) and changes in SOM composition (Schulten et al., 1990). Potential changes in SOM fractions were here further investigated by assessing the distribution of SOM in size separates and POM fractions of the 0- to 10-cm depth layer.

Surface Soil Organic Carbon and Nitrogen Distribution in Isolated Size and Particulate Organic Matter Fractions

Table 2 gives a comparison of the OM-free dry matter (DM) distribution as obtained by the soil textural analysis and soil physical fractionation (0–10-cm depth layer). Overall, in spite of a difference in sieving size, DM yields of the 0- to 63-, 0- to 53-, and 0- to 50-μm fractions were comparable. The fact that both the >63-μm fraction of the ultrasonication–sedimentation method and the >250- plus >53-μm heavy fraction of the microaggregate-isolation method did not yield higher sand-sized DM yields than the textural analysis suggests that no silt or clay particles from >53- and >63-μm aggregates were left in these fractions. There was a strong similarity between the DM percentages of silt and clay size separates and the textural analysis, which indicates that the ultrasonication at 440 J mL^{-1} completely dispersed all <63-μm particles. These results show that the two different physical fractionation methods applied here give comparable results and can be complementarily used for further separation of POM fractions and isolation of silt and clay size separates.

Results of the fractionation of OM in particle sizes by ultrasonication–sedimentation (0–10-cm depth layer) are presented in Table 3. The general distribution of OC and N across the particle size fractions was sand < silt < clay. The clay fraction thus contained the bulk of the SOM in both CT and RT soils in spite of the fact that 74% of the soil DM was located in the 2- to 63-μm fraction. A 40 to 60% contribution of clay-sized SOC appears to be typical for Luvisols, however, as demonstrated in a review of the literature on this subject by Schulten and Leinweber (2000). The amounts of OC and N in all three size fractions were, on average, 59 and 53% higher, respectively, under RT than CT management. Since this difference in OC and N was proportional across all three size fractions, there was almost no shift in the relative OC and N distribution of

the RT soil compared with the CT soil. At first sight, these data suggest no particular enrichment of either labile or stable OM in the RT field compared with the CT field.

The physical fractionation of OM according to the micro-aggregate isolation methodology by Six et al. (2000) showed a similar distribution of OC and N between sand and silt + clay particle size fractions (Table 4) as the particle size separation by ultrasonication and sedimentation. Higher amounts of OC and N were found for all isolated fractions in the RT soil than in the CT soil, except for OC in the coarse fPOM. Among the isolated fractions, this difference was disproportional, as there was a higher contribution of POM OC and N in the RT soil (Fig. 2). Mainly, the amount of OC in the iPOM was almost three times larger in the RT than in the CT soil (Table 4). This larger amount of iPOM contributed 39% of the difference in SOC content between the RT and the CT fields. This result is in agreement with findings by Six et al. (1999) and Denef et al. (2004), who reported 20 and 90% of the increase in SOC following no-till management from an accumulation of iPOM. Our results thus support the current theory that reduced disturbance of soil enhances microaggregation and occlusion of POM therein. It cannot be excluded, however, that the larger amounts of iPOM, OC, and N have at least partly resulted from redistribution from fPOM to iPOM. Following the conceptual model of Six et al. (2002), the proportion of the "physically protected" SOM (i.e., the iPOM) was twice as large in the RT soil (Fig. 2). The amount of fPOM, which is assumed to be "unprotected" against microbial decomposition, was 15% higher in the RT soil, accounting for only 7% of the difference in SOC, with most (70%) of this difference attributed to the fine fPOM.

Pyrolysis–Field Ionization Mass Spectroscopy of Surface Soil Intramicroaggregate and Free Particulate Organic Matter, Particle Size Fractions, and Whole Soil Samples

The measured total ion intensity (TII) and percentage of volatilized matter in Py–FIMS analysis are given in Table 5. Sorge et al. (1993) showed that the TII as measured by Py–FIMS is proportional to the OC content and volatilized matter. Therefore, it is possible to

Table 2. Distribution of soil organic-matter-free dry matter across the isolated size fractions of the 0- to 10-cm depth layer of the reduced tillage (RT) and conventional tillage (CT) fields at Villers-le-Bouillet and comparison with the soil texture analysis.

