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Total lipid extracts from characteristic soil horizons in a podzol profile

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Summary

The podzolization process is studied through lipids in nine characteristic podzol horizons. Organic matter accumulates particularly with aluminium in the Bh horizon, while the hard, cemented Bs horizon below this is formed mainly by iron oxides. The low soil pH seems to have no great influence on the preservation of lipids as reflected by the absolute amounts present and the presence of bacterial lipid markers throughout the profile. Independent of soil pH, lipids accumulate in organically enriched horizons. Albeit, high molecular weight organic compounds accumulate to a relatively greater extent than lipids in these horizons. A lipid signal related to the aerial parts, i.e. leaves and flowers, of *Calluna* is observed only in the O horizon. This '*n*-alkane, steroid and triterpenoids' signal is quickly lost in the underlying Ah horizon due to (bacterial) oxidation. The other total lipid extracts obtained are dominated by root-derived compounds. In subsoil horizons rich in organic matter, i.e. the Ahb and Bh horizons, root-derived friedooleanan and steroid compounds dominate the total lipid signal. Degraded horizons, poor in organic matter, i.e. the E2, Bhs, Bs and B/C horizons, are dominated by C_{22} and C_{24} ω-hydroxy acids, long-chain ($> C_{20}$) *n*-alkanoic acids with a strong even-over-odd predominance and C_{22} and C_{24} *n*-alkanols. Steroid and root-derived triterpenoids with a friedooleanan structure have been removed from these horizons through degradation. Based on total organic carbon content and lipid composition, the formation of an E1 horizon has started, but is not yet complete. In the Ahb horizon, a contribution from buried vegetation to the total lipid signal is still present, although degradation and an input from roots have significantly altered the original signal. Overall, lipid data indicate that degradation (microbial oxidation) is an important process that should be taken into account, in addition to leaching, when describing podzolization processes in soils.

Introduction

Soil lipids are, by definition, organic compounds insoluble in water, but soluble in common organic solvents. They include *n*-alkanoic acids, *n*-alkanols, hydroxy acids, ketones, steroids, terpenoids, acyl glycerols and hydrocarbons, as well as phospholipids and lipopolysaccharides (Stevenson, 1982). These compounds originate from both plants and animals as products of deposition, decomposition and exudation, as well as from various other pedogenic sources, including fungi, bacteria and mesofauna (Bull *et al*., 2000a). Lipids accumulate in acid soils such as podzols (Jambu *et al*., 1985). In these, as well as in other soils, their composition is influenced by a wide range of processes, including bioturbation, oxidation, microbial degradation and hydrolysis. The rate of these processes is directly affected by soil pH, moisture, microbial biomass, etc. (van Bergen *et al*., 1997, 1998). So far, lipid research in podzols has focused on specific lipid fractions, e.g. *n*-alkanes, *n*-alkanols and *n*-alkanoic acids, mainly in litter and A horizons (e.g. Jambu *et al*., 1993; Amblès *et al*., 1998). To our knowledge, no studies on the composition of total lipid extracts throughout the whole podzol profile have been undertaken.

In this paper, the composition of total lipid extracts from nine characteristic podzol horizons from the Veluwe area (a natural park, in the centre of The Netherlands) was determined by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS). In addition, soil pH (H_2O), and ammonium oxalate- and sodium pyrophosphate-extractable aluminium and iron were determined. Results are discussed in terms of the origin of the compounds identified and the processes that effect their distribution in the podzol profile.

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Materials and methods

Site description

Samples were taken from a Haplic Podzol (FAO, 1998) at the Veluwe area near Kootwijk/Assel, The Netherlands, which has formed in wind blown sands, deposited during the Pleistocene. Vegetation is dominated by heather (*Calluna vulgaris*) and moss together with small birch trees. The profile is characterized by a litter layer (O) underlain by several horizons typical of podzols (Figure 1). Recognizable roots and root fragments were found only to a depth of about 52 cm (start of the Bs horizon).

Sampling, sample pretreatment and inorganic analyses

Samples were taken in early September 1999 from each horizon and from in between the Bh and Bs horizons (referred to as the Bhs horizon in this paper). Samples were dried at 60°C and sieved over a 2-mm and a 250-μm sieve to remove roots. Soil pH (H_2O) of the soil was measured in the supernatant of a suspension (1:2.5 sieved ($< 250\,\mu$m) sample:water) with a Scott Geräte pH meter CG 805. Sodium pyrophosphate-extractable Fe and Al were determined by shaking 0.5 g of sieved ($< 250\,\mu$m) soil overnight (16 hours) with 50 ml of 0.1 M Na pyrophosphate solution. Oxalate-extractable Fe, Al and Si were determined by shaking (4 hours in the dark) 0.5 g of sieved ($< 250\,\mu$m) soil with 25 ml 0.2 M ammonium oxalate solution. Concentrations of Al, Fe and Si were measured with a Perkin Elmer Optima3000 ICP-OES.

