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Podzolisation and exportation of organic matter in black waters of the Rio Negro (upper Amazon basin, Brazil)

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Abstract In the Rio Negro basin, podzols develop at the expense of clay-depleted laterites through localized and spectacular weathering fronts. This natural process leads to the remobilization of previously accumulated organic matter (OM) which is redistributed within soil profiles and exported towards rivers, hence their typical black coloration. We investigate the fate of OM in the soil–water continuum in the Rio Negro basin through description of the composition of OM potentially mobile at different stages of podzol development and exploration of evidence for contributions from different soil horizons to the exportation of OM into waters. OM was water-extracted from seven key soil samples from a sequence representative of the transition between latosol and podzol on the low elevation plateaux of the Rio Negro basin, thus enabling following both vertical and lateral differentiations. The chemical structure of freeze-dried samples, investigated using ^{13}C nuclear magnetic resonance and thermally assisted hydrolysis and methylation (THM), shows contrasting features depending on the horizon considered. The bulk features of water extracts were first compared with samples collected in the water-tables and rivers draining the soil sequence. A molecular level comparison was then performed with groundwater draining the well-developed podzol. This approach evidenced a contribution from deep horizons of well-developed podzols. It highlights that OM is certainly remobilised after being accumulated in Bh horizons during the development of podzols through accumulation of OM redistributed from surface horizons. The identification of specific compounds opens new perspective to trace a "podzolic origin" of OM in drainage networks.

Keywords Amazon basin · Black waters · Podzols · Water-extractable organic matter

Introduction

The Rio Negro is one of the most famous black-water rivers in the world. A few studies have investigated

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the properties of organic matter in its waters, showing that it is responsible for its relatively strong acidity (pH $\leq$ 5), mostly colloidal (Eyrolle et al. 1996) and that it exhibits a rather high aromaticity (Benedetti et al. 2002). Chauvel et al. (1987) and McClain et al. (1997) suggest that the properties of OM in these black waters are directly linked with soil-forming processes occurring in this region. Within the whole Rio Negro basin, podzols develop at the expense of lateritic formations (e.g. Lucas et al. 1987; Fritsch et al. 2002). The podzolisation process leads to the relative accumulation of Si and organic matter, remobilization and translocation of previously accumulated Fe, Al through organic complexation. Due to the high porosity of podzols and high rainfalls in this region, organic matter can easily be transferred from podzols to river networks and cause the black coloration of waters.

Downstream the Rio Negro, at the margin of the strongly podzolized area, podzols were shown to occur in two different landscape positions. They were first described in footslope position (e.g. Lucas et al. 1987; Righi et al. 1990). They are freely drained and develop upwards at the expense of lateritic covers. In such podzols, Briand (1995) used sterols as biomarkers to show that OM is remobilized from podzolic Bh horizons and exported towards rivers. More recently, waterlogged podzols lying in depressions of low elevation plateaux were studied by do Nascimento et al. (2004). They are formed at the expense of higher elevated and well-drained clay-depleted laterites. These dynamics are linked to the development of reducing and acidic conditions in shallow perched groundwater seated on a weakly permeable layer and connected to the permanent river. During the wet season, the perched groundwater reaches the surface whereas in dry season, only the deep podzolic horizons are in contact with water (do Nascimento et al. 2008). Previous investigations of a soil sequence representative of the transition between latosols and podzols (do Nascimento et al. 2004; Bardy et al. 2007, 2008a) enabled highlighting the main steps involved in the podzolisation of these clay-depleted laterites. It further showed that within soils, podzolisation leads to a strong variation of OM characteristics and it led to the determination of key samples in three different pits of the sequence (Bardy et al. 2008a, b). However, little is known about the structure of OM transferred to rivers and about the contributions of the different soil horizons to this exportation.

The present study aims at investigating the fate of OM in the soil–water continuum in waterlogged podzols of the Rio Negro basin with two main objectives:

(i) Describing the composition of OM water extracted from soils likely to be exported at different stages of podzol development, as no such reference exists for tropical podzols.
(ii) Identifying horizons acting as a source of exportation of OM from soils to rivers. In particular, it is possible to evidence contributions of OM from (a) surface or deep podzolic horizons and (b) poorly-developed podzols in transition zones where podzolisation is most active or from downslope and better-differentiated, waterlogged, podzols? A priori deep podzolic horizons are formed through accumulation of OM and should act as a sink more than as a source of OM. Surface horizons are therefore expected to act as a major provider of OM to the drainage network.

To achieve this, the study focuses on the representative soil sequence previously described by do Nascimento et al. (2004, 2008) and Bardy et al. (2007, 2008a, b). OM was water-extracted from seven key samples that enable following both vertical and lateral differentiations. Samples were also collected in the perched groundwater and brooks draining the soil sequence, both in the wet and dry seasons. Different properties of water-extracted OM (WEOM) were determined and compared with those of OM present in groundwater and surface water of the drainage network. Specific UV Absorbance (SUVA), pH and conductivity of aqueous samples were measured. Freeze-dried samples were then investigated using solid-state ^{13}C nuclear magnetic resonance (NMR) and thermally assisted hydrolysis and methylation (THM) to gain detailed structural information. Taken together, the results enable highlighting contributions from the different podzolic horizons to the exportation of OM to the drainage network, and add to our understanding of the fate of OM of the soil–water continuum in the Amazon basin.

Materials and methods

Soil samples

A precise description of the investigated site can be found in do Nascimento et al. (2004). Briefly, it is located on the low elevation plateaux of the Jau National Park (upper Amazon basin, Fig. 1a). It is preserved from human activity and has a hot humid tropical climate with mean annual temperature and precipitation of respectively 26°C and 2,000 mm. The studied samples belong to a 200 m long and 2–3 m deep soil sequence along which podzolic soils develop at the expense of yellow clay-depleted laterites (Fig. 1b). Podzols are located in a depression while laterites remain in higher positions.

Seven organic-rich horizons were selected in two profiles (II, III) along the sequence (Fig. 1b). They belong either to subsurface organo-mineral horizons (A) or deep illuviated horizons (B, BC) with accumulations of OM (suffix h) and/or metals (Fe and Al, suffix s). Two (II A12, II Bhs) come from the upper part of pit II and belong to horizons of weakly expressed podzols at the margin of the depression. The other five (II Bh, II 2BCs, III A12, III Bh, and III 2BCs) are specific to horizons of well expressed podzols sampled at the bottom part of pit II and towards the centre of the depression on the whole profile of pit III. Soil samples were collected from the freshly cleaned faces of soil pits. They were air-dried, sieved through a 2-mm screen and stored in the dark in sealed glass jars prior to analyses.

Water-extractable organic matter

Water-extractable organic matter can be obtained using different methods: by centrifugation of field-moist samples or by shaking soil with deionised water and subsequent centrifugation. This second approach was chosen so that the yields were sufficient for analysis (Zsolnay 1996; Nierop and Buurman 1998).

Soil samples were shaken end-over-end overnight with a soil:water ratio of 1:3 w:w. After centrifugation at 2,200*g* for 20 min. the residue was shaken end-over-end in the same conditions for 2 h and submitted to centrifugation at 2,200*g* for 20 min. The supernatants were combined to form WEOM. An aliquot of WEOM was freeze-dried for further analysis. WEOM was not filtered so as to include the colloidal/particulate OM and allow comparison with groundwater.

Water sampling and physico-chemical parameters

Groundwater from pits II and III was sampled as well as waters from eight brooks draining the soil sequence or connected to the Jau River in the area, both in the low- and high-water seasons (resp.

