

Composition of organic matter in sandy relict and cultivated heathlands as examined by pyrolysis-field ionization MS

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Received: 17 September 2007 / Accepted: 10 June 2008 / Published online: 24 June 2008
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Abstract Unusually high SOC levels have been reported for sandy cropland soils in North-Western Europe. A potential link with their general heathland land-use history was investigated by comparing two soil pairs of relict heathland and cultivated former heathland in the Belgian sandy region. A sequential chemical fractionation yielded similar sizes in corresponding SOM fractions between the heathland and cropland soils (i.e. NaOCl resistant: 12.3–15.0 g C kg⁻¹ and NaOCl + HF resistant: 2.6–5.3 g C kg⁻¹). Higher amounts of clay sized N in the cropland plots can be attributed to N additions from mineral fertilizers and animal manure. Temperature resolved Pyrolysis Field Ionization Mass Spectroscopy analysis showed that the composition of both relict heathland and cultivated soils was surprisingly similar, in spite of over 60 years of intense cropland management. The mass spectra of SOM in both heathland-cropland soil pairs investigated was dominated by signals from lipids, alkylaromatics and sterols. The accumulation of this SOM rich in aliphatics

was logically linked to the high input of lipids, long-chain aliphatics and sterols from heathland vegetation and the low soil pH and microbial activity. Based on the relatively high OC surface loadings of HF-extractable OM (13–44 mg C m⁻² Fe and 1.2–2.3 mg C m⁻² clay), direct organo-mineral bonds between OM and Fe-oxides or clay minerals seem to be only partly involved as a stabilization mechanism in these soils. The distinct bimodal shape of the thermograms indicates that OM-crosslinking could furthermore contribute substantially to SOM stabilization in these soils. This study therefore corroborates the previously proposed view that lipids may be bound in networks of alkylaromatics, the structural building blocks of OM macromolecules. We hypothesize that such binding is able to explain the measured retention of these OM components, even under several decades of cropland management.

Keywords Soil Organic Matter · Heathland · Analytical Pyrolysis · Chemical fractionation · Land-use

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Abbreviations

CLSM	Confocal laser scanning microscopy
MOC and MN	Mineral protected organic C and N
OC	Organic carbon
Py-FIMS	Pyrolysis Field Ionization Mass Spectroscopy
ROC and RN	Recalcitrant organic C and N
SOM	Soil organic matter

Introduction

Numerous reports in literature indicate that given the same climate and organic matter (OM) input, soils of a finer texture usually contain a larger microbial biomass and exhibit slower OM turnover than soils containing less silt and clay. In spite of this generally accepted consensus, large surfaces of sandy arable soils were found to have unusually high soil organic carbon (SOC) levels in North-West Europe (Germany: Springob and Kirchmann 2002; Belgium: Sleutel et al. 2006). Based on a large SOC database (over 100,000 fields sampled between 1989 and 2000) Sleutel et al. (2006) found sandy cropland soils in Flanders, the Northern half of Belgium, to have an average SOC percentage of 1.9% OC compared to only 1.2% OC in silt soils. The higher amount of OM present in these soils may be explained by specific types of earlier land use, e.g. heathland and the use of plaggen manure over centuries, which was practised in Northwest Europe from Schleswig-Holstein to Belgium. Springob and Kirchmann (2002) found that OC in the OM fraction remaining after a 6 M HCl treatment accounted for 50%–90% of the whole soil OC in 23 soil samples taken from the Ap horizon of sandy-textured soils in Germany. They concluded that old sandy Ap horizons may have high levels of refractory constituents. This conclusion was backed by data on comparatively very low microbial biomass and low OC and N mineralisation from such sandy soils with a historical heathland or plaggen land-use (Springob and Kirchmann, 2003). Still, the study by Springob and Kirchmann (2002) cannot be considered to unequivocally have assessed the nature or proportion of such a large stable SOM fraction.

A thorough understanding of the mechanisms involved in the retention of the SOM in these sandy soils with unusually large SOC contents is the key to a correct interpretation of the origin of this SOC build-up and prediction of future SOM evolution. However, to date very little is known neither on the chemical composition of the OM in these soils, nor on the distribution of SOM over fractions. Therefore the objectives of our research were: (1) To verify the hypothesized presence of a large chemically refractory SOM fraction in sandy soils having a historical heathland land-use. (2) To further elucidate the chemical structure of SOM in cropland soils with such historical heathland land-use and in current relict

heathland soils and compare it with the molecular-chemical composition of heather vegetation. (3) To assess the potential contribution of recently put forward SOM stabilization mechanisms in the retention of SOM in these sandy soils.

We postulated that investigation of SOM in pairs of relict heathland and cultivated soils in the Belgian sandy region by pyrolysis-field ionization mass spectrometry (Py-FIMS) and chemical and physical fractionation has a strong potential for addressing these objectives. Particularly so, because Py-FIMS uniquely yields a detailed chemical characterization of SOM and relevant SOM compound classes, combined with data on the thermal stability of these compounds, which depend strongly on the strengths of the chemical bonds within organic macromolecules or between organic materials and minerals.

Materials and methods

Study area description and sampling

The study area comprised the sandy region located in the North-West of Belgium. Precise historical land-use maps are available for this region dating back to 1775 (map of Ferraris) and 1850 (map of Vandermaelen). There has been drastic conversion of former heathland into arable land or forest in this region with only 10% of the 1775 surface left in 1850 and less than 1% of relict heathland remaining at present (Piessens 2006). The climate is temperate (9.8°C) and humid (780 mm). Podzols and postpodzols are the most prominent soil types. Cultivation of podzols created a 20–30 cm thick Ap horizon, which is composed of the former thin A and underlying E horizons of these soils. In some cases, past deeper rotating tillage operations (up to 60 cm depth) often have mixed parts of the Bh/s horizons into this Ap horizon leaving a fragmented Bh horizon (i.e. so called postpodzols). Plaggen application has never been practiced in the region and can therefore be excluded as a potential SOM forming factor. Topsoil samples were collected from two relict heathland soils and two cropland soils with a historical heathland use from the 11th until the end of the 18th century at two different sites, namely Wingene (Lat. 51°4′–Long. 3°20′) and Knesselare (Lat. 51°8′–Long. 3°27′). Neither of these two sites has been under heathland

permanently, but from the end of the 18th century forestation periods alternated with heathland use. This recurrent shift between periods of heathland and forestation is very typical for the study area. The vegetation of the ‘permanent’ heathland plot at Wingene (WH) consisted mainly of *Erica tetralix*, *Molinia caerulea* and *Calluna vulgaris*. At Knesselare (KH) *Calluna vulgaris* dominated. The sampled croplands at Wingene (WC) and Knesselare (KC) were situated at approximately 300 m from the WH and KH plots. Both fields have been put into cultivation about 60 years ago and have been under maize monoculture since three to four decades. Fifteen soil samples (0–30 cm) were taken at these four plots by means of an auger (\varnothing 2.5 cm) within a 25×40 m rectangle. Litter layers in the relict heathland soils were removed. Bulk samples were then mixed and dried to the air. Samples of above- and below-ground-*Calluna vulgaris* vegetation were collected at the KH plot. Plant material was dried at 50°C and ground in a ball mill. Three undisturbed soil cores (\varnothing 5 cm, H 5 cm) were taken per plot to measure soil bulk density at a depth of 10 cm.

Characterization of the soil mineral matrix and particle size fractionation

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). Al and Fe in $<0.45 \mu\text{m}$ filtrates were measured with atomic absorption spectroscopy (AAS). Soil texture was determined by the pipette-sedimentation method (Table 1). Air dried whole soil samples were separated into three particle size fractions: clay ($<2 \mu\text{m}$), silt (2–63 μm) and sand (63–2,000 μm). For this purpose, 30-g soil samples were dispersed in a two step procedure according to Amelung (1997) using an ultrasonic vibrator (Sonics Vibracell 600 with Sonotrode CV 26, Sonics & Materials Inc., Newton, CT). After soft ultrasonication at 60 J ml^{-1} , the $>63 \mu\text{m}$ fraction was removed by wet sieving, and a second ultrasonication at 440 J ml^{-1} was used to disperse the silt and clay sized fraction. The probe energy supply was calibrated from the heating of water inside a dewar vessel (Sleutel et al. 2007).