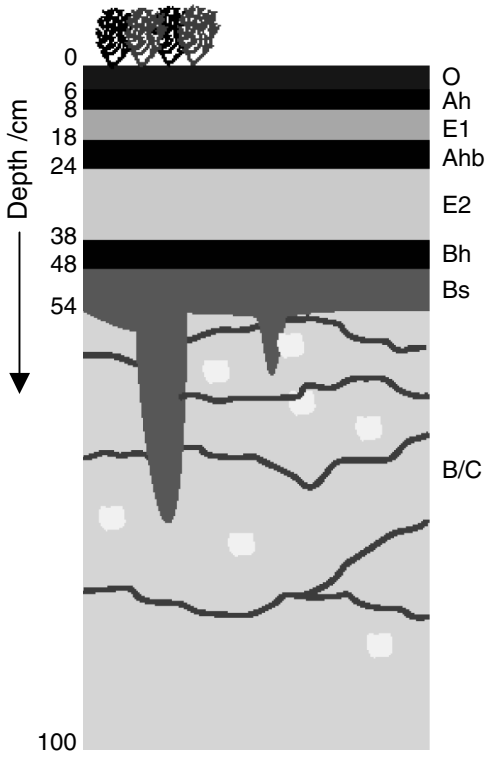

Figure 1 Schematic representation of the podzol profile sampled. Characteristic horizon symbols are indicated at the right.

Organic analyses

Total organic carbon contents of sieved ($< 250\,\mu$m) soil were measured with a Fisons Instruments NA 1500 NCS analyser, with a cycle time of 180 s, a source temperature of 190°C and an oxygen flow of *c*. 30 litres minute^{-1}. Approximately 15–25 g of each sieved ($< 250\,\mu$m) sample was extracted on a Soxhlet apparatus with dichloromethane/methanol (DCM/MeOH) (9:1 by volume) for 24 hours. The DCM/MeOH extract collected was taken to complete dryness in a rotary evaporator. The dry residue obtained was dissolved in approximately 2 ml DCM/isopropanol (2:1 by volume), filtered in a Pasteur pipette packed with defatted wool, 0.5 cm Na_2SO_4 and 2 cm SiO_2, and dried in a stream of N_2. Free hydroxyl and carboxylic acid groups present in an aliquot were derivatized to their corresponding trimethylsilyl (TMS) ethers and esters, respectively, by heating for 1 hour at 70°C with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane. The derivatized aliquots were dried under N_2 and dissolved in hexane. An aliquot of a standard solution containing 0.18 μg μl^{-1} 10-nonadecanone was added. The remaining part of the extract was evaporated to dryness in air.

Gas chromatography (GC)

Derivatized total lipid extracts in hexane (1 μl) were analysed by GC on a Hewlett-Packard 6890 instrument equipped with a CP-sil 5CB silica column (50 m × 0.32 mm, film thickness 0.12 μm). Extracts were injected into the column. The oven temperature was programmed from 70°C to 130°C at 20°C minute^{-1}, and from 130°C to 320°C (isothermal for 20 minutes at 320°C) at 4°C minute^{-1}. Compounds were detected with a flame ionization detector (FID) at 325°C. Helium was used as carrier gas.

Gas chromatography–mass spectrometry (GC/MS)

GC/MS analyses were performed on a Hewlett-Packard 5890 series II gas chromatograph connected to a Fisons Instruments VG platform II mass spectrometer operating at 70 eV, scanning the range *m*/*z* 50–650 in a cycle of 0.65 s. The capillary column and temperature programme were as described for the GC analyses. The compounds were identified from their mass spectral data and a NIST library or by interpretation of the spectra and the GC retention times. In addition, compound identification was based on published data (e.g. Holloway, 1982; Killops & Frewin, 1994; van Bergen *et al.*, 1997).

Results

Inorganic soil analyses

Values for pH (H_2O) ranged from 4.6 to 3.5 (Table 1). From the O horizon (4.6) to the Ah horizon (3.7), the pH (H_2O) decreased, only to increase again to 4.6 in the E2 horizon. There was most active iron (Fe_{ox}) in the Bs horizon and least in the E2 horizon. The amount of organically complexed Al (Al_{py}) was greatest in the Bh horizon and, like the organically complexed Fe, was least in the E2 horizon.

Total organic carbon and quantification of total lipid extracts

The largest total C contents (> 5%) were found in the O, Ah, Ahb and Bh horizons, while both E horizons together, with the Bs and B/C horizons, contained less than 2% (Table 2). The C contents of residues after solvent extraction showed a similar trend to those of the unextracted samples. The yields of total lipid extracts (mg g^{-1} soil) ranged from 0.02 to 2.8, the highest amounts again being found in the humic horizons. The Ah horizon made the largest contribution of lipids to the total C on a mass basis.

