

Organic Matter in Clay Density Fractions from Sandy Cropland Soils with Differing Land-Use History

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Land-use history is often overlooked when assessing soil fertility of intensive cropland production systems. The unusually high organic carbon (OC) content of many sandy cropland soils in Northwestern Europe is unexpected given their general low clay content (3–8%) and organic matter (OM) input typical of cropland, but appears to be related to historical heathland land-use. Clay fraction OM composition was compared between two groups of sandy cropland soils with (HC) or without (CC) a history of heathland/forest land-use. Light (1.6–2.2 g cm⁻³) and heavy (>2.2 g cm⁻³) clay fractions in HC soils were nearly twice as rich in OC (on average 199 g kg⁻¹) compared with those of CC soils (on average 109 g kg⁻¹). The hypothesized preferential presence of stable heathland derived OM in light soil fractions, was not supported by our data. Pyrolysis-field ionization mass spectrometry of the clay fractions revealed a more decomposed character of OM in the CC soils and lasting long-term influence of land-use history on SOM composition. This could be concluded from higher proportions of lipids and sterols, a lower thermostability in the HC compared with the CC soils, and enrichment of alkylaromatics and heterocyclic N-containing compounds in the latter. The density fractionation methodology separated organic-mineral particles with similar OM loadings but lower proportions of sterols and medium to long-chained lipids in the heavy compared with the light clay fraction. Given the very high clay OC loadings (6–16 mg C m⁻²) and low binding capacity of the quartz/kaolinite/mica dominated clays, we hypothesize that OM–OM interactions are involved as an OM stabilization mechanism. However, contrary to our hypothesis high clay OC loading (and hence thick OM layering) were found in all sandy croplands regardless of land-use history or density fraction.

Abbreviations: CC, continuous cropland; HC, cropland with a history of heathland/forest land use; OC, organic C; OM, organic matter; Py-FIMS, pyrolysis-field ionization mass spectrometry; SOC, soil organic carbon; SOM, soil organic matter; SSA, specific surface area; TII, total ion intensity; XRD, x-ray diffraction.

Despite decades of research attempting to predict the stability of soil organic C (SOC) and N by means of both soil organic matter (SOM) fractionation methods and spectroscopic techniques, these have had only varying success. Management in terms of crop rotation and fertilization is considered to have a dominant impact on SOC and N levels under intensified cropland production. Management, soil texture, and climate are therefore casually translated into common 'site input variables' in simulation models of C and N cycling. Land-use history, however, is often overlooked as a contributing factor to differences in soil quality in otherwise apparently similar cropland soils. Nevertheless, an important part of the SOM present is of older age and derived from periods that precede cropland use, with possibly very contrasting conditions in terms of the amount and quality of OM inputs, soil tillage, fertilization, and drainage. For example, Schulp and Veldkamp (2008) confirmed that historical land use, up to 230 yr ago, influenced present-day soil OM content variability in the Dutch Sand region.

Large areas of sandy arable soils were found to have unusually high SOC levels in Northwest Europe (Germany: Springob and Kirchmann, 2002; Belgium: Sleutel et al., 2006). Specific types of earlier land use, like heathland (i.e., shrubland dominated by *Ericaceae* often extensively grazed by sheep) and the use of plaggen

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manure may explain the higher stability of OM present. Both were commonly practiced over centuries in Northwest Europe from Schleswig-Holstein to Belgium (Blume and Leinweber, 2004). Studies employing incubation experiments, physical and chemical fractionation methods (Springob and Kirchmann, 2002; Sleutel et al., 2010) and detailed spectroscopic characterizations (Sleutel et al., 2008) have confirmed both the similarity in soil OM stability of relict and cultivated heathlands on the one hand, and the lower stability of soil OM in sandy cropland soils without this historical land use on the other. Specifically, lipids, sterols and to a lesser extent N-containing compounds were selectively preserved from previous heathland land-use (Sleutel et al., 2008). In addition, the high OM loadings of the clay-sized fraction (Sleutel et al., 2010) suggested more than ‘mono-OM-layer-equivalent’ covering of soil particles, that is, an OM loading equivalent to what would be expected for a single layer of moderately sized organic molecules. Because variation in organic-mineral particle density may reflect variations in the thickness of organic accumulations (Sollins et al., 2006) we hypothesize that such thick OM layered particles are preferentially fractionated in lower density clay fractions. Accumulation of the aforementioned lipids, sterols, and N-containing compounds in such lighter particles with thicker ‘OM layers’ would consequently indicate that OM–OM interactions are of importance in the stabilization of soil OM here. We build on these studies by investigating soil OM in heavy ($>2.2 \text{ g cm}^{-3}$) and light ($1.6\text{--}2.2 \text{ g cm}^{-3}$) clay fractions of a series of three sandy cropland soils with and three soils without historical heathland by means of Pyrolysis-field ionization mass spectrometry (Py-FIMS). The objectives were (i) to test whether accumulation and stabilization of OM in thickly coated clay particles, namely in the lighter clay fraction, occurred in croplands with historical heathland coverage and not in the permanent croplands and (ii) to assess if indeed lipids, sterols and N-containing compounds are enriched in this fraction.

MATERIALS AND METHODS

Soils and Sampling

Six sandy cropland soils were sampled in the sandy region located in the North of Belgium. The sites were selected so as to include three cropland soils with a historical heathland land use (hereafter termed cultivated heathlands, HC) and three ‘permanent’ cropland soils (termed CC) without a heathland history. Topsoil samples (0–30 cm) were collected from the three HC soils, now cropland, at Kalmthout (HC1, Lat. $51^{\circ}23'$ and Long. $4^{\circ}28'$), Knesselare (HC2, Lat. $51^{\circ}8'$ and Long. $3^{\circ}27'$), and Wingene (HC3, Lat. $51^{\circ}4'$ and Long. $3^{\circ}20'$), and from the three CC soils without a heathland history at Anzegem

(CC1, Lat. $51^{\circ}4'$ and Long. $3^{\circ}20'$), Oudenburg (CC2, Lat. $51^{\circ}4'$ and Long. $3^{\circ}20'$), and Koekelare (CC3, Lat. $51^{\circ}4'$ and Long. $3^{\circ}20'$). Using precise historical land-use maps available for this region (map of de Ferraris [Gemeentekrediet van België, 1965]) the land use history of the HC and CC sites was deduced as heathland and cropland in 1775, respectively (Fig. 1). Based on a reconstructed land-use map (Piessens, 2006), the heathland use at sites HC2 and HC3 could even date back to 1000. From the 18th century, forestation periods alternated with heathland use on the HC sites, followed by their cultivation about 60 to 100 yr ago with maize monoculture or maize-temporary pasture rotations in the last three to four decades. Cultivation of these soils created a 30-cm thick Ap horizon, which is composed of the former thin A and underlying E horizons of these soils. In some cases, deeper rotating tillage operations (up to a 60-cm depth) even have mixed parts of the Bh/ir horizons into this Ap horizon. Additionally at site HC1, historical plaggen application may have been practiced. The three CC sites in contrast were put into cultivation at least 230 yr ago and have also been under maize monoculture or maize-temporary pasture for three to four decades. Fifteen soil samples were taken at each plot by means of an auger ($\varnothing 2.5 \text{ cm}$) within a $25 \text{ m} \times 40 \text{ m}$ rectangle. Bulk samples were then mixed and air-dried.