Tillage	Soil texture analysis			Ultrasonication–sedimentation particle-size separation			Microaggregate-isolation method	
	>50 μm	2–50 μm	<2 μm	>63 μm	2–63 μm	<2 μm	>53 μm	0–53 μm
					g kg^{-1} soil			
RT	79	722	198	52	734	214	76	924
CT	94	746	160	93	742	165	72	928

Table 3. Amount of organic C (OC) and N in isolated particle size fractions by ultrasonication–sedimentation particle size separation of the 0- to 10-cm depth layer of the reduced tillage (RT) and conventional tillage (CT) fields at Villers-le-Bouillet (average ± standard deviation).

Tillage	Soil fraction						Sum of fractions	
	>63 μm		2–63 μm		<2 μm			
	OC	N	OC	N	OC	N	OC	N
	g OC kg^{-1}	g N kg^{-1}	g OC kg^{-1}	g N kg^{-1}	g OC kg^{-1}	g N kg^{-1}	g OC kg^{-1}	g N kg^{-1}
RT	3.76 ± 0.73	0.20 ± 0.05	4.18 ± 0.06	0.52 ± 0.03	8.85 ± 0.15	1.23 ± 0.05	16.79 ± 0.64	1.96 ± 0.08
CT	2.25 ± 0.56	0.11 ± 0.03	2.50 ± 0.14	0.36 ± 0.08	5.83 ± 0.26	0.82 ± 0.03	10.59 ± 0.75	1.29 ± 0.09

Table 4. Amount of organic C and N in isolated soil organic matter fractions obtained by physical fractionation of the 0- to 10-cm depth layer of the reduced tillage (RT) and conventional tillage (CT) fields at Villers-le-Bouillet (average ± standard deviation) and their C/N ratio.

Tillage	>250 µm (coarse sand + coarse fPOM†)	53–250 µm (microaggregates + fine fPOM + fine sand)				<53 µm (silt + clay)
		Light fraction (fine fPOM)	Heavy fraction			
			>53 µm (iPOM‡ + fine sand)	<53 µm (silt + clay)		
			Organic C, g OC kg⁻¹			
RT	2.61 ± 0.14	0.58 ± 0.25	3.67 ± 0.26	4.07 ± 1.02		7.46 ± 1.55
CT	2.48 ± 1.99	0.28 ± 0.18	1.33 ± 0.15	3.06 ± 0.77		5.30 ± 0.75
			N, g N kg⁻¹			
RT	0.14 ± 0.01	0.02 ± 0.01	0.25 ± 0.03	0.37 ± 0.08		0.68 ± 0.17
CT	0.08 ± 0.04	0.01 ± 0.01	0.11 ± 0.01	0.36 ± 0.09		0.62 ± 0.08
			C/N ratio			
RT	19.3	29.9	14.6	11.1		10.9
CT	32.6	20.6	12.3	8.6		8.6

† fPOM, free particulate organic matter.

‡ iPOM, intramicroaggregate particulate organic matter.

calculate from Py–FIMS data the relative distribution of the SOM across several important compound classes. Approximately 52% of the TII from pyrolysate signals could be explained (Table 5). If the contributions of low-mass signals (16% of TII), isotope peaks (13% of TII), and unknown pyrolysis products (6% of TII) are added, in total 87% of the produced signals were assigned. Application of Py–FIMS to the CT and RT soils showed that the OM in these soils primarily contained the following compound classes (in order of abundance): phenols + lignin monomers > alkylaromatics ≥ carbohydrates = N-containing compounds. Figures 3 and 4 show the Py–FIMS mass spectra and thermograms of the iPOM, fPOM, and silt and clay fractions of the RT and CT soils. Visual observation of the mass spectra shows significant differences between the RT and CT spectra of the iPOM and clay fractions. Intense peaks at m/z 58, 172, and particularly 326 and 354 were found only in the spectrum of the CT soil and not in the RT soil. The sand fraction spectra were both characterized by intense peaks in the m/z 150 to 300 range, but the dominant peaks in the RT soil (m/z 194 and 208) were different from the CT soil (m/z 96 and 192). The clay fractions were characterized by peaks in the m/z 125 to 225 range but, again, dominant peaks differed (RT: m/z 96 and 180; CT: m/z 186 and 210). The mass spectra of the fPOM and silt fractions showed much closer