The composition of total lipid extracts from all nine characteristic horizons

The extract obtained from the O horizon was dominated by a series of long-chain ($> C_{25}$) odd-over-even dominated *n*-alkanes (Figure 2a), the most abundant being C_{31} and C_{33}. In addition, steroids and triterpenoids (see Table 3 for characteristic mass fragments and IUPAC names) were detected and included taraxerol, camposterol, stigmasterol, β-sitosterol, C_{29} ketosteroid, α-amyrin, lupeol, friedoolean-3-one, an unidentified triterpenoid and a C_{30} triterpenyl acid. Other compounds, present in smaller concentrations, were even-over-odd dominated C_{18}–C_{26} *n*-alkanols, *n*-alkanoic acids, ranging from C_{14} to C_{26}, C_{15} *iso* and *anteiso* acids, C_{18} isoprenoid methylketone and short-chain ($< C_{25}$) *n*-alkanes without an odd-over-even predominance.

C_{31} and C_{33} *n*-alkanes, together with their co-eluting C_{29} and C_{31} methylketones, were also fairly abundant in the extract of the Ah horizon (Figure 2b). In addition, a C_{29} ketosteroid together with triterpenoids including taraxerone, friedoolean-3-one and an unknown steroid, dominated the last part of the chromatogram. Even-over-odd dominated C_{12}–C_{32} *n*-alkanoic acids and C_{23}–C_{28} odd-over-even dominated methylketones were found in relatively large concentrations. Apart from saturated linear fatty acids, C_{15} *iso* and *anteiso* acids and one C_{18} *n*-alkenoic acid were found. β-sitosterol was present only in smaller concentrations, together with even C_{20}–C_{26} *n*-alkanols, the C_{18} isoprenoid methylketone, C_{22} and C_{24} ω-hydroxy acids, and short-chain ($< C_{25}$) *n*-alkanes without an odd-over-even predominance.

The only difference between the extract obtained from the E1 horizon (Figure 2c) and that from the Ah horizon (Figure 2b) was the further increase in relative concentration of the *n*-alkanoic acids. In contrast, *n*-alkanoic acids were much less important in the Ahb extract (Figure 2d), which extract was characterized both by β-sitosterol and by the C_{29} ketosteroid. Other important compounds were friedoolean-2-ene, T2, friedoolean-3-one, T9, stigmasterol (Table 3), C_{22} and C_{24} *n*-alkanols, and the co-eluting C_{31} *n*-alkane and C_{29} methylketone.

In the E2 horizon, the relative contribution of steroids and triterpenoids was smaller again, whereas the even-over-odd dominated *n*-alkanoic acids, *n*-alkanols and C_{22} and C_{24} ω-hydroxy acids were more abundant (Figure 2e). Two short-chain, i.e. C_8 and C_9, ω-hydroxy acids were identified together with phytol, C_{23}–C_{31} methylketones and C_{12} and C_{14} *n*-alkanoic acids.

The compound β-sitosterol and a C_{29} ketosteroid, together with several triterpenoids including friedoolean-2-ene, taraxerone, taraxerol and friedoolean-3-one, characterized the extract obtained from the Bh horizon (Figure 2f), similar to the Ahb horizon extract (Figure 2d). Other fairly abundant compounds included C_{22} and C_{24} *n*-alkanols and *n*-alkanoic acids. In addition to a series of odd-over-even dominated ketones (C_{23}–C_{31}), *n*-alkanes without a clear odd-over-even dominance (C_{20}–C_{23}) were identified.

The C_{22} and C_{24} ω-hydroxy acids were found to dominate the extract obtained from the Bhs horizon in addition to an even-over-odd dominated series of *n*-alkanoic acids (C_{10}–C_{28}) and *n*-alkanols (C_{14}–C_{28}; Figure 2g). Compounds present in

Table 1 Inorganic soil analyses

Horizon:	O	Ah	E1	Ahb	E2	Bh	Bhs	Bs	B/C
pH (H_2O)	4.6	3.7	3.9	3.8	4.6	3.5	3.5	4.0	4.1
Al_{ox}[a] /%	0.11	0.31	0.02	0.23	0.02	1.81	0.33	0.84	0.41
Al_{py}[b] /%	0.12	0.14	0.04	0.10	0.02	0.82	0.28	0.39	0.15
Fe_{ox} /%	0.07	0.23	0.02	0.18	0.01	0.87	0.61	1.54	0.07
Fe_{py} /%	0.07	0.17	0.03	0.07	0.02	0.42	0.52	0.75	0.03

[a]Oxalate extractable.

[b]Pyrophosphate extractable.