General Soil Properties and Mineralogical Characterization

The OC and N content of the soils was determined by dry combustion with a Variomax CNS-analyzer (Elementar Analysensysteme, Germany). Total Fe and Al in pedogenic oxides were estimated by the dithionite–citrate–bicarbonate (DCB) method (Blakemore et al., 1987) (Fe_d and Al_d). Pyrophosphate extractable Fe (Fe_p) and Al (Al_p) were determined at a soil/solution ratio of 1:100 (wt/v) in 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ (Blakemore et al. 1987). Aluminum and Fe in $<0.45\text{-}\mu\text{m}$ filtrates were measured with atomic absorption spectroscopy. Soil texture was determined by the combined sieve and pipette-sedimentation method. Soil microbial biomass OC was determined by the fumigation-extraction method (Vance et al., 1987). Hot water-extractable OC was determined according to Leinweber et al. (1995).

The mineralogical composition of the bulk clay-size fraction was determined by x-ray diffraction (XRD). Samples were hand ground with a mortar and pestle, treated with dilute H_2O_2 (3%, w/w) overnight at 80°C in 85-mL Nalgene centrifuge tubes to remove OM and washed three times with 50 mL of deionized H_2O by centrifugation and decantation. The washed clays were then fully dispersed by shaking for 2 h in 30 mL Na-metaphosphate ($1.773 \text{ g } 100 \text{ mL}^{-1}$) and washed again three times with 50 mL of deionized H_2O . X-ray diffraction analysis of the clay fraction was performed using oriented samples on glass slides. The clay fractions were first used without any further treatment and therefore they were Na^+ saturated (N). Glycol solvation of the Na^+ -treated samples was performed in vacuum with glycol vapor during 24 h. All XRD patterns were collected on a Philips X'pert System with a PW 3710 based diffractometer, equipped with a Cu tube anode, a secondary graphite beam monochromator, and a proportional xenon filled detector. The XRD data were collected in a theta, 2-theta geometry from 3.00° onward, at a step of 0.020° 2-theta, and a count time of 1 s per step.

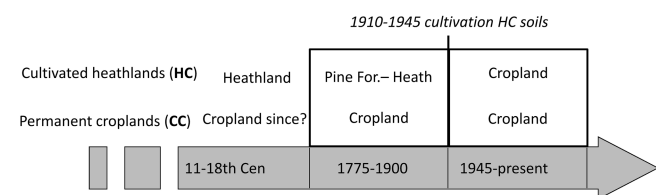


Fig. 1. Land-use history of the sampled cropland soils with historical cropland land-use (CC) and with historical heathland land-use (HC).

Physical Fractionation

Air-dried whole soil samples were separated into three particle-size fractions: clay (<2 μm), silt (2–53 μm) and sand (53–2000 μm) by gravity sedimentation in water following Stoke's law. Particle-size fractionations were performed in duplicates. For this purpose, 60 g of soil was first dispersed in a two step procedure at 60 J mL^{-1} and at 440 J mL^{-1} according to Amelung (1997) using an ultrasonic vibrator (Sonics Vibracell 600 with Sonotrode CV 26, Sonics and Materials Inc., Newton, CT) (Sleutel et al., 2007).

The clay-sized fractions were subjected to a density fractionation to separate a light and heavy fraction. First, about 1 to 3 g of clay were suspended in 50 mL of sodium polytungstate (SPT) at a density of 1.6 g cm^{-3} inside 85-mL centrifuge tubes to remove free particulate OM. After lateral shaking and centrifugation, the supernatant was aspirated onto a nylon filter. In accordance with Sollins et al. (2006) the centrifuge tubes were then filled with a SPT solution of which the density was adjusted to reach a final density of 2.2 g cm^{-3} taking into account dilution by residual lighter 1.6 g cm^{-3} SPT. A density of 2.2 g cm^{-3} was selected to yield a cut-off between theoretically mono-OM-layered and multi-OM-layered clay particles. According to Mayer et al. (2004) an OC loading of about 1 mg C m^{-2} corresponds to such a 'mono-OM-layer' equivalent. Assuming a relative surface area of about 85 $\text{m}^2 \text{g}^{-1}$ for the typical illitic clay type soils (Kahle et al., 2003), this corresponds to 0.085 mg C mg clay^{-1} (or 0.17 $\text{mg OM mg clay}^{-1}$). Considering the densities of OM and mineral matter to be 1.4 and 2.65 g cm^{-3} , respectively, the density of a mono-OM-layered organic-mineral particle is calculated at 2.44 g cm^{-3} (with density particle = $1.4 \times 0.17 \text{ mg OM mg clay}^{-1} + 2.65 [1 - 0.17 \text{ mg OM mg clay}^{-1}]$). A threefold OM loading could then arbitrarily be assigned as 'thick' multi-OM-layered organic-mineral particle, which has a theoretical density of 2.01 g cm^{-3} .

After addition of 50 mL of 2.2 g cm^{-3} SPT to the centrifuge tubes, the samples were centrifuged for 29 h at $18500 \times g$ at 40°C. Under these centrifugation conditions, only mineral particles with diameters < 0.02 μm (at density 2.65 g cm^{-3}) and organic-mineral particles < 2.2 g cm^{-3} were left in suspension. Supernatants and pellets were collected, rinsed thoroughly and dried at 60°C.

Subsamples (200–800 mg) of the bulk soil and physical fractions were analyzed for total C and N content by dry combustion with a Variomax CNS-analyzer (Elementar Analysensysteme, Germany). The supernatant of the particle-size fractionation was analyzed for OC concentration with a TOC analyzer (Shimadzu, Japan).

The specific surface area (SSA) of the clay density fractions was determined by the N_2 -BET approach, using a Area-Max I surface area analyzer (CIS Ingenieurbüro Seifert, Germany). Before analysis, OM was removed from the clay fractions by treatment with sodium peroxodisulfate ($\text{Na}_2\text{S}_2\text{O}_8$) in the presence of sodium bicarbonate (NaHCO_3) according to a method proposed by Meier and Menegatti (1997) modified by Kiem and Kögel-Knabner (2002). The surface area of the previously outgassed samples was derived from the adsorption isotherm of N_2 at 77 K, at p/p_0 0.30 (single point BET).

Pyrolysis-Field Ionization Mass Spectrometry

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. The samples (three replicates) were heated under a high vacuum from ambient temperature to 700°C at a heating rate of 10 K per magnetic scan ($\approx 1.7 \text{ K s}^{-1}$). After about 20 min of total registration time, 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. The summed spectra of four replicates (2 lab-replicates \times 2 Py-FIMS replicates) were averaged to give the final survey spectrum. 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). Assignment of marker signals to chemical compound classes was interpreted according to Schnitzer and Schulten (1992) and Schulten and Leinweber (1999), modified to include nine compound classes by considering all free fatty acids as lipids. 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.

Independent-samples *t* tests were used to detect significant differences in the recorded Ion Intensity proportions of the individual compound classes between the land-use groups and paired-samples *t* tests were used to detect significant differences between the clay density fractions using SPSS 15.0.

RESULTS

General Soil Properties and Mineralogical Composition

Data for bulk soil OC and N, texture, pH (KCl), and extracted Al_d , Fe_d , Al_p , and Fe_p are given in Table 1. There were only minor insignificant differences in physical soil properties between the CC and HC soils (Table 1). Contents of Fe_p , Fe_d , Al_p , and Al_d were similar and low in the CC and HC soils. Higher contents of C ($P < 0.05$) and C/N ratios (but only significant at $P < 0.1$) were measured in the HC soils.

X-ray diffraction analysis showed that all studied Na^+ -saturated clay fractions are composed of a variable admixture of open 2:1 phyllosilicates, illite (peaks at 1.0, 0.500, and 0.334 nm), kaolinite (0.713 and 0.358 nm) and quartz (0.426 and 0.334 nm); especially the amount of open 2:1 phyllosilicates, showing clear swelling after glycolation, seems to be highly variable. This swelling confirms the presence of smectite. The studied samples, except CC1 and CC3, contain a small amount of smectitic layers, indicated by the broad, weak, and somewhat asymmetrical reflection at 1.2 to 1.3 nm, shifting to the lower angle side after glycolation (for example soil HC1 Fig. 2a). Smectite is the likely dominant clay mineral component in samples CC1 and CC3, shown by the strong reflection at 1.23 nm in the Na^+ -saturated

Table 1. Selected properties of the sampled sandy cropland soils with historical cropland land-use (CC) and historical heathland land-use (HC).