Table 1 Selected soil properties of the sampled heathland-cropland soil pairs at Wingene and Knesselare in the Belgian sandy region

Site	Soil particles (%)	OC (kg OC m ⁻²)			N (kg N m ⁻²)	OC _{mic} ^a (g C kg ⁻¹)	OC _A ^b (g C g C ⁻¹)	k ^b (day ⁻¹)	pH _{KCl} (–)	Extractable Fe ^c (g Fe kg ⁻¹)		Extractable Al ^c (g Al kg ⁻¹)	
		>50 μm	2–50 μm	<2 μm						Fe _P	Fe _d	Al _P	Al _d
<i>Wingene</i>													
WH	83.8	9.8	6.4	7.07	0.34	0.107	0.011	0.013	3.8	1.18	0.86	1.07	0.47
WC	85.3	8.1	6.5	7.80	0.62	0.141	0.022	0.015	5.8	1.16	1.52	1.68	1.22
<i>Knesselare</i>													
KH	87.3	5.8	6.9	8.73	0.35	0.117	0.014	0.009	3.1	0.24	0.32	0.95	0.68
KC	87.5	6.0	6.5	8.98	0.54	0.688	0.021	0.009	4.9	0.74	1.26	1.23	1.47

^a OC_{mic} = microbial biomass OC

^b OC_A = mineralizable OC (expressed as g OC per g SOC present) and k mineralization rate constant as determined in a 120-day mineralization experiment

^c Fe_d, Al_d = Na-dithionite-citrate-bicarbonate-extractable Fe and Al; Fe_p, Al_p = Na-pyrophosphate-extractable Fe and Al

Chemical fractionation of SOM

In search for a chemical fractionation procedure to isolate a stable SOM pool for process studies of SOM stabilization, Siregar et al. (2005) concluded that threefold sequential 6 h 6% NaOCl treatments at pH 8 was most suitable for selective removal of OM, whilst minimally affecting the mineral soil constituents and hence mineral-associated OM. HF reacts with silicates by forming soluble fluoride complexes. Mikutta et al. (2006) therefore further isolated a non-mineral bound fraction within the oxidation residue using HF-acid extraction. Dissolution of these minerals by HF treatment will lead to a release of mineral bound OM into solution. We used this oxidation-extraction fractionation procedure to sequentially isolate 1° a SOM fraction composed of mineral-protected as well as biochemically recalcitrant OM and 2° a biochemically non-bound recalcitrant SOM fraction. A ball-milled 5 g soil sample was reacted three times for 6 h with 50 ml 6% NaOCl adjusted to pH 8.0 inside 65 ml nalgene centrifuge tubes. Samples were centrifuged and decanted in between oxidation cycles and were ultimately washed one time with 1 M NaCl and three times with deionized H₂O. After drying and weighing, a subsample was used for total C and N analysis.

Next, 3 g of the oxidation residue were treated four times with 20 ml 10% HF in order to dissolve and remove mineral constituents and mineral-bound OM. Extraction residues were washed five times with deionized H₂O to remove salts and residual HF, and were dried and weighed. Chemical fractionations were carried out in duplicates. Sub samples (200–800 mg) of the original samples, the NaOCl-oxidation and the HF-extraction residues were analyzed for total C and N content by dry combustion with a Variomax CNS-analyzer (Elementar Analysensysteme, Germany).

Soil respiration and microbial biomass

Two hundred g air dried soil was weighed into 7.5 cm PVC tubes. The soil columns were slightly compacted in a vertical plane with a plastic cylinder to obtain a bulk density of 1.3 g cm⁻³. The water content was adjusted to 50% water filled pore space by adding distilled water. The soils were incubated in duplicate at 20 ± 1°C for 120 days in airtight closed jars. Amounts

of evolved CO₂, captured in NaOH, were regularly measured by titration of the NaOH with 1 M HCl to pH 8.3 in the presence of BaCl₂ (Anderson 1982). After removal of the vials containing NaOH, the glass jars were left open for 2 h to allow replenishment of oxygen. Soil moisture content was adjusted, fresh vials containing NaOH were added, and the jars were sealed again to continue the C mineralization measurements. A first-order kinetic model:

$$C(t) = C_A(1 - e^{-kt})$$

was fitted to the C-mineralization data, expressed as C mineralized per g SOC, with $C(t)$, the cumulative amount of substrate (i.e. carbon) mineralized at time t (days), C_A the amount of mineralizable C and k the first-order mineralization rate constant. Soil microbial biomass OC was determined on freshly collected soil samples with the fumigation-extraction method (Voroney et al. 1993). K₂SO₄ extracts before and after fumigation were filtered through paper filters (Whatman 5). The K₂SO₄ extracts were analyzed for dissolved OC (TOC analyzer, Shimadzu, Japan).

Pyrolysis-field ionization mass spectroscopy

For temperature-resolved Py-FIMS, about 2–5 mg of sample material was thermally degraded in the ion-source of a modified Finnigan MAT 731 high-performance mass spectrometer. The samples were heated under a high vacuum from ambient temperature to 700°C at a heating rate of 10 K per magnetic scan (≈ 1.7 K s⁻¹) (three replicates). After about 20 min of total registration time, 60 magnetic scans were recorded for the mass range 16–1,000 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 Schnitzer and Schulten (1992), modified to include 10 compound classes. These are carbohydrates with pentose and hexose subunits (CHYDR), phenols and lignin monomers (PHLM), lignin dimers (LDIM), lipids, alkanes, alkenes, bound fatty acids and alkylmonoesters (LIPID), alkylaromatics (ALKY), mainly heterocyclic N-containing compounds (NCOMP), sterols (STEROL), peptides (PEPTI), suberin (SUBER) and free fatty acids (FATTY). In addition to a 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. The average ion intensities for each class of compound were plotted against the pyrolysis temperature, giving characteristic thermograms. All samples were weighted before and after Py-FIMS to normalize ion intensities per mg 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).

Confocal laser scanning microscopy

We made a preliminary investigation on the presence of OM coatings on the soil's 50–250 μm fraction by means of confocal laser scanning microscopy (CLSM), a readily used technique in cellular biochemistry. Free OM was firstly removed from this size fraction by density separation in 1.85 g cm^{-3} sodium polytungstate. Dry sample material was suspended in immersion oil (Cargille oil Type DF, $n = 1.515$ Fluorescence very very low). The visualization of organic versus mineral components in CLSM relies on the auto-fluorescence of specific OM-components such as lipids and lignin. The CLSM experiments were performed on a BIORAD Radiance system combined with a Nikon eclipse TE300 microscope. The 488 nm band of an argon-ion laser was used to excite the fluorescing OM components. The filters in the experimental set-up were chosen to allow the measurement of the fluorescence at 528 nm with a bandwidth of 50 nm.

Results and discussion

Physical and chemical fractionation of SOM

Compared to the whole soil the silt (40.0 g OC kg^{-1} ; 2.1 g N kg^{-1}) and clay ($201.9\text{ g OC kg}^{-1}$; 11.6 g N kg^{-1}) fractions were enriched with OC and N and the sand size fraction (6.1 g OC kg^{-1} ; 0.3 g N kg^{-1}) was strongly depleted in all four sandy soils. Surprisingly, the largest proportions of OC and N were present in the clay size fraction (57% and 60% of the whole soil OC and N) (Fig. 1) in spite of the low percentage of clay in these soils (Table 1). These proportions of clay sized OC and N are relatively

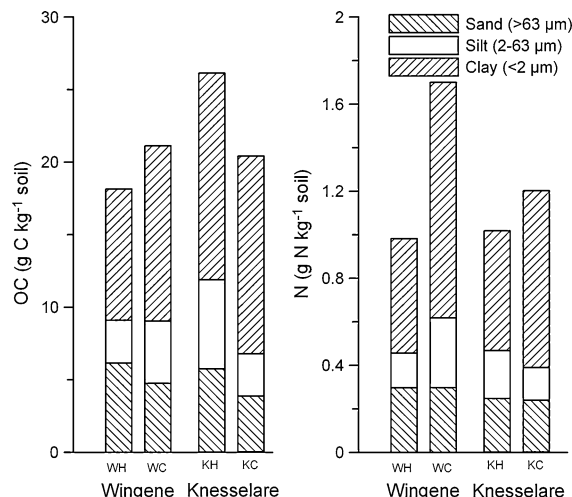


Fig. 1 Summed amounts of OC and N in isolated particle size fractions of the Wingene and Knesselare heathland and cropland soils

high when compared to other studies. For example, Schulten and Leinweber (2000) reported about 30% of the topsoil OC in Cambisols to be present in the clay size fraction. Elucidation of the role of organo-mineral interactions, which are suggested by these high clay SOM proportions, was further derived from the chemical fractionation methodology according to Mikutta et al. (2006).