Table 2 Analyses of total organic carbon (TOC) and total lipid extracts (TLEs)

Horizon:	O	Ah	E1	Ahb	E2	Bh	Bhs	Bs	B/C
TOC /wt %	10.1	7.8	2.0	5.3	0.6	6.6	1.7	1.2	0.4
TOC Sox. Res.[a] /wt %	9.6	7.3	1.6	4.9	0.5	5.7	1.2	1.1	0.4
TLEs /mg g^{-1} soil	2.2	2.8	0.3	1.1	0.1	1.6	0.2	0.1	0.1
TLEs /mg g^{-1} TOC	21.9	35.7	13.0	20.8	22.3	24.2	12.9	3.9	6.7

[a]Soxhlet residue.

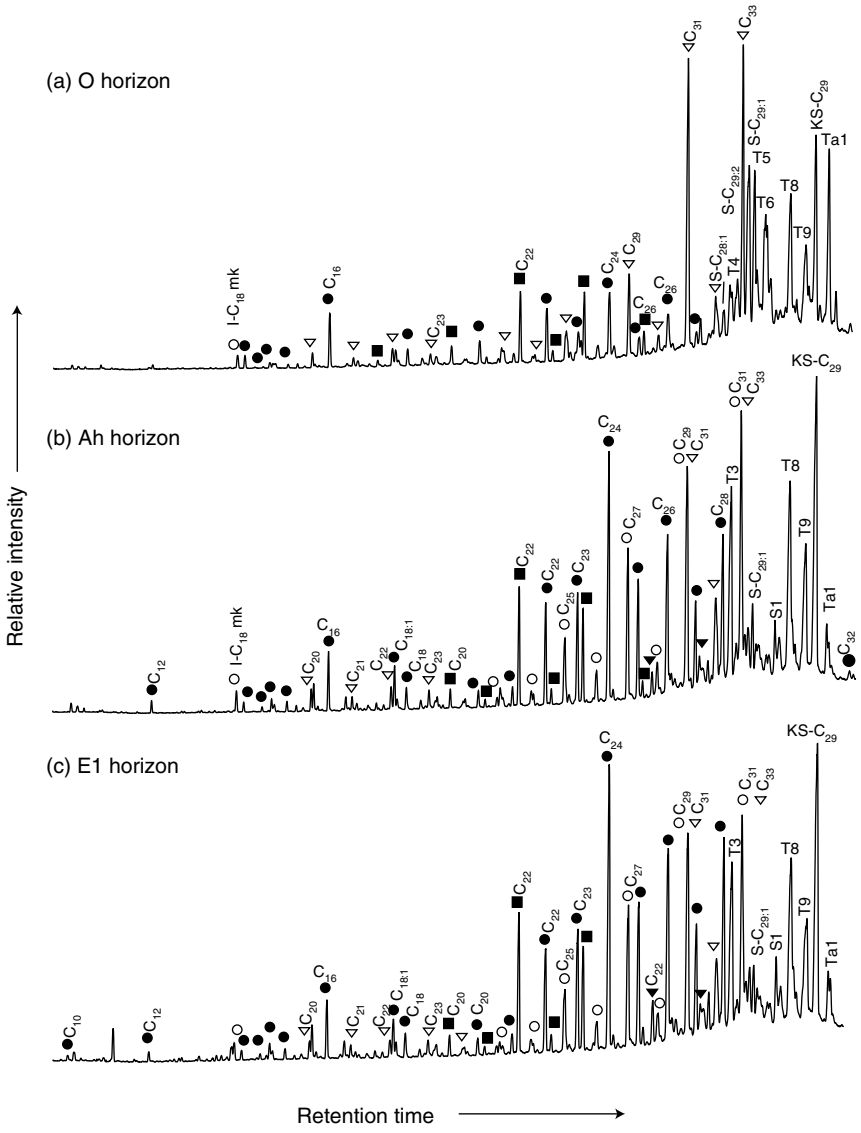

Figure 2a–c

smaller concentrations included *n*-alkanes (C_{18}–C_{23}), β-sitosterol, taraxerone, friedoolean-3-one, the C_{29} ketosteroid, as well as ketones (C_{23}–C_{29}) and two saccharides.

The Bs horizon total lipid extract was characterized by a series of even-over-odd dominated C_{12}–C_{28} *n*-alkanoic acids maximizing at C_{18}, C_{22} and C_{24} (Figure 2h). In addition, a series of even-over-odd dominated *n*-alkanols ranging from C_{14} to C_{28} and maximizing at C_{20}, C_{22} and C_{24}, was found. Other compounds detected were β-sitosterol, C_{22} and C_{24} ω-hydroxy acids, C_{27} and C_{29} ketones, a saccharide, taraxerone and friedoolean-3-one.

Like the sample obtained from the Bhs horizon (Figure 2g), long-chain ($> C_{20}$) *n*-alkanoic acids together with C_{22} and C_{24} ω-hydroxy acids characterized the B/C horizon extract (Figure 2i). Although there was a small increase in the relative concentration of C_{20} *n*-alkane and the long-chain *n*-alkanoic acids, the B/C extract resembled to a great extent the extract obtained from the overlying Bhs horizon (Figure 2h).