Soil	Soil Texture			OC	N	C/N	pH _{KCl}	Extractable Fe and Al†				Clay Surface area	
	>50 μm	2–50 μm	<2 μm					Fe _p	Fe _d	Al _p	Al _d	< 2.2 g cm ⁻³	> 2.2 g cm ⁻³
	%							g kg ⁻¹				m ² g ⁻¹	
CC1	83.5	9.8	6.7	12.8	1.2	11	5.80	1.16	2.94	0.26	0.67	41	–
CC2	92.6	3.6	3.8	14.5	1.3	11	3.99	1.62	1.34	1.31	1.04	10	–
CC3	86.8	5.0	8.2	14.7	1.1	13	5.14	1.72	1.42	0.79	0.67	29	26
HC1	89.7	6.2	4.2	20.9	1.3	17	4.94	0.74	1.18	1.18	1.40	9	32
HC2	89.8	6.1	4.1	21.8	1.2	18	4.19	1.1	1.06	2.11	1.71	15	13
HC3	86.7	7.0	6.4	24.7	2.0	12	5.42	1.38	2.70	1.21	1.19	22	15
<i>t</i> test land-use‡	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	–

* $P < 0.05$.

† Fe_d and Al_d, Na-dithionite-citrate-bicarbonate-extractable Fe and Al; Fe_p and Al_p, Na-pyrophosphate-extractable Fe and Al.

‡ Result of independent samples *t* tests between the CC and HC land-use groups with NS not significant.

clay, shifting to 1.67 nm after glycolation (Fig. 2b). The reflections at 1.00 and 0.500 nm indicate illite.

Specific surface area analysis revealed the clay density fractions, SSA to be rather low (light clay fraction on average 20.9 m² g⁻¹ and heavy clay fraction on average 21.6 m² g⁻¹). Insufficient sample material was available to obtain a SSA measurement for the heavy clay fraction of soils CC1 and CC2. Samples CC1 and CC3 had the highest SSAs, in agreement with the predominance of smectite as indicated from the XRD analysis (Table 1).

Organic Carbon and Nitrogen Distribution over Isolated Size Fractions

Most of the OC and N was present in the silt and clay soil fractions (Table 2). Both clay density fractions had comparable very high OC contents (on average 156 and 147 g kg⁻¹ clay in light and heavy fractions, respectively [data not shown]). Very low quantities of <1.6 g cm⁻³ material were recovered from the clay fractions, insufficient for further analysis. Chenu and

Plante (2006) also found that free OM with a density lower than 1.6 g cm⁻³ had a very low contribution to the soil OM in clay-size fractions. Therefore we refer to the 1.6 to 2.2 g cm⁻³ fraction as the light clay fraction. On average over all soils, heavy clay OC amounted to just about 20% of the light clay OC (Table 2). The dry matter weight of the light clay fraction (42.5 ± 19.2 g kg⁻¹) exceeded the heavy clay fraction (7.4 ± 3.0 g kg⁻¹) by far, a fractionation that corresponds well with that found by Chenu and Plante (2006) of a silt cropland and forest soil. Kiem and Kögel-Knabner (2002) on the other hand found a larger contribution of the >2 g cm⁻³ clay fraction (40% of the clay OC) in a fertilized sandy soil. This discrepancy with our data can likely be explained by the lower density cut-off and much lower OC concentrations (47–55 g OC kg⁻¹ clay compared with 80–231 g OC kg⁻¹ clay in our soils). Average clay OC loadings (i.e., average OC concentration [in mg C g⁻¹]/specific surface area [in m² g⁻¹]) by far exceeded the mono-OC-layer equivalent in both CC (on average 6.6 mg OC m⁻²) and HC soils (on average 15.7 mg OC m⁻²). Average C/N ratios followed the

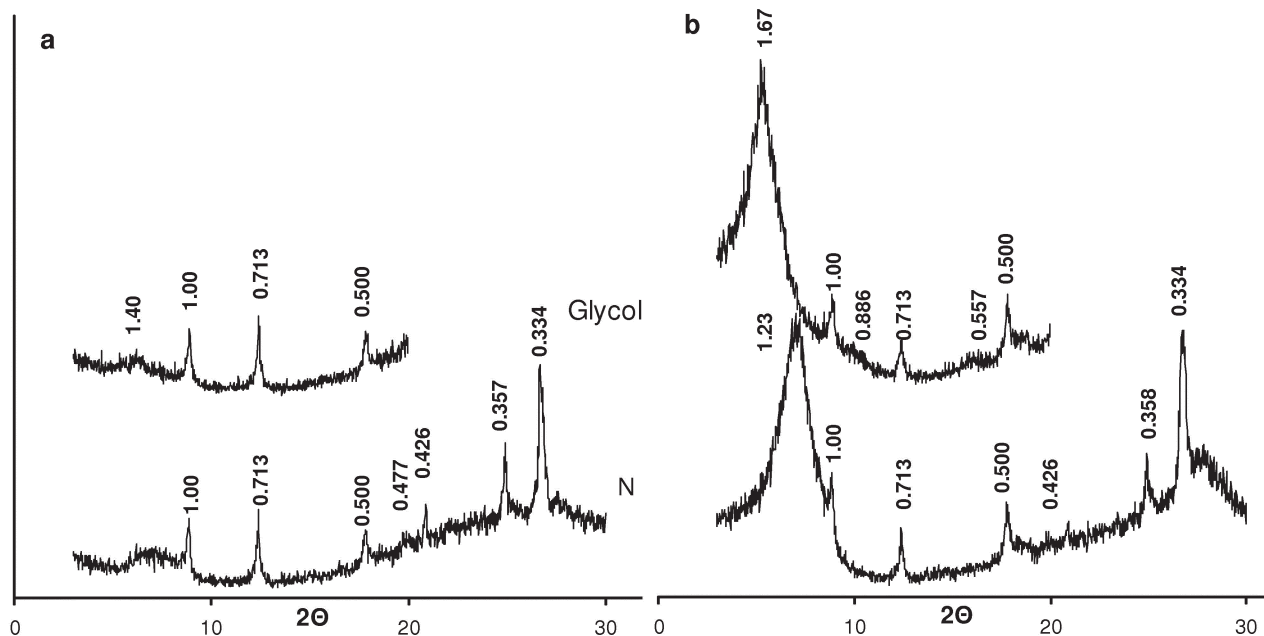


Fig. 2. X-ray diffraction patterns of the Na⁺-saturated (N) and glycolated (Glycol) clay fractions of selected samples (a) HC1 and (b) CC3; spacing given in nm.

order sand (15.0 ± 2.7) \approx silt (14.5 ± 3.4), light clay (11.8 ± 3.1), heavy clay (9.6 ± 2.8), and they were significantly different ($P < 0.01$) between all fractions except between the sand and silt fractions. A non-negligible share of the OC was dissolved in the deionized H₂O used for the particle-size fractionation (5.9% of SOC) and most likely derives from labile water soluble OM (Schulten and Leinweber, 1999).

Since contents of sand OC were comparable between CC and HC soils, significant higher SOC contents in the HC compared with the CC soils was mainly due to twofold higher contents of silt OC ($P < 0.01$) and two to threefold higher contents of heavy and light clay OC ($P < 0.05$). These differences were also confirmed for a larger soil set studied by Sleutel et al. (2010). Differences in N content of the physical fractions were smaller and insignificant. Among all fractions OC and N in the heavy clay-size fraction were considerably higher in the HC soils group (1.88 g C kg^{-1} , 0.17 g N kg^{-1}) compared with the CC soils group (0.56 g C kg^{-1} , 0.08 g N kg^{-1}).

