INSOLUBLE ORGANIC MATTER FRACTIONS IN INCIPIENT PODZOL B HORIZONS: PRESERVATION OF ALIPHATIC BIOPOLYMERS FROM ROOTS

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Abstract

Organic matter in podzol B horizons is generally considered as the result of illuviation from upper horizons. This illuviated organic matter is thought to be largely, if not completely, extractable by sodium pyrophosphate. In incipient podzols from the Leuvenum Forest, 17-37% of the B organic matter remained insoluble after extraction with NaOH. Extraction of these insoluble fractions by sodium pyrophosphate hardly enlarged the amounts of soluble organic matter. Combining pyrolysis, thermochemolysis and SEM revealed that the insoluble organic matter consisted almost completely of root remnants. It appeared to be highly aliphatic, being composed of suberin and a 'suberan-like' polymer, whereas lignin and polysaccharides were virtually absent. This suggested that the aliphatic biopolymers were (highly) selectively preserved, not transported by illuviation and that root organic matter is an important constituent in B horizons.

Keywords: humus, humic substances, fractionation, podzols, illuviation.

INTRODUCTION

Humin is defined as the organic residue after extraction of soils and sediments with 0.5M NaOH or other basic solutions (Schnitzer and Kahn 1972). It may consist of fresh or slightly altered plant materials, clay-organic matter complexes, fungal melanins and highly condensed humic matter. The latter is sometimes called the 'real humin' (Stevenson 1994). Because of lack of proper methods to separate the several components, the concepts about the origin of this material are not equivocal: on one hand it is considered as the final product of the humification process in the condensation theory, whereas on the other hand, in the degradative biopolymer model, humin is the starting material (Hatcher and Spiker 1988). The main chemical characteristics of the humin faction are less controversial: it has generally a highly aliphatic composition (Beyer 1996).

Large portions, 50-90%, of organic matter in podzol B horizons can be extracted with NaOH (Mokma and Buurman 1982), and thus constitute fulvic and humic acid fractions. Up to 50% remains insoluble. 0.1 M of sodium pyrophosphate ($Na_4P_2O_7$) is considered to be more effective in extracting organic matter from podzols. In general, no humin fractions are obtained, using a sample:solution ratio of 1:100 (w:w) (Mokma and Buurman 1982). This is due to the ability of $Na_4P_2O_7$ to complex with Fe and Al, leaving the organic matter previously associated with these metals as soluble remnants.

In a study about the nature and origin of organic matter in a vegetation sequence, it appeared that the organic matter in incipient podzol B horizons was predominantly aliphatic. Furthermore, we found that about 17-37% of the organic matter was not soluble in NaOH. FTIR and DRIFT spectroscopic data of these 'humin' fractions revealed that considerable amounts of clay was present in those fractions (Buurman et al., unpublished results). The organic matter in the humin fraction proved to be highly aliphatic. Also Theng et al. (1986) and Schulten et al. (1996) found that organic matter associated with clay in podzol B horizons was predominantly aliphatic. So, clay-organic matter complexes may be present in the insoluble fractions of the incipient B horizons.

To determine the origin of the organic matter, the humin fractions were subjected to further fractionation to distinguish between organic matter complexed with metals, associated with clay, or present as plant residues. Extracts and residues were analyzed by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) in the absence or presence of TMAH, while they were also studied by SEM.

MATERIALS AND METHODS

Profiles

The B horizons were sampled from sites at the Leuvenum Forest. This is a drift sand area, which is nowadays largely covered by a forest. Samples 1, 2 and 3 were collected from sites in the same Scots pine (*Pinus sylvestris* L.) stand, that differed in undergrowth: *Deschampsia flexuosa* (L.) Trin., *Vaccinium myrtillus* L. and *Empetrum nigrum* L., respectively. Under pine and *Deschampsia*, weakly developed podzols occur, whereas under the Ericaceae, podzolization is more pronounced (Emmer 1994). When deciduous trees are introduced, they eventually tease away the pines and undergrowth. Sample 4 is under beech (*Fagus sylvatica* L.) with pines in the surroundings, and sample 5 is from a site with only beech. Both beech sites have a more developed podzol profile than in sites 1-3. All B horizons had only a few (living) roots.

Fractionation

Before use, the soil samples were air-dried and sieved (2 mm) to remove roots. Next, they were fractionated according to the scheme shown in Figure 1. About 100 g of soil was extracted with 1 L of 0.5M NaOH for 24 h. under nitrogen. After centrifugation, the solution was decanted and the residue was shaken again with 0.5M NaOH for 24 h. under nitrogen. The residue after centrifugation and decantation (residue 1) was acidified with HCl, washed several times until pH was neutral, and freeze-dried. Carbon contents were determined by dry combustion using an Interscience Elemental Analyzer EA1108.