Discussion

Inorganic and bulk organic soil analyses

The largest amounts of aluminium and iron complexed with organic matter, i.e. Al_{py} and Fe_{py}, are found in the B horizons (Bh, Bs; Table 1). According to these data, soluble organic matter transported down the profile is immobilized in particular by aluminium in the Bh horizon (Lundström *et al*., 1995; Wesselink *et al*., 1996). The Al_{ox} and Fe_{ox} data, in combination

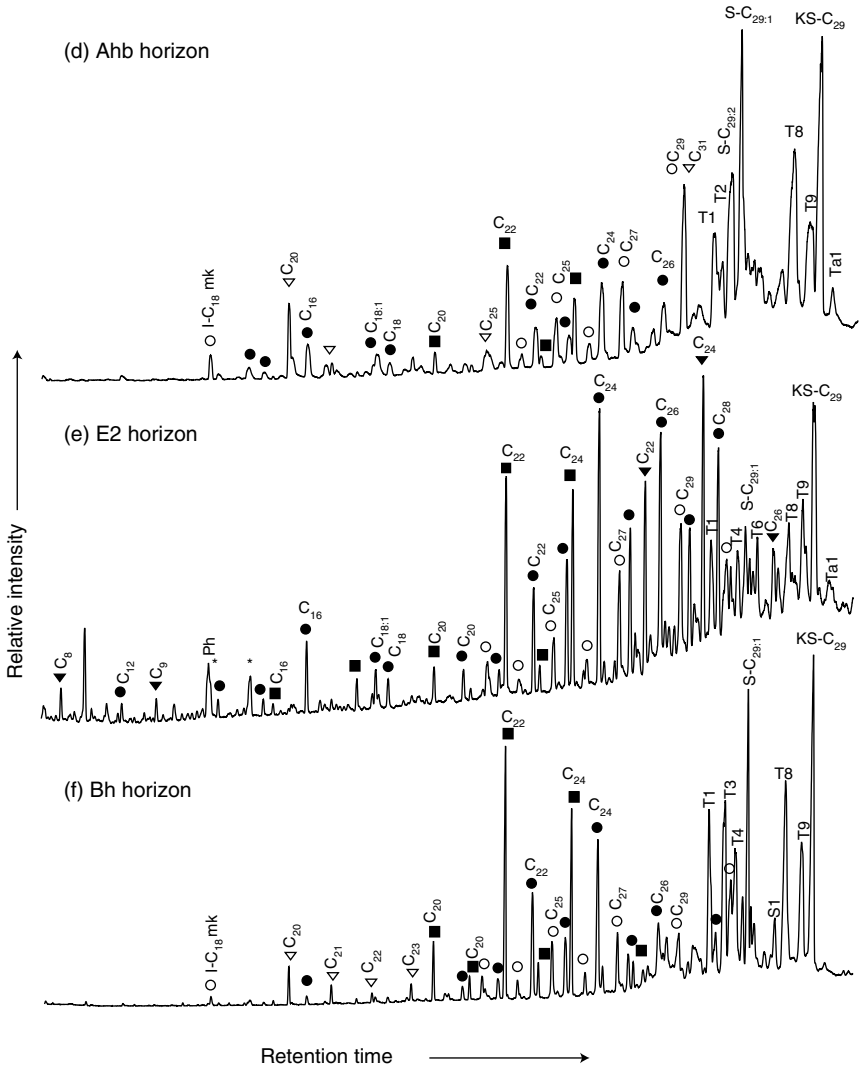

Figure 2d–f

with Al_{py} and Fe_{py} data, show that the Bs horizon is mainly formed by Fe oxides (Table 1).

Acid soils are known to enhance the preservation of lipids (Jambu *et al.*, 1985; van Bergen *et al.*, 1998; Bull *et al.*, 2000a). All concentrations of extractable lipids, with the exception of the Ah sample, where it is $35.7\,mg\,g^{-1}$ total C (Table 2), lie within the range of $2–25\,mg\,g^{-1}$ normally found for soils (Stevenson, 1982). The low soil pH therefore seems to have no great influence on the preservation of lipids in the soil profile studied.

The change of total C with depth (Figure 3) clearly indicates the presence of layers (E1 and E2) containing little organic carbon, an accumulation of organic matter (Ah, Bh), and a litter layer (O). The Bhs, Bs and B/C samples contain small amounts of organic matter, indicating that its accumulation occurs mainly above the hard, cemented Bs horizon (De Coninck, 1980). In addition to these characteristic horizons (van Breemen & Buurman, 1998), accumulated organic matter was found in the buried A horizon (Ahb). The newly formed E1 horizon on top of this Ahb horizon (Figure 1) still contains 2% total C (Table 1 and Figure 3), suggesting that the formation of this horizon has started, but is not yet complete.