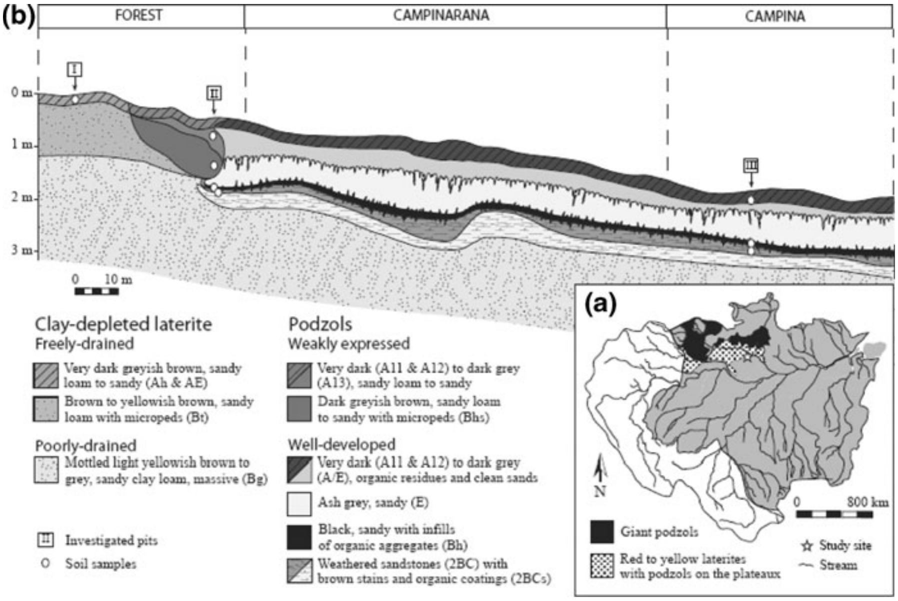

Fig. 1 **a** Site location (*star*) on a simplified soil map of the Amazon basin, **b** simplified representation of the soil catena from a high to low elevation point of the plateau with investigated horizons (*white dots*). Adapted from do Nascimento et al. (2004)

October and May). Groundwater was sampled in soil pits after emptying them and allowing them to replenish. Pits II and III were sampled in the high-water season while only pit III was sampled in the low water season, since no permanent perched groundwater could be observed in pit II. Groundwater level fluctuation is discussed in detail in do Nascimento et al. (2008).

Conductivity and pH were measured in the field on fresh bulk samples of both groundwater and river water. Groundwater was concentrated in the field using reverse osmosis, with a concentration factor of about 15. Aliquots were freeze-dried for further analysis.

Soil pH measurement

Soil pH was measured in water with a 1:2.5 soil:water ratio.

SUVA

SUVA is defined as UV absorbance at 254 nm divided by dissolved organic carbon (DOC) content. UV absorbance at 254 nm was measured using a Varian Cary 5G spectrometer using 1 cm plastic tubes. DOC was measured using a Shimadzu Vsh total organic carbon analyser.

Solid-state ^{13}C nuclear magnetic resonance

Solid-state cross polarisation (CP) magic angle spinning (MAS) ^{13}C NMR spectra were recorded on a Bruker AV-400 NMR spectrometer operating at 100.6 MHz with a spinning-speed of 15 kHz. A ramped ^{1}H pulse was used to minimise Hartmann–Hahn mismatching (Metz et al. 1994). The experiments were carried out with a contact time of 1 ms, a 90° pulse of 3.4 µs and a recycle delay of 3 s. Chemical shifts were expressed relative to tetramethylsilane (= 0 ppm) and calibrated with glycine (= 176 ppm).

Thermally assisted hydrolysis and methylation: gas chromatography–mass spectrometry (GC–MS)

Prior to pyrolysis, the sample (ca. 2 mg) was thoroughly crushed in a mortar with a large excess (ca. 80 µl) of tetramethylammonium hydroxide (TMAH, 2.5 wt% in water) and freeze-dried prior to loading onto the ferromagnetic wire. Thermally assisted hydrolysis was performed using a Pylodist CPP Curie point flash pyrolyser for 10 s using ferromagnetic wires with a Curie temperature of 610°C under a 15 psi He flow. The pyrolysis unit was directly coupled to the GC/MS system: Thermo Electron Trace GC ultra gas chromatograph (30 m RTX5 Sil MS capillary column, i.d. 0.25 mm, film thickness 0.5 µm) and Thermo Electron Trace DSQ mass spectrometer (electron energy 70 eV, ion source temperature 220°C, scanning from *m/z* 40 to 650, 0.7 scan/s). The temperature of the GC oven was programmed from 50 to 300°C at a rate of 2°C min^{-1} after a first stage at 50°C for 10 min to allow better separation of the most volatile pyrolysis products.

Results and discussion

Spectroscopic and pyrolytic features of water-extracted organic matter

Solid-state ^{13}C NMR was used to reveal the main functional groups of OM, while pyrolytic studies enabled molecular-scale investigations.

Solid-state CP/MAS ^{13}C NMR

The spectrum of WEOM from surface II A12 (Fig. 2a) shows a sharp resonance at 33 ppm, indicative of long-chain aliphatic structures. However, it is dominated by an intense signal in the *O*-alkyl-C region centred at 72 ppm, associated with weaker signals around 62 and 102 ppm. These peaks dominate the spectra of polysaccharides like cellulose (e.g. Pastorova et al. 1994; Gauthier et al. 2003) and are attributed to C-2, C-3 and C-5 (72 ppm), C-6 (62 ppm) and to the anomeric C-1 (102 ppm) carbons. They thus reflect a major contribution from carbohydrates. A decrease in the ratio of the abundance of the 102 ppm peak to that of the 72 ppm indicates carbohydrate decomposition. In addition, a weak contribution at 128 ppm reflects the presence of aromatic compounds, while the peak at 174 ppm derives from carboxylic acids, esters or amides.

On the spectrum of WEOM from II Bhs (Fig. 2a), the sharp aliphatic peak is less intense than in II A12,

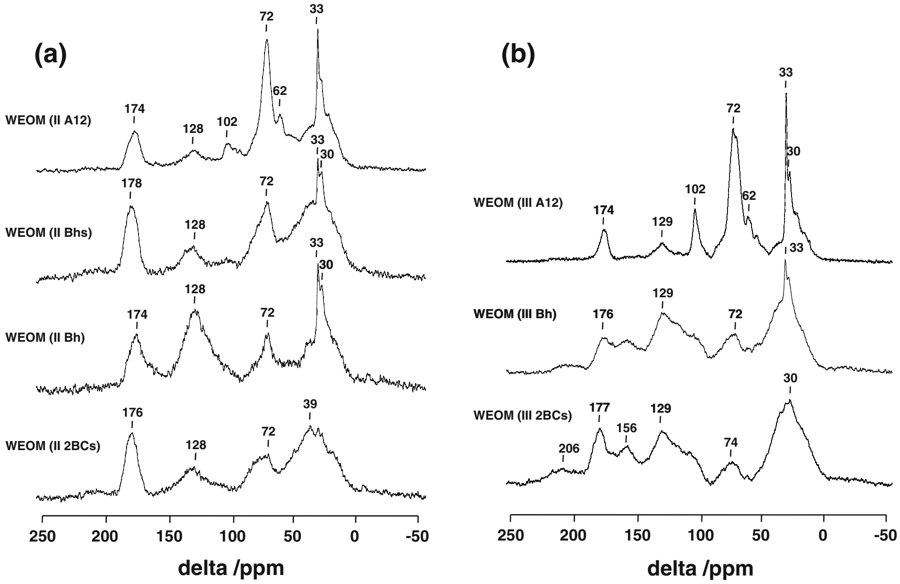

Fig. 2 Solid-state ^{13}C NMR spectra of water-extractable OM from **a** the transition zone (pit II) and **b** the well-developed podzol (pit III)

reflecting a lower abundance of long-chain polymethylenic structures. This peak is split into two resonances at 30 and 33 ppm that correspond respectively to amorphous and crystalline polymethylenic chains (Hu et al. 2000). Such patterns have been reported in peat humin and humic acids (Hu et al. 2000), and in the vegetal biopolymer cutin (Deshmukh et al. 2005). According to these authors, the occurrence of rigid chains reflects the cross-linked character of OM. A large resonance maximizing around 40 ppm is additionally superimposed to these sharp peaks, suggesting an important contribution from groups with tertiary and quaternary carbons (Skjemstad et al. 1992) as well as from ether bonds or amine groups. According to Skjemstad et al. (1992), this feature again reflects the presence of cross-linked OM. In WEOM from II Bhs, the major contributions arise from *O*-alkyl- and carboxylic-C (including amide groups). The aromatic signal at 128 ppm, although more intense than in II A12, is still moderate.

