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Shifts in soil organic matter composition following treatment with sodium hypochlorite and hydrofluoric acid

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ABSTRACT

A renewed interest in chemical fractionation of soil organic matter (SOM) originates from the premise that it enables to isolate labile SOM from SOM protected through mineral binding and recalcitrant SOM. Both selective removal of labile non-bound SOM through oxidation or hydrolysis as well as selective removal of minerals and attached SOM are often applied. Molecular-level SOM characterization by means of temperature resolved Pyrolysis-Field Ionization Mass Spectroscopy analysis (Py-FIMS) was used here as an approach to obtain insight into the fate of SOM upon wet chemical treatment with regard to composition and thermal stability. The applied sequential chemical treatment with 6% NaOCl and 10% HF yielded similar sizes in stable SOM fractions between sandy semi-native heathland and cultivated cropland soil pairs (i.e. NaOCl resistant OC: 12.3–15.0 g C kg^{-1} and NaOCl+HF resistant OC: 2.6–5.3 g C kg^{-1}). Py-FIMS spectra of bulk SOM in both heathland–cropland soil pairs were dominated by signals assigned to lipids, alkylaromatics and sterols. Difference spectra and thermograms showed selective loss of signals from sterols, lignin dimers and thermolabile lipids. This matches advancing SOM decomposition as derived from previously reported gradients in SOM composition as decomposition proceeds from plant material over particulate organic matter (OM) to SOM in silt and clay particle sizes. However, increased ion intensity attributed to carbohydrates, peptides and short-chained lipids after NaOCl treatment indicates that biologically labile SOM components were also enriched, and they may possibly have been protected through mineral binding or encapsulation in macromolecular OM structures. Subsequent HF treatment yielded increased volatilization in the thermostable region for mass signals tentatively assigned to phenols and lignin monomers and of heterocyclic N-containing compounds and thermostable alkylaromatics. The resistance to chemical treatment of the latter two components matches with their hypothesized structural function in macro OM molecules. However, even for the sites investigated here, with a very similar soil texture, climate, land-use, drainage and contents of pedogenic oxides, contrasting and not readily explainable results were found for other SOM constituents. Therefore, chemical fractionations seem to yield very site specific and less distinct patterns and this study demonstrated that derivation of useful information regarding SOM stabilization mechanisms from such experiments is by no means straightforward.

1. Introduction

The study of soil organic matter (SOM) is impeded by its complexity, its heterogeneity and its fundamental association with the soil mineral phase. Pool based SOM models have been used as simplifications of these complex chemical, biological and physical characteristics of SOM (Von Lützow et al., 2006). More recently, efforts have been directed towards measuring SOM fractions in which the organic matter (OM) is protected against decomposition by distinct stabilization mechanisms. Chemical methods which are based on either oxidative extraction of labile SOM (e.g. by H_2O_2, NaOCl or $Na_2S_2O_8$) (Kaiser and Guggenberger, 2003; Eusterhues et al., 2005; Kleber et al., 2005; Zimmermann et al., 2007) or on the extraction of mineral-associated SOM (e.g. by HF or Dithionite Citrate Bicarbonate (DCB)) (Schmidt and Gleixner, 2005; Mikutta et al., 2006) have increasingly been used in combination with ^{14}C-dating, the latter confirming the higher mean residence time of OM in those fractions (Trumbmore and Zheng, 1996; Eusterhues et al., 2003; Kleber et al., 2005). Analyses of the composition of chemically isolated fractions have almost exclusively been carried out by solid-state ^{13}C NMR spectroscopy and by Fourier Transform Infrared spectroscopy (FTIR) on some occasions. Chemical oxidation has often been found to remove lignin and carbohydrates, and residues were dominated by alkyl-C and enriched in N-containing compounds (e.g. Chefetz et al., 2002; Mikutta et al., 2006). Studies using A horizons pointed to only minor changes in SOM composition following treatment with HF. However, solid-state ^{13}C NMR and FTIR do not provide information at the molecular level (Rumpel et al., 2006). Furthermore, the applicability of solid-state ^{13}C NMR is limited for samples with low OC

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content or with appreciable amounts of paramagnetic species (Skjemstad et al., 1994). Pyrolysis-field Ionization Mass Spectroscopy (Py-FIMS) is not subject to these limitations and has been established as a successful method for the molecular level characterization of bulk SOM, OM in particle size fractions, lipid extracts, dissolved OM and hot-water extractable OM (Leinweber and Schulten, 1999).

Here we studied the chemical composition of SOM in oxidized and HF-treated samples derived from previous studies. A recently developed chemical fractionation procedure, that involves sequential oxidation in 6% NaOCl and treatment with 10% HF for isolation of recalcitrant and mineral-protected SOM (Mikutta et al., 2006), was used to fractionate the topsoil of two native heathland — cultivated sandy soil pairs. A previous study revealed a similar SOM composition in both these relict heathland as well as cultivated sandy soils (Sleutel et al., 2008). Here we expand this research as for the first time Py-FIMS was applied to simultaneously obtain molecular-level SOM compositional data and measure shifts in the thermal stability of SOM fractions isolated by such a chemical fractionation.

2. Materials and methods

2.1. Soil samples

Topsoil samples (0–30 cm) were collected from two relict heathland podzols and two cropland degraded podzols, that were formerly also under heathland, at Wingene (Lat. 51°4′–Long. 3°20′) and Knesselare (Lat. 51°8′–Long. 3°27′) in the North-West of Belgium. Neither of these two sites has been under heathland permanently, but from the end of the 18th century forestation periods alternated with heathland use. Both croplands (Wingene: WC, Knesselare: KC) were put into cultivation about 60 years ago and have been under maize monoculture since 3 to 4 decades. Fifteen soil samples were taken at these four plots by means of an Auger (Ø 2.5 cm) within a 25 m × 40 m rectangle. Litter layers in the relict heathland soils (Wingene: WH, Knesselare: KH) were removed prior to sampling. Bulked samples were then mixed and air dried. Soil texture was determined by the pipette-sedimentation method (Gee and Bauder, 1986). Total Fe and Al in pedogenic oxides were estimated by the DCB-method (Blakemore et al., 1987). Pyrophosphate extractable Fe and Al (mainly Fe and Al bound in humus complexes) were determined at a soil:solution ratio of 1:100 (wt:v) in 0.1 M $Na_4P_2O_7$ (Blakemore et al., 1987). Al and Fe in <0.45 µm DCB and $Na_4P_2O_7$ extracts were measured by atomic absorption spectroscopy. Data for total OC, texture, pH (KCl), DCB- and pyrophosphate extracted Al and Fe are given in Table 1.

2.2. Chemical fractionation of SOM

An oxidation–extraction fractionation procedure according to Mikutta et al. (2006) was used to sequentially isolate (1) a stable SOM fraction composed of mineral-protected as well as biochemically recalcitrant OM and (2) a stable SOM fraction consisting primarily of biochemically non-bound recalcitrant OM. 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. Dried samples were centrifuged at 8500 g, decanted in between oxidation cycles and washed 1 time with 1M NaCl and three times with deionized H_2O. After drying at 60 °C, cooling down in a desiccator and weighing, a subsample was analyzed for total C and N content. 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 H_2O, and were dried at 60 °C, transferred to a desiccator and weighed. The chemical fractionation by Mikutta et al. (2006) has mainly been applied to subsoils. A larger residue of particulate organic matter present in the investigated A horizons, possibly resistant to oxidation might bias results of the Py-FIMS analysis. The fate of this particulate organic matter was therefore investigated in a separate fractionation of the light fraction (LF). LF material (<1,85 g cm^{-3}) was isolated by density fractionation of 5 g soil in 50 ml sodium polytungstate inside 85 ml centrifuge tubes. After centrifugation at 8500 g, LF material was aspirated on a nylon filter, rinsed with deionized water and dried at 60 °C. Then, 0,2 g LF material was treated with 6% NaOCl as described above. Chemical fractionations were carried out in twofold. Sub samples (100–800 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 Analysesysteme, Germany).

2.3. 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 (three replicates) 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}). 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 three replicates were averaged to give the final survey spectrum. 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).

3. Results

3.1. OC and N contents in isolated chemical fractions

The distribution of the SOC and N among the chemical fractions is given in Table 1 (data taken from Sleutel et al. (2008)). Briefly, the 6% NaOCl treatment removed 36±12% and 44±7% of the whole soil OC and N, respectively. This result matches the low end of the 28 to 88% range of OC resistant to 6% NaOCl, measured by Siregar et al. (2005). The LF constituted 31±13% of the SOC and 36±21% of the N and was oxidized to a substantial extent as 70±5% of the LF OC and 80±5% of

Table 1
Selected properties of the sampled topsoils in the Belgian sandy region and distribution of OC and N over isolated chemical soil fractions (average±standard deviation) (data taken from Sleutel et al. (2008))

Soil	Soil texture			pH	SOM fractionation						Extractable Fe and Al[a] (g kg^{-1})			
					Bulk Soil		NaOCl-resistant		NaOCl+HF-resistant					
	>50 µm (%)	2–50 µm (%)	<2 µm (%)		OC (g kg^{-1})	N (g kg^{-1})	OC (g kg^{-1})	N (g kg^{-1})	OC (g kg^{-1})	N (g kg^{-1})	Fe_P	Fe_d	Al_P	Al_d
WH	83.8	9.8	6.4	3.8	19.3	0.91	15.0±2.6	0.6±0.3	2.6±0.2	0.1±0.0	1.18	0.86	1.07	0.47
WC	85.3	8.1	6.5	5.8	20.9	1.66	13.1±3.0	0.9±0.3	3.2±0.5	0.2±0.0	1.16	1.52	1.68	1.22
KH	87.3	5.8	6.9	3.1	25.3	1.01	12.3±3.3	0.5±0.0	5.3±0.1	0.2±0.0	0.24	0.32	0.95	0.68
KC	87.5	6.0	6.5	4.9	20.9	1.25	14.2±1.0	0.7±0.0	4.2±0.1	0.1±0.1	0.74	1.26	1.23	1.47

[a] Fe_d, Al_d=Na–dithionite–citrate–bicarbonate–extractable Fe and Al; Fe_p, Al_p=Na–pyrophosphate–extractable Fe and Al.

the LF N were removed by the 6% NaOCl. Hence, most particulate organic matter is contained in the NaOCl oxidizable fraction, which is presumed labile, and this material should therefore not be assigned to an additional SOM fraction. Subsequent mineral dissolution by 10% HF reduced the sample dry matter by 43 ± 7 wt.% and released 71 ± 11% of the 6% NaOCl resistant OC, which is higher than the 38 to 57% range given by Helfrich et al. (2007). Following Mikutta et al. (2006), the NaOCl + HF-resistant OM can be assumed to constitute primarily biochemically stable SOM whereas the HF-extractable OM can serve as a proxy for stable mineral-protected SOM. This potentially mineral-protected OM pool held a much larger proportion of the soil OC (47 ± 15%) and N (43 ± 7%) than the recalcitrant OC (17 ± 4%) and N (12 ± 3%). A first observation is that the amounts of NaOCl + HF-resistant OC and N were similar between the heathland and cropland A horizons. Moreover, there were also strong similarities in the amounts of HF-extractable OC and N between the cropland and heathland soils. The low content of DCB-extractable Fe and Al (Table 1) indicates that this HF extractable OM was not associated with poorly crystalline minerals. Instead the HF extracted OM may have originated from OM associated with silicate clay minerals or from unbound OM with a low solubility in NaOCl (Sleutel et al., 2008).

3.2. Characterization of bulk soil samples by Py-FIMS

Table 2 shows the measured total ion intensity (TII) and percentage volatilized matter in Py-FIMS analysis. Data for bulk soil samples were discussed previously by Sleutel et al. (2008). 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 a number of important compound classes that are relevant for SOM degradability. On average 51.6% of the pyrolysate signals could be assigned to these classes (Table 2). If the contributions of low-mass signals (2.1%) and isotope peaks (14.7%) are accounted for, in total 68.4% of the produced mass signals were assigned. Application of Py-FIMS to the untreated WH, WC, KH and KC soils showed that their macromolecular SOM building blocks were primarily composed of the following compound classes (in order of abundance): lipids > alkylaromatics > lignin dimers > sterols. All four soil samples were characterized by bimodal thermograms, which suggests that at least two thermally different OM fractions, differing in biological stability and controls for stabilization are present. 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 (Sleutel et al., 2008). Based on these findings and the bimodal shape of the thermograms it was suggested that next to mineral association, the structural composition of the OM must have furthermore contributed substantially to SOM stabilization in these soils (Sleutel et al., 2008).

3.3. Changes in the Py-FIMS data sets after NaOCl treatment

Changes in the chemical composition of the OM in the samples by 6% NaOCl treatment were visualized by plotting difference Py-FIMS mass spectra (spectra of non-treated minus 6% NaOCl-treated samples) (Fig. 1). In all four soils there was a consistent selective loss of high mass signals, mainly situated between *m*/*z* 278 and *m*/*z* 454. Most pronounced losses were found for masses that can tentatively be assigned to sterols (*m*/*z* 392 ethylcholestatetraene, 394 dehydroergosterol and 408 C3-alkylcholestatriene), long-chained lipids, namely *n*-C_{29} alkene (*m*/*z* 406), *n*-C_{29} to *n*-C_{31} alkanes (*m*/*z* 408, 422, 436), *n*-C_{27} to *n*-C_{29} fatty acids (*m*/*z* 410, 424, 438) and to a lesser extent for other lipids (such as *m*/*z* 278, 296). On the contrary, low-mass signals were selectively enriched in the 6% NaOCl-oxidized samples (Fig. 1). Negative %TII-difference signals from alkylnaphthalenes (*m*/*z* 142, 156, 170, 184), alkylphenanthrenes (*m*/*z* 206, 220) and to a lesser extent alkylbenzenes (*m*/*z* 190, 192, 204, 218) show that alkylaromatics were selectively retained in the NaOCl-residues. Furthermore, negative % TII-difference signals from carbohydrate hexose and pentose subunits (*m*/*z* 84, 112, 126 and 144) and from phenols and lignin monomers (mainly at *m*/*z* 168, 180, 182, 196, 210 and to a lesser extent at *m*/*z* 166, 194, 212) suggest their relative enrichment in the 6% NaOCl-residues. Several smaller negative signals could be attributed to heterocyclic N containing compounds (*m*/*z* 153, 167, 183, 195, 257) and peptides (*m*/*z* 57, 70, 84, 99) and indicate the relative enrichment of specific organic N in the 6% NaOCl-residues.

3.4. Changes in the Py-FIMS data sets after HF treatment

Changes in the chemical composition of the OM in the samples by subsequent 10% HF-treatment were visualized by plotting difference

Table 2
Total ion intensity, percentage of volatilized matter and mean proportions of SOM compound classes[a] (percentage of total ion intensity: %TII) in whole soil samples, 6% NaOCl treated samples and 6% NaOCl + 10% HF treated samples of the sampled topsoils in the Belgian sandy region as determined by Py-FIMS

	TII[b]	%VM[c]	CHYD	PHLM	LDIM	LIPID	ALKY	NCOMP	STER	PEPTI	SUBE	FATTY	Sum
Bulk soil													
WH	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	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
KH	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	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
6% NaOCl treated soil													
WH	13.7	7.5	2.4	6.8	5.2	13.1	11.9	4.1	3.7	1.1	0.3	3.9	52.4
WC	10.3	8.8	3.6	8.4	5.4	10.3	12.2	5.8	2.1	1.5	0.1	2.5	51.8
KH	15.8	8.3	2.1	4.9	4.6	12.7	9.5	4.0	4.4	1.4	0.5	4.4	48.4
KC	21.3	9.7	1.8	5.0	4.9	14.0	10.1	4.3	4.8	1.0	0.5	5.1	51.7
6% NaOCl + 10% HF treated soil													
WH	7.4	4.0	1.9	3.8	6.0	14.2	9.7	3.0	5.0	1.3	0.6	5.0	50.6
WC	10.2	8.8	2.4	4.5	4.9	12.9	8.9	4.2	4.0	1.6	0.5	4.9	48.8
KH	19.3	2.6	2.3	6.0	3.9	13.5	10.9	3.7	4.0	1.0	0.3	5.6	51.1
KC	11.2	6.0	2.3	7.3	3.8	14.2	11.9	3.9	3.2	1.0	0.2	6.4	54.2

Bulk soil data taken from Sleutel et al. (2008).

[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-esters; ALKY: alkylaromatics; NCOMP: mainly heterocyclic N-containing compounds; STER: sterols; PEPTI: peptides; SUBE: suberin; FATTY: free fatty acids.

[b] TII: Total ion intensity expressed in 10^6 counts mg^{-1} sample.

[c] %VM: Percentage matter volatilized during pyrolysis.

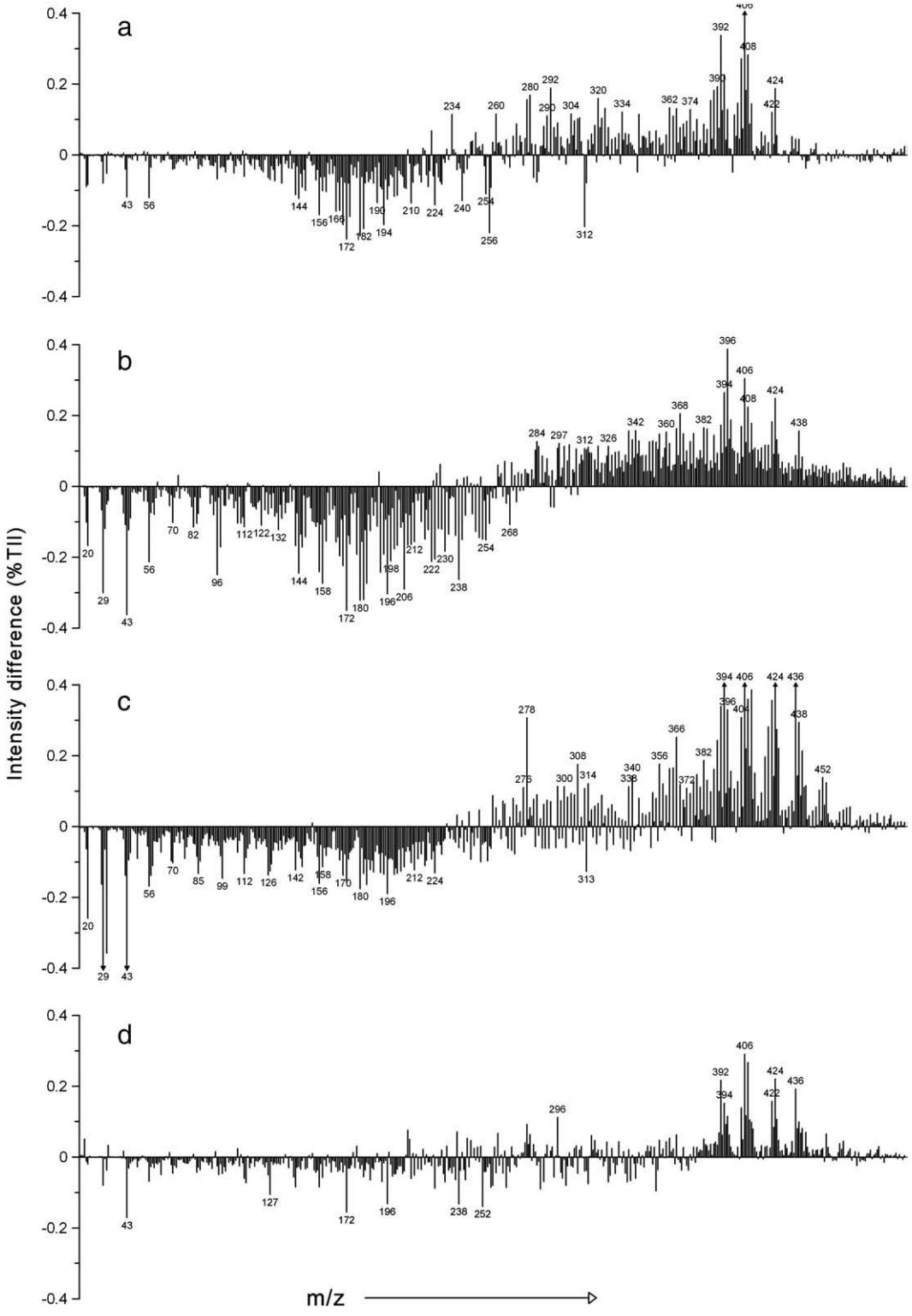

Fig. 1. Difference pyrolysis-field ionization mass spectra of 4 sandy topsoils (a: WH, b: WC, c: KH, d: KC): spectra before oxidation with 6% NaOCl minus spectra after oxidation. (Positive values indicate larger relative ion intensities in the non-oxidized samples; negative values indicate larger relative ion intensities in the oxidized samples).

Py-FIMS mass spectra (spectra of NaOCl-treated minus NaOCl+HF-treated samples) (Fig. 2). There were no consistent shifts in the difference mass spectra among all four soils but instead these shifts were clearly site specific. For the WH and WC soils (Wingene site) there was a prominent selective loss of low-mass signals, mainly situated between *m*/*z* 96 and *m*/*z* 280 and a selective enrichment of higher masses between *m*/*z* 300 and *m*/*z* 500. Pronounced losses were found for signals tentatively assigned to phenols and lignin monomers (*m*/*z* 168, 178, 180, 182, 196, 208), homologous series of shorter-chained lipids, namely *n*-C_{15} to *n*-C_{21} alkenes (*m*/*z* 210–294), *n*-C_{15} to *n*-C_{19} alkanes (*m*/*z* 212–268) and C_{10} to C_{15} *n*-alkyl diesters (*m*/*z* 202–272), and to a lesser extent for other lipids (such as *m*/*z* 278, 296). Increases were found for mass signals attributable to long-chained lipids, namely *n*-C_{20} to *n*-C_{29} fatty acids (*m*/*z* 312–438), C_{18} to C_{24} *n*-alkyl diesters (*m*/*z* 314–398) and

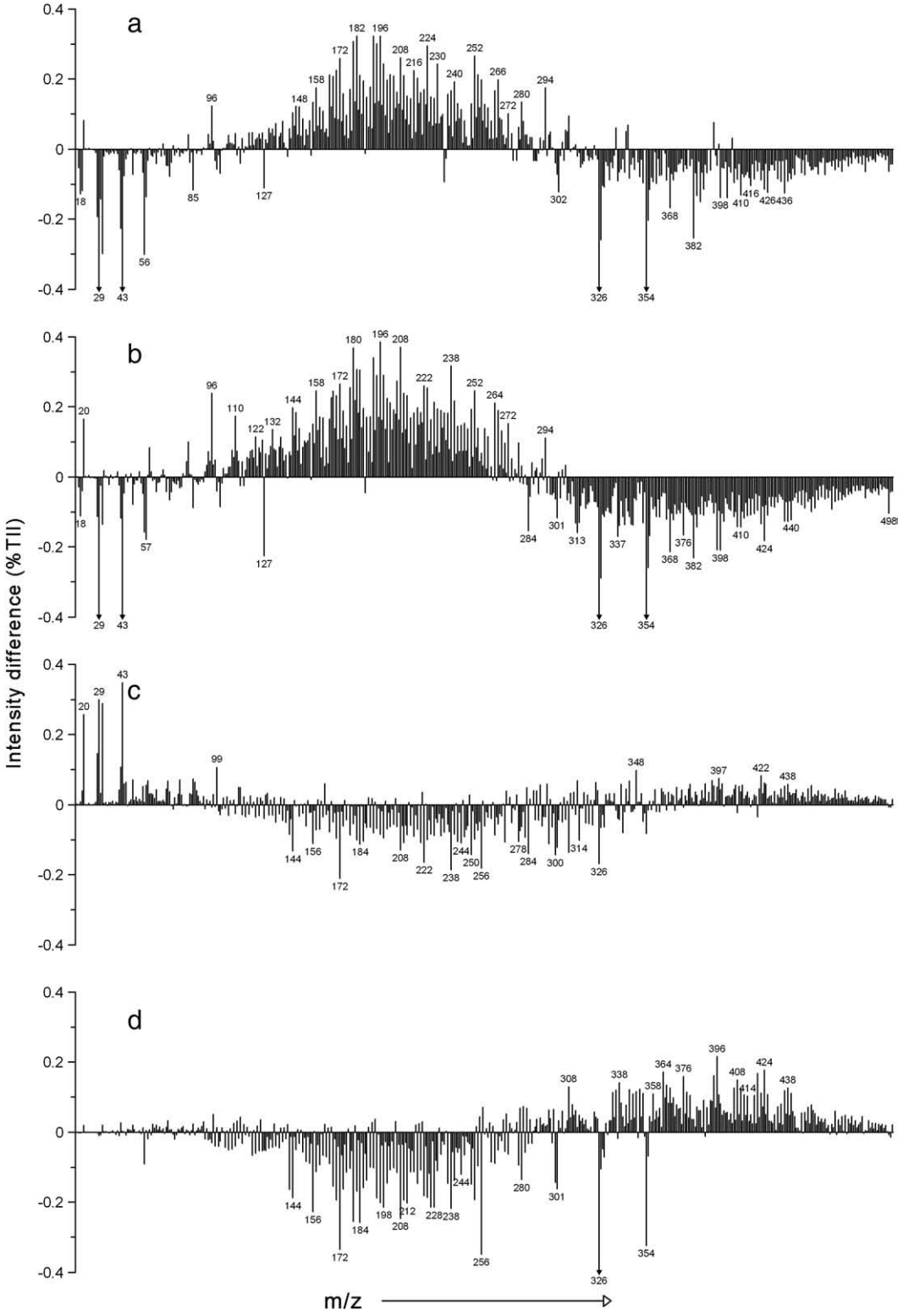

Fig. 2. Difference pyrolysis-field ionization mass spectra of 4 sandy topsoils (a: WH, b: WC, c: KH, d: KC): spectra after oxidation with 6%NaOCl minus spectra after oxidation with 6% NaOCl and subsequent 10% HF extraction. (Positive values indicate larger relative ion intensities in the non-extracted samples; negative values indicate larger relative ion intensities in the extracted samples).

n-C_{24} to *n*-C_{31} alkanes (*m*/*z* 338–436), the latter mainly in WC. Several of these masses overlapped with marker peaks for lignin dimers and sterols.

As opposed to the WH and WC soils, 10% HF treatment of the KH and KC soils resulted in enrichment of signals in the range *m*/*z* 140–300. These included several signals attributable to phenols and lignin monomers (*m*/*z* 178, 182, 196, 208, 210, 212), alkylaromatics (*m*/*z* 142, 144, 156, 170, 184, 198, 220, 246) and lipids (*m*/*z* 224, 226, 238, 240, 244, 252, 256) and to signals that could be attributed to either lipids or lignin dimers (*m*/*z* 272, 248, 296, 300, 314). In the higher mass range there was a selective loss of masses that can be assigned to long-chained lipids, namely *n*-C_{24} to *n*-C_{29} fatty acids (*m*/*z* 368–438), C_{22} to C_{27} *n*-alkyl

diesters (*m*/*z* 370–440) and *n*-C_{24} to *n*-C_{31} alkanes (*m*/*z* 338–436), mainly in the KC and to a much lesser extent in the KH soil. Several intense negative low-molecular weight difference peaks for WH and WC might indicate the presence of OM fragments in the HF-treatment residues (*m*/*z* 29: ethyl ion, 43: propyl ion, 57: butyl ion). Lastly, two intense negative peaks at *m*/*z* 326 and 354 (*n*-C_{21} and *n*-C_{23} fatty acid respectively) dominated the difference spectra of the WH, WC and KC soils (Fig. 2).

3.5. Thermogram data

The temperature course of the detected ion intensity of individual mass signals, as derived from Py-FIMS, gives an indication of the thermal energy required for the volatilization of individual biomarkers of SOM. In the untreated WH and WC soils the detection of ion intensity started at 280 °C and in the KH and KC soils at 240 °C (Fig. 3). An earlier volatilization was found in both the 6% NaOCl treated as well as in the NaOCl+HF treated samples, namely at 230 °C for the WH and KH soils and 210 °C for the WC and KC soils. Thermograms had a clear bimodal profile in all samples investigated. A first volatilization maximum occurred at 350 to 400 °C and a second one at 480 to 510 °C. There were no distinct shifts in the peak temperature of these maxima after either 6% NaOCl or subsequent 10% HF treatment, however the size of the first peak was clearly reduced in the 6% NaOCl treated samples as compared to the untreated samples, especially in the WH and KC soils. There was a tendency for higher temperature volatilization, i.e. at >550 °C in the 6% NaOCl treated samples as compared to the untreated samples. Specific thermograms of integrated masses were tentatively assigned to the major biomacromolecules present in these soils, namely lignin dimers, alkylaromatics, phenols and lignin monomers and lipids (Fig. 4). Shifts by 6% NaOCl treatment in thermograms for lignin dimers and alkylaromatics marker signals were limited, except for accumulation of very thermostable matter (>550 °C) in the WH and KC soils, but this OM was removed by subsequent HF extraction. Oxidation by 6% NaOCl lead to pronounced increased volatilization of phenol and lignin monomer marker signals in the 400 to 550 °C range except for the KC soil. In this temperature range, treatment with HF greatly reduced the volatilization of phenols and lignin monomers in the WH and WC soils but increased volatilization in the KH and KC soils. Consistent lower volatilization of marker peaks tentatively assigned to lipids in the 300 to 400 °C temperature range occurred in the 6% NaOCl treated samples compared to the untreated soils, but subsequent 10% HF treatment caused a relative increase of these signals. For all but the KH soil an increased volatilization in the lower temperature range (170 to 300 °C) was observed for lipid markers.

4. Discussion

The difference mass spectra (Figs. 1 and 2) revealed a clear cut-off between trends in low and medium molecular weight SOM building blocks and high molecular weight SOM building blocks upon treatment by both NaOCl and HF. Both are therefore discussed separately.

4.1. Shifts in low and medium molecular weight SOM building blocks

A relative enrichment of phenols and lignin monomers in the 6% NaOCl oxidized samples when compared to the untreated soils was suggested from the %TII difference spectra. Mikutta et al. (2006) observed that treatment with 6% NaOCl dramatically reduced lignin-derived phenols, and these were preferentially removed when compared to bulk OC. These seemingly contradictory observations might be explained by cleavage of lignin dimers into monomers as will be discussed below. Because of the gradual volatilization combined with soft field ionization used in Py-FIMS, separate measurement of dimeric and monomeric lignin is uniquely possible by Py-FIMS. Other techniques such as Curie point flash pyrolysis GC-MS and Cu-oxidation combined with GC-MS only analyze monomers whereas ^{13}C NMR only allows quantification of C functional groups.

The observed %TII increase of marker signals assignable to alkylaromatics would be logical since these molecules have been put forward as fundamental building blocks of humus macromolecules (Schulten et al., 1991). Consequently, these may therefore be less prone to chemical degradation by wet chemical oxidation. Oxidative degradation of OM by NaOCl, in the case of aromatic compounds, involves electrophilic substitution of chlorine, followed by loss of aromatic character and oxidative cleavage of the ring (Chefetz et al., 2002). The relative stability of alkylaromatics towards NaOCl can be explained by their alkyl-chain substitution whereas activated aromatics substituted with electron-withdrawing substituents like nitro, hydroxy, methoxy, cyano, and carboxyl substituents are more prone to oxidation (Chakrabartty and Kretschmer, 1974).

The large relative increase in the sum of carbohydrate marker peaks %TII by 43% and peptide marker peaks by 71% on average upon 6% NaOCl treatment seems surprising, since these compounds are often presumed to be biologically labile. However, potential explanations for relative inertness of carbohydrates and peptides to oxidation may involve mineral protection and their occlusion in the macromolecular structure of humic substances. As such, it is clear that any simple chemical fractionation such as oxidation by 6% NaOCl will fail to remove specific labile SOM constituents exclusively.

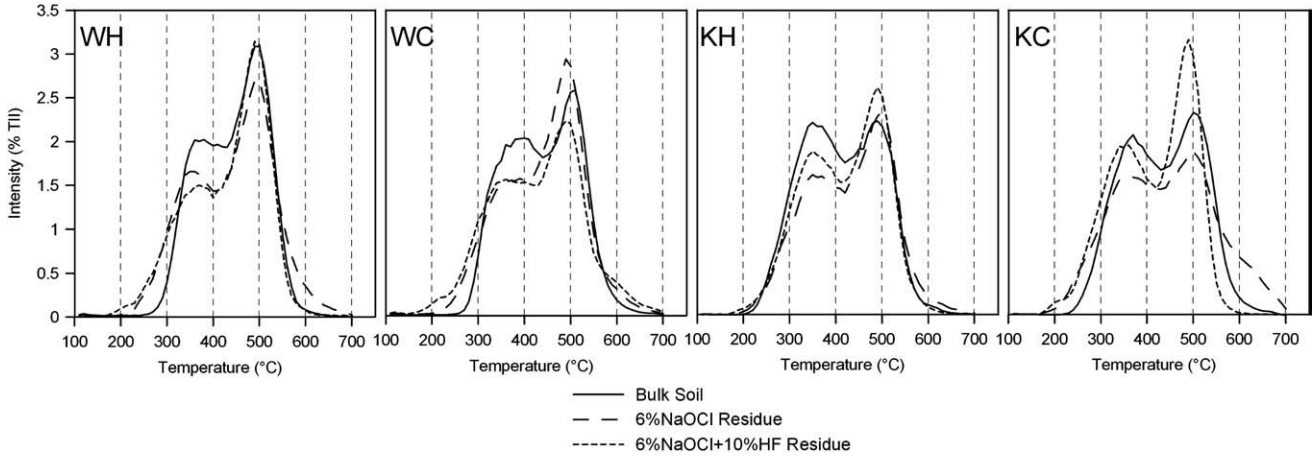

Fig. 3. Thermograms for the volatilization of SOM for the WH, WC, KH and KC bulk soils, oxidation residues of 6% NaOCl treatment and residues of the extraction of 6% NaOCl residues by 10% HF.

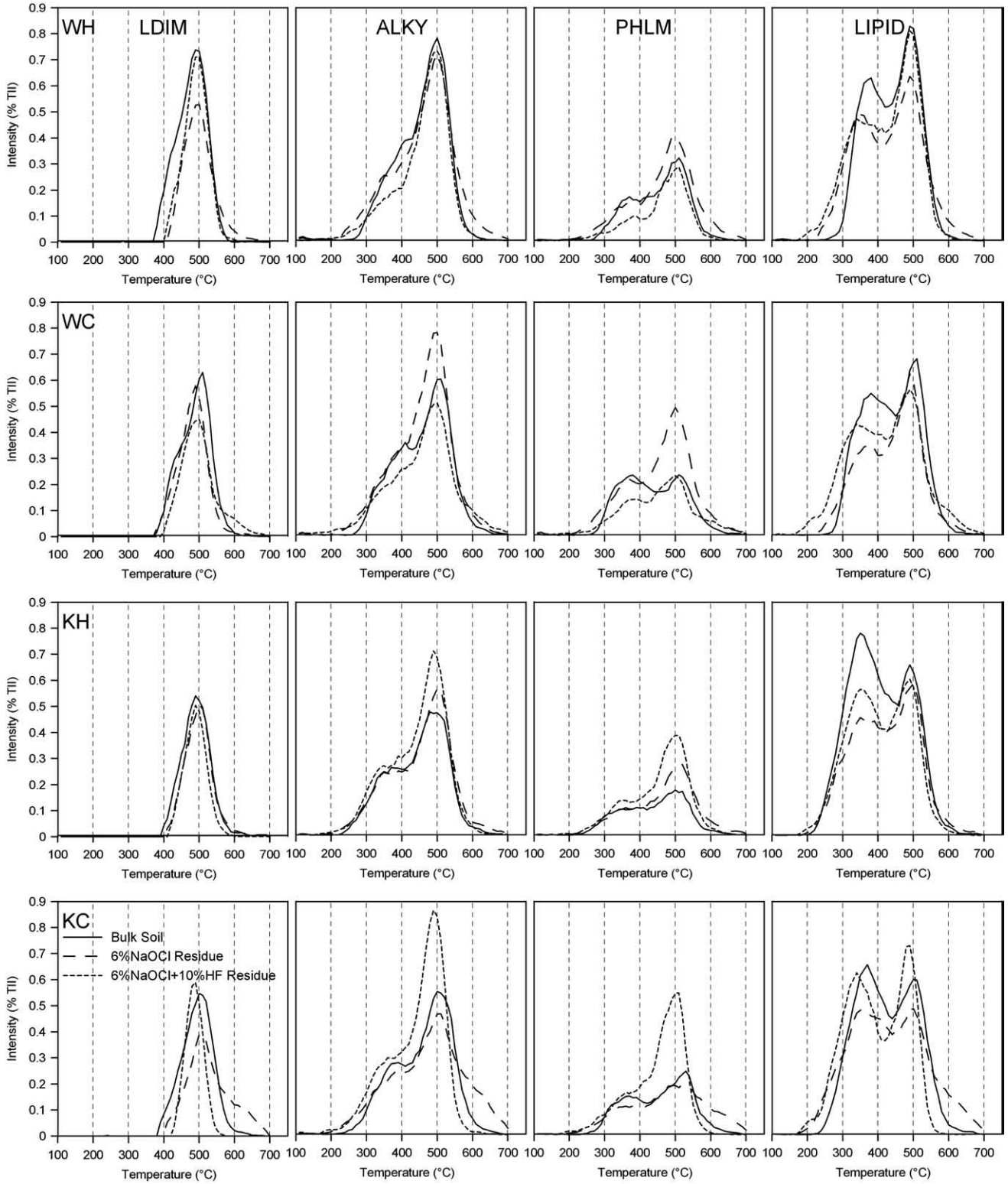

Fig. 4. Thermograms for the volatilization of selected tentatively assigned compound classes (LDIM: Lignin Dimers; ALKY: alkylaromatics; PHLM: Phenols and Lignin Monomers; LIPID: Lipids, alkanes, alkenes, fatty acids and *n*-alkyl esters for the WH, WC, KH and KC bulk soils, oxidation residues of 6% NaOCl treatment and residues of the extraction of 6% NaOCl residues by 10% HF.

Further information was derived from characterization of the 10% HF-treatment residues by Py-FIMS. The strong relative decreases in % TII of marker peaks tentatively assigned to phenols and lignin monomers and carbohydrates by treatment with 10% HF in the WH and WC soils could indeed indicate that they were mineral-bound, which would explain their stability against oxidation by NaOCl. However, concomitantly considerable selective removal of alkylaromatics occurred with 10% HF treatment. Because these are considered to be structural building blocks of humic compounds, it cannot be excluded that instead the HF just extracted humic compounds and

carbohydrates occluded in humus macromolecules. It cannot be concluded that the same mechanisms operate in the KH and KC soils, in which there was a relative increase in TII for carbohydrate maker signals and no relative increase of peptide maker signals following extraction with 10% HF. Instead, HF treatment of these soils yielded enrichment of almost all signals in the *m*/*z* 140 to *m*/*z* 300 range. Included were ample signals that are assignable to phenols and lignin monomers, alkylaromatics and lipids. Mikutta et al. (2006) found that after 6% NaOCl oxidation the remaining small proportion of lignin-derived phenols was virtually completely removed by 10% HF treatment, which suggests a small stable lignin pool to be protected by mineral association. While in line with the data for the WH and WC soils, the observations for the KH and KC soils are completely contradictory. A possible explanation for the contrasting results of the HF treatment between the two sites may be that the efficiency of removal of SOM constituents by the preceding NaOCl treatment affects the outcome of the HF treatment. For example, in contrast to all three other soils, for the KC soil a selective removal of high molecular weight SOM components by HF treatment was preceded by a non-selective destruction of SOM by NaOCl treatment. A poor efficiency of the NaOCl oxidation to remove these compounds was compensated by a selective removal by subsequent HF-treatment. In contrast, for the WH and WC soils, NaOCl treatment did result into a selective removal of high molecular weight compounds which then was not followed by any further selective removal upon HF treatment. At the very least, our results show that reactions of SOM with HF can be very much site specific. Apart from N in peptides, the difference spectra suggested that N was also enriched in the 6% NaOCl-residues in heterocyclic N containing compounds, in spite of the higher C:N ratios of these residues compared to untreated samples. No selective loss of peptides by HF extraction was observed here, in contrast to other studies in literature. Chesire et al. (2000) concluded that some amino acids were protected against degradation possibly within microaggregates or by interaction with mineral surfaces. Mikutta et al. (2006) came to a similar conclusion for N which survived 6% NaOCl-treatment, because it was effectively and preferentially removed during mineral dissolution with 10% HF. The combined selective enrichments of heterocyclic N-containing compounds and alkylaromatics in the 6% NaOCl residues match the conceptual vision on the structure of humic substances by Leinweber and Schulten (1998). If N heterocycles and alkylaromatics are structural parts of larger humus molecules, their resistance to oxidation is straightforward.

4.2. Shifts in high molecular weight SOM building blocks

Upon oxidation with 6% NaOCl, there was a clear decrease in %TII of mass signals assigned to lignin dimers in all four soils (Table 2 and Fig. 1), which suggests these SOM components to be selectively oxidized by NaOCl. These results are in line with previous findings by Norwood et al. (1987), who noted that chlorination of aquatic fulvic acid produced chlorinated aliphatics and resulted in preferential removal of lignin phenols. Westerhoff et al. (2004) as well observed a higher reactivity of aqueous chlorine for aromatic compounds than for aliphatic compounds in natural organic matter. The ratio of %TII attributed to mass signals from phenols and lignin monomers to signals from lignin dimers has been found to increase with progressive decomposition of SOM (Leinweber and Schulten, 1995). This ratio was 0.7 in both WH and KH soils and 0.8 in both WC and KC soils. The 6% NaOCl treatment increased this ratio to 1.3 and 1.1 in the WH and KH soils, respectively, and to 1.6 and 1.0 in the WC and KC soils, respectively. This shift from dimeric to monomeric lignin may explain the relative increase in %TII assigned to phenols and lignin monomers, as discussed above. Our study thereby refines results by previous workers (Mikutta et al., 2006; Rumpel et al., 2006) in that not all lignin components are equally oxidized. Furthermore, from this perspective, treatment with 6% NaOCl does seem to be able to mimic biological degradation of lignin.

Additional decreases in high mass peaks, consistent over all four soils, were mainly derived from losses of marker signals assignable to sterols and long-chained lipids. Many sterols can be accounted for as very labile components of SOM and hence their removal by 6% NaOCl is in line with the vision that this chemical oxidation removes younger, labile SOM. Several lipids as well have shorter residence times than SOM (Wiesenberg et al., 2004). Particularly free fatty acids and short chained lipids (alkenes, alkanes, alkyl-esters, bound alkanoic acids) do not contribute to a stable SOM pool. Difference spectra did not suggest any of these short chained lipid components to have been selectively lost upon oxidation by 6% NaOCl. By comparing fatty acid patterns from OM inputs (manure, plant residues) and the whole soil, Jandl et al. (2005) concluded that long-chained fatty acids (n-C_{21} to n-C_{34}), probably derived from plant waxes, seem to be the only source of stable fatty acids in soil. The presence of considerable proportions of homologous series of C_{10} to C_{20} n-alkyldiesters, n-C_{20} to n-C_{30} alkanes and alkenes and n-C_{16} to n-C_{30} fatty acids in the KC and WC soils, even after long-term cultivation, indeed proves their biological stability in these soils (Sleutel et al., 2008). Schulten and Schnitzer (1991) as well found these compounds to occur in natural waxes that are preserved in SOM. Treatment with 6% NaOCl, however, lead to a selective loss of signals attributable to long-chained fatty acids (n-C_{26} to n-C_{30}) and to a lesser extent long-chained alkanes (n-C_{26} to n-C_{30}). In this sense, 6% NaOCl partly removed the stable assumed lipids while leaving the labile shorter chained lipids relatively unaffected.

Treatment with 10% HF showed a different pattern for the Wingene (WH and WC) and Knesselare (KH and KC) sites, as was the case for the low to medium molecular weight-part of the Py-FIMS spectra. Completely opposite to the action of 6% NaOCl, %TII of signals tentatively assignable to long-chained fatty acids, alkanes and alkyldiesters was relatively increased in the 10% HF-residues of the WH and WC soils, although a partial overlap with marker peaks from lignin dimers and sterols cannot be excluded. Based on pyrolysis GC/MS analysis Rumpel et al. (2006) as well found that aliphatics dominated the pyrolysis products of a 10% HF-treated forest Cambisol Ah horizon. In contrast, Zegouagh et al. (2004) observed that the distribution patterns of those moieties in OM of a silt loam cropland soil were unaffected by 2% HF. However, thick coatings of OM in clay fractions of such OM rich A horizons may protect the minerals from dissolution in HF (Eusterhues et al., 2007), and hence absence of compositional change could also be due to incomplete action of the HF as a mineral dissolving agent. A clear interpretation of these and our results is also hampered by the possibility that long-chained lipids may have a low reactivity because of their poor solubility in HF. In addition, as stated above, opposite results were found for the KC soil with decreases in high-molecular weight mass signals assignable to longer-chained lipids making data interpretation even more problematic.

4.3. Thermal stability

The bulk soil thermograms (Fig. 3) revealed the relative accumulation of both very thermolabile (<300 °C) as well as very thermostable (>550 °C) material (mainly in WH and KC) by treatment with 6% NaOCl. The increased ion intensity above 550 °C might, however, not be solely attributed to a relative enrichment of specific thermostable SOM components because volatilization above 550 °C was absent in the untreated samples. Instead, this high temperature volatilization might also originate from organic matter, that was newly formed by chemical treatment. Leifeld and Zimmermann (2006) showed for <63 µm fractions of eight mineral soils that the susceptibility of OM towards oxidation with NaOCl does not relate to its thermal stability. Our results for the bulk SOM are partly in line as just a weak consistent rise in thermal stability was observed after 6% NaOCl treatment. Subsequent 10% HF treatment resulted in very inconsistent shifts in thermal stability among the four investigated soils, which hampers interpretation of these data. Since thermal

stability of SOM may be associated with its biological stability (Plante et al., 2005; Leifeld et al., 2006; Leinweber et al., 2008) the absence of a clear expected enrichment of thermostable OM might suggest that 6% NaOCl or 10% HF treatment did not specifically isolate biologically stable SOM fractions.

Regarding individual SOM compound classes, first, the selective loss of %TII assigned to thermolabile lipids (300 to 400 °C) with 6% NaOCl treatment, that occurred in all soils (Fig. 4), indicates that weakly bound lipids were selectively removed. Second, at the same time a relative increase in %TII assigned to thermostable phenols and lignin monomers (450 to 600 °C) was found for the WH, WC and KH soils. These observations, in contrast to bulk SOM data, would confirm the success of oxidation by 6% NaOCl as a means for isolation of a more biological stable fraction. Yet, unequivocal verification of this biological stability, e.g. by incubation experiments with NaOCl residues, is problematic due to a severe risk of artifact generation. Subsequent 10% HF treatment resulted in relative enrichment of thermostable phenols and lignin monomers (450 to 550 °C) and of thermostable alkylaromatics (400 to 500 °C) for the KH and KC soils only. No similar observations were made for other important SOM constituents such as lignin, carbohydrates or N-containing compounds.

4.4. Considerations on the molecular level characterization of chemical SOM fractions

In theory, CO_2 and H_2O are the end products of complete organic matter oxidation but a variety of by-products form during the chemical treatments. For example, side chain cleavage and adsorption of fragments might explain the prominent negative peaks of ethyl, propyl and butyl carbocations in the (bulk soil–6% NaOCl) residue difference spectra. Another indication is given by the consistently higher %TII ratios of acid to aldehyde side chain-containing vanillin lignin monomers in the NaOCl residues (1.75) compared to the whole soil samples (2.27), which suggests increased side-chain oxidation of the phenylpropane unit (Hedges et al., 1988). Next, when natural OM is treated with hypochlorite species (HOCl, OCl), high-molecular-weight chlorinated compounds form (Li et al., 2000). Chlorination of OM by NaOCl was probably confined because the pH was maintained at 8 (Mikutta et al., 2006), but nonetheless must have been significant.

Changes following HF treatment may include hydrolysis of organic compounds and melanoidin formation by recondensation of proteins and polysaccharides (Rumpel et al., 2006). The increased volatilization of very thermostable (>550 °C) material (mainly in WH and KC) after treatment with 6% NaOCl could point to newly formed SOM during chemical fractionation. Leifeld and Zimmermann (2006) suggested instead this thermostable SOM remaining after NaOCl to be 'black carbon' or other condensed aromatic structures.

An additional issue on the HF extraction of mineral and mineral-bound SOM concerns the differing solubility of specific organic molecules. It is well known that all common amino acids and many proteins and cellulose, starch and D-glucose are highly soluble in HF, (Lenard, 1969). Lipids in contrast, could either be expected to be initially mineral-bound, but remain insoluble after dissolution of the mineral matrix (Eusterhues et al., 2007) or could intrinsically not be mineral-bound but rather stabilized in HF-resistant SOM structures.

Finally, next to potential artifacts remaining in the chemical fractions, differences in detectability of SOM constituents might bias results. In the case of Py-FIMS, a lower sensitivity in fractionation residues might occur. When the TII was recalculated relative per mass unit C, however, no loss of signal for the NaOCl (1121 10^6 counts mg^{-1} C) and NaOCl + HF treated samples (3085 10^6 counts mg^{-1} C) compared to the untreated samples (918 10^6 counts mg^{-1} C) was found. Instead, the much higher sensitivity for the HF-treated samples may reveal a general destabilization of the SOM, probably by dissolution of complexing Fe and Al ions or other mineral constituents.

5. Conclusions

Pyrolysis Field Ionization Mass Spectroscopy analysis showed that the composition of SOM is severely altered both by oxidation by 6% NaOCl as well as by subsequent 10% HF extraction. The selective loss of signals from sterols, dimeric lignin components and thermolabile lipids upon NaOCl oxidation, as suggested from the difference spectra and thermograms, makes sense from a biological perspective. The increased ion intensity attributed to carbohydrates, peptides and short-chained lipids shows that none of these often labile assumed SOM constituents are completely removed by treatment with NaOCl. The 10% HF treatment yielded some expectable findings such as increased volatilization in the thermostable region for marker signals of phenols and lignin monomers and of heterocyclic N-containing compounds and thermostable alkylaromatics, both presumed structural building blocks of humic substances. However, even for the sites in this study, which are very similar in soil texture, climate, land-use, drainage and contents of pedogenic oxides, very contrasting results were found for other SOM constituents. Hence, this study revealed the difficulties of interpreting SOM transformations that take place with wet chemical fractionations aimed at selective removal of SOM components by oxidation or extraction. This stresses a need for improved fractionation schemes and suggests more emphasis might need to be put on studies of thermal stability of SOM.

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