Pyrolysis-Field Ionization Mass Spectrometry

The total ion intensity (TII), that is, the summed count of molecular ions detected, expressed in 10^6 counts mg^{-1} sample, was generally higher in the HC compared with CC soils and this is linked to the higher OC content of the former (Table 3). As shown earlier by Sorge et al. (1993), the TII as measured by Py-FIMS is proportional to the OC content and volatilized matter. Therefore, it is possible to calculate from Py-FIMS data the relative distribution of the soil OM over a number of important compound classes that are relevant to soil OM degradability. On average 45.1 and 44.7% of the pyrolysis signals could be assigned to these classes (Table 3), in the light and heavy clay fractions, respectively. If the contributions of low-mass signals (7.4 and 7.7%) and isotope peaks (16.8 and 17.0%) are accounted for, in total 72.7 and 71.9% of the produced mass signals could be assigned to the light and heavy clay fractions, respectively. The percentage of volatile matter showed no consistent difference between both clay fractions or between CC and HC land-use

groups. Application of Py-FIMS to the clay density fractions of the HC and CC soils showed that their SOM building blocks were primarily composed of the following compound classes (in descending order of abundance): lipids > alkylaromatics > phenols + lignin monomers = (mainly) heterocyclic N-containing compounds

Detailed Comparison of the Pyrolysis-Field Ionization Mass Spectrometry Chemometric Dataset of the Light and Heavy Clay Fractions

In general, the Py-FIMS spectra of the two isolated clay density fractions were largely similar (Table 3) and the order of differences of identified mass signals was smaller than between the CC and HC land-use history groups (see below). Main differences concerned a significantly higher TII proportion of lipids ($P < 0.05$) and sterols (however only significant at $P < 0.1$) in the light clay fraction. Summed %TII of the N-containing compounds and peptides classes and the phenols and lignin monomers compound classes were significantly higher ($P < 0.01$) in the heavy compared with the light clay fraction (Table 3).

Closer inspection of the difference mass spectra (Fig. 3 and 4) showed a shift toward high mass signals in the light compared with the heavy clay fraction (CC1: between m/z 183–438; CC2: between m/z 294–420; CC3: between m/z 288–500; HC3: between m/z 338–500). There was only a slight tendency toward high mass signals for the HC1 and HC2 soils (Fig. 4). Dominant differences in masses tentatively assigned to N-containing compounds were recorded at m/z 59 (acetamide), 81 (methylpyrrole), 123 (3-acetoamide-6-methyl-pyrone), 125 (acetamidofuran), and 161 (indoethanol) and for peptides at m/z 70, 84, and 97 (acidic amino acids), m/z 74, 120 (neutral aromatic amino acids) and m/z 73 (neutral amino acids). For the CC soils mainly marker signals of long-chained alkanes (n -C₂₈ to n -C₃₀) at m/z 394, 408, 422, and medium to long-

Table 2. Distribution of organic carbon (OC) and N and dissolved OC (DOC) over isolated particle size and density fractions (in g kg^{-1} bulk soil) and corresponding C/N ratios of sandy cropland soils with historical cropland land-use (CC) and historical heathland land-use (HC).

Soil	Sand			Silt			Clay						DOC†
							Light ($< 2.2 \text{ g cm}^{-3}$)			Heavy ($> 2.2 \text{ g cm}^{-3}$)			
	OC	N	C/N	OC	N	C/N	OC	N	C/N	OC	N	C/N	
	g kg^{-1}			g kg^{-1}			g kg^{-1}			g kg^{-1}			g kg^{-1}
CC1	5.92	0.42	14.0	3.17	0.28	11.5	5.25	0.59	9.0	0.47	0.08	6.1	0.96
CC2	6.24	0.55	11.3	3.62	0.31	11.7	1.93	0.20	9.7	0.53	0.06	8.4	0.88
CC3	3.57	0.24	14.8	3.70	0.29	12.8	4.17	0.44	9.5	0.69	0.09	8.1	0.94
HC1	4.38	0.31	14.1	7.69	0.58	13.3	9.70	0.82	11.8	2.48	0.26	9.5	1.26
HC2	3.37	0.21	16.4	7.39	0.39	18.8	7.48	0.45	16.8	1.80	0.13	14.1	1.12
HC3	4.92	0.25	19.4	7.08	0.37	18.9	6.38	0.45	14.3	1.36	0.12	11.4	1.07
t test land-use‡	NS	NS	NS	**	NS	NS	*	NS	*	*	NS	*	*

* $P < 0.05$.

** $P < 0.01$.

† DOC, dissolved OC measured in the H₂O used in the sequential sedimentation cycles of the particle size fractionation.

‡ Result of independent samples t tests between the CC and HC land-use groups with NS not significant.

chained fatty acids (*n*-C₁₉, *n*-C₂₁ to *n*-C₂₅ and especially *n*-C₂₆ to *n*-C₂₉) at *m/z* 284, 326, 340, 354, 396, 410, 424, 438 contributed to the higher percentage of TII of the lipids compound class in the light clay fraction. About half of this difference in percentage of TII was explained by signals for thermolabile (volatilized at 110 to 400°C) fatty acids. For the HC soils mainly masses attributed to medium to long-chained fatty acids (*n*-C₁₈, *n*-C₂₁ to *n*-C₂₃, *n*-C₂₈ and *n*-C₃₀) at *m/z* 298, 326, 340, 354, 368, 382, 424, 452 contributed to the higher percentage of TII of lipids in the light clay fraction and these differences were unspecific with respect to volatilization temperature. Higher TII proportions of sterols in the light clay fractions derived mainly from *m/z* 394 (dehydroergosterol), 396 (ergosterin), 398 (ethylcholestene), 408 (C3-alkylcholestatriene), 426 (taraxasterin). Since overlap with marker signals from the lipids compound class is substantial for sterols, their assignment is tentative here. Nonetheless this trend in the TII proportions of sterols was consistent over all six soils.

Detailed Comparison of the Pyrolysis-Field Ionization Mass Spectrometry Chemometric Dataset According to Land-use History

Both clay density fractions of the HC soils had significantly higher TII proportions attributed to longer chained (>C₂₀) fatty acids (*m/z* 312, 340, 354, 368, 382, 396, 410, 424, 438, 452) (*P* < 0.05), other lipids (*m/z* 394, 408, 422) (*P* < 0.05) and sterols (*m/z* 394, 396, 398, 408, 410, 426) than the CC soils. However, contrary to the prominent higher percentage of TII of the lipids compound class as a whole, specific shorter chained fatty acids and alkanes had higher TII proportions in the CC compared with the HC soils (C₁₆ to C₁₉ fatty acids *m/z* 256, 270, 284, 298 (but not significantly) and C₁₀ to C₁₆ alkyldiesters *m/z* 202, 216, 230, 244, 258, 272, 286 [*P* < 0.05]). Significantly lower TII contributions of masses attributed to phenols and lignin monomers (*P* < 0.05) (*m/z* 94, 108, 110, 124, 150, 180), alkylaromatics (*P* < 0.01) (*m/z* 148, 162, 176, 192, 198, 206, 220, 232, 234) and N-containing compounds (*P* < 0.05) (*m/z* 59, 67, 79, 81, 95, 109, 137, 167, 195, 233) were

Table 3. Total ion intensity (TII), percentage of volatilized matter (%VM) and percentage of total ion intensity (%TII) of soil OM compound classes† (means ± standard deviation) determined by Py-FIMS in light (<2.2 g cm⁻³) and heavy (>2.2 g cm⁻³) clay fractions of sandy cropland soils with historical cropland land-use (CC) and historical heathland land-use (HC).