Aliquots of the humins were subjected to extraction with 0.1 M of sodium pyrophosphate ($Na_4P_2O_7$), (C:solvent ratio 1:50 (w:w)). After 15 minutes of ultrasonic treatment, the samples were shaken for 24 h. After centrifugation, both solution (extract 2) and precipitate (residue 2) were acidified with HCl, dialysed, and freeze-dried.

To destroy clay, aliquots of residue 2 were treated for 24 h. with HF/HCl (3:1 v/v). After centrifugation, the solid phase was extracted by 0.5M NaOH for 24 h. Centrifugation, acidification of the solution, and freeze-drying, yielded residue 3a. Similar procedures were carried out on aliquots of residue 2 using refluxing HF/HCl, followed by NaOH extraction, giving extract 3b and residue 3b, and refluxing in 0.5M NaOH, resulting into extract 3c and residue 3c. These 'hot' steps were also done after ball-milling aliquots of residue 2.
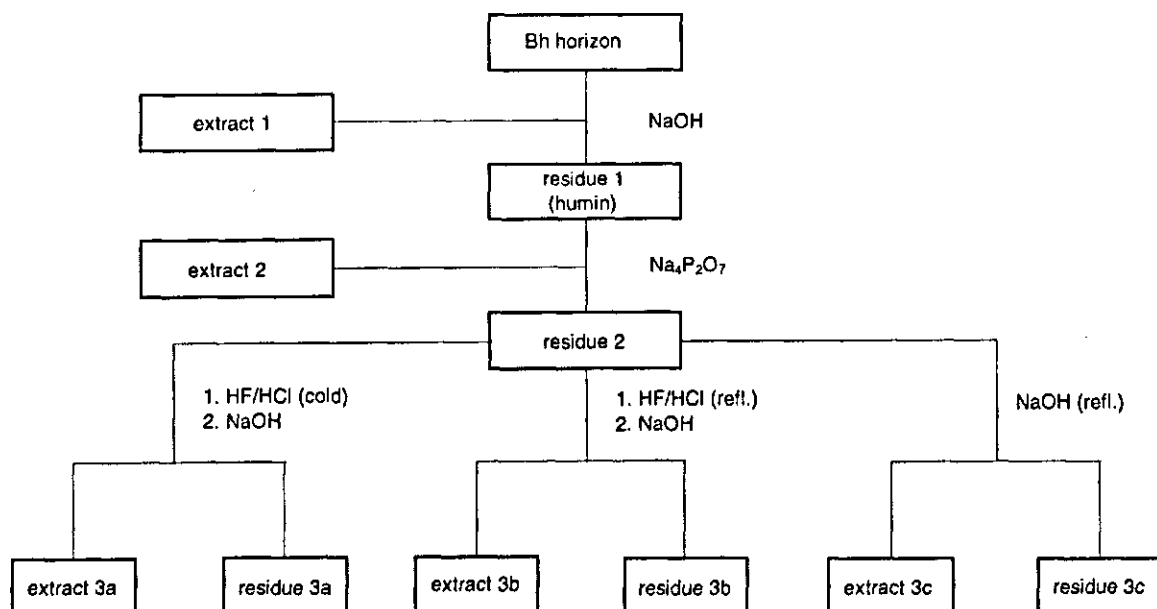

Figure 1: Fractionation scheme of organic matter in B horizons

Pyrolysis-GC/MS and thermochemolysis

Pyrolysis was carried out on a Horizon Intruments Curie-Point pyrolyser. Samples were heated for 5 s at 610°C. The pyrolysis unit was connected to a CE gas chromatograph and the products were separated by a fused silica column (Chrompack, 25 m, 0.25 mm i.d.) coated with CP-Sil 5 (film thickness 0.40 μm). Helium was used as carrier gas. The initial oven temperature was 40°C. After pyrolysis, the oven was heated at a rate of 7°C/min to 320°C and held for 20 min. The end of the GC column was coupled to a Fisons MD 800 mass spectrometer (mass range m/z 45-650, cycle time 1 s). Pyrolysis-methylation (thermochemolysis) was performed by adding one or two drops of a solution of tetramethylammonium hydroxide (TMAH) in water (25%) onto the sample, dried for 1-2 minutes, and immediately followed by pyrolysis, using the same GC program as with conventional pyrolysis.

SEM

Scanning Electron Microscope (SEM) analyses were performed with a Philips 535 SEM at IPO-DLO Wageningen.