similarities, suggesting a smaller difference in fPOM and silt-sized OM between the RT and CT soils. Volatilization maxima were quite similar between the RT and CT soils and approached 450°C for the iPOM and fPOM fractions, 400°C for the silt fraction, and 500°C for the clay fraction. The higher thermal stability of the clay-sized OM is directly indicative of the advanced crosslinking of molecular OM building blocks and their stronger bonding to mineral surfaces with decreasing particle size (Leinweber and Schulten, 1995; Schulten et al., 1993).

Sand and Particulate Organic Matter Fractions

Sand-sized OM in the RT and CT soils was mainly composed of phenols + lignin monomers > alkylaromatics > carbohydrates > lipids > N-containing compounds = lignin dimers (Table 5). This composition compares with previous Py–FIMS analyses (Leinweber and Schulten, 1995), from which it can be concluded that sand fractions are generally characterized by large proportions of phenols, lignin monomers, lignin dimers, and carbohydrates. Looking at both RT and CT soils, the fPOM had more volatile matter, lignin dimers, lipids, sterols, and free fatty acids than the iPOM, silt, clay, and whole-soil samples (Table 5). The iPOM showed changes in the compound classes with fPOM toward the composition of the whole soil, such as lower volatile matter and proportions of lignin dimers, lipids, and sterols, and larger proportions of carbohydrates, phenols and lignin monomers, peptides, and fatty acids. Studies by Golchin et al. (1994) and Kölbl and Kögel-Knabner (2004), based on ¹³C NMR, found the occluded LF to contain more alkyl C (fatty acids, lipids, and proteins) and aryl C (lignin), and less O-alkyl C (carbohydrates and polysaccharides) than the free LF. Although these LF fractions were derived from different methods than our isolated POM, both these and our observations designate intraaggregate OM to be further transformed than free OM.

Pronounced differences in the chemical composition of sand-sized OM between the RT and CT soils were larger proportions of sterols, free fatty acids, N-containing compounds, and lipids (Table 5) in the RT soil. Similar observations were also made for the fPOM and the iPOM, which together constitute the sand-sized OM. Proportions to the TII of n-C₁₆ to n-C₃₄ fatty acids were larger in the sand fraction of the RT soil than CT soil. This corroborates results from Dinel et al. (1998), who found no-till soils to be relatively rich in n-fatty
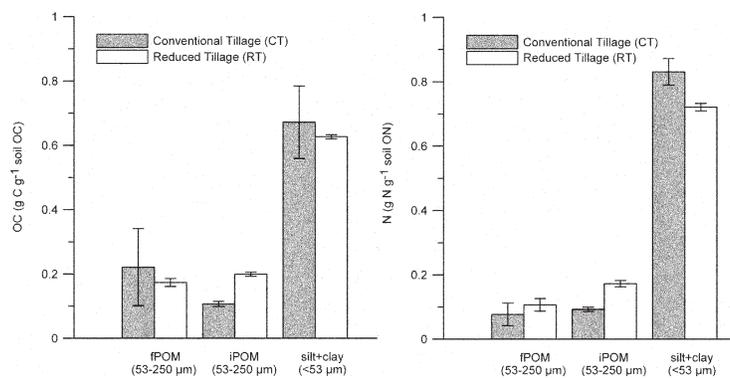

Fig. 2. Relative distribution of the whole-soil organic C (OC) and N across coarse and fine free particulate organic matter (fPOM, >53 µm), intra-microaggregate particulate organic matter (iPOM, 53–250 µm) and silt + clay associated (<53 µm) soil organic matter fractions (0–10-cm depth layer) of the reduced tillage (RT) and conventional tillage (CT) fields at Villers-le-Bouillet, Belgium. Columns represent average values of triplicates; error bars represent corresponding standard deviations.