By contrast, WEOM from II Bh (Fig. 2a) exhibit a major peak of aromatic carbons (128 ppm), reflecting the aromaticity of organic matter already observed in the corresponding soil of these waterlogged podzols (Bardy et al. 2008a). The aliphatic signal, still arising from both amorphous (30 ppm) and crystalline (33 ppm) chains, is increased relative to II Bhs. On the opposite, the contribution at 72 ppm significantly decreased with respect to II A12 and II Bhs.

At last, WEOM from II 2BCs (Fig. 2a) has virtually no contribution from long polymethylenic chains, leaving only a larger resonance maximizing around 39 ppm, as the one mainly assigned to tertiary and quaternary carbons in WEOM from II Bhs. It additionally displays an important contribution from carboxylic functions at 176 ppm.

Like in IIA12, WEOM from III A12 (Fig. 2b) is dominated by a polysaccharidic contribution, with a sharper peak at 102 ppm, and a doublet at 72 ppm, as described for poorly-decomposed carbohydrates. Its spectrum is otherwise similar to that of WEOM from II A12, except for a weak resonance at 56 ppm, which could reflect the occurrence of C–N bonds in proteinaceous material, or the presence of epoxide groups like those observed in cutin (Deshmukh et al. 2005). This peak is also commonly assigned to methoxyl groups in lignin; however, such a contribution would be at most minor according to the low aromaticity of WEOM. It can be noticed that this peak at 56 ppm occurs throughout the profile in pit III.

The spectrum observed for WEOM from III Bh (Fig. 2b) is also similar to that of II Bh, except for an increased contribution of tertiary and quaternary carbons (around 39 ppm) relative to long-chain aliphatics (33 ppm), and an additional signal from phenolics at 156 ppm.

As for WEOM from III 2BCs (Fig. 2b), like WEOM from II 2BCs, it has a broad resonance in the

10–65 ppm range and a small contribution from *O*-alkyl carbons maximizing at 74 ppm, as well as a carboxylic resonance at 177 ppm. But it also additionally displays a larger aromatic resonance around 129 ppm, and contributions from phenolics at 156 ppm and carbonyls at 206 ppm.

^{13}C NMR spectroscopy offers a qualitative overview of the different functions borne by WEOM. The presence of amorphous polymethylenic chains along with broad resonances centred around 40 ppm points to the highly cross-linked character of the WEOMs, as the latter was suggested by Skjemstad et al. (1992), to be associated with highly cross-linked material. This cross-linked character is especially pronounced for WEOM from II Bhs, II 2BCs and III 2BCs. In both pits, ^{13}C NMR spectra of WEOM from the different horizons exhibit quite contrasting features reflecting diversity in the nature of their functional groups. It can first be noticed that the spectra of WEOM present many similarities with those of OM in clay-size fractions of the corresponding horizons (Bardy et al. 2008a). This is not contradictory, since WEOM can be considered as a subfraction of clay size OM. The abundance of carbohydrate-derived WEOM in surface horizons is in agreement with a dominant contribution of fresh organic matter. The decrease of the peak at 72 ppm with depth in both profiles reflects the fact that these compounds are easily metabolised by microorganisms, hence a rapid decrease in their concentration with depth (Nierop et al. 2001). The observation of large aliphatic resonances in ^{13}C NMR spectra of WEOM can be surprising due to the low solubility of such compounds. However, CP sequence is known to overestimate aliphatic contributions, which may thus not be as important as displayed on the spectra. Anyway, Piccolo et al. (1996) showed that aliphatic molecules could be dispersed in solution with a micelle-like organization providing enough hydrophilic moieties, like carboxylic groups, were present. Such aliphatic units might also reflect a root contribution as suggested by Buurman and Jongmans (2005). However, the root density is very low in those deep podzolic horizons and it must be noted (see below) that no suberin-derived products could be detected through THM. Concerning aromatic contributions, they are most abundant in Bh and BCs horizons. The phenolic resonances observed in spectra of WEOM from III Bh and III 2BCs point to the contribution of altered lignin moieties or other polyphenols. Lignin-derived moieties usually contribute to dissolved OM from terrestrial environments (e.g. Nierop and Buurman 1998), and even act as markers for OM of terrestrial origin (e.g. Hedges and Parker 1976). However, the study of mixtures of lignin and carbohydrates showed that CP/MAS ^{13}C NMR highly underestimates the former compared with the latter (Gauthier et al. 2003). As a consequence, we cannot rule out a contribution from lignin-derived moieties or polyphenols like tannins in samples whose ^{13}C NMR spectra do not display a significant phenolic contribution. This highlights the importance of confronting several analytical techniques in order to investigate OM structure.

Thermally assisted hydrolysis and methylation

Thermally assisted hydrolysis and methylation is widely used to study the structure of natural OM (e.g. Martin et al. 1995; Saiz-Jimenez and Hermosin 1999; Chefetz et al. 2000; van Heemst et al. 2000; Nierop 2001; Frazier et al. 2003; Nierop and Buurman 2007). TMAH, which is a base and a methylating agent, both enables releasing structural units of macromolecules and methylating polar groups. They are thus better detected through GC.

The total ion current (TIC) traces of products released upon THM of WEOM are shown in Fig. 3. Numbered compounds are identified in Table 1. It is first important to note that no free hydroxyl or carboxyl groups could be detected, showing that complete methylation took place. In the following, the origin and evolution of the identified compounds are discussed. They mainly derive from lignin, tannins, carbohydrates, proteins, or correspond to aliphatic moieties.

Lignin/tannin-derived products A number of aromatic compounds detected in the samples after THM are classically released during THM of lignin (e.g. Challinor 1995; Clifford et al. 1995; McKinney et al. 1996; del Rio et al. 1998; Gauthier et al. 2003). They correspond to methylated derivatives of the three types of lignin units: *p*-hydroxyphenyl (**21**: 4-methoxybenzoic acid methyl ester), guaiacyl (**11**: 1,2-dimethoxybenzene; **27**: 3,4-dimethoxybenzaldehyde; **31**: 3,4-dimethoxybenzoic acid) and syringyl (**28**: 3,4,5-trimethoxytoluene; **32**: 3,4,5-trimethoxybenzoic acid),