RESULTS

Fractionation

Between 17 and 37% of the total organic matter present in the B horizons appears to be insoluble in 0.5M NaOH (see Table 1). Further extraction of these humin fractions by $Na_4P_2O_7$ removes another 1-2% (samples 1-3) or 3-4% (samples 4 and 5) of the total C originally present.

Virtually no yields are obtained when treating residue 2 with HF/HCl to destroy the mineral matrix to set free the clay-bound organic matter. From this it can be concluded that the amounts of organic matter bound to clay is negligible. The final residue 3a is therefore similar to residue 2 and will not be discussed further.

When residue 2 is boiled in HF/HCl for 1 hour, the organic matter does not dissolve at all, and after adding NaOH the solution remains colourless: thus no significant amounts are soluble. Also when residue 2 is refluxed in 0.5M NaOH for 1 hour, the organic matter is still in the residue. However, the organic matter in ball-milled aliquots of residue 2 almost completely dissolves in 0.5M of NaOH.

Humins (residue 1)

Figure 2 shows the pyrolysis-GC traces of the humin fractions of samples 1 and 5. Pyrolysis results of the humin fractions of samples 2 and 3 are similar to that of sample 1. Dominant signals in the pyrolysate of sample 1, 2 and 3 can be attributed to polysaccharides (peaks *1, 2, 4, 6, 8, 10, 13* and *16*), benzene derivatives (peaks *3, 5* and *7*), phenols (peaks *9, 11* and *14*), methoxyphenols (peaks *12* and *15*) and aliphatic compounds such as fatty acids and alkene/alkane pairs (consult Table 2 for the identified peaks). The latter are supposed to be pyrolysis products derived from highly aliphatic biopolymers, such as cutan and suberan (Nip *et al.* 1986, Tegelaar *et al.* 1995, Augris *et al.* 1998).

Very abundant pyrolysis products in the humin fraction of sample 5 are C_{22} and C_{24} alcohols, derived from suberin of beech (Holloway 1983, Matzke and Riederer 1991, Tegelaar *et al.* 1995). These alcohols are also identified in the Py-GC trace of sample 4, but in lower amounts. Benzene and phenol derivatives (peaks *3, 5, 7, 9, 11*) and alkenes/alkanes are encountered as well in the insoluble organic matter of samples 4 and 5.

$Na_4P_2O_7$ extracts (extract 2) and residues 2

Fatty acids are enriched in the $Na_4P_2O_7$ soluble fractions (extracts 2) compared to the residues 2 obtained from samples 1, 2 and 3 (see Figure 3).

Similarly, samples 4 and 5 (Fig. 4) have a relatively high concentration of fatty acids in the $Na_4P_2O_7$-extract. In addition, higher amounts of polysaccharide-derived pyrolysis products have

Table 1: Carbon contents (mass fractions of air-dried soil) and distribution of extractable and residual C (mass fraction of total C) in B horizons of profiles 1-5

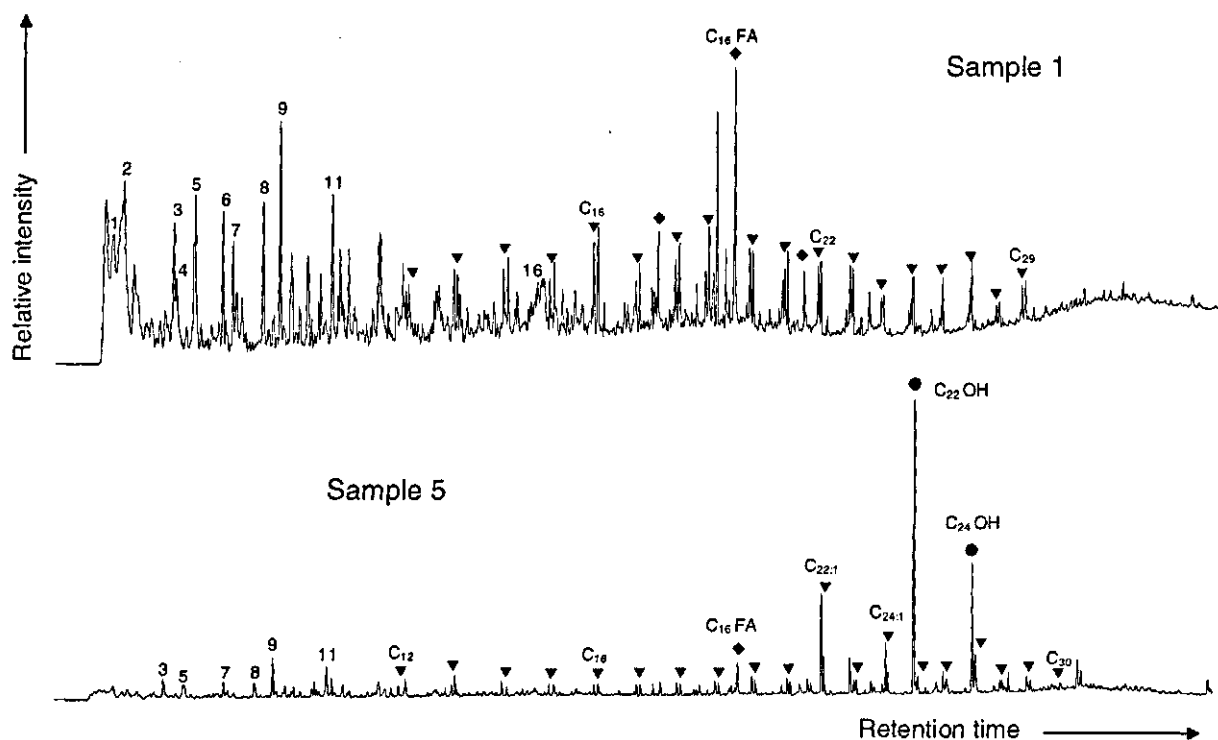| Profile no	C	NaOH extract 1	residue 1	$Na_4P_2O_7$ extract 2	residue 2
	mg/kg	%	%	%	%
1	2.7	79	21	1	20
2	4.0	64	36	2	34
3	4.1	63	37	2	35
4	4.4	83	17	3	14
5	8.0	68	32	4	28