Table 5. Total ion intensity (TII), percentage of matter volatilized in pyrolysis (VM), and relative contributions of soil organic matter compound classes to the TII as determined by pyrolysis–field ionization mass spectroscopy in free particulate organic matter, intramicroaggregate particulate organic matter, size separates, and the whole soil of the 0- to 10-cm depth layer of the reduced tillage (RT) and conventional tillage (CT) fields at Villers-le-Bouillet.

Tillage	TII	VM	Relative contribution to TII of compound class[†]										
			CHYDR	PHLM	LDIM	LIPID	ALKY	NCOMP	STERO	PEPTI	SUBER	FATTY	Sum
	10^{12} counts kg^{-1}	%	———————————————————— % ————————————————————										
			Free particulate organic matter										
CT	11.86	23.8	6.9	11.1	3.2	6.9	10.4	6.6	1.1	3.8	0.1	1.1	51.3
RT	18.12	27.7	7.1	12.3	2.9	7.6	10.2	6.7	1.4	3.7	0.1	1.8	53.8
			Intramicroaggregate particulate organic matter										
CT	1.21	20.1	11.3	10.7	0.9	5.8	8.0	5.9	0.7	4.4	0.0	3.0	50.7
RT	7.69	10.6	7.7	13.1	3.1	6.2	10.2	7.1	1.0	3.9	0.1	1.1	53.4
			≥63-µm particle size fraction										
CT	0.14	6.0	6.0	8.3	3.8	5.1	8.9	3.1	0.1	1.8	0.0	0.2	37.4
RT	0.38	4.9	4.9	12.4	4.4	7.7	9.9	5.0	0.9	2.1	0.1	2.0	49.4
			2–63-µm particle size fraction										
CT	0.29	9.4	9.4	16.4	2.1	3.9	12.4	8.0	0.1	4.3	0.0	0.0	56.6
RT	1.22	7.6	7.6	15.5	2.7	4.6	11.8	7.4	0.1	3.4	0.0	0.1	53.3
			≤2-µm particle size fraction										
CT	0.221	8.1	8.1	19.3	0.9	3.0	18.9	6.9	0.0	3.1	0.0	0.0	60.2
RT	0.460	7.8	7.8	17.4	0.9	2.8	15.3	9.7	0.0	2.5	0.0	0.0	56.5
			Dispersed whole soil										
CT	0.048	8.0	11.7	14.9	1.1	2.2	7.9	10.8	0.0	2.9	0.3	0.0	51.7
RT	0.126	11.7	8.0	12.7	2.1	3.8	13.2	8.8	0.1	3.3	0.0	0.1	52.1

† CHYDR, carbohydrates with pentose and hexose subunits; PHLM, phenols and lignin monomers; LDIM, lignin dimers; LIPID, lipids, alkanes, alkenes, bound fatty acids, and alkyl monoesters; ALKY, alkylaromatics; NCOMP, mainly heterocyclic N-containing compounds; STERO, sterols; PEPTI, peptides; SUBER, suberin; FATTY, free fatty acids.

acids. When averaging the fatty acid compound class proportions of the fPOM and iPOM, a similar higher content was found in the RT soil, although differences between the RT and the CT soils were less pronounced than in the sand size fraction. According to Schnitzer (1986), n-C$_4$ to n-C$_{26}$ fatty acids originate predominantly from microorganisms and n-C$_{16}$ to n-C$_{38}$ fatty acids from the waxes of plants and insects. Confirmations that crop-originated long-chain free fatty acids are traceable in soils were found by Jandl et al. (2005) and for other lipids by Jandl et al. (2006). Based on those findings, the particular higher ion intensity of signals at m/z 256, 284, 289, 312, 326, 340, 368, and 396, which come from n-C$_{16}$ to n-C$_{27}$ fatty acids, thus indicate that, more specifically, there is a higher proportion of plant-residue-originated fatty acids in the RT soil. Moreover, long-chain fatty acids appeared to be important agents that helped stabilize macro-aggregates under continuous wheat (Monreal et al., 1995). Jandl et al. (2004) as well suggested new evidence for the aggregate-stabilizing function of n-C$_{21:0}$ to n-C$_{34:0}$ fatty acids by their characteristic pattern