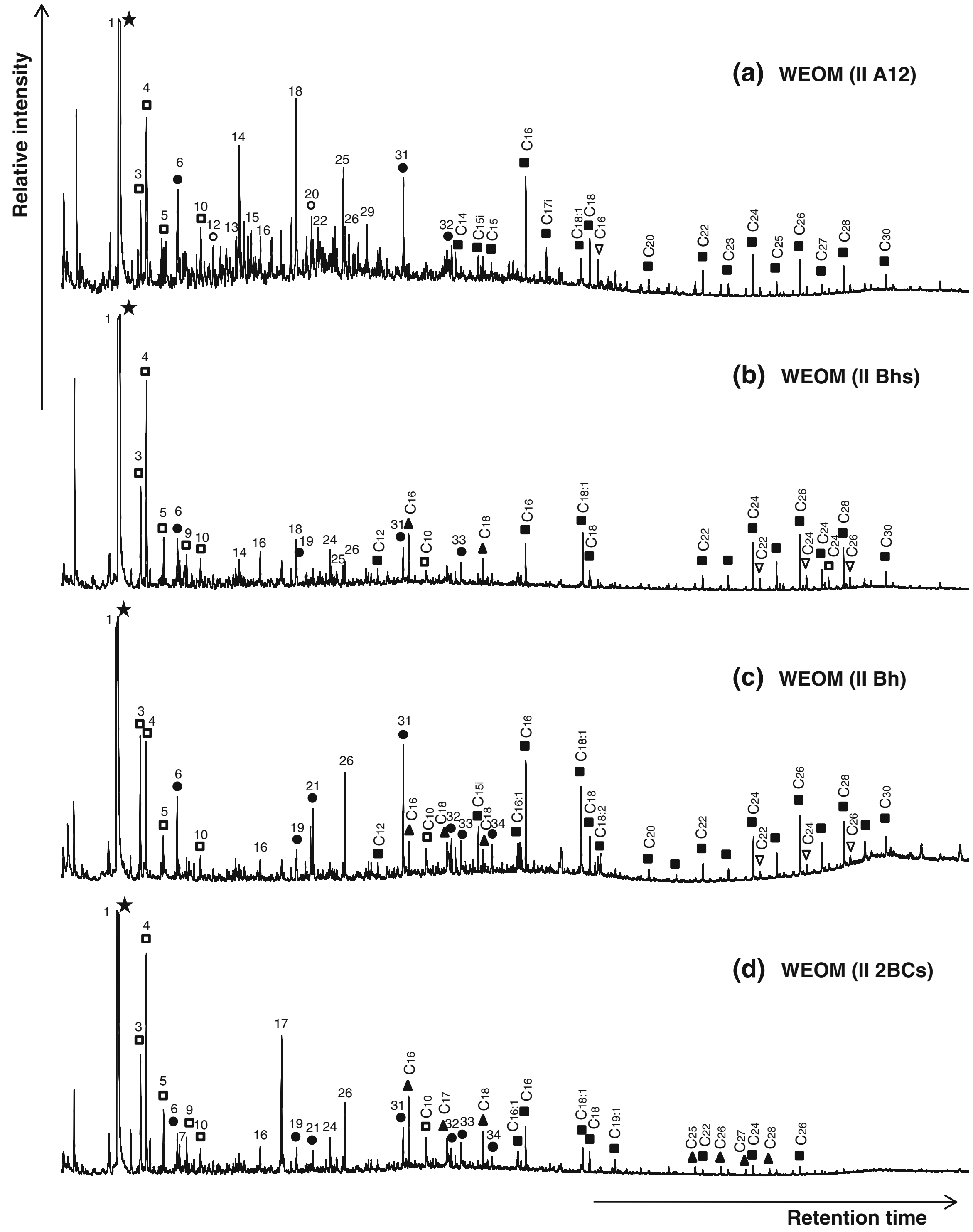

Fig. 3 TIC traces of 610°C TMAH/Curie point pyrolysate of WEOM from pit II (**a**–**d**) and pit III (**e**–**g**). *Key*: (▲) alkanes, (▼) alkenes, (■) alkanoic acids, (▣) diacids, (▽) ω-hydroxyacids, (⊽) alkanols, (○) phenolic compounds, (●) benzoic acids, (★) 1,3,5-trimethylhexahydro-1,3,5-triazine. In Cx:y, x refers to the total number of carbon atoms and y to the total number of double bonds. i stands for *iso*. See Table 1 for identification of numbered peaks

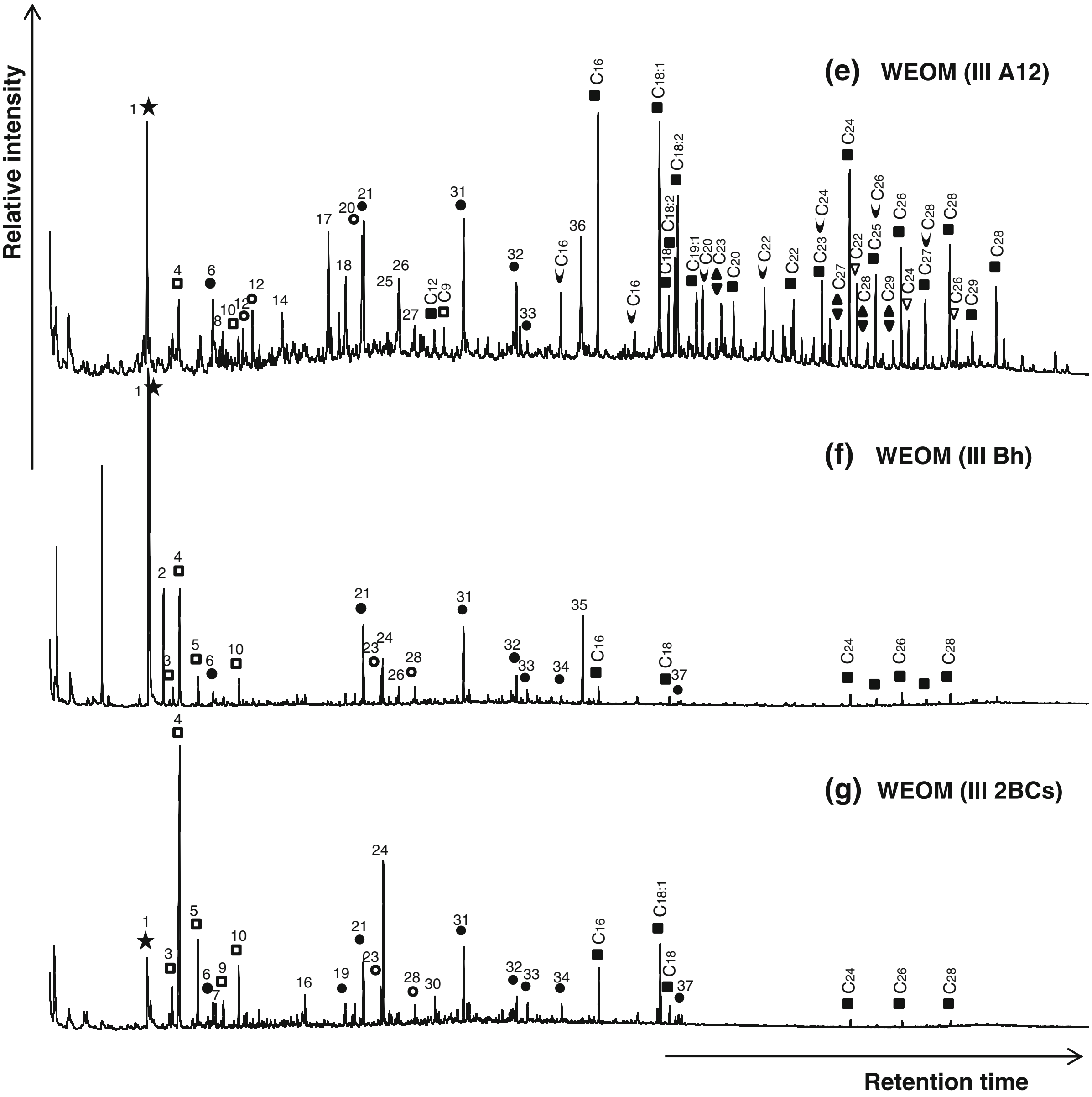

Fig. 3 continued

as well as cinnamyl (**36**: 3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester) units. Guaiacyl lignins are typical for woody gymnosperms (softwood), woody angiosperm (hardwood) lignin contains both guaiacyl and syringyl units, while the presence of cinnamyl units reveals a contribution from non-woody higher plants like grasses (Hedges and Mann 1979). Methylated benzene(poly)carboxylic acids were also detected in the samples (**26**: 1,2-benzenedicarboxylic acid dimethyl ester; **34**: 1,2,4-benzenetricarboxylic acid trimethyl ester; **37**: 1,2,4, 5-benzenetetracarboxylic acid tetramethyl ester). Although compound **26** (dimethylphtalate) is a well-known contaminant, it also belongs to the benzenepolycarboxylic acids along with **34** and **37**. Such benzenepolycarboxylic acids are commonly observed upon THM of DOM and humic acids by e.g. Saiz-Jimenez and Hermosin (1999) and Saiz-