Figure 2: Pyrolysis-GC traces of humin fractions (residue 1) of samples 1 and 5. Numbers and symbols correspond to compounds listed in Table 2.

Table 2: Major identified pyrolysis products

No.	Compound	M
1	Furan	68
2	Acetic acid	60
3	Toluene	92
4	(2H)-Furan-3-one	84
5	Dimethylbenzene	106
6	2-Furaldehyde	96
7	Styrene	104
8	5-Methyl-2-furaldehyde	110
9	Phenol	94
10	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	114
11	Methylphenol	108
12	Guaiacol	124
13	Levoglucosenone	126
14	4-Vinylphenol	120
15	4-Vinylguaiacol	150
16	Levoglucosan	162
▼	n-Alkene/n-alkane doublet	
◆	Fatty acid	
●	Alcohol	

been found in extract 2 than in residue 2. Apart from the C_{22} and C_{24} alkenes and the corresponding alcohols, the pyrolysate of the residue 2 contains phenols, especially 4-vinylphenol and 4-vinyl-guaiacol, levoglucosan and alkene/alkane pairs. Both vinylic compounds are most probably pyrolysis products from esterified p-coumaric acid and ferulic acid (de Leeuw and Baas 1993), linked

to e.g. polysaccharides, lignin and suberin (Kolattukudy 1980, de Leeuw and Largeau 1993). The alcohols (C_{22} and C_{24}) are important in extract 2, and even more so in residue 2. The presence of relatively high peaks of C_{22} and C_{24} alkenes, indicative for esters of the corresponding alcohols in the pyrolysates of both the extract as the residue, suggests that three days of basic treatment (twice with NaOH and once with $Na_4P_2O_7$) hydrolyses the ester bonds far from completely.