in size-fractionated, disaggregated and aggregated samples. Based on our measured Py–FIMS data, however, we cannot conclude a direct link between the higher content of iPOM, i.e., aggregate-protected OM, and the larger proportion of free fatty acids under RT.
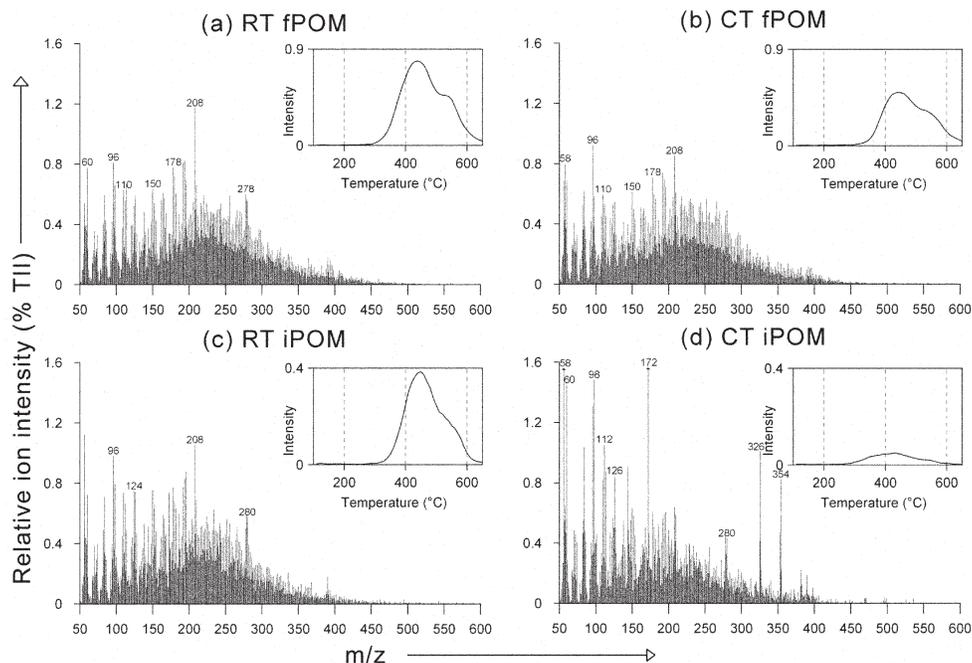