Table 1 Products formed upon 610°C Curie point THM of WEOM for the different soil horizons

Peak number	Compounds
1	1,3,5-Trimethylhexahydro-1,3,5-triazine
2	Unknown (44,102,132)
3	2-Butenedioic acid, dimethyl ester
4	Butanedioic acid, dimethyl ester
5	Methyl-butanedioic acid, dimethyl ester
6	Benzoic acid, methyl ester
7	Methylene-butanedioic acid, dimethyl ester
8	Methylated carbohydrate derivative[a]
9	2-Pentenedioic acid, dimethyl ester
10	Pentanedioic acid, methyl ester
11	1,2-Dimethoxybenzene
12	1,4-Dimethoxybenzene
13	1,3-Dimethyl-2,4(1H, 3H)-pyrimidinedione
14	57,71,82/83,113,127,142[b]
15	53,68,83,125,154[b]
16	1,2,6-Trimethoxyhexane
17	Unknown (57,59,85,117,75,100,131)
18	*N*-Methyl-5-oxoproline, methyl ester
19	3-Methoxybenzoic acid, methyl ester
20	1,2,4-Trimethoxybenzene
21	4-Methoxybenzoic acid, methyl ester
22	Methylated carbohydrate derivative[a]
23	1,3,5-Trimethoxybenzene
24	1,2,3-Propanetricarboxylic acid, trimethyl ester
25	1,3,5-Trimethyl-2,4(1H,3H)-pyrimidinedione
26	1,2-Benzenedicarboxylic acid, dimethyl ester
27	3,4-Dimethoxybenzaldehyde
28	3,4,5-Trimethoxytoluene
29	Methylated carbohydrate derivative[a]
30	1,2,4-Butanetricarboxylic acid, trimethyl ester
31	3,4-Dimethoxybenzoic acid, methyl ester
32	3,4,5-Trimethoxybenzoic acid, methyl ester
33	Dimethoxy-dimethyl-benzoic acid, methyl ester
34	1,2,4-Benzenetricarboxylic acid, trimethyl ester
35	Dodecylbenzene
36	3-(3,4-Dimethoxyphenyl)-2-propenoic acid, methyl ester
37	1,2,4,5-Benzenetetracarboxylic acid, tetramethyl ester

[a] Fabbri et al. (1996) and Fabbri and Helleur (1999)

[b] Schwarzinger et al. (2002)

Jimenez et al. (2006) and are generally considered as highly oxidized lignin degradation products. Such products were also recently identified in THM of old charcoal, reflecting the presence of oxidized black carbon (Kaal et al. 2008).

The predominance of carboxylic acid functions within methoxybenzene derivatives, already observed in soil solutions by e.g. Guggenberger and Zech (1994) could indicate the presence of lignin with oxidized side chains. This is likely, since the α-carbon is oxidized preferentially, and a high degree of lignin oxidation increases its solubility (Guggenberger and Zech 1994) but as argued by Hatcher and Minard (1995), side chains can also be oxidized during THM. It is thus impossible to know if the short side chain lengths observed result from the presence of oxidized lignin or if the oxidation is due to TMAH.

Moreover, THM does not enable distinguishing between initially present hydroxyl and methoxyl groups. Using ^{13}C-labelled TMAH, Filley et al. (2006) recently showed that the above cited aromatic compounds could also be derived from fully hydroxylated precursors and demethoxylated counterparts appear to be more soluble than their methoxylated homologues (Guggenberger and Zech 1994). Compounds **11**, **31**, **32** and **36** could thus be for a large part methylated versions of 1,2-benzenediol (catechol), 3,4-dihydroxybenzoic acid (protocatechuic acid), 3,4,5-trihydroxybenzoic acid (gallic acid) and 3-(3,4-dihydroxyphenyl)-2-propenoic acid (caffeic acid), respectively and therefore derive from lignin demethoxylation. Such compounds were also suggested to be building blocks of polyphenolic molecules like tannins (e.g. Derenne and Largeau 2001). Such an origin is consistent with the presence of trimethoxybenzenes **20**, **23**, trimethoxytoluene **28** as the latter were identified upon THM of purified tannins along with dimethoxybenzene **11** and di- and trimethoxybenzoic acids **31**, **32** (Nierop et al. 2005a). At last, it must be noted that 3,4,5-trimethoxytoluene (**28**) was also shown to be released from cutan upon THM (McKinney et al. 1996) but cutan was suggested to be restricted to drought-adapted plants (Boom et al. 2005).

It can first be noticed that on the whole, the evolution of lignin- or tannin-derived structures agrees with ^{13}C NMR data regarding aromatic contributions. These compounds are most abundant in WEOM from Bh horizons, which have prominent aromatic contributions. However, it is still unclear why no phenolic resonance can be observed in the ^{13}C NMR spectrum of WEOM from II Bh. Cinnamyl unit **36** is detected in WEOM from III A12 only. Since a vegetation change is observed along the sequence (do Nascimento et al. 2004), these units are likely grass-derived. Pit II is covered with a *Campinarana* vegetation (forest) whereas pit III is overlain by *Campina* (shrub savannah), in which non-woody plants are much more abundant relative to woody plants. Cinnamyl units are not detected any more in deeper horizons, either showing that their side-chain becomes oxidized with depth, or that WEOM from III Bh and III 2BCs derives from OM from a previous woody vegetation. We can observe that benzenecarboxylic moieties present in WEOM are increasingly oxidized with depth and podzol development. Benzenetricarboxylic acid (**34**) is detected in Bh and 2BCs horizons only, while in pit III, Bh and 2BCs additionally display contributions from benzenetetracarboxylic acid (**37**).

Carbohydrate-derived products Several studies have shown that THM tends to underestimate carbohydrate-derived contributions relative to lignin-derived contributions (e.g. Clifford et al. 1995; Gauthier et al. 2003). The previous authors found that this underestimation was less marked than that for conventional pyrolysis, while Nierop (2001) found the opposite. However, THM of model compounds enabled determination of characteristic carbohydrate-derived THM products (e.g. Fabbri et al. 1996; Fabbri and Helleur 1999; Schwarzinger et al. 2002; Gauthier et al. 2003). These studies further suggest that carbohydrate THM is highly sensitive to experimental conditions such as amount of TMAH used, pyrolysis temperature, nature of the solvent (methanol vs. water), etc. According to Fabbri et al. (1996) and Fabbri and Helleur (1999), peaks **8**, **22** and **29** were tentatively assigned to methylated carbohydrate derivatives since their mass spectra present high contributions of ions at $m/z = 101$, 127 and 129. As for peaks **14** and **15**, their structure is unknown but they were found as major products of cellulose upon THM by Schwarzinger et al. (2002). At last, the previous studies indicate that THM of carbohydrates can release substantial amounts of methoxybenzenes **11** (1,2-dimethoxybenzene) and **20** (1,2,4-trimethoxybenzene). However, as stated in the previous section, and suggested by Chefetz et al. (2000), these methoxybenzenes can also originate from lignin or tannins.

Carbohydrate-derived compounds are found almost exclusively in surface horizons, except for compound **15**, also present in WEOM from II 2Bhs. This is consistent with prominent carbohydrate contributions on their ^{13}C NMR spectra and further indicates that peaks around 72 ppm in WEOM from the other horizons are certainly associated with ether or amine bond in other structures. Owing to its presence upon THM of WEOM from surface horizons only, 1,2,4-trimethoxybenzene (**20**) is probably carbohydrate-derived only in the present study. Carbohydrates are most likely part of lignocellulose degradation products released by microbial activity (Guggenberger and Zech 1994). These authors have already observed a sharp decrease of carbohydrate concentration in DOM from a forest floor and attributed it to microbial preferential mineralization of these compounds, to their physical adsorption, or to their precipitation.