The absolute amounts of extracted lipids (Figure 3) show a strong correlation with the total C contents (Figure 4 in which $r = 0.94$). When the Ah horizon is excluded, the correlation is even stronger ($r = 0.98$), showing that lipids accumulate in organic-rich horizons. Comparison of the contribution of lipids to the total C with the C content itself reveals that lipids do not accumulate relative to other forms of soil organic matter in these horizons. In other words, organic compounds other than lipids have accumulated even more than lipids in podzol B horizons (Schmidt *et al.*, 2000). The relatively large contribution of lipids to the total amount of organic carbon in the E2 horizon (Table 2) could arise because hydrophobic

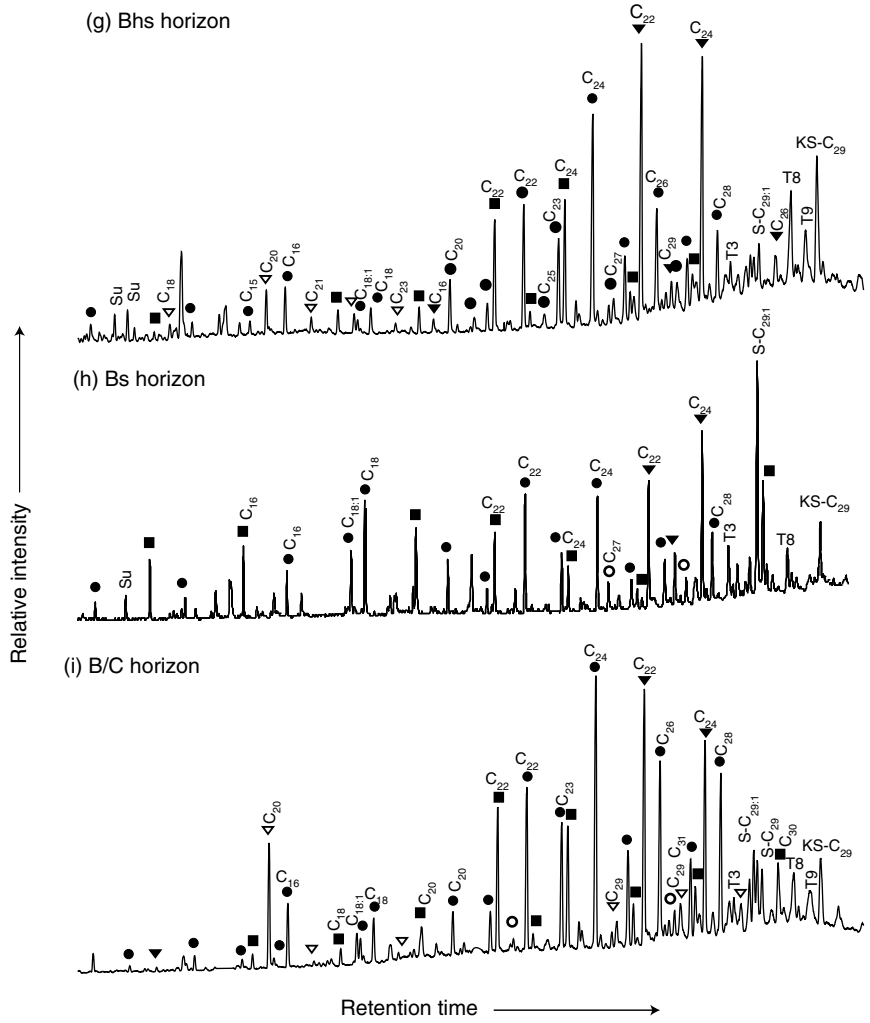

Figure 2 Gas chromatograms of total lipid extracts (TLEs) obtained from: (a) O horizon, (b) Ah horizon, (c) E1 horizon, (d) Ahb horizon, (e) E2 horizon, (f) Bh horizon, (g) Bhs horizon, (h) Bs horizon, and (i) B/C horizon. Key: ■, *n*-alkanols; ●, *n*-alkanoic acids; ▽, *n*-alkanes; ▼, *ω*-hydroxy acids; ○, ketones; Su, saccharide; I-C_{18} mk, isoprenoid methylketone; Ph, phthalate. C_x refers to the total number of carbon atoms. S-C_x refers to steroids with *x* being the total number of carbon atoms. KS-C_x refers to ketosteroids with *x* being the total number of carbon atoms. The number after the colon refers to the total number of double bonds. T*x* and Ta*x* refer to triterpenoids and triterpenoic acids, respectively, with *x* referring to their number as assigned in the text and Table 3. Co-eluting compounds are indicated according to their relative contribution, i.e. the most abundant compound is indicated first.

compounds, such as lipids, are less susceptible to leaching than are hydrophilic organic compounds. Alternatively, it could be due to the very small total C content which would exaggerate any errors in this ratio. The difference between the theoretical yield calculated using the difference in total C% between the unextracted and solvent extracted samples (Table 2), and the absolute weight of the total lipid extracts, is caused by losses during the preparation of the extract. The large contribution of free lipids found in the Ah horizon matches the accumulation of aliphatic compounds in A horizons of acidic soils observed previously (Jambu *et al.*, 1993) and is most likely due to the hydrolysis of wax esters (Jambu *et al.*, 1993).