Mineral dissolution by 10% HF reduced the sample dry matter by 39–50 wt% (mean value $43 \pm 7\text{ wt}\%$). Mikutta et al. (2006) found larger losses of soil dry matter after HF-treatment of 92%, but most soils used in their study contained much higher clay contents than the sandy soils used here. The distribution of the OC and N over the obtained chemical fractions, expressed on a bulk soil basis (g kg^{-1} soil), are given in Table 2. Following Mikutta et al. (2006), we assumed the NaOCl + HF-resistant OM to constitute a recalcitrant SOM pool (ROC and RN) and the HF-extractable OM to coincide with a mineral-protected SOM pool (MOC and MN). The MOC and MN are calculated as the difference between the NaOCl-resistant OC and N and the ROC and RN, respectively. A first observation is that the amounts of ROC and RN were very similar between the heathland and cropland soils in spite of differences in their bulk soil OC and N contents (Table 2). This result suggests a similar resistant OM pool to be present in the current and former heathland soils, but the size of this fraction

Table 2 Distribution of OC and N over isolated chemical soil fractions and comparison with the bulk soil OC and N balances (average \pm standard deviation)

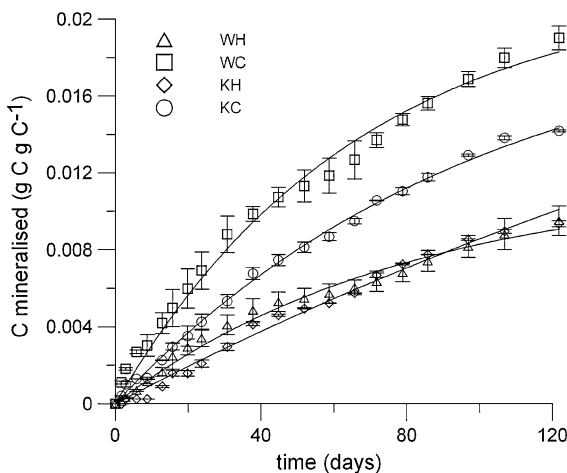
Site	Untreated		NaOCl-resistant					
			Total		Extracted by HF		HF-resistant	
	OC (g C kg ⁻¹)	N (g N kg ⁻¹)	OC (g C kg ⁻¹)	N (g N kg ⁻¹)	MOC (g C kg ⁻¹)	MN (g N kg ⁻¹)	ROC (g C kg ⁻¹)	RN (g N kg ⁻¹)
<i>Wingene</i>								
WH	19.3	0.91	15.0 \pm 2.6	0.6 \pm 0.3	12.4 \pm 2.6	0.5 \pm 0.3	2.6 \pm 0.2	0.1 \pm 0.0
WC	20.9	1.66	13.1 \pm 3.0	0.9 \pm 0.3	9.9 \pm 3.0	0.7 \pm 0.3	3.2 \pm 0.5	0.2 \pm 0.0
<i>Knesselare</i>								
KH	25.3	1.01	12.3 \pm 3.3	0.5 \pm 0.0	7.0 \pm 3.3	0.3 \pm 0.0	5.3 \pm 0.1	0.2 \pm 0.0
KC	20.9	1.25	14.2 \pm 1.0	0.7 \pm 0.0	10.0 \pm 1.0	0.5 \pm 0.1	4.2 \pm 0.1	0.1 \pm 0.1

(14–21% and 10–16% of bulk soil OC and N respectively) is not large enough to explain the high SOC contents of the sandy cropland soils in the study area. Instead, the mineral-protected OM pool held a much larger proportion of the soil OC (28–65%) and N (34–51%). This seems to suggest that binding of OM to the mineral phase would be involved as an important stabilization mechanism here. Strong similarities in MOC and MN were measured for both land-use types and this suggests that the cropland soils approximately contain an equally large pool of mineral-bound assumed OM as their initial heathland precursors. Because of the low content of both crystalline and poorly crystalline Fe and Al (Table 1), the HF extractable pool might, however, also be

interpreted by either the association of OM with clay minerals other than Fe and Al-(hydr)oxides or by a low solubility of the SOM in NaOCl. Nonetheless, similarities in the amounts of MOC and MN between the cropland and heathland soils were striking.

Soil respiration and microbial biomass

The estimates of the parameter OC_A , expressed as g mineralizable OC per g SOC and k that were fitted to the C-mineralization data (Fig. 2) are given in Table 1. While these parameters may not be realistic estimates of truly biologic quantities, the results clearly demonstrate the cropland soils to contain a relatively larger fraction of labile OC than the heathland soils. A closer estimate may be obtained by recalculation to the SOC mineralization under field conditions. By means of the temperature dependence model of De Neve et al. (1996) it was determined that a 1-year mineralization at 9.8°C (the average year temperature) corresponds to a 137-days incubation under the lab conditions of this experiment. Expressed as a relative fraction of the total SOC mineralized in a 1-year period under field conditions, the C mineralization of WH and KH was 2 and 1.5 times lower than of WC and KC respectively. Correspondingly, the OC_{mic} was also higher in the cropland soils (Table 1). These data show that the SOM and particularly vegetation residues under the heathland soils were less decomposable in comparison to the cropland soils. Cornelissen (1996) compared leaf decomposition rates of 125 temperate climate terrestrial vascular plants in 8-weeks and 20-weeks litter bag

**Fig. 2** Cumulative CO₂ evolved during aerobic incubation of the Wingene and Knesselare heathland and cropland soils, with a first-order kinetic model fitted (solid lines) to the data. Bars represent ± 1 standard deviations

experiments. *Calluna vulgaris* and *Erica cinerea* leaves ranked as the fifth and fifteenth least decomposable species out of this selection, which included the main woody and herbaceous species of Europe. Thus, breakdown of heathland plant litter is clearly restricted by its very poor decomposability. The larger amounts of sand fraction OC in the KH and WH compared to the KC and WC soils respectively (Fig. 1) are most likely related to this poor decomposability. However, it should also be considered that the acidity of the heathland soils (Table 1) may have inhibited the C mineralization. The low pH_{KCl} 's in the KH and WH soils compare to the average pH_{KCl} of 3.5 that was previously measured in 173 A horizons under heathland in our study area (Van Orshoven et al. 1988). Van der Wal et al. (2006) also observed that fungal biomass was able to degrade added glucose, cellulose or sawdust in heathland soils only at a slow rate in contrast to a rapid decomposition under cropland. These authors assigned the inhibition of fungal activity to the low pH or to phenolic compounds originating from *Calluna vulgaris* roots. Furthermore, field and laboratory studies have found little or no apparent soil N mineralization in heathland soils, resulting in mineral N pools being maintained at very low content (Kristensen and

McCarty 1999). N availability can be a limiting factor for decomposition (Trinsoutrot et al. 2000), particularly so for plant materials with high C:N ratio, such as *Calluna vulgaris*. Consequently, we cannot rule out a limiting N condition to have inhibited the C mineralization. Thus, under heathland relatively untransformed SOM is stored not only because of poor litter quality but possibly also by poor conditions for microbial growth.

Pyrolysis-field ionization mass spectroscopy (Py-FIMS)

The measured total ion intensity (TII) and percentage volatilized matter in Py-FIMS analysis are given in Table 3, whereas the Py-FI mass spectra and thermograms are given in Fig. 3 (soils) and Fig. 4 (plant litter). Visual observation of the mass spectra from soils clearly showed similarities between the heathland and cropland soil spectra of both the Wingene and Knesselare sites. All spectra were characterized by intense peaks in the molecular mass to charge ratio (m/z) range of 200–425. Dominant peaks in the Wingene heathland and cropland soils (m/z 208, 252 and 280) were similar. The mass spectra of the Knesselare soils showed fewer similarities with

Table 3 Average molecular weight, Total ion intensity, percentage of volatilized matter and mean proportions of SOM compound classes^a (percentage of total ion intensity) identified in whole soil samples of heathland and cropland soil pairs at

Wingene and Knesselare in the Belgian Sandy Region and of above and below ground *Calluna vulgaris* plant parts as determined by Py-FIMS

	M_w^b	M_n^b	TII ^c	%VM ^d	CHYD	PHLM	LDIM	LIPID	ALKY	NCOMP	STER	PEPTI	SUBE	FATTY	Sum
<i>Wingene</i>															
WH	311.3	282.6	16.4	14.6	1.9	5.2	7.9	14.2	11.4	3.6	5.8	0.8	0.3	2.1	53.2
WC	314.3	279.3	16.0	7.7	2.2	5.4	6.4	13.2	9.9	5.1	4.5	1.1	0.4	3.0	51.2
<i>Knesselare</i>															
KH	350.8	318.7	28.3	14.5	1.3	3.5	5.3	17.0	8.7	2.8	7.8	0.5	0.7	6.8	54.4
KC	333.1	298.2	19.6	11.7	1.5	4.6	5.9	14.5	9.6	4.0	6.0	0.7	0.5	4.6	52.0
<i>Vegetation</i>															
Above ground			241.0	74.8	4.8	8.2	3.0	11.0	7.7	2.1	4.8	2.1	1.0	0.7	50.2
Below ground			218.0	74.6	7.1	11.9	3.1	11.3	9.4	3.8	5.0	2.3	0.8	4.5	59.2

^a CHYD, 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; STER, sterols; PEPTI, peptides; SUBE, suberin; FATTY, free fatty acids

^b M_w , Weight-average molecular weight; M_n , number-average molecular weight

^c TII, Total ion intensity expressed in 10^6 counts mg^{-1} sample

^d %VM, Percentage matter volatilized during pyrolysis

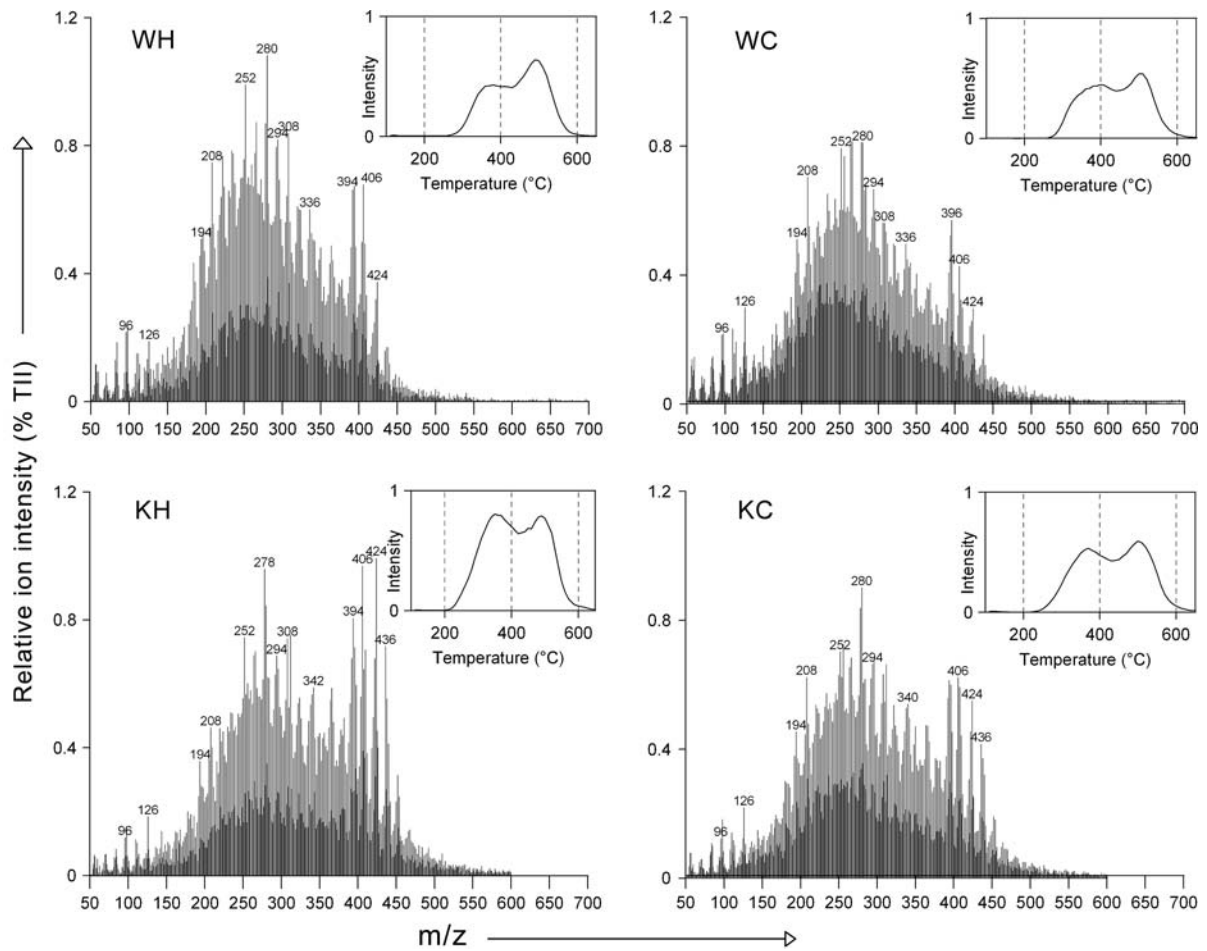


Fig. 3 Thermograms of the total ion intensities (upper right) and summed, averaged pyrolysis-field ionization mass spectra of whole soil samples of heathland and cropland soil pairs at Wingene and Knesselare in the Flemish Sandy Region

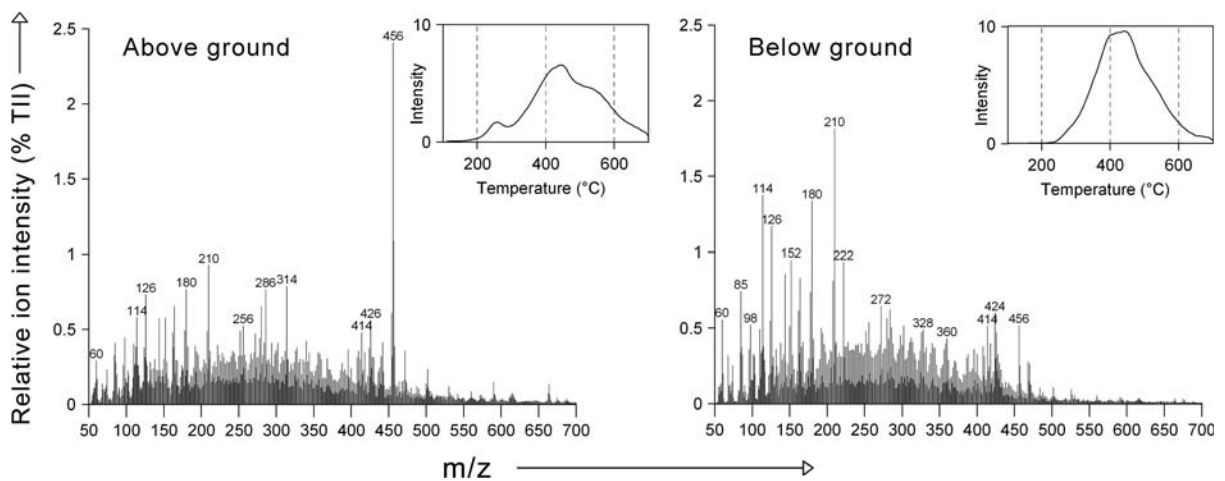


Fig. 4 Thermograms of the total ion intensities (upper right) and summed, averaged pyrolysis-field ionization mass spectra of above-ground and below-ground *Calluna vulgaris* plant material

dominant peaks at m/z 278, 406 and 424 for KH and at m/z 252, 280 and 294 for KC. The above and below ground *Calluna vulgaris* material mass spectra had dominant peaks at m/z 210 and 180. A very intense peak at m/z 456 was measured only in the above ground material.

As has been shown earlier (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 SOM over several important compound classes. Roughly 53% of the pyrolysate signals could be assigned to these classes (Table 3). If the contributions of low-mass signals (1%) and isotope peaks (14%) are accounted for, in total 68% of the produced mass signals were assigned. Application of Py-FIMS to the WH, WC, KH and KC soils showed that pyrolyzed biopolymer building blocks of SOM were primarily derived from the following compound classes (in order of abundance): lipids > alkylaromatics > lignin dimers > sterols. This composition generally matches best that of podzolic soils. Leinweber and Schulten (1999) compiled Py-FIMS data for soil groups and reported that strongly developed podzols were characterized mainly by Py-FIMS signals from lignin-dimers, long-chain lipids and sterols. Instead, the SOM of Phaeozems, Chernozems and Regosols under agricultural use was similar and showed the order carbohydrates = phenols > lignin monomers > N-containing compounds > alkylaromatics > lignin dimers > lipids, which clearly differs from the Wingene and Knesselare soils. While no field replicate samples were taken, a general picture of the natural variability of SOM composition in our soils may be derived from an extensive Py-FIMS dataset compiled by Leinweber et al. (2008), particularly from podzolic soils that are similar to the ones investigated here. Based on Leinweber et al. (2008), a low variability can be expected for the main OM components, namely lipids and lignin dimers, as the coefficient of variation (C.V.) of their TII-proportion amounted 17% for these compound classes. The TII-proportions of alkylaromatics, N-containing compounds and sterols appeared to be more variable (C.V. 30%–50%). A large variability may be expected for carbohydrates, phenols and lignin monomers, peptides, suberin and fatty acids (coefficient of variation >60%).

Py-FIMS spectra of above and below ground *Calluna vulgaris* parts showed signals of (in order of abundance): lipids > alkylaromatics > phenols and lignin monomers > carbohydrates \geq sterols (Table 3). An unusually large proportion of fatty acids was present in the below ground plant material. In contrast, very low TII-proportions of carbohydrates (Table 3) were measured when compared to other studies. For example, Nierop et al. (2001) found carbohydrate derived pyrolysates to be generally present (pyrolysis-GC/MS) and Huang et al. (1998) quantified 46% of all C to be O-alkyl C (CPMAS ^{13}C -NMR). However, other studies measured much lower carbohydrate contents in *Calluna vulgaris*: for example, Anderson and Heterington (1999) measured cellulose contents of 22% and lignin contents of 41% and Iason and Hester (1993) measured 35%–38% acid detergent fibre (i.e. cellulose + lignin + cutin) and 17%–23% lignin. The wet chemical methods (Klason lignin and acid detergent fibre lignin) used in both studies may, however, have yielded imprecise lignin content estimates (Preston et al., 1997). However, one should be careful when interpreting lignin data, since lignin can be measured in a variety of ways, each yielding very different absolute values. Still, the lignin:carbohydrate ratios of 2.3 and 2.1 in roots and above ground plant parts as determined in the present study (Table 3) match this composition. While it would seem that Py-FIMS underestimated the carbohydrate contents, it should be noted that not all pyrolysates from carbohydrates were accounted for: in particular low-molecular weight compound signals (m/z 15 to 56 which are unspecific and can derive from various compound classes), the ^{13}C isotope contribution to signals, and the contribution of carbohydrate building blocks other than the selected hexose and pentose-subunit marker signals. Consequently, the TII-proportions of OM compound classes cannot be translated directly into absolute amounts of these substances and a comparison to carbohydrate contents measured by other techniques is tentative. The proportions of lipids and alkylaromatics were very high when compared to e.g. *Phleum pratense* (Schulten et al. 1992) and *Zea mais* (Gregorich et al. 1997). The true source for the alkylaromatic signals is uncertain but it seems very unlikely that they are present as such in the *Calluna vulgaris* plant materials. Some overlap with marker signals of the same nominal mass from lignin-dimers, $n\text{-C}_{10}\text{--C}_{14}$ alkanes and levoglucosan exists, but their contribution could explain at most 52% of the TII assigned to

alkylaromatics. Furthermore, thermal evolution of lignin dimers typically occurs at a higher temperature than the volatilization maximum of the alkylaromatics at 380°C. It is well known that *Calluna vulgaris* contains very high quantities of condensed tannins, up to 20% of the dry matter (e.g. Iason and Hester 1993), yet assignment of tannin pyrolysis products is tentative. Possibly, intact monomers (flavanols) with masses ranging between 238 and 372 or their fragments contributed substantially to the alkylaromatics TII, leading to an unreliable assignment of alkylaromatics in polyphenol rich plant species such as heather.

In order to characterize the structural building blocks of SOM biomacromolecules, weight average (M_w), number-average (M_n) molecular weights and their M_w/M_n ratio (=PDI, the polydispersity index) of the pyrolysis products were calculated from the summed Py-FIMS spectra (Table 3). The PDI has a value always greater than 1, but as the polymer chains approach uniform length, the PDI approaches unity. In the case of SOM, comparisons of M_w , M_n and the PDI reflect the size of structural building blocks of SOM biomacromolecules and their average repeatability (Sorge et al. 1994). The PDI was only slightly lower in the KH and WH soils than in the KC and WC soils, which indicates the SOM under both land-uses to be characterized by biomacromolecules that yield pyrolysis subunits with an equal structural similarity.

Thermogram data

Temperature-resolved Py-FIMS gives an indication of the thermal energy required for the evolution of individual biomarkers of SOM. In the WH and WC soils the release of molecules started at 250°C and in the KH and KC soils at 210°C and 220°C respectively (Fig. 5). The whole soil OM thermograms had a clear bimodal profile in all samples investigated. A first volatilization maximum occurred at 350–400°C and a second at 480–510°C. In correspondence to numerous previous Py-FIMS analyses of soils (Schulten and Leinweber 1999), these bimodal thermograms reflect the different OM bond types. The volatilization of OM at 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 bonds. The high temperature volatilization at 480–510°C in contrast is indicative of a thermally stable fraction with strong organic-mineral or organic-organic bonds. Thermograms of both the above as well as the below ground *Calluna vulgaris* material had maxima at 350–400°C, very similar to the first thermogram peak of the soil samples.

The thermograms of the N-containing compounds, phenols and lignin monomers, lipids and alkylaromatics

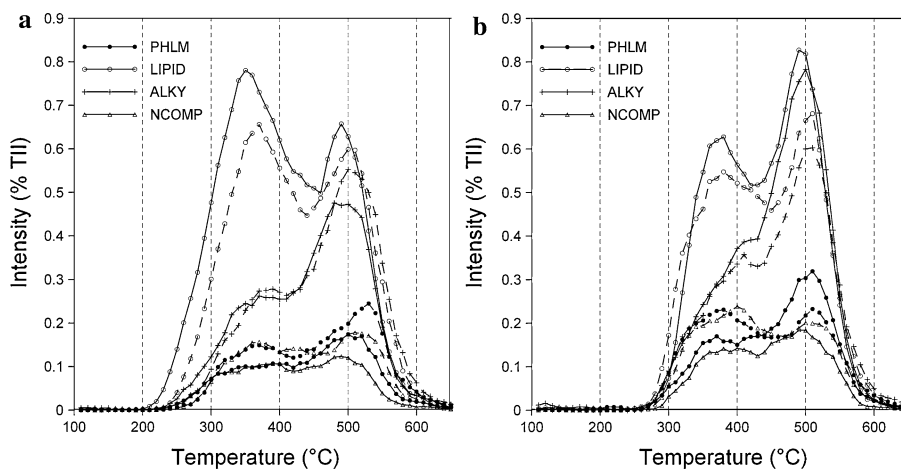


Fig. 5 Thermograms for the volatilization of selected compound classes (PHLM: Phenols & Lignin Monomers; LIPID: Lipids, alkanes, alkenes, fatty acids & *n*-alkyl esters; ALKY: alkylaromatics; NCOMP: mainly heterocyclic N-containing

compounds) for the KH and KC (a) and WH and WC (b) soils. The heathland plots are represented by solid lines and the cropland plots by dashed lines

clearly followed such a bimodal course. In correspondence, Sorge et al. (1994) observed bimodal thermograms for carbohydrates, phenols and lignin monomers, alkylaromatics, and N-containing compounds in the Ap horizon of a Haplic Podzol, whereas no bimodal thermograms for these compounds were found for the Bh horizon. Both first and second thermogram maxima occurred consistently at about 20–30°C higher for the cropland compared to the heathland soils. Plante et al. (2005) found that thermal properties of clay associated OM are related to biological decomposition. Using differential scanning calorimetry they found the thermally resistant SOM fraction to be preferentially retained after land-use conversion from forest to arable land. This linkage between SOM quality and its thermal stability has been investigated in detail and confirmed by Schulten and Leinweber (1999). Consequently, the volatilization at higher pyrolysis temperatures indicates a smaller fraction of thermally labile compounds (i.e. weakly bound) in the SOM of the WC and KC cropland soils, and a stronger biological stabilization. At first sight, these findings seem to be at odds with the results of the lab incubations. The generally low microbial biomass and activity due to acidic conditions and poor litter quality under heathland, as discussed above, could however have precluded the decomposition of the thermally labile OM measured here by temperature resolved Py-FIMS.

Principal component analysis

The results of Py-FIMS were analyzed by principal component analysis (PCA). Principal components

(p.c.'s) were calculated from the integrated mass spectra in the range m/z 50–500. The result of the PCA is visualized as a plot of the first versus the second component (Fig. 6). While no separate field replicates were analyzed by Py-FIMS, p.c. scores of individual measurements are shown in Fig. 6, to represent the analytical variability. The first two p.c.'s account for 71.9% of the total variance and the soil samples could be clearly separated by these two p.c.'s (Fig. 6). The KH and KC soils had negative scores on p.c.1, in contrast to the WH and WC soils (except for one replicate of KC). For both Wingene and Knesselare sites the scores of the croplands on p.c. 2 were more positive than the scores of the corresponding heathland samples. Therefore p.c.1 seems to discriminate both sites, while p.c.2 appears to be applicable to distinguish between land-use. For a more detailed interpretation of the PCA, selected signals with loadings $>|0.075|$ were listed in Table 4. Strong positive loadings of p.c.1 came from phenols and lignin monomers and alkylaromatics. Dominant negative loadings of p.c.1 came from sterols and lipids (m/z 406, 410, 422, 424, 436, 438, 440), specifically from fatty acids. Many of these long-chain fatty acids and sterols are directly plant-derived and may be linked to vegetation. Wilcken et al. (1997) as well found that the SOM composition of B-horizons with podzol features can be related to the composition of organic precursors (e.g. *Calluna vulgaris* vs. *Picea abies*). The substantially large proportion of free fatty acids and lower proportions of phenols and lignin monomers and alkylaromatics of the KH and KC samples were thus the main basis for

Table 4 Py-FIMS signals which load the first two principal components (p.c.) with loadings $>|0.075|$

p.c.	1	2
m/z Positive loadings	184 ^b , 192 ^b , 194 ^a , 196^a , 198 ^b , 208^a , 210 ^c , 212 ^a , 218 ^b , 222 ^b , 224 ^c , 234 ^b , 236, 266 ^c	–
m/z Negative loadings	312, 366 ^c , 382 ^c , 392 ^d , 394 ^c , 396 ^{cd} , 398 ^c , 404, 406^c , 407, 408 ^c , 409, 410^{cd} , 412 ^d , 418 ^c , 420 ^c , 422^{cd} , 423, 424^{cd} , 425, 426 ^d , 436^c , 437, 438^c , 440^c , 441, 442, 452 ^c , 454	222, 224 ^c , 230 ^c , 236, 252^c , 280 ^c , 292 ^{bc} , 308^{bc} , 324 ^c , 390 ^d , 392 ^d , 404, 406^c

m/z which dominantly load the p.c.'s are given in bold

^a Signals related to lignin monomers and phenols

^b Signals related to alkylaromatics

^c Signals related to lipids

^d Signals related to sterols

^e Signals related to lignin dimers

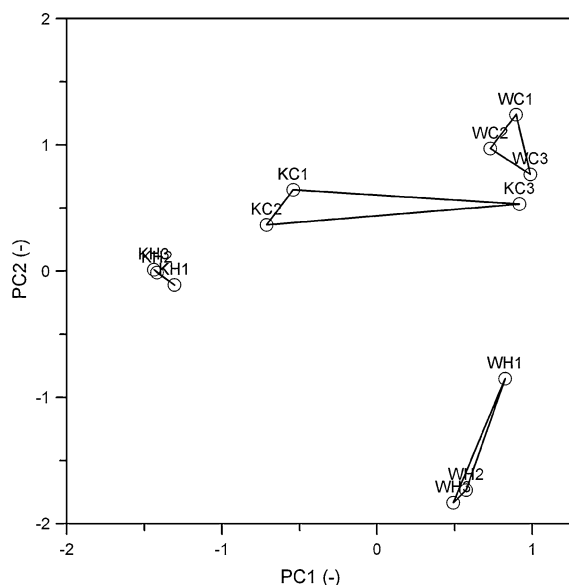


Fig. 6 Score plots of the first two p.c. (p.c. 1 versus p.c. 2) calculated from the set of pyrolysis-field ionization mass spectra of whole soil samples of heathland and cropland soil pairs at Wingene and Knesselare in the Flemish Sandy Region. Individual points of repeated Py-FIMS measurements per plot are enclosed by a line

discriminating these spectra from the Wingene soils. The high loadings of lipid mass signals for p.c.2 indicate their power to discriminate SOM of contrasting land-uses for these soils.

Chemometric evaluation of the pyrolysis data set

General inspection of the proportions of SOM compound classes identified through Py-FIMS yields useful information on the shifts in OM composition associated with conversion of heathlands to croplands. First, increased TII-proportions of carbohydrates, N-containing compounds, peptides and phenols and lignin monomers (Table 3) all seem to reflect a general shift of the SOM towards that of cultivated soils. In correspondence to Schnitzer et al. (2006), who compared a virgin and cultivated prairie soil in Canada, higher proportions in carbohydrates and N-containing compounds in the WC and KC soils compared to the WH and WC soils may be the result of microbial synthesis, while increasing proportions of phenols + lignin monomers and low-molecular weight organic molecules may have arisen from the degradation of complex lignins, lipids, sterols and suberins. The relative enrichment of biologically recalcitrant

N-heterocyclics due to intensive soil management with reduced input of fresh organic matter was also observed in long-term agricultural field experiments at Halle (Schulten and Leinweber 1991) and Bad Lauchstädt (Leinweber et al. 1994). In contrast, in plots of these field experiments receiving large inputs of fresh OM such as farmyard manure, there was a relative enrichment of other compound classes such as phenols and lignin monomers, lignin dimers, fatty acids and alkyl-aromatics leading to lower relative proportions of N-containing compounds. Next, various evidence was reported that mineral fertilizer N may be effectively incorporated into ‘unknown’, mainly heterocyclic N-containing compounds (Schulten and Leinweber 1996). The combined lower input of fresh OM under cropland with silage maize production and the abundant additions of mineral N fertilizers and N in slurry, of which about 50% is NH_4 and 25% is easily mineralizable ON, may thus well explain the higher proportions of N-containing compounds in the KC and WC soils. Still, when considering the average C:N ratio of 13.9 in sand textured arable soils in Flanders (Van Hove 1969) the C:N ratio of the KC (17.0) soil was relatively high. Springob and Kirchmann (2003) observed that SOM under heathland in German sandy soils has very large C:N ratios (often >20) and that these C:N ratios were preserved in these soils even after 50 years conversion to arable land. Our results confirm that C:N ratios tend to decrease only slowly after such land use changes. Next, the thermogram (Fig. 5) and the chemical fractionation data (Table 2) both point to binding of N-containing compounds to minerals or humus. Oxidation in NaOCl increased the C:N ratios by 2.7. Subsequent HF-treatment of the NaOCl oxidation residues led to a stronger increase by 5.3 on average. Nitrogen is thus preferentially eliminated during HF-extraction isolation, suggesting the liberation N-containing components by mineral dissolution with HF. In correspondence, Mikutta et al. (2006) found that N remaining after NaOCl treatment was effectively removed (87%) by mineral dissolution with HF.

The ratio of phenols + lignin monomers : lignin dimers has been proposed to increase with progressive decomposition of SOM (Leinweber and Schulten 1995) and was 0.66 in both WH and KH soils and 0.84 and 0.78 in the WC and KC soils, respectively. These data suggest the SOM in the cropland soils to be further transformed. Since typical values of this ratio for Ap horizons of historically cultivated whole

soils (Anthrosols) are higher, i.e. in the range of 1.1–1.2, this result might furthermore indicate the transformation process to be still ongoing.

As indicated by the PCA, main differences between the cropland and heathland soils were found for lipids, sterols and free fatty acids. The largest differences between the KH and KC spectra were found for homologous series at m/z 410, 424, 438, 452 (n -C₂₇ to n -C₃₀ alkanic acids) and m/z 394, 422, 436 (n -C₂₈, n -C₃₀ and n -C₃₁ alkanes), 392, 406, 420 (n -C₂₈ to n -C₃₀ alkenes) and m/z 388 and 390. Relative intense signals of m/z 410 and 424 which may as well represent squalene and taraxerone are characteristic for *Ericacea* rootlets (Wilcken et al. 1997) and were also prominent in the *Calluna vulgaris* below ground plant parts as well. The predominant peak at m/z 456 likely originates from the triterpene ursolic acid. For WH and WC differences were less pronounced and mainly restricted to m/z 252, 280, 308, 392, 406 (n -C₁₈, n -C₂₀, n -C₂₂, n -C₂₈, n -C₂₉ alkenes). The strong signals at m/z 388 (cholesterol), 390 (ethylcholestanepentaene), 392 (ethylcholestatetraene), 394 (dehydroergosterol) indicate the presence of sterols in the KH and WH soils, which were partly lost under cropland. The Py-FIMS analysis also showed the presence of large amounts of sterols in the vegetation. Nonetheless, considerable proportions of homologous series of n -C₁₀–C₂₀ alkyldiesters, n -C₂₀–C₃₀ alkanes and alkenes and n -C₁₆–C₃₀ fatty acids were present in the KC and WC soils. According to Schulten and Schnitzer (1991) these compounds occur in natural waxes that are preserved in SOM. Homologous series of C₁₄–C₁₉ alkyldiesters, n -C₁₆– n -C₂₈ alkanic acids, C₁₅–C₂₄ alkenes and alkanes, present in both above as well as belowground *Calluna vulgaris* parts, directly confirm this. Apparently, heathland vegetation leads to an accumulation of a lipid and sterol rich SOM and specific lipid components are lost following cultivation. Beyer (1996) compared the composition of SOM in Bh horizons of Podzols under differing vegetation and found the Bh horizon in two podzols under *Calluna vulgaris* indeed to be extremely enriched in alkyl units (derived from CPMAS ¹³C-spectra) and 1:1 ethanol:benzene-extractable lipids compared to Podzols under pine and cropland. We measured lower proportions of the lipids compound class in the WC and KC soils, which is largely composed of alkyl-C, than in the WH and KH soils.

However, it is clear that both WC and KC still hold very large proportions of lipids (Table 3) when the relative TII proportions of the lipids compound classes of these soils are compared to average proportions for other soil types commonly found in temperate regions. Leinweber et al. (2008) reported TII proportions of $2.2 \pm 1.3\%$ TII for lipids in Luvisols; $10.3 \pm 1.3\%$ TII for Phaeozems and $11.2 \pm 0.4\%$ TII in Cambisols and Regosols. Wiesenberger et al. (2004) investigated SOM turnover of several agricultural soils after maize monoculture was introduced. While bulk soil-C had residence times up to 250 years, lipid fractions in dichloromethane/methanol were less stable with turnover times of 21 to maximal 60 years. Consequently, the inherent biochemical composition of lipids seems unable to explain their resistance against rapid biodegradation after land-use conversion. Prominent lipids in maize are primarily n -C_{16:0}, n -C_{18:0}, n -C_{24:0} and n -C_{18:1}, n -C_{18:2} fatty acids in leaves and shoots and n -C_{16:0}, n -C_{18:0}, n -C_{20:0}, n -C_{24:0} and C_{18:1} and n -C_{16:1} fatty acids and n -C_{23:0}, n -C_{27:0} branched fatty acids in roots (Jandl et al. 2005; Jandl et al. 2006). To a much lesser extent n -C₁₆ to n -C₂₀, n -C₂₂, n -C₂₇ to n -C₂₉ and n -C₃₁ alkanes are present in maize leaves and shoots and n -C₁₆ to n -C₁₉ and n -C₂₇ to n -C₃₁ alkanes in maize roots (Jandl et al. 2006). Some of these lipids are present in the KC and WC soils (n -C_{16:0}, C_{18:0}, n -C_{24:0} fatty acids and n -C₁₆ and n -C₂₀ alkanes) and may thus be maize-derived. However, n -C_{17:0}, n -C_{19:0}, n -C_{20:0}, n -C_{21:0}, n -C_{27:0}, n -C_{29:0} fatty acids and n -C₁₆ to n -C₂₆, n -C₂₈ and n -C₃₀ alkanes are low in maize cropped soils (Jandl et al. 2006; Wiesenberger et al. 2004) but were abundant in the KC and WC soils.

Jandl et al. (2005) assessed the origin of fatty acids in a cropland soil under maize for 38 years that was formerly cropped under monoculture rye. The contributions from soil biota, from farmyard manure applications and from maize were limited compared to the long-chain fatty acid pool still originating from rye. Likewise, Quéneé et al. (2006b) found that the bulk (75% to ca. 90%) of alkanes in a 22 year maize cropped sandy soil was not derived from maize. They concluded that based on n -alkane distributions, maize roots and shoots are not major sources of n -alkanes in the cropped soil. Our results and these studies strongly suggest that large portions of the lipids in the cultivated soils were not maize derived. Whilst

the KC and WC plots still received an input of lipids through OM additions from animal slurry and harvest residues after cultivation, it is clear that only a significant contribution of heath-derived lipids may explain the lipid proportions that we measured here. Furthermore, Jandl et al. (2005) concluded that plant waxes seem to be the only source of stable long-chain fatty acids in soil. This seems to be confirmed by longer chain lengths of lipids identified in the KH soil as compared to the *Calluna vulgaris* plant material.

Next to vegetation, external factors such as the low pH and low soil microbial activity of the KH and WH soils may also have favored the accumulation of a lipid-rich SOM. Bull et al. (2000) concluded that soil pH has a significant effect on the preservation of certain lipids. *n*-alkane preservation was enhanced under more alkaline conditions whilst *n*-alkanoic acids and ω -hydroxy acids accumulated, relative to other components, in more acidic soils. Accumulated *n*-alkanoic acids were found to persist in soils even after a change to more alkaline conditions. The pH of the KC and WC soils was about higher by 2 pH units than the KH and WH soils (Table 1) and, amongst others, *n*-alkanoic acids were indeed partly preserved. Soils exhibiting a low microbial activity have furthermore been shown to possess a relatively higher lipid content than those exhibiting high levels of activity (Bull et al. 2000). While mechanisms resulting in such preservation are poorly understood, our data indeed confirms this.

Mechanisms for SOM stabilization

The question arises as to which factors support the retention of high levels of apparently stable SOM that is characterized by lipids and alkylaromatics in these soils. We will discuss several potential tracks:

(1) Firstly, lipids have often been reported to be enriched in the clay fraction of soils (Leinweber 1995; Jandl et al. 2004; Qu  n  a et al. 2004). *n*-C₁₁ to *n*-C₉₄ alkanes, *n*-C₁₃ to *n*-C₃₄ fatty acids, *n*-C₁₆ to *n*-C₂₄ diols and *n*-C₃₈ to *n*-C₆₇ alkyl monoesters were unequivocally shown to be important compounds in fine clay (Schulten and Schnitzer 1990). Thus, a possible preferential link between the lipid rich SOM and clay minerals imposes itself. Schulten and Leinweber (1995) found lipids and lignin dimers marker signals to be selectively enhanced in Py-FIMS spectra of DCB extracts, which suggests biopolymers

of these compounds to be preferentially associated with trivalent Fe and Al. Recently, Wang and Xing (2005) provided direct evidence that aliphatic fractions are preferentially adsorbed by clay minerals. Possibly, the COOH and OH groups present in these molecules renders them more suitable for such linkages. On the one hand, the very pronounced bimodal course of the lipids compound class thermograms (Fig. 6) would seem to confirm a part of the lipids to be involved in organo-mineral associations. On the other hand, a predominant influence of metal-induced organo-mineral associations seems unlikely here, given the low quantities of Fe and Al present in all four investigated soils (Table 1). Whilst many recent studies have unequivocally confirmed the importance of such metal species for SOM stabilization in acid subsoil horizons, conflicting observations have been made for topsoil layers. For example, Eusterhues et al. (2005a) observed high relative proportions of oxidation-resistant OC relative to the content of Fe oxides in a Haplic Podzol under forest. Assuming an average specific surface area of about 500 m² g⁻¹ Fe in oxides (goethite has 200 m² g⁻¹ Fe and ferrihydrite 800 m² g⁻¹ Fe; Eusterhues et al. 2005b), we calculated surface loadings between 13–44 mg C m⁻² Fe for the mineral-protected OC pool (the MOC). This by far exceeds the experimentally measured (Mayer et al. 2004) ~1 mg C m⁻² equivalent to a “monolayer OM coating” of moderately sized organic molecules. Eusterhues et al. (2005a) therefore concluded that mineral protection was not the main mechanism for the resistance of OM against oxidation in topsoils and that rather the chemical recalcitrance of OM and bonding to other minerals is involved as well. Using an average 85 m² g⁻¹ clay, which is typical for illitic soils (Kahle et al. 2003), the MOC surface loadings of our soils calculate to 1.2–2.3 mg C m⁻² clay, which is much closer to the 1 mg C m⁻² monolayer equivalent. OM adsorption to phyllosilicates therefore seems to be a relevant protection mechanism here. However, according to these estimations still up to about half of the MOC and furthermore the complete HF-resistant OM fraction (the ROC) may not be bound directly to the clay sized mineral phase. Additionally, Mayer and Xing (2001) found that in A horizons of low pH sandy soils the occluding OM is present in a low-surface area configuration, such as in organo-clay aggregates, rather than as dispersed

coatings on mineral grains. OM-OM interactions, i.e. more than monolayer coverage, are thus needed to explain the estimated large C loadings per m² sorbent.

(2) Consequently, we hypothesize stabilization of OM through cross linking and inherent chemical recalcitrance to hold a non-negligible share next to mineral bonding. A first large proportion of the lipids compound class volatilized at relatively low temperatures (300–400°C) in both cropland and heathland soils (Fig. 6). Sorge et al. (1994) found that, surprisingly, the contribution of the thermolabile lipid fraction increased with decreasing particle size fractions in a spodosol Bh soil horizon. This proves the importance of OM-crosslinking for lipids, even for the clay size fraction. Accordingly, the substantial low temperature volatilizable lipid fraction could well be derived from OM which is not mineral bound and not incorporated in strong OM bonds. Rumpel et al. (2004) also considered that alkyl-C in the A horizon clay fraction of a Haplic Podzol was protected as alkyl structures entrapped in the organic matrix rather than through organo-mineral bonding. Independent proof for such crosslinking of lipids was furthermore given by Quénéa et al. (2006a). These authors investigated the composition of the non-hydrolysable SOM fraction of a forest soil by means of double-shot pyrolysis. They concluded that sterical protection from microbial degradation of alkanes, alkenes, *n*-alkan-2-ones and fatty acids, mostly originating from plants, was provided by entrapment in the macromolecular structure of non-hydrolysable SOM. The first low temperature maximum in the lipids class thermogram may coincide with such a pool.

Next, the thermal evolution maxima of the alkyl-aromatics and lignin dimers and the second maximum of the lipids were in the 500°C range, which proves biopolymers of these OM components to be stabilized in thermostable bonds. Dinel et al. (1990) reported that lipids undergo direct transformations during humification, resulting in alkyl carbon structures with a high degree of cross-linking. Moreover, Quénéa et al. (2006a) unequivocally assessed that cracking of lipid moieties, which are incorporated into the non-hydrolysable macro-molecular humus structure through the formation of covalent bonds, occurred during pyrolysis in this temperature range. Such incorporation would result from condensation

reactions, as observed for the humin of some soils via chemical degradation. The proportions of alkyl-aromatics and lipids evolved at higher temperatures may well correspond to these fractions. In summary, our data and these findings all support the previously proposed view (Schulten et al. 1991) that alkylaromatics are significant structural building blocks of OM networks that can bind carbohydrates, proteins, lipids as well as inorganics. This line of thought is able to link the here predicted considerable OM fraction that is not directly stabilized through organo-mineral bonds on the one hand to the observed bimodal lipid thermograms on the other. Since dimeric lignin seems to be inherently thermally resistant up to high temperatures, we are not able to conclude a similar stabilization through either organic-mineral or organic-organic bonds for lignin from the high temperature evolution of the lignin dimers compound class. Recently, Mikutta et al. (2006) found that treatment with NaOCl dramatically and preferentially reduced lignin-derived phenol contents (Mikutta et al. 2006). They concluded, as did several other authors (e.g. Kiem and Kögel-Knabner 2003), no quantitatively important contribution of lignin to mineral-protected and recalcitrant SOM.

(3) We cannot exclude that deep-ploughing (i.e. up to 60 cm depth), a generally applied measure for soil amelioration in the Belgian sandy region since the Middle Ages, was used on our soils. If so, it is well possible that underlying material from Podzol B horizons has been mixed into the here sampled upper 30 cm layers. From both CPMA¹³C-NMR and Py-FIMS analyses on nine soils throughout Germany, Wilcken et al. (1997) concluded a prominent presence of lipids, sterols and lignin dimers in podzol B horizons. A possible extra line of reasoning for explaining the aliphatic character (long chain lipids, sterols, alkyl chains in alkylaromatics) of the topsoil SOM of the KC and WC soils would thus be the presence of such relict Bh horizon material in the topsoil. Microbial decomposition of OM in podzols results in a selective preservation of more resistant organic fragments, especially aliphatic ones (Buurman et al. 2007). Hence the evident further gradual decay of mixed Bh horizon OM would only further lead towards a lipid-rich composition.

(4) Lastly, given the substantial proportion of sand sized OM present, we looked into adsorption to sand sized mineral particles as well. Recent research by

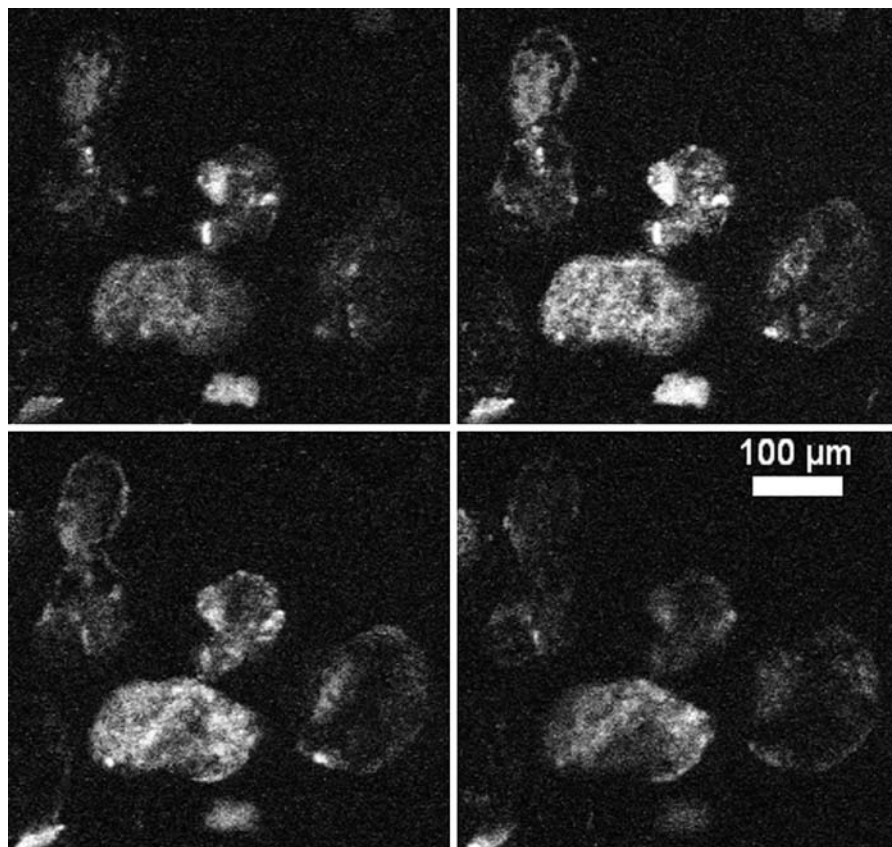


Fig. 7 Confocal laser scanning microscopy scan (4 out of a total of 25 focal depths) of the $>1.85 \text{ g cm}^{-3}$ 50–250 μm fraction of the KH soil. The subsequent 4 scans are spaced 20.4 μm apart from each other into the z-direction. Emitted

light due to fluorescence of OM-components covering the soil particles is white. Sand mineral particles are invisible because quartz is not fluorescent and reflectance was eliminated using an immersion oil

means of X-ray photoelectron spectroscopy has shown that Al-organic complexes generally occur on soil particle surfaces (Yuan et al. 1998; Gerin et al. 2003) even in soil horizons where the OC content is less than 0.1 g kg^{-1} . Other authors as well have suggested hydrophobic interactions at low pH or van der Waals forces (Von Lützow et al. 2006) to potentially induce strong bindings between OM molecules and uncharged mineral surfaces, including sand. First scans (Fig. 7) at a resolution of $0.53 \mu\text{m}$ proved the presence of OM coatings on the sand particles, which occurred in patches rather than as a continuous coating. Some sand grains were not coated at all. Any possible artifacts due to reflectance were eliminated by the addition of the immersion oil, which rendered the sand grains fully transparent. While our results prove that OM is bound to the sand size fraction in a patchy configuration, quantification

of this SOM fraction's volume and thus relevance is still lacking. However, quartz proved to be a particularly fit carrier for observation of OM coatings since its transparency to the fluorescent light allows generating 3D images through computed combination of sequential microscopic scans at different focus. High resolution 3D images of coatings generated by CLSM may therefore ultimately be used for such a quantification.

Conclusions

Pyrolysis Field Ionization Mass Spectroscopy (Py-FIMS) analysis primarily detected pyrolysis products of lipids, alkylaromatics and sterols in SOM of sandy topsoils with a historical heathland land-use. The accumulation of this aliphatic rich

SOM was logically linked to the high input of lipids and sterols from heath vegetation and the low soil pH and microbial activity under heathland. On the basis of a comparison of relict and cultivated heathland plots, we can conclude that conversion to cropland leads to increased proportions of carbohydrates, N-containing compounds, peptides and phenols and lignin monomers SOM biopolymer building blocks. However, SOM constituents that yield lipids and alkylaromatics upon pyrolysis appear to be largely stabilized since their presence in Py-FIMS spectra is still pronounced after long-term cropland management. The presence of such a substantial relict SOM fraction indeed suggests a stable aliphatic SOM pool in these sandy soils. Based on the bimodal course of Py-FIMS thermograms and high mineral-surface loadings of oxidation resistant C, we hypothesize contrasting stabilization mechanisms to be involved. Next to mineral association to oxides and clay minerals, which have frequently been referred to as important processes in subsoil horizons, advanced cross-linking of SOM should be considered. We hypothesize lipids, that are otherwise readily degradable, to be sterically protected by entrapment in the macromolecular SOM structure. Next, a substantial quantity of lipids and alkylaromatics evolved at higher temperatures, and this suggests SOM constituents from which they are pyrolyzed to be intensely cross-linked by covalent bonds. Ultimately, this line of thought supports the previously proposed reasoning that they form the building blocks of macromolecular aryl-alkyl OM dominated networks that constitute humified soil material.

Acknowledgements S. Sleutel is working as a post-doctoral researcher for the Flemish Research Foundation (FWO). The authors wish to thank R. Beese and K.-U. Eckhardt, University of Rostock, for carrying out the Py-FIMS analyses. We gratefully acknowledge C. Verschueren, E. Kuycken and K. De Kesel for providing access to relict heathland plots in the Gulke Putten and Maldegemveldt nature reserves.

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