Soil	TII	%VM	CHYD‡	PHLM	LDIM	LIPID	ALKY	NCOMP	STER	PEPTI	SUBE	Sum
	10 ⁶ counts mg ⁻¹ sample	%	%TII									
Light (<2.2 g cm ⁻³) clay fraction												
CC1	47.2	21.3	5.6	7.8	4.4	7.6	9.5	7.4	2.4	3.2	0.2	48.1
CC2	104.1	34.1	4.4	5.8	4.4	10	8.8	6.3	3.3	2.4	0.4	47.4
CC3	74.3	27.7	4.4	6.1	4.7	10.5	8.8	6.1	4.0	2.5	0.3	45.8
HC1	115.3	31	3.9	5.0	3.7	9.7	7.3	5.7	3.6	3.0	0.5	42.4
HC2	178.3	29.8	3.2	4.0	3.1	11.6	6.6	4.6	5.2	2.8	0.6	41.7
HC3	119.2	27.9	3.3	4.5	3.8	11.3	7.1	5.2	4.9	2.4	0.5	43.0
mean	106.4 ± 44.6	28.6 ± 4.3	4.1 ± 0.9	5.5 ± 1.4	4.0 ± 0.6	10.1 ± 1.4	8.0 ± 1.2	5.9 ± 1.0	3.9 ± 1.0	2.7 ± 0.3	0.4 ± 0.1	47.7 ± 2.1
CC mean	75.2 ± 28.5	27.7 ± 6.4	4.8 ± 0.7	6.6 ± 1.1	4.5 ± 0.2	9.4 ± 1.6	9.0 ± 0.4	6.6 ± 0.7	3.2 ± 0.8	2.7 ± 0.4	0.3 ± 0.1	49.3 ± 0.8
HC mean	137.6 ± 35.3	29.6 ± 1.6	3.5 ± 0.4	4.5 ± 0.5	3.5 ± 0.4	10.9 ± 1.0	7.0 ± 0.4	5.2 ± 0.6	4.6 ± 0.9	2.7 ± 0.3	0.5 ± 0.1	45.9 ± 1.3
<i>t</i> test	NS	NS	*	*	*	NS	**	*	NS	NS	*	*
land-use§												
Heavy (> 2.2 g cm ⁻³) clay fraction												
CC1	43.5	22.6	6.5	8.2	3.7	6.4	9.8	7.4	1.8	3.7	0.1	47.6
CC2	116.1	35.2	4.5	6.1	4.3	9.8	8.5	6.5	3.1	2.6	0.4	48.2
CC3	79.1	22.9	4.7	6.3	5.3	9.8	9.2	6.3	3.8	2.5	0.3	45.8
HC1	122.0	37.3	4.1	5.5	3.7	9.8	7.5	6.0	3.7	3.1	0.5	43.9
HC2	198.7	34.9	3.3	4.2	3.5	11.2	6.6	4.8	5.1	2.8	0.6	42.1
HC3	113.4	27.9	3.3	4.4	4.6	10.6	7.1	5.3	4.6	2.5	0.4	42.8
mean	112.1 ± 51.8	30.1 ± 6.5	4.4 ± 1.2	5.8 ± 1.5	4.2 ± 0.7	9.6 ± 1.7	8.1 ± 1.3	6.0 ± 0.9	3.6 ± 1.2	2.9 ± 0.5	0.4 ± 0.2	48.0 ± 1.1
CC mean	79.6 ± 36.3	26.9 ± 7.2	5.2 ± 1.1	6.8 ± 1.2	4.4 ± 0.8	8.7 ± 2.0	9.2 ± 0.7	6.7 ± 0.6	2.9 ± 1.0	2.9 ± 0.7	0.3 ± 0.2	48.9 ± 1.0
HC mean	144.7 ± 47.0	33.4 ± 4.9	3.6 ± 0.5	4.7 ± 0.7	3.9 ± 0.6	10.5 ± 0.7	7.1 ± 0.5	5.4 ± 0.6	4.5 ± 0.7	2.8 ± 0.3	0.5 ± 0.1	47.2 ± 0.4
<i>t</i> test	NS	NS	*	*	NS	NS	**	*	NS	NS	NS	*
land-use§												
<i>t</i> test	NS	NS	NS	*	NS	*	NS	*	NS	NS	NS	NS
density§												

* *P* < 0.05.

***P* < 0.01.

† NS, not significant.

‡ CHYD: Carbohydrates with pentose and hexose subunits; PHLM: phenols and lignin monomers; LDIM: lignin dimers; LIPID: lipids, alkanes, alkenes, bound and free fatty acids and alkyl-esters; ALKY: alkylaromatics; NCOMP: mainly heterocyclic N-containing compounds; STER: sterols; PEPTI: peptides; SUBE: suberin.

§ Result of independent samples *t* tests between the CC and HC land-use groups and paired samples *t* test between the <2.2 and >2.2 g cm⁻³ clay fractions.

measured in the HC compared with the CC soils as well. While percentage of TII differences between both land-use groups for marker signals of lignin dimers were smaller, the ratio of phenols + lignin monomers: lignin dimers were consistently higher in the CC soils (1.5 ± 0.4) than in the HC soils (1.3 ± 0.2). It has been proposed that this ratio increases with progressive decomposition of primary plant materials (Wiedow et al., 2007) and soil OM (Leinweber and Schulten, 1995). Therefore, these data indicate a more decomposed character of the soil OM in the CC soils.

Thermogram Data

Next to cumulative mass spectra, Py-FIMS gives an indication of the thermal energy required for the evolution of

individual biomarkers of soil OM. Thermograms of both clay density fractions had a distinct bimodal shape in all soils, reflecting the presence of two soil OM pools of differing thermostability, namely at 250 to 400 and 450 to 600°C (data not shown). An earlier volatilization of the thermolabile OM pool occurred in the HC soils with a peak maximum at 350°C compared with 370°C for the CC soils. Closer inspection of the individual thermograms of the compound classes (Fig. 5) also demonstrated this bimodal shape in the lipids, alkylaromatics, phenols + lignin monomers and N-containing compounds classes and to a lesser extent also in the carbohydrates and peptides classes. Compared with numerous previous Py-FIMS analyses of soils (Schulten and Leinweber, 1999), these bimodal thermograms reflect different OM bond types. The volatilization of OM at