Thermochemolysis

Pyrolysis with in situ methylation using tetramethylammonium hydroxide (TMAH) has been recently proven to be complementary to conventional pyrolysis in the analysis of polymers (Challinor 1989), cutin (de Leeuw and Baas 1993), lignin (Clifford et al. 1995, Hatcher et al. 1995, McKinney et al. 1995), cutan (McKinney et al. 1996) and humic substances (Hatcher and Clifford 1994). Relatively polar pyrolysis products are poorly chromatographable as such, but can be separated well by GC after methylation in the presence of TMAH as methyl esters and/or methyl ethers methylated. Because the reactions are chemolytic rather than pyrolytic (de Leeuw and Baas 1993), the procedure is more often referred to as thermochemolysis (McKinney et al. 1995, Clifford et al. 1995).
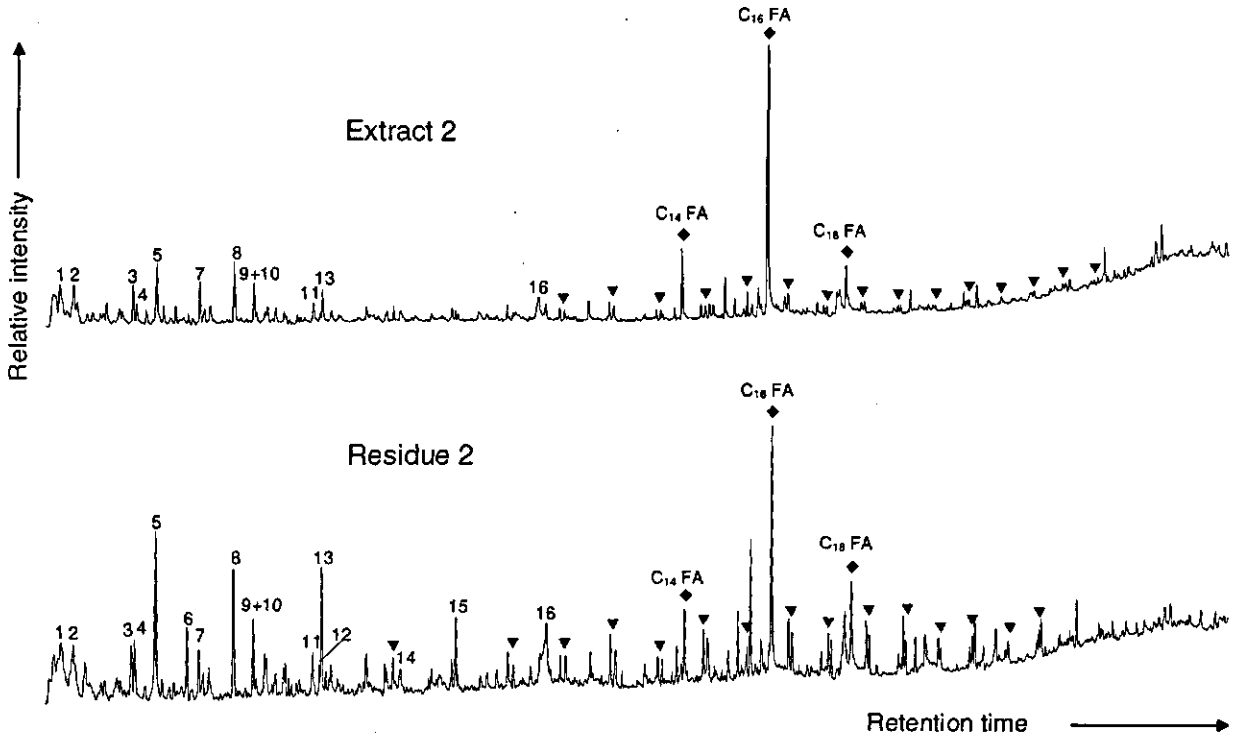
Figure 3: Pyrolysis-GC traces of $Na_4P_2O_7$-soluble (extract 2) and insoluble (residue 2) fractions of sample 1. Numbers and symbols correspond to compounds listed in Table 2.
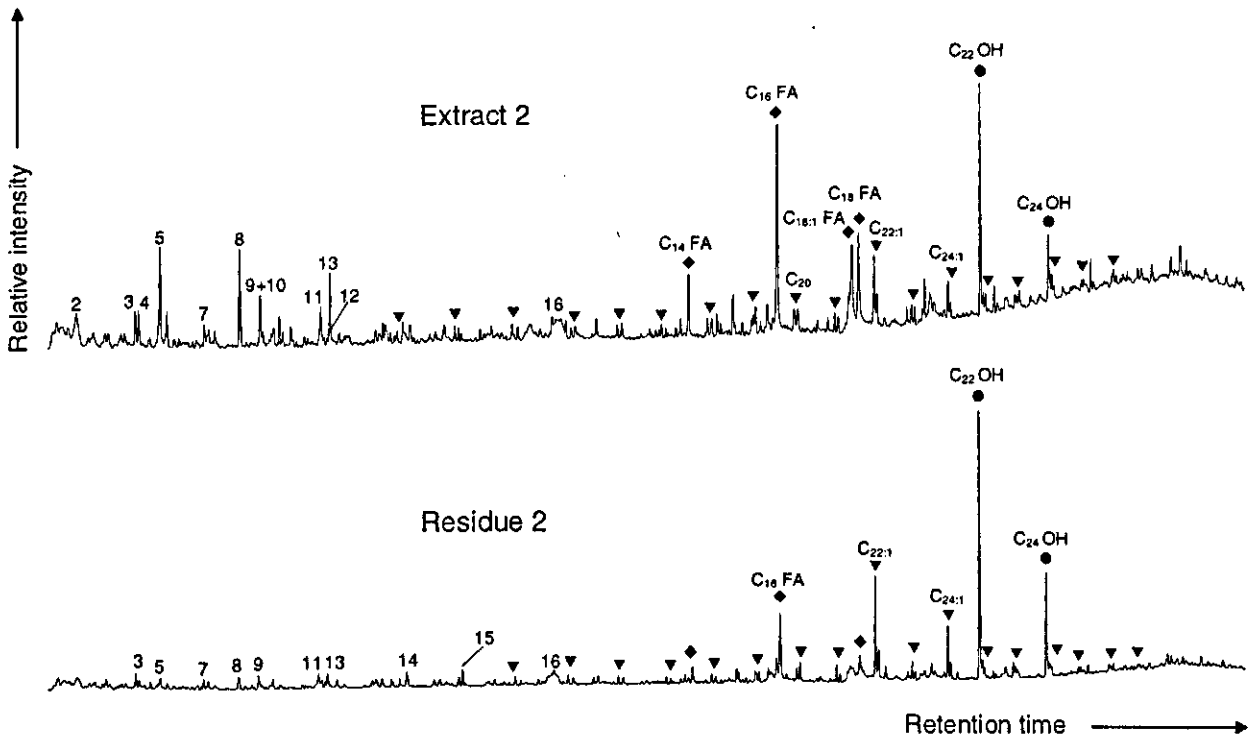