Fig. 3. Thermograms of the total ion intensities (TII) and summed averaged pyrolysis–field ionization mass spectra of the (a, b) free particulate organic matter (fPOM) and (c, d) intramicroaggregate particulate organic matter (iPOM) fractions of the 0- to 10-cm depth layer of the reduced tillage (RT) and the conventional tillage (CT) fields at Villers-le-Bouillet, Belgium.
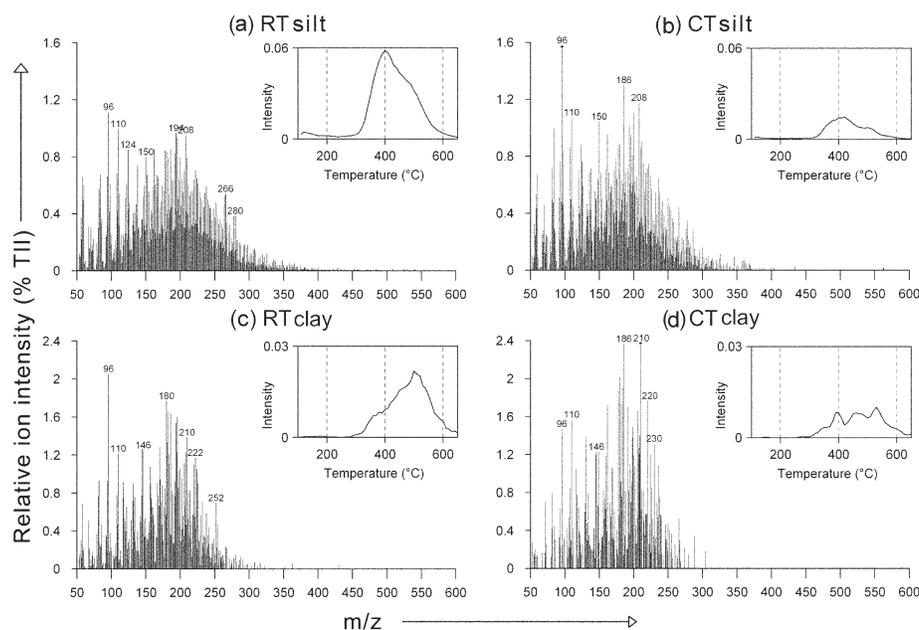
Fig. 4. Thermograms of the total ion intensities (TII) and summed averaged pyrolysis–field ionization mass spectra of the (a, b) silt and (c, d) clay fractions of the 0- to 10-cm depth layer of the reduced tillage (RT) and the conventional tillage (CT) fields at Villers-le-Bouillet, Belgium.

OM, whereas fPOM is continuously replaced by fresh plant-derived OM. As a consequence, the composition of iPOM and fPOM could well deviate again in the RT field.

Silt and Clay Fractions and Whole-Soil Samples

As the relative amount of lignin dimers and lipids decreased, the relative proportions of N-containing compounds and peptides to the TII increased in the silt fraction compared with the sand-sized fraction (Table 5). In correspondence with earlier observations (Leinweber and Schulten, 1995), this reflects the progressive decomposition of OM with decreasing particle size. Surprisingly, in spite of the significantly higher SOC content of the silt fraction under RT (Table 3), the composition of the OM in the silt-sized soil fraction was similar between the RT and the CT fields. No indications were found for any of the substantial relative enrichments of OM compound classes that were observed for the RT soil's sand-sized fraction. These results suggest that the OM composition of the silt-sized fraction is less affected by tillage management.

In accordance with observations throughout the literature (Leinweber and Schulten, 1995), the clay fractions of both RT and CT soils contained larger proportions of N-containing compounds, carbohydrates, phenols, and lignin monomers and fewer lignin dimers and lipids than the sand fraction (Table 5). Much smaller differences in chemical composition were found between the RT and CT soils than for the sand fraction. Calculation of the TII percentage of either the peptide or N-containing compound classes is based on the assignment of marker peaks from the Py–FIMS mass spectra. Potential synthesis of peptide-derived molecular ions into heterocyclic N-containing compounds during pyrolysis makes a clear separation between both compound classes tentative. Therefore, both compound classes should instead be considered jointly. There was a limited relative enrichment in N-containing compounds (mainly at m/z 67, 79, 81, 167, 181, 183, and 233) plus peptides in the RT soil. Possibly, the much larger microbial biomass present in the RT soil (Table 1) and a consequently higher continuous input of microbial metabolites has driven this enrichment. This result agrees with observations by Szajdak et al. (2003) of higher humic-acid-bound amino acids in no-till soils.

Next, there was a reduction in the proportion of alkylaromatics in the TII of the clay fraction of the RT soil (Table 5). Alkylaromatics are considered to be a result of the humification of plant materials and microbial metabolites and form the skeletons of humic substances (Schulten and Schnitzer, 1993). Consequently, a lower proportion of alkylaromatics suggests that the OM in the clay fraction of the RT soil contains relatively higher amounts of less-decomposed components than the clay-sized OM in the CT soil. This lower proportion corresponds with findings by other researchers (Ding et al., 2002; Szajdak et al., 2003), who found humic acids

In contrast, the higher TII percentage of N-containing compounds in the RT field sand fraction was largely due to a higher proportion of N-containing compounds in the iPOM fraction of the RT soil than the CT soil. Mainly, marker peaks at m/z 153, 195, 203, 257, and 271, which originate from n-alkyl nitriles, i.e., mostly pyrolysis products from amines, contributed to this difference.