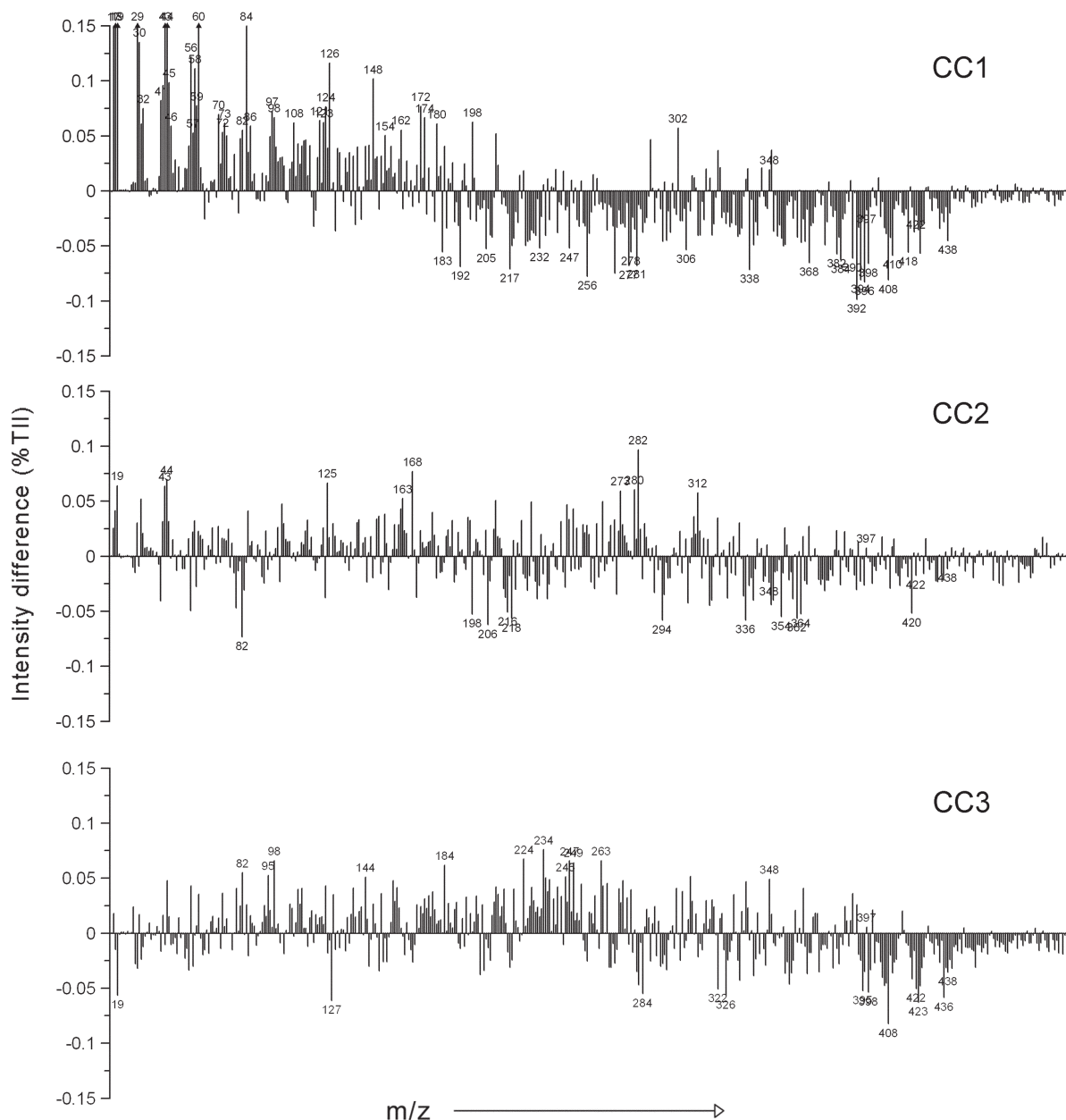


Fig. 3. Difference pyrolysis-field ionization mass spectra of clay fractions of three sandy cropland soils with historical cropland land-use (CC): spectra of heavy (>2.2 g cm⁻³) clay fraction minus the light (<2.2 g cm⁻³) clay fraction (Positive values indicate larger relative ion intensities in the heavy [>2.2 g cm⁻³] clay fraction; negative values indicate larger relative ion intensities in the light [<2.2 g cm⁻³] clay fraction).

lower temperatures originates from both fairly undecomposed free plant fragments as well as from a thermally labile fraction consisting of carbohydrates, N-containing compounds, peptides and phenols and lignin monomers, which are associated with humified OM by weak organic-organic bonds. The contribution of heavy clay thermolabile material (0–400°C) to the TII was on average higher in the HC soils ($45.5\% \pm 2.3\%TII$) compared with the CC soils ($38.4 \pm 4.8\%TII$). An analogous observation was made for the light clay fraction with $46.4 \pm 0.4\%TII$ and $40.9 \pm 4.5\%TII$ thermolabile OM in the HC and CC soils, respectively. The high temperature volatilization at 450 to 600°C in contrast is indicative of a thermally stable fraction with strong organic-mineral or organic-organic bonds.

Differences in the shapes of the thermograms of the light and heavy clay fractions were minor and largely inconsistent over all soils and within the CC and HC groups. Exceptions were (i) a selectively higher TII contribution of thermolabile lipids in the light clay fraction of the CC soils and (ii) a selective higher TII contribution of thermostable carbohydrates in the heavy clay fraction of the CC soils.

DISCUSSION

This study aimed to investigate the relevance of OM-OM interactions in SOM stabilization of the unusually SOM rich sandy soils in Western-Europe. In the discussion here below, first we compare clay OM composition in relation to land-use

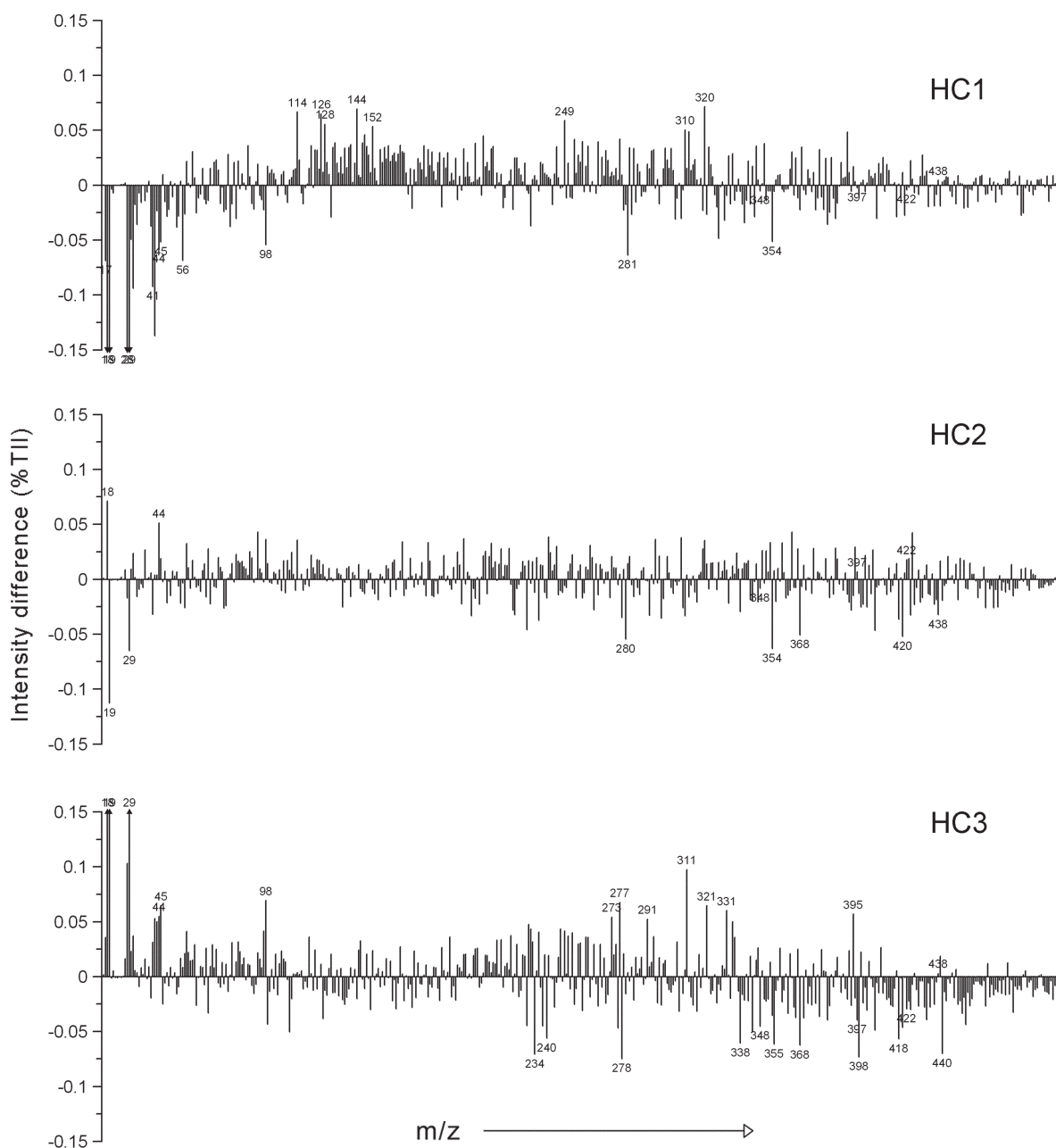


Fig. 4. Difference pyrolysis-field ionization mass spectra of clay fractions of three sandy cropland soils with historical heathland land-use (HC): spectra of heavy ($>2.2 \text{ g cm}^{-3}$) clay fraction minus the light ($<2.2 \text{ g cm}^{-3}$) clay fraction (Positive values indicate larger relative ion intensities in the heavy [$>2.2 \text{ g cm}^{-3}$] clay fraction; negative values indicate larger relative ion intensities in the light [$<2.2 \text{ g cm}^{-3}$] clay fraction).

history to verify the hypothesized stability of OM accumulated under historical heathland land-use.