Protein-derived products Only a few N-containing compounds are released upon THM of WEOM. *N*-Methyl-5-oxoproline methyl ester (**18**) is produced upon THM by cyclisation of amino acids, especially glutamine or glutamic acid (Gallois et al. 2007). This compound has already been detected upon THM of soil and sediment samples (Knicker and Hatcher 1997; Zang et al. 2000; Garcette-Lepecq et al. 2001) and evidences the presence of proteinaceous material. 1,3-dimethyl-2,4-(1H,3H)-pyrimidinedione (**13**: 1,3-dimethyluracil) and 1,3,5-trimethyl-2,4-(1H,3H)-pyrimidinedione (**25**, also called 1,3,5-trimethyluracil or 1,3-dimethylthymine) have been found by numerous authors in soil (e.g. Chefetz et al. 2000; Saiz-Jimenez et al. 2006) and aqueous samples (e.g. Mannino and Harvey 2000; Frazier et al. 2003). These compounds are methylated nucleic acids. Nucleic acids can account for a significant part of soil nitrogen (Cortes and Schnitzer 1979). In the present study, they are most probably released from microbial cells since they are mainly present in surface, biologically-active horizons.

Other compounds, though nitrogen-free, can be derived from proteinaceous material. However, they are not characteristic of this compound class. For instance, Gallois et al. (2007) showed that aspartic acid deamination led to production of butenedioic acid dimethyl ester (**3**) and butanedioic acid dimethyl ester (**4**). Mannino and Harvey (2000) also suggest that a part of butanedioic acid dimethyl ester (**4**), methyl-butanedioic acid dimethyl ester (**5**) are protein-derived THM products. However, in the present study, their occurrence and abundance of these THM products do not seem to be related with these of N-containing products.

Like polysaccharide-derived compounds, N-containing products are only released from WEOM from surface (II A12 and III A12) and subsurface horizons (II Bhs). This is in agreement with microbial decomposition of fresh organic matter in surface horizons.

Aliphatic compounds Aliphatic compounds released upon THM can either be part of a macromolecular network, or occur as lipids trapped within the sample. Among the dominant aliphatic products released upon THM is a bimodal series of aliphatic acids in the C_{12}–C_{32} range with an even-over-odd predominance. Short-chain acids are dominated by the C_{16} or C_{18} homologues while C_{26} or C_{28} are the most abundant long-chain counterparts. Mono-unsaturated ($C_{16:1}$, $C_{18:1}$ and $C_{19:1}$), di-unsaturated ($C_{18:2}$) and branched (iso C_{15} and C_{17}) acids are also identified. Such a bimodal distribution of linear alkanoic acids is commonly found when analysing soil lipids (e.g. Jambu et al. 1985; Amblès et al. 1994; Naafs et al. 2004; Quénéa et al. 2004; Nierop et al. 2005b). In aqueous environments, long-chain homologues up to C_{34} have been reported (e.g. Frazier et al. 2003). Long-chain homologues with even predominance are typical of higher plant waxes (Kolattukudy et al. 1976), while short-chain *n*-alkanoic acids are ubiquitous. Unsaturated counterparts can originate from plant triglyceride hydrolysis (e.g. van Bergen et al. 1997) or from microorganisms (e.g. Haack et al. 1994). The occurrence of low amounts of C_{16} and C_{22}–C_{26} even carbon-numbered ω-hydroxyacids and C_{16} and C_{24} α,ω diacids point to the contribution of cutin and suberin biopolymers. Cutin covers the aerial parts of vascular plants and suberins are mostly present in roots and some suberized tissues of plants (Kolattukudy 1980). Cutin and suberin are biopolyesters composed of carboxylic acids, ω- or α-hydroxylated, diacids and alcohols. Whereas the chain length of the cutin monomers is C_{16} and C_{18}, that of suberin monomers expand to C_{26} with a maximum around C_{24}. The ω-hydroxyacids and α,ω diacids detected upon THM likely result from the solubilisation of degradation products of the aforementioned biopolyesters. Once again, the presence of long-chain aliphatic compounds, especially alkanoic acids, among THM products of WEOM is not contradictory due to their amphiphilic character. Long-chain acids are released from WEOM from II A12, II Bhs, II Bh and III A12. It cannot be excluded that part of the observed alkanoic acids occur as free lipids. Indeed, in a previous study, Bardy et al. (2008b) showed that free fatty acids extracted from the same horizons have similar distributions to these observed upon THM. However, no diunsaturated $C_{18:2}$ acid was observed, probably owing to the high sensitivity of poly-unsaturated fatty acids to degradation. Indeed, a better preservation was shown to occur for acids released upon pyrolysis (tightly bound acids) when compared to free ones (e.g. Zegouagh et al. 1996). As a consequence, WEOM certainly encompasses both free acids and plant-derived macromolecules from which bound lipids like the $C_{18:2}$ are released.

Another prominent contribution comes from short-chain diacids, mainly C_4 (**3**, **4**, **5**, **7**) and C_5 (**9**, **10**), along with lower amounts of C_9 and C_{10} homologues. Such compounds have already been found in THM (e.g. Garcette-Lepecq et al. 2001; Templier et al. 2005). The C_9 and C_{10} diacids can result from microbial oxidation of double bonds in unsaturated monoacids, like the ubiquitous oleic acid ($C_{18:1\omega9}$) for the former. As previously stated, C_4 and C_5 diacids can be partly released upon THM of proteinaceous material (Gallois et al. 2007). They are also likely to act as bridges between phenolic units in macromolecular network, reflecting its cross-linking level (Martin et al. 1995). It can be noted that the contributions of C_4 and C_5 diacids are major in the TIC traces of WEOM from II Bhs, II 2BCs, III 2BCs and to a lesser extent III Bh. A high level of cross-linking in these horizons is consistent with the high contents of organometallic complexes measured in the soils of these horizons (Bardy et al. 2007), since binding with metallic cations increases OM cross-linking.

The aliphatic tricarboxylic acids 1,2,3-propanetricarboxylic acid (**24**) and 1,2,4-butanetricarboxylic acid (**30**) are also present in some of the THM. The former has already been recovered upon THM of aquatic organic matter (e.g. Lehtonen et al. 2000). Like short diacids, these compounds could act as bridges in a highly cross-linked macromolecular network. It can be noted that compound **24** is released upon THM of WEOM from II 2BCs, III Bh, and III 2BCs only. Its contribution is major in the latter and is associated with that of compound **30**.

Other aliphatic compounds released upon THM are long-chain *n*-alkanols in the C_{16}–C_{28} range, with even chain-length. Their even predominance points to their vegetal origin (e.g. Kolattukudy 1980). Lastly, alkanes and alkenes are also observed. III A12 displays low amounts of alkane/alkene doublets. Such doublets are classically formed upon pyrolysis of natural macromolecular organic matter (e.g. Largeau et al. 1986) like the highly resistant aliphatic biopolymers cutan (e.g. Nip et al. 1985) or suberin (Tegelaar et al. 1995). They originate from the homolytic cleavage of C–C bonds in polymethylenic chains. In soils, such doublets have already been observed upon pyrolysis of macromolecular lipids (Gobé et al. 2000), macromolecular non-hydrolysable fractions (Augris et al. 1998; Quénéa et al. 2005), and WEOM (Nierop and Buurman 1998). In the investigated samples, these doublets are rather scarce, pointing to the low abundance of highly aliphatic structures in WEOM. In WEOM from II Bhs, II Bh and II 2BCs, C_{16}–C_{18} *n*-alkanes are additionally detected without co-occurrence of the corresponding alkenes. These compounds are therefore most likely free alkanes trapped in the macromolecular network of WEOM (Deniau et al. 2004).