Figure 4: Pyrolysis-GC traces of $Na_4P_2O_7$-soluble (extract 2) and insoluble (residue 2) fractions of sample 5. Numbers and symbols correspond to compounds listed in Table 2.
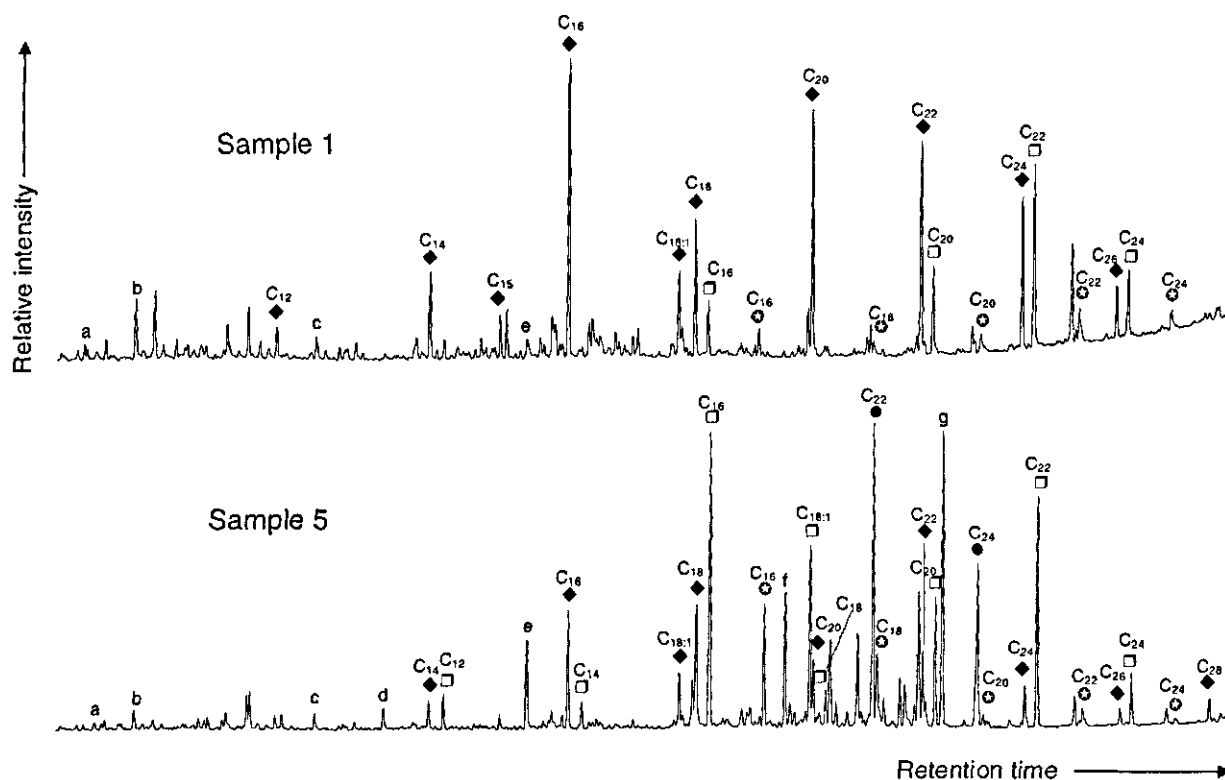
Figure 5: Thermochemolysis-GC traces of residue 2 of samples 1 and 5. For symbol legend, see Table 3.