Molecular composition of total lipid extracts (TLEs) from characteristic horizons

Composition of the TLE from the O horizon. The dominant long-chain ($> C_{27}$) alkanes with a strong odd-over-even predominance (Figure 2a) are usually ascribed to epicuticular waxes and protective layers on vascular plants and commonly observed in lipid extracts from aerial vegetation and (top) soils (Amblès *et al.*, 1989; Jambu *et al.*, 1991). Moreover, the relatively large amounts of C_{31} and C_{33} alkanes (Figures 6c and 7c), with smaller contributions of C_{27} and C_{29}, are characteristic of heather (*Calluna*) flowers and leaves (Nierop *et al.*, 2001) that grow on top of the profile. A heather-derived input is also reflected in the *n*-alkanol distribution, that

Table 3 Characteristic mass fragments of steroid and known and unknown triterpenoid compounds (analysed as their trimethylsilyl (TMS) derivatives)

Compound (in order of elution)	Characteristic fragment ions (m/z)	$[M]^+$
Friedoolean-2-ene[a] (T1)[b]	69, 95, 205, 218, 231, 243, 257, 274, 287, 395	410
Unidentified triterpenoid (T2)	73, 121, 190, 204, 218, 269, 359, 393, 483	498
24-Methylcholest-5-en-3β-ol[a,c] (campesterol) (S-$C_{28:1}$)	75, 129, 213, 255, 261, 343, 367, 382, 457	472
Friedoolean-14-en-3-one (T3) (taraxerone)[a]	69, 81, 95, 109, 189, 191, 203, 205, 273, 409	424
Friedoolean-14-en-3β-ol (T4) (taraxerol)[a]	121, 135, 189, 204, 269, 284, 359, 374, 393, 408, 483	498
24-Ethylcholest-5, 22-dien-3β-ol[a,c,d] (stigmasterol) (S-$C_{29:2}$)	83, 129, 255, 351, 379, 394, 469	484
24-Ethylcholest-5-en-3β-ol[a,c,d] (β-sitosterol) (S-$C_{29:1}$)	129, 255, 275, 357, 381, 396, 471	486
24-Ethylcholestan-3β-ol (S-C_{29})[a]	75, 95, 107, 215, 257, 305, 359, 383, 398, 473	488
Ursan-12-en-3β-ol[c] (α-amyrin) (T5)	73, 95, 189, 190, 203, 218, 279, 393, 408, 483	498
Lupeol[c,d] (T6)	73, 189, 190, 203, 218, 369, 393, 408, 483	498
Steroid[a] (S1)	69, 95, 109, 134, 187, 205, 259, 274, 286, 393	408
Friedoolean-3-one[a] (T8)	69, 81, 95, 109, 123, 273, 302, 341, 379, 411	426
Triterpenoid[a] (T9)	69, 81, 95, 109, 121, 137, 205, 218, 313, 327, 341, 409, 424, 481	496
24-Ethyl-5-cholestan-3-one (KS-C_{29})[a] (C_{29} ketosteroid)	69, 81, 95, 109, 123, 147, 163, 177, 207, 231, 257, 275, 317, 395	414
C_{30} Triterpenyl acid[d] (Ta1)	73, 133, 189, 203, 279, 320, 483, 585	600

[a]Van Smeerdijk & Boon (1987).
[b]Letters (Tx, Sx) used in Figure 2 shown in parentheses.
[c]Killops & Frewin (1994).
[d]Van Bergen *et al.* (1997).

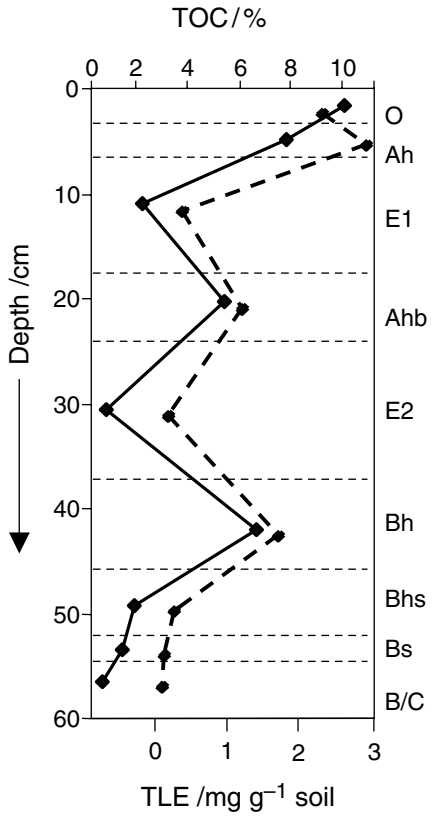

Figure 3 Total organic carbon (TOC) and total lipid extract (TLE) plotted against depth. The TOC is represented by the solid line, and the TLE by the dashed line. Characteristic horizons occur between dashed lines; horizon codes are found at the right.