Other compounds 1,3,5-Trimethylhexahydro-1,3,5-triazine (**1**) is a common by-product of TMAH (e.g. Templier et al. 2005; Nierop and Buurman 2007). Benzoic acid methyl ester (**6**), 1,2,6-trimethoxyhexane (**16**) and dimethoxydimethylbenzoic acid (**33**) cannot be associated with a particular class of source compounds. At last, *n*-dodecylbenzene (**35**) is the only alkylbenzene moiety detected in significant amounts. It only appears in one of the THM, namely WEOM (III Bh) in Fig. 3b, and its peak is rather intense. The same contribution is also detected upon conventional pyrolysis (not shown). Alkylbenzenes are formed through cyclisation of unsaturated acids upon conventional pyrolysis (Saiz-Jimenez 1995a), but not upon THM, since carboxylic groups are protected (Saiz-Jimenez 1995b). They can also result from cyclisation and aromatisation of radicals formed upon cleavage of long alkyl chains. However, in that case the alkylbenzenes occur along with the corresponding alkane/alkene doublets, the latter being much more abundant. The high abundance of the dodecylbenzene thus rules out this origin and suggests that the benzene ring is not formed upon pyrolysis. In fact, it is likely that this product is derived from a contaminant, as alkylsulfonate and sulphate with a C_{12} alkyl chain are well-known surfactants.

The comparison of the THM of the different WEOMs described above allows the determination of some markers that are specific for given soil horizons:

- WEOM from surface horizons have a high polysaccharidic content, and produce compounds specific to cellulose (**14** and **15** on Fig. 3). Two compounds derived from proteinaceous material (**18** and **25**) are detected in significant amounts in the two surface horizons only. These can therefore be considered as markers for II A12 and III A12. The latter is also the only horizon from which a methylated cinnamyl unit (**36**) is produced upon THM, probably due to the development of *Campina* vegetation.
- WEOM from deep II Bh, III Bh and III 2BCs horizons are much more aromatic than WEOM from the other horizons. This is reflected in their THM products, especially by the presence of compounds **19**, **21** and **26**. It must be noted that these products are also detected in the THM of II Bhs and II BCs but their abundance with respect to the other peaks is lower than in II Bh. As previously mentioned, **26** may be a contaminant and cannot be used alone as a marker.
- When compared to II Bh THM, that of III Bh also contains **21** and **26** (the latter in much lower relative abundance) but it exhibits additional peaks such as **23**, **24** and **28**. The latter peaks are also present in the THM of III 2BCs, although with a different distribution, along with **19** and **21**. The presence of compounds **23**, **24** and **28** is thus characteristic of III Bh and III 2BCs.

It must be noted that the aforementioned compounds that can be used as markers for a given horizon all elute in the same range of the GC trace.

Exportation of soil organic matter to the river network

We have defined WEOM as "potentially mobile OM", and showed that it had contrasting characteristics depending on the considered horizon and the stage of podzol development. However, comparison with OM in ground- and river-water is useful to investigate if there is evidence for their actual mobility. This was done using techniques with various levels of complexity.

Hints from bulk features of soil water extracts, ground- and river-waters

pH, conductivity and Specific UV Absorbance (SUVA) were measured for water extracts from the different soil horizons (Fig. 4). They were compared with the ranges of values (grey areas on Fig. 4) measured in the field, in the water tables of the different soil pits and in the streams directly draining the soil sequence.

pH In agreement with the variations observed for soil pH, pHs of the soil water extracts are globally low (<5.5, Fig. 4a). Water extracts from the well-developed podzol (pit III) exhibit lower values than those from the transition area (pit II). In III Bh and III 2BCs, they even drop under 4. Such low values of pH (around 4) are commonly found in black waters draining podzols in the Amazon basin (e.g. Eyrolle et al. 1996). Consistently, in the present drainage network, waters exhibit very acidic pHs ranging between 3 and 4.

Conductivity Conductivities are moderate in water extracts from the transition area (pit II), where they range from 10 to 21 μS cm^{-1} (Fig. 4b). It can be noticed that conductivity is higher in WEOM from the II A12 and II Bh horizons. It is much higher in water extracts from the well-developed podzol, where it reaches values above 40 μS cm^{-1} in deep III Bh and III 2BCs horizons. Waters from the drainage network have a conductivity that is even higher and ranges from 60 to 80 μS cm^{-1}.

SUVA SUVA is commonly used as an indicator of the aromaticity of dissolved organic matter (DOM). Laor and Avnimelech (2002) also suggest that high SUVA values are associated with poorly-degradable material, the latter being rich in aromatic moieties. Soil water extracts have very contrasting SUVA (Fig. 4c), ranging from 1.1 to 6.4. The lowest values (<1.6) are found in water extracts from II Bhs, II 2BCs and III A12 horizons, while that from II A12 displays a higher value (3.1). By contrast, SUVA is very high in water extracts from II Bh, III Bh and III 2BCs, where they reach values above 5. This is consistent with the aforementioned NMR data (Fig. 2) confirming the relationship between high SUVA and aromaticity The SUVA is also very high in waters from the drainage network, ranging from 5 to 7. Benedetti et al. (2002) already measured such high SUVA for colloids of the Rio Negro. These data thus suggest a strong aromatic character of OM both in water extracts from II Bh, III Bh and III 2BCs, and in the drainage network.

Taken together, the comparison of these bulk features in water extracts and waters from the soil drainage network gives a first indication of the possible origin of OM exported from podzols to the drainage network. Indeed, only water extracts from III Bh and III 2BCs have a pH lower than 4, a high conductivity, although lower than that in the streams,

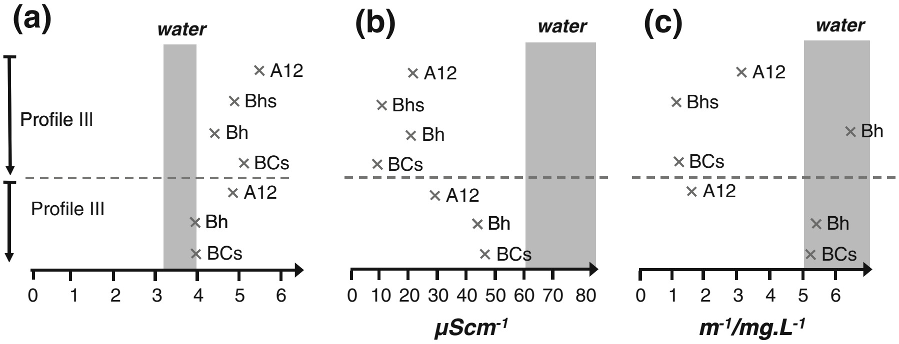

Fig. 4 **a** pH, **b** conductivity and **c** SUVA of soil water extracts (*crosses*) and streams draining the soil sequence (*grey areas*). *Grey areas* show the extent of the range of data measured for the eight brooks sampled around the investigated site

and a high SUVA. This suggests that these two horizons contribute significantly to the exportation of OM towards black rivers associated with podzols. This is in agreement with findings by Briand (1995) on sandy podzols of the Manaus region. The results of this first section seem to show that the same happens in podzols occurring on plateaux as in those located in footslope position, and further tend to indicate that podzols in the transition zone contribute little to the exportation of OM to the river network. This is also in agreement with data from do Nascimento et al. (2008), who showed that there is much more organic carbon in soil solutions from the well-developed podzol (pit III) than from the transition area (pit II).

Hints from spectroscopic and pyrolytic data on soil water extracts and groundwaters

NMR The solid state ^{13}C NMR spectrum of the OM from the groundwater draining the well-developed podzol during the wet season, pit III is shown on Fig. 5. Similar spectra were obtained for the wet and dry season samples with a slightly lower contribution of the carbohydrate signal in the dry season, consistent with lower level of the water-table. This is consistent with previous investigations showing a lower aromatic contribution from the topsoil A horizons to the exportation of matter to river systems, when compared to suspended loads during the dry season (Rodriguez-Zuniga et al. 2008).