The combination of physical fractionation and Py-FIMS analysis of isolated clay density fractions of the selected three CC and three HC revealed a clear difference in SOM composition between HC and CC soils. Previously, we demonstrated the

strong similarity in soil OM composition (Sleutel et al., 2008) and stability (Sleutel et al., 2010) between soil OM in native heathlands and in two of the cultivated heathland soils (HC2 and HC3). Dominant soil OM contributions by lipids, long-chained aliphatics and sterols could be logically linked to a large input from heathland vegetation (Sleutel et al., 2008). Based on

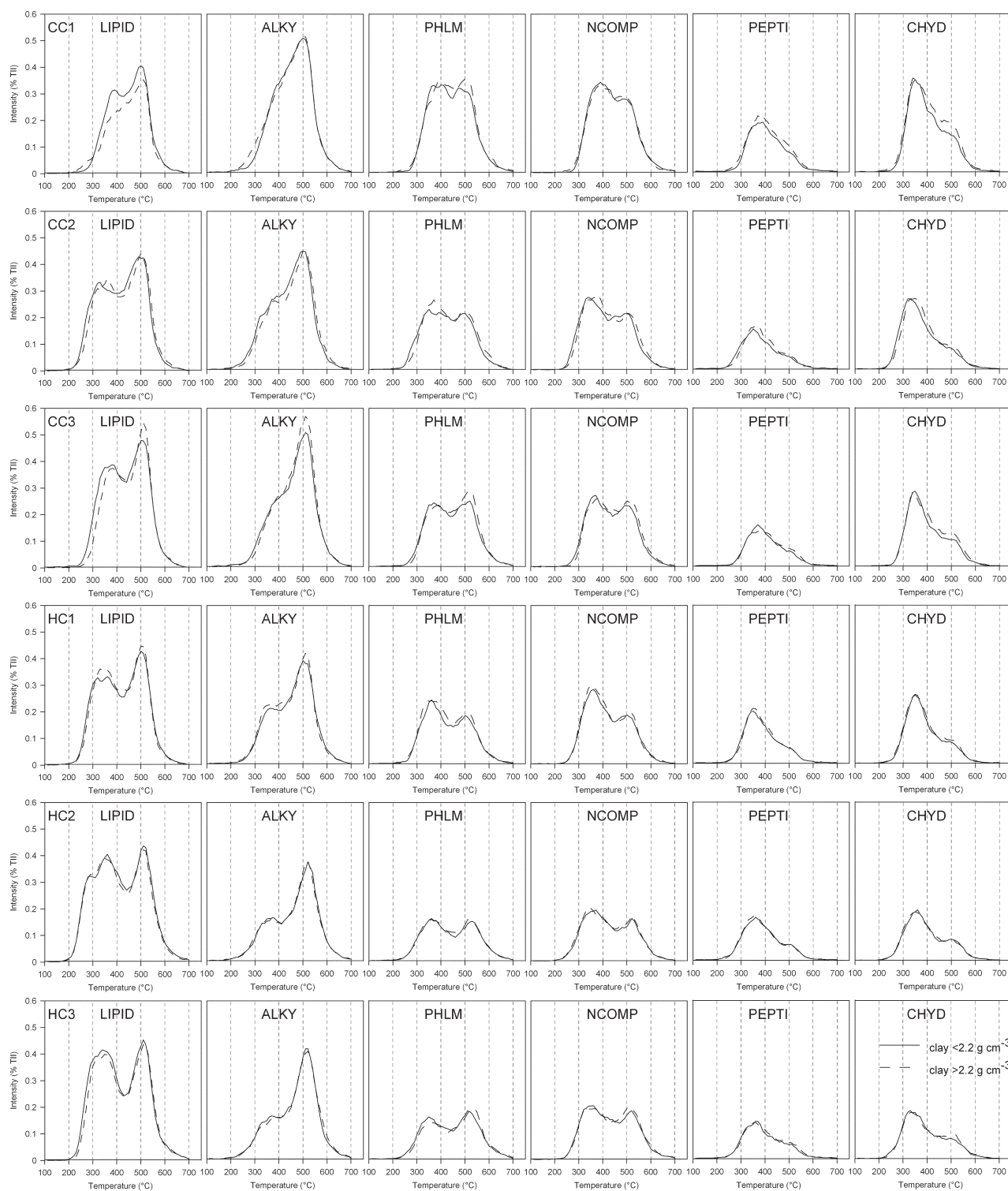


Fig. 5. Thermograms for the volatilization of selected compound classes (LIPID: Lipids, alkanes, alkenes, fatty acids and *n*-alkyl esters; ALKY: alkylaromatics; PHLM: Phenols and Lignin Monomers; NCOMP: mainly heterocyclic N-containing compounds; PEPTI: peptides; CHYD: carbohydrate hexose and pentose subunits) of the light (<2.2 g cm⁻³) and heavy (>2.2 g cm⁻³) clay fractions of sandy cropland soils with historical cropland land-use (CC) and with historical heathland land-use (HC).

the Py-FIMS data and the higher clay OC and N contributions in the HC compared with the CC soils (Table 2) compiled in this study, it is reasonable to state that also a share of the clay-sized OM in the HC soils is derived from soil OM formed during the former heathland land-use.

Consequently, a more decomposed character of the soil OM in the CC soils (demonstrated by higher phenols + lignin monomers to lignin dimers ratios, significantly higher ($P < 0.05$) %TII of N-containing compounds, phenols and lignin monomers and alkylaromatics and consistent lower %TII of sterols and fatty acids) is in line with similar studies for cultivated prairies (Schnitzer et al., 2006; Leinweber et al., 2009). These observations indirectly confirm the presence and stability of relatively untransformed soil OM in the HC soils. Other observations support this reasoning, such as two to three times higher %TII of suberin and of odd numbered n -C₂₉ to n -C₃₃ alkanes (typically rich in *Calluna vulgaris* leaves [Nierop et al., 2001]) mass signals in the HC compared with the CC soils (significant at $P < 0.05$ for the light clay fraction).

The Py-FIMS data also revealed that heterocyclic N-containing compounds (and to a lesser, not significant extent also peptides) had higher relative TII proportions compared with other compounds in the clay OM of the CC soils compared with the HC soils (Table 3). This is in agreement with the C/N ratios (Table 2), and can be explained by the cycling and metabolization of more N-rich biomass in these soils in historical times. By contrast, OM in the HC soils was characterized by larger proportions of lipids and sterols, resulting in clay fractions with wider C/N ratios (Table 2). Since N inputs under historical heathland land-use can be expected to have been very low, a selective historical enrichment and retention of N in this or any of the isolated fractions during heathland seems unlikely. Differences in the quality of N between cultivated heathlands and permanent croplands on such sandy cropland soils were also indicated previously by a lower N mineralization (Springob and Kirchmann, 2003; Heumann et al., 2003) and higher total N contribution of 6% NaOCl-oxidation resistant N (Sleutel et al., 2010) in the former. This quality difference, however, was not reflected in the thermogram data as there were no differences in thermal stability of peptides nor of non-proteinaceous N-containing compounds between HC and CC soils.