revealed a relatively large abundance of the C_{22} and C_{24} *n*-alkanols characteristic for *Calluna* stem wood and roots (Nierop, 1998; Nierop *et al.*, 2001) as well as Ericaceae rootlets (van Smeerdijk & Boon, 1987).

The *n*-alkanoic acids identified can have various natural origins such as plant, fungal or bacterial origins (Amblès *et al.*, 1994b). Straight-chain compounds of fungal origin are similar to those of plant origin, but they range only from C_{10} to C_{24}, often characterized by the presence of unsaturated C_{16} and C_{18} acids (Weete, 1976; Ruess *et al.*, 2002). Furthermore, long-chain ($> C_{20}$) *n*-alkanoic acids in this topsoil layer may originate from the hydrolysis of wax esters (van Bergen *et al.*, 1998) or the oxidation of a variety of other compounds such as *n*-alkanes and *n*-alkanols (Mouçawi *et al.*, 1981; Amblès *et al.*, 1994a,b), processes which are enhanced by a low soil pH (van Bergen *et al.*, 1998) and the presence of hydrous iron oxides (Mouçawi *et al.*, 1981). The strong even-over-odd dominance and chain-length distribution observed (Figure 2a) imply that odd-chain compounds such as *n*-alkanes are unlikely sources. Based on the same criteria, and the dominance of both C_{22} and C_{24} acids, a contribution from the oxidation of *n*-alkanols is more likely.

The *n*-alkanes ranging from C_{20} to C_{27}, with a carbon preference index close to unity, are more probably of microbial origin (Amblès *et al.*, 1989), although they have also been identified in association with Ericaceae rootlets (van Smeerdijk & Boon, 1987). A microbial input is also obvious from the *iso*- and *anteiso*- C_{15} fatty acids (Boon *et al.*, 1977), and possibly by the detection of C_{18} alkenoic acid (Parlanti *et al.*, 1994).

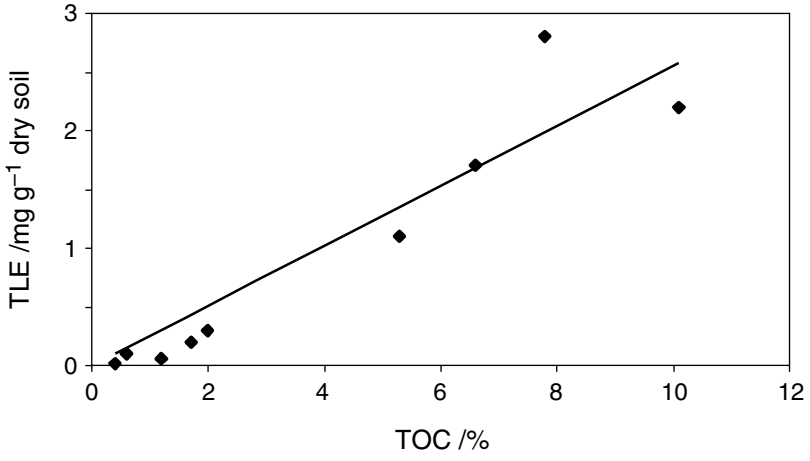

Figure 4 Scatter of total lipid extract (TLE) plotted against total organic carbon (TOC), for which the correlation coefficient, *r*, is 0.94.

The steroids and triterpenoids identified have frequently been detected in soil and leaf or litter extracts (Killops & Frewin, 1994; van Bergen *et al*., 1997; Bull *et al*., 1998). However, one should note that lupeol, taraxerol, α-amyrin and the triterpenoic acid are associated more with aerial vegetation (Killops & Frewin, 1994). On the other hand, all steroid compounds identified, together with friedoolean-3-one and T9, have been recognized as significant constituents of heather roots (van Smeerdijk & Boon, 1987; Nierop *et al*., 2001), although heather leaves and flowers do not contain these compounds in significant amounts (Nierop *et al*., 2001). In addition to their vascular plant origin, C_{29} steroids are derived from fungi (Weete, 1976; Grandmougin-Ferjani *et al*., 1999). Ketosteroids are oxidation products of sterols (Goad, 1991) and have been identified in association with heather rootlets (van Smeerdijk & Boon, 1987). The C_{18} isoprenoid methylketone is a degradation product of phytol, which has been found before in total lipid extracts from aerial vegetation (van Bergen *et al*., 1997; Bull *et al*., 2000a).