Table 3: Major identified thermochemolysis products

No.	Compound	M
a	1,2,3-Trimethoxybenzene	168
b	1,2,4-Trimethoxybenzene	168
c	3,4-Dimethoxybenzoic acid, methyl ester	196
d	3-(4-Methoxyphenyl)-2-propenoic acid, methyl ester	192
e	3-(3,4-Dimethoxyphenyl)-2-propenoic acid, methyl ester	222
f	9,16 and 10,16-Dimethoxyhexadecanoic acid, methyl ester	330
g	9,10,18-Trimethoxyoctadecanoic acid, methyl ester	388
◆	Fatty acid, methyl ester (FAME)	
▢	ω-Methoxy fatty acid, methyl ester	
◎	α,ω-Alkanedioic acid, dimethyl ester	
●	1-Methoxyalkane	

All thermochemolysis-GC traces of the residues 2, after NaOH and $Na_4P_2O_7$ extractions, are dominated by methyl esters and/or ethers of fatty acids (C_{12}-C_{28}), ω-hydroxy fatty acids (C_{12}-C_{24}) and α,ω-alkanedioic acids (C_{16}-C_{24}). Sample 4, and in particular sample 5 (Fig. 5 and Tab. 3), have abundant signals of methyl ethers and/or esters of 1-alcohols (C_{22} and C_{24}), 9,16- and 10,16-dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic

acid. Also the ω-hydroxy fatty acids and, α,ω-alkanedioic acids are more important in the GC traces of samples 4 and 5 than in those of samples 1-3. Only small traces of other compounds are present, confirming the results of conventional pyrolysis that the residues are predominantly aliphatic. All aliphatic compounds mentioned above can be related to the biopolyester suberin, present in the periderms that cover secondary stems, wounds and secondary roots (Kolattukudy 1980, Matzke and Riederer 1991, Riederer et al. 1993, Tegelaar et al. 1995), with the 1-alcohols (C_{22} and C_{24}) in samples 4 and 5 as marker of beech suberin in the soil. Although data about the chemical composition of suberin in roots are lacking, the ratio of the C_{22} to C_{24} alcohol of about 2 resembles that of values measured in suberin from beech bark (Holloway 1983).

Furthermore, the relatively high abundance of 22-hydroxydocosanoic acid, hexadecanedioic acid and 9,10,18-trihydroxyoctadecanoic acid refers to suberin derived from beech (Holloway 1983, Matzke and Riederer 1991). The relatively high contributions in the pyrolysates of methylated versions of p-coumaric and ferulic acid in relation to other aromatic compounds suggest that these acids are derived from, or esterified with suberin.

Fatty acids with chain lengths < C_{20} are uncommon constituents of suberin (Kolattukudy 1980, Holloway 1983). Therefore, they will have originated from other compounds, most probably from triglycerides or waxes. The alkene/alkane patterns observed in the Py-GC traces of all residues are not encountered after thermochemolysis. This resembles the pyrolytic and thermochemolytic behaviour as encountered for the aliphatic biopolymer cutan from the cuticle of *Agave americana*. It gave a homologous series of alkadiene/alkene/alkane triplets upon pyrolysis, whereas, among others a series of fatty acid methyl esters (FAMEs) is obtained after thermochemolysis (McKinney *et al.* 1996). Thus, the FAMEs obtained in the residues can to some extent also be derived from an aliphatic polymer similar to cutan or suberan.

In addition to the FAMEs, McKinney *et al.* (1996) identified also a number of aromatic compounds, mainly 1,3,5-trimethoxybenzene derivatives and, in lower amounts, 1,2,3-trimethoxybenzene derivatives and dimethoxybenzoic acid methyl esters. We did not observe 1,3,5-trimethoxybenzenes, whereas 1,2,3- and 1,2,4-trimethoxybenzenes were clearly present. It cannot be excluded that these trimethoxybenzenes are indeed derived from a suberan-like aliphatic biopolymer with building blocks similar to those of cutan from *Agave americana*, but structurally different.

Scanning Electron Microscope (SEM)

SEM (not shown) was applied to assess whether the observed chemical data obtained with pyrolysis and thermochemolysis derive from recognizable plant remnants or from completely humified, i.e. unrecognizable amorphous material.

In the humin fractions, intact and partly altered plant fragments are recognized by SEM. Some of the fragments can be identified as parts from roots, but all fragments consisted predominantly of cell walls without inner cell material. $Na_4P_2O_7$ extractable organic matter (extracts 2) does not contain such remnants, but forms fibrous and flagged structures, most likely caused by freeze-drying. As the humin fractions, the residues 2 contain predominantly plant structures, albeit more degraded than in the humin fractions. Apparently extraction with $Na_4P_2O_7$ removes parts of (degraded) plant tissue.