In conclusion, we can state that differences exist in clay OM composition between sandy cropland soils with different historical land-use. However, regardless of historical land-use, all studied sandy soils had remarkably high clay OC loadings (between 6 and 16 mg C m⁻² clay) and the SOM in all soils was rich in lipids, alkylaromatics and heterocyclic N containing compounds. Hence, it thus appears that OM accumulation in clay fractions far above the 'mono layer equivalent' does not occur exclusively in sandy soils with historical heathland land-use, contradicting our research hypothesis. Nonetheless, very high proportions of relatively labile SOM constituents such as lipids and sterols in the clay fraction point to their presence

in thick OM coatings and begs for explanations regarding a mechanism of stabilization against decomposition.

The primary objective of this study was to investigate if hypothesized accumulations of OM, characterized by high contents of lipids, sterols, heterocyclic N containing compounds and alkylaromatics, occurred specifically in the light clay fraction, which presumably comprises these thick OM layers. The data, in general, indicate that physical fractionation seems to have had limited success in isolating two clay fractions with distinct OM coating thickness. Similar OC contents were found in both investigated clay density fractions (on average grams in light and heavy fractions), in contrast to previous studies involving density fractionation of soil clay fractions (Chenu and Plante, 2006; Kiem and Kögel-Knabner, 2002). Differences in their soil OM composition were also relatively small as derived from the %TII assigned to major soil OM constituents such as lignin dimers, carbohydrates, and alkylaromatics (Table 3) and their thermal volatilization patterns (Fig. 5). It is possible that the very high OM loadings of both light and heavy clay fractions may have obstructed good physical separation of primary organic-mineral complexes from OM-free particles and clay sized microaggregates.

Our hypothesis that heathland derived OM accumulated and remained preferentially in light clay fractions could not be confirmed. Instead, the physical fractionation data showed that differences in OC and N between the HC and CC soils were found in both silt and the clay density fractions and were even largest in the heavy clay fraction. Stability of OM in heavy clay compared with other soil fractions has been observed previously by Chenu and Plante (2006), Schulten and Leinweber (2000) and Kiem and Kögel-Knabner (2002) as well. The latter authors attributed this OM stabilization to mineral binding, particularly through interaction with oxides in sandy soils. A similar reasoning seems less plausible for the HC and CC soils because of (i) their very low Fe_d contents, (ii) the very high OC loadings of the light and heavy clay fractions, and (iii) the relatively low SSA of the quartz, muscovite, and kaolinite dominated clay mineralogy. These observations suggest that other stabilization mechanisms operate as well in the clay fractions. A difference in thermolabile OM, that is, non-mineral bound OM, between HC and CC soils suggests that a share of the soil OM may be stabilized through OM-OM interactions, especially so for thermolabile lipids (0–400°C) which were enriched in the clay fractions of the HC soils. The mechanisms underlying these OM-OM interactions could be physical entrapment in larger macromolecular humic structures, following the humic substances model by Schulten et al. (1991), or alternatively hydrophobic interactions between individual smaller molecules proposed by the layering-model by Kleber et al. (2007). Such mechanisms don't appear to be exclusive for sandy soils with historical heathland land-use, as hypothesized, but would appear to be general in sandy soils in Northwestern Europe.

Although our experimental set up failed to fully isolate thick OM layered clay fractions and compare these between

CC and HC soils, some consistent differences of interest in soil OM quality could still be observed between the two isolated clay density fractions. Higher TII proportions of marker signals from lipids (mainly long chained fatty acids) (significant at $P < 0.05$) and sterols (though only significant at $P < 0.1$) in the light clay fraction in both CC and HC soils can be explained by different hypotheses. First, by the selective accumulation of lipids and sterols in 'thicker' OM layers in organic-mineral particles; second by their presence in OM contained in clay-sized microaggregates that are supposedly concentrated in this fraction; third, by a selective binding and hence concentration in the heavy clay fraction of other soil OM building blocks. The first hypothesis would imply stabilization of lipids through entrapment in the organic matrix and such a mechanism has been forwarded previously by other authors (Sorge et al., 1994; Rumpel et al., 2004; Quénéa et al., 2006). The lower-temperature volatilization peak of the prominent bimodal lipids thermogram indeed most likely derives from non-mineral bound OM (Schulten and Leinweber, 1999; Sleutel et al., 2008). The second hypothesis could be explained by presence of particulate OM in clay fractions, as confirmed by Chenu and Plante (2006), this fraction being rich in lipids and sterols (Sleutel et al., 2007). The third hypothesis may be explained for instance by selective binding in the heavy fraction of aromatic compounds (significantly higher [$P < 0.05$] %TII alkylaromatics and phenols and lignin monomers) and N-containing compounds (significantly higher [$P < 0.05$] summed %TII heterocyclic N-containing compounds and peptides) relative to sterols and lipids (Table 3). The available data, however, do not allow to conclude on the relative importance of these three hypothesized mechanisms.

Another observation was that the C/N ratios of the heavy clay fractions were on average (9.6) lower than the C/N ratios of the light clay fractions (11.8). Relative higher N proportions in heavier density fractions are well documented (Sollins et al., 2006) and have been attributed to preferential strong and stable binding of amino acids to clay minerals (Leinweber and Schulten, 2000). Such selective binding of organic N compared with C was indeed suggested by the significantly ($P < 0.05$) higher summed percentage of TII of N-containing compounds and peptides in the heavy clay fraction. Possibly, available mineral surfaces at silicates and pedogenic oxides were covered by relatively N-rich OM in early stages of soil formation, followed by the formation of lipid and sterol rich clay OM during centuries of heathland. Thick OM-layered organic-mineral particles, which are preferentially fractionated in the light clay fraction (although not fully in this study), could hold a larger share of lipid rich OM and hence lower relative contribution of N-rich OM, as seen from the Py-FIMS data. Our data are also in line with a recent OM layering model for low-charge clays proposed by Kleber et al. (2007) and with data from bulk soil sequential density fractionation by Sollins et al. (2009). Possibly there is limited neof ormation of clay minerals (and reactive surface area) in the studied sandy soils and hence, new lipid rich plant derived OM may only undergo OM-OM bonding.

CONCLUSIONS

From both physical fractionation and Py-FIMS data, we conclude that there is a distinct and lasting influence of historical land-use on OM composition in sandy cropland soils. The hypothesis of preferential historical accumulation of OM mainly in thick OM-layered organic-mineral particles in the cultivated heathlands was, however, not confirmed. Nevertheless, the unusually high clay fraction OC loadings (6–16 mg C m⁻²) and comparatively low binding potential of the mineral phase in these soils demonstrate a relatively limited involvement of organic-mineral binding as a soil OM stabilization mechanism regardless of land-use. A potential alternative stabilization mechanism, for intrinsically labile soil OM constituents, such as thermolabile lipids and sterols enriched in the sandy soil's clay fractions, would involve OM-OM crosslinking. Of particular interest is the modest but significant relative higher proportion of N-containing compounds in the heavy clay fraction. Possibly preferential binding of organic N predates the heather vegetation-driven accumulation of the lipid and sterol dominated clay OM. Verification of such a SOM building pathway would, however, require specific isolation and dating of such mineral bound organic N from the rest of the clay-sized OM. Finally, our findings clearly demonstrate that historical heather land-use needs to be considered as a site factor when analyzing cropland production processes and C-storage on sandy soils in North-West Europe, since current SOM composition appears to be partly inherited.

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