The TII percentage of the sterols compound class in the sand size fraction was a factor of nine higher in the RT soil than in the CT soil. The TII percentage of sterols in the fPOM and iPOM was also higher in the RT soil than in the CT soil, but this difference was less pronounced. Up to three quarters of the difference in the TII percentage of sterols in the sand fraction could be attributed to signals at m/z 396, 398, 400, 402, 408, 410, and 412, which originate from phytosterols, indicating a plant origin. In addition, prominent signals were found for m/z 372, 386 (cholesterol), and 390. The latter signal has been shown to be enriched in composted manure (Smidt et al., 2004) and was only higher in the fPOM fraction, which might thus point at the presence of farmyard-manure-derived fPOM in the RT surface soil layer. Gregorich et al. (1997) found sterols to be enriched in the LF compared with maize (*Zea mays* L.) leaf tissue and the whole soil. Our measured data confirm a connection between the higher proportions of fPOM and iPOM under RT management and an increased proportion of sterols in the RT soil compared with the CT soil.

When considering the distribution of the TII of all 10 compound classes, the composition of the iPOM and fPOM were in closer correspondence in the RT soil than in the CT soil. A conceivable explanation would be that reduction of soil disturbance under RT has led to inclusion of more POM into microaggregates. The chemical composition of this recently formed iPOM evidently more closely matches that of fPOM, i.e., higher contents of lipids, lignin dimers, sterols, and N-containing compounds. With time, transformation of this new iPOM may alter its composition closer to that of the whole-soil

in no-till soils to be less aromatic than in CT soils, although their analytical methodologies differed from the one used here.

Schulten and Leinweber (1999) previously reported that strong bonds to silt particles are possibly the main reason for the often-observed low decomposability of silt-sized OM rather than a specific molecular composition. Given the silt texture of our soils, we further compared the thermograms of the individual compound classes, as they may provide information on the degree of mineral association of the SOM. The thermal stability of the compound classes was investigated by compiling difference thermograms of the ion intensities between CT and RT. For the silt fractions, the thermal energy required to volatilize OM from the phenols + lignin monomers, lignin dimers, and lipids compound classes was lower in the RT soils (Fig. 5), but an opposite shift was observed for carbohydrates and peptides. No clear trends were found for any of the remaining compound classes. No shifts toward lower or higher volatilization temperatures were found for the RT soil's clay fraction either. In conclusion, these results do not suggest a considerable difference in association of the OM with the soil mineral phase between the RT and CT soils.

Differences in the whole-soil SOM composition between RT and CT were limited (Table 5) and the OM composition of the whole-soil samples approximately matched the silt-sized fraction. The substantial accumulations of fatty acids and sterols that were observed in the sand-sized soil fraction were not reflected in the Py–FIMS analyses of the whole-soil samples. While the difference was small, there was a decreased proportion of carbohydrates to the TII in the RT compared with the CT soil for the whole-soil samples as well as for all three size fractions. These differences were relative, however, as the TII from carbohydrate marker peaks (in counts of molecular ions per kilogram of sample) increased strongly by a factor of 2 to 3. This result seems to contradict an expected relative increase in carbohydrates under RT management due to an enhanced input from microbially derived metabolites or due to the higher input of fresh plant OM. The carbohydrates compound class represents pyrolysis products of cellulose and hemicellulose. These are marked by mass signals at m/z 114 and 132 (pentose units) and at m/z 126, 144, and 162 (hexose subunits). The ratio of hexose/pentose subunits, i.e., (galactose + mannose)/(arabinose + xylose), has frequently been used as an indicator for either the plant (<0.5) or microbial (>2) origin of carbohydrates (e.g., Guggenberger et al., 1994). The CT soil size separates all had high ratios (>3) and were consistently higher than in the RT soil (a factor of 1.6, 1.3, and 2.7 for the sand, silt, and clay fractions, respectively). These results indicate a more plant-derived signature of the carbohydrates in the RT topsoil, which may readily be explained by the increased input of plant material. A rapid decomposition of carbohydrates, mediated by the much larger microbial biomass in the RT soil, might have precluded a relative increase of carbohydrates.

The contribution to the TII of marker peaks assigned to phenols + lignin monomers was lower in the silt and clay fractions and in the dispersed whole-soil samples of the RT soil than the CT soil (Table 5). More important is the ratio of phenols + lignin monomers to lignin dimers, which has been proposed to increase with progressive decomposition of SOM (Leinweber and Schulten, 1995). This ratio increased very strongly from sand (2–3) through silt (6–8) to clay (19–21) size fractions in both RT and CT soils. Lower ratios were found in the silt and clay fractions for the RT soil than the CT
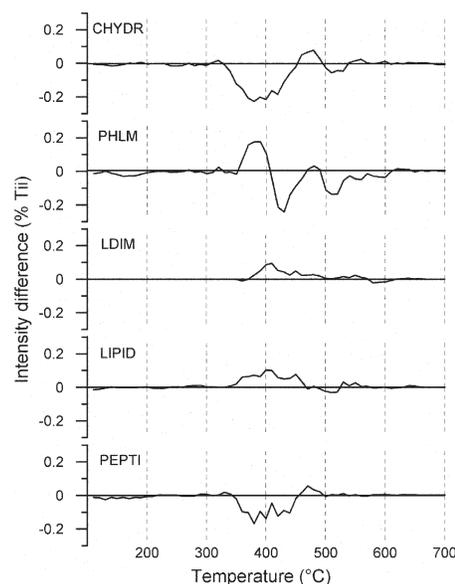

Fig. 5. **Difference thermograms (reduced tillage [RT] minus conventional tillage [CT]) of the total ion intensities (TII) for the volatilization of selected pyrolysis–field ionization mass spectroscopy compound classes (CHYDR: carbohydrates with pentose and hexose subunits; PHLM: phenols and lignin monomers; LDIM: lignin dimers; LIPID: lipids, alkanes, alkenes, fatty acids, and *n*-alkyl esters; PEPTI: peptides) of the silt size fraction. Positive values indicate that relatively more molecules from the respective compound class were volatilized from the RT soil than from the CT soil.**

soil, which again points to the less decomposed character of SOM under RT in these size fractions.

CONCLUSIONS

By comparison of a pair of RT and CT managed fields, tillage management was shown to alter the chemical composition of sand-sized SOM in the surface soil layer (0–10 cm). We found indications for selective accumulation of free fatty acids, sterols, and N-containing compounds in the sand fraction under RT compared with CT management. Although the difference in the amounts of sand-fraction SOC and N between the RT and CT soils was mainly due to a higher amount of iPOM in the RT soil, we cannot conclude a direct link between enrichments in fatty acids or sterols and this higher proportion of iPOM. Based on Py–FIMS analysis of silt and clay size fractions, no substantial differences in their OM composition may be expected under RT and CT management at the time scale studied here, in spite of the measured large differences in the amount of OM in these fractions between the RT and CT fields. Mineral stabilization of specific OM compounds or the whole-soil OM also does not seem to be influenced by tillage management in these silt soils. A lower proportion of alkylaromatics in the RT soil and distributions of Py–FIMS marker peaks of carbohydrates, phenols + lignin monomers, and lignin dimers compound classes, however, all point to a shift toward a less decomposed and more directly plant-residue-dominated composition of the SOM in silt and clay size separates under RT compared with CT management. A relative accumulation of N-containing compounds in the clay fraction of the RT surface soil could possibly have resulted from the higher microbial activity measured here under RT management. We conclude that

reduction of tillage has probably driven these observed changes in surface soil OM between the RT and CT fields both directly through a reduction of mixing of fresh OM to deeper soil layers and indirectly through enhanced microbial activity under RT.

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