DISCUSSION

Pyrolysis, thermochemolysis and SEM results all point to a predominantly root origin of the insoluble organic matter. Emmer (1995) determined the relative root densities of the species present at sites 1-3. Except for *Empetrum*, all species have roots in the mineral horizons. The similarity of the humin fractions, the extracts 2 and the residues 2 of the organic matter in the B horizons of the profiles 1, 2 and 3 strongly suggests that the (root) input is independent of the understorey vegetation. In profiles 4 and 5, later stages in the vegetation sequence, the influence of beech as indicated by the C_{22} and C_{24} alcohols is clearly present in the B horizons. The relative increase of mono, di and trihydroxy fatty acids and α,ω-alkanedioic acids in the pyrolysates of the two samples under beech points to an increasing concentration of suberin in the B horizon. According to SEM, the organic part of the residues is composed of intact or partly altered cell walls. About half a century ago, Kononova (1943, cited by Kononova 1961) established the rapid degradation of medullary rays, cambium, phloem and parenchyma of the primary cortex in humified lucerne roots, leaving the periderm and xylem vessels unaffected. As determined by pyrolysis, polysaccharides and lignin are hardly present in the residues 2, suggesting that xylem is almost completely decomposed. This means that the periderm layer (suberin and a suberan-like polymer) of the cell wall are selectively preserved, giving rise to the predominant aliphatic character of the organic matter in the B horizons, and in the insoluble fractions in particular.

Considering the vegetation sequence and history of the Leuvenum Forest, pine trees and other species have abundant fine roots in the Ah horizon during the first succession stages. With time, the ectorganic layer develops and becomes the main rooting compartment. The old and dead roots originally present in the Ah horizon remain in the newly formed E and B horizons. Upon decay, microbial readily decomposable compounds, such as polysaccharides and lignin, decompose, while the less accessible aliphatics are selectively preserved. Under beech, not only roots from previous species contribute to the organic matter in the B horizons, but also roots from the current trees. This is clearly manifested in samples 4 and 5, where the presence of typical beech root components, such as the already mentioned C_{22} and C_{24} alcohols, is established. Site 5 has older beech trees than site 4, but with the same amount of living roots. Hence, the

relatively higher concentration of e.g. the C_{22} and C_{24} alcohols in sample 5 is most likely derived from a greater accumulation of dead beech roots.

In principle it could be possible that part of suberin in the B horizons is derived of illuviation of water soluble organic matter. However, the solubility of suberin in water is negligible (Kögel-Knabner *et al.* 1989), which is also shown by Nierop and Buurman (1999) for the WSOM fractions of the investigated profiles here. In very old podzols (e.g. thousand years old) the possible contribution of suberin in B horizons due to illuviation may be significant, but within the time span of two centuries in the soils of the Leuvenum Forest illuviation of suberin may be neglected.

Assuming that NaOH does not dissolve Al- and Fe-organic matter complexes, increasing amounts of $Na_4P_2O_7$ extractable C in the sequence from sample 1 to sample 5, suggest that effects of podzolization also increase in that direction. Nevertheless, metal-organic matter complexes seem to be of minor importance in these incipient podzols. $Na_4P_2O_7$ soluble fractions also contain aliphatic compounds. As seen with SEM, the residues after $Na_4P_2O_7$ extraction exhibit more degraded plant remnants, whereas pyrolysis suggests that the alcohols are to some extent present as esters in these fractions. This probably means that, in addition to metal-organic matter complexes, some polymerized parts are solubilized in 0.1M of $Na_4P_2O_7$.

Cold treatment with HF/HCl, followed by NaOH extraction of residue 2 does not induce much effect in dissolving organic matter. This most likely means that little of the organic matter was bound to clay. Neither hot treatment with HF/HCl followed by extraction with NaOH gives a soluble fraction, nor hot NaOH only. However, when prior to treatment with hot NaOH residues 2 are ball-milled, the bulk of the organic matter becomes soluble. This can be explained by an increasing surface area of the plant fragments in the residues 2, so that the base-catalyzed hydrolysis of ester bonds of the suberin polymers takes much faster place.

CONCLUSIONS

In conclusion, in incipient podzol B horizons, the organic matter appears to be highly aliphatic, particularly the more recalcitrant, insoluble fractions. These fractions mainly contain outer cell wall remnants without inner cell wall material. Pyrolysis and thermochemolysis confirm this, showing that this material is practically devoid of lignin and polysaccharides, and mostly consisted of aliphatics,

which can be related to a suberan-like polymer and the biopolyester suberin, present in the periderm layer of the cell wall. Periderms have a protective function in living plant roots – and apparently this function still exists –, while other plant components containing biopolymers, such as polysaccharides and lignin, are virtually absent. Hence, these root remnants can be a more important source of carbon sink in the B horizons than the illuvial organic matter caused by podzolization.

Acknowledgements

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