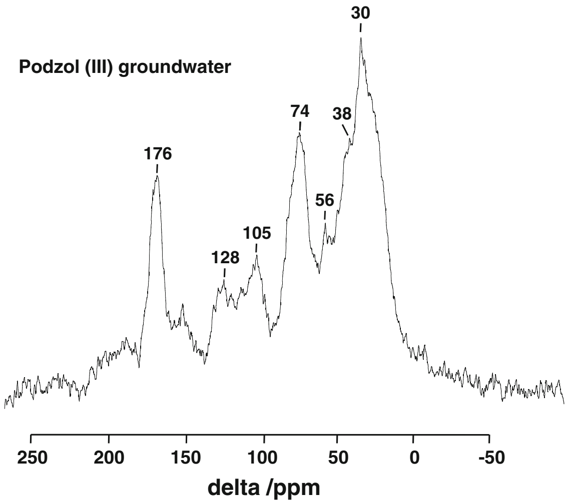

Fig. 5 Solid-state ^{13}C NMR spectra of OM from pit III groundwater (high level water season)

These spectra show a rather broad signal in the aliphatic region, peaking at 30 ppm and exhibiting a marked shoulder at 38 ppm. This suggests a significant contribution of quaternary aliphatic carbons. A significant carbohydrate contribution is indicated by the peaks at 74 and 105 ppm. The aromaticity of the OM in the drainage water is revealed by the peak at 128 ppm, which along with the peak at 56 ppm suggests a lignin and/or tannin contribution. Carboxylic functions are also shown to be present based on the large peak at 176 ppm.

When compared with Fig. 2, the shape of the aliphatic signal is more similar to that of the Bh and BCs horizons than to that of the surficial samples. The intensity of the carboxylic peak along with the location of its maximum (176 ppm) also supports a contribution of the well-developed horizons. However, a significant carbohydrate signal typical of the surface horizons is also observed, indicating that both surface and deep horizons supply OM to groundwater.

THM THM allows a comparison between the WEOMs and the groundwater at the molecular level. It must be noted that similar chromatograms were found for groundwater sampled in the wet and dry seasons.

The THM of the groundwater draining pit III (Fig. 6) is dominated by compounds likely derived from lignin and/or tannins, namely **21**, **28**, **31**, **32**. The latter are present along with several methoxy aromatic products such as **23**, **19**, **12** and traces of **16**. Benzoic acid **6** also contributes to the pyrolysate. Taken together, these products indicate a higher aromaticity than expected based on the NMR spectrum (Fig. 5). However, as stressed above, CP/MAS NMR is known to overestimate the aliphatic contribution and SUVA data are consistent with such an aromaticity. In addition to these compounds, the groundwater pyrolysate contains several short aliphatic diacids (**3**, **4**, **5**, **9**, **10**) and triacid (**24**). These compounds are assumed to reflect the high level of cross-linking in this OM, in agreement with the NMR spectrum. Finally, substantial amounts of C_{16} and C_{18} fatty acids are identified. These compounds are ubiquitous but their abundance in the pyrolysate is consistent with the abundance of the 176 ppm peak.

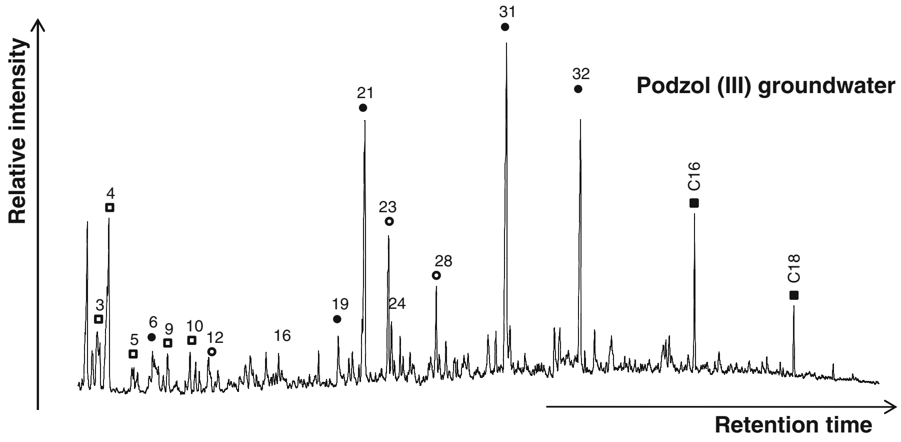

Fig. 6 TIC trace of 610°C TMAH/Curie point pyrolysate of OM from pit III groundwater (high level water season). *Key*: (○) phenolic compounds, (●) benzoic acids. See Table 1 for identification of numbered peaks

The nature and abundance of the THM products identified in the groundwater can be compared with those from the different WEOMs. A special attention will be paid to the specific compounds that were shown to be typical of WEOM from a given horizon. However, it must be noted that a direct comparison is only possible assuming that no diagenetic changes occur. This is obviously not the case but the presence of a marker or a combination of markers of a given horizon strongly indicates the contribution of OM from that horizon to the drainage water. In contrast, the lack of some markers may only reflect their diagenetic degradation.

The pyrolysate of groundwater OM exhibits peaks **19** and **21** that were also found for WEOM from II Bh, III Bh and III 2BCs. This likely reflects the contribution of OM from deep horizons. This is further supported by the presence of the short chain diacids and triacid. These compounds were shown to be relatively more abundant in II Bhs, II 2BCs, III 2BCs and to a lesser extent III Bh. The presence of peaks **23**, **24** and **28** which were only detected in the pyrolysate of III Bh and III 2BCs even points to the mobilization of OM from deep horizons of the well-developed podzol (pit III) itself.

On the opposite compounds **18** and **25** are not observed in the pyrolysate of the groundwater OM. Of course their absence does not rule out any contribution from surface horizons. The latter is especially expected in the wet season when the groundwater table reaches the soil surface. As mentioned above, such a contribution is certainly reflected by the carbohydrate signal in NMR is consistent with the presence of labile material in the groundwater. Although **18** and **25** are derived from amino acids and nucleic acids that were observed in various natural environments including sediments, in the present study, they have most likely undergone diagenetic transformations.

Taken together, bulk features, NMR and THM point to a non-negligible contribution of horizons III Bh and III 2BCs to the exportation of OM from podzols to black rivers. It also points out specific compounds (**23**, **24** and **28**) that could be used to evidence for a podzolic source of the OM in these rivers.

Conclusion

The present study aims at understanding the fate of OM in the soil–water continuum in the Rio Negro basin. It focuses on a soil sequence representative of the lateral and vertical differentiation of podzols at the expense of latosols on low elevation plateaux of the Rio Negro basin. Water extracts from seven key samples were analyzed for pH, conductivity and SUVA. Freeze-dried OM was additionally subjected to ^{13}C NMR and THM in order to gain structural information. These features were compared with those of OM from natural surface and groundwaters of the soil sequence drainage network.

^{13}C NMR and THM data are in general agreement and show that WEOMs from the different horizons and from the two considered pits are contrasting. They are highly cross-linked and exhibit a significant aromatic character especially in Bh horizons as well as in 2BCs horizon of the well-developed podzol, most probably related to the presence of altered lignin or polyphenolic moieties. This point is in agreement with high SUVA measured for water extract. THM also produced series of compounds specific to surface (e.g. cellulose- and protein-derived products) or to some of the deep horizons (e.g. polycarboxylic aliphatic and characteristic benzene-derived products). They could be used as markers to trace a "podzolic origin" of OM in black-water rivers of the Rio Negro basin.

The comparison of data obtained for water extracts with those of natural waters draining the soil sequence shows that both surface and deep horizons of the well-developed podzol contribute to the exportation of OM towards black waters of the rivers. After a first stage during which Bh horizons are formed through accumulation of illuvial OM, there thus seems to be a second stage during which previously immobilized OM is remobilized and exported towards the river network. This is certainly made easier by the large pore space of this sandy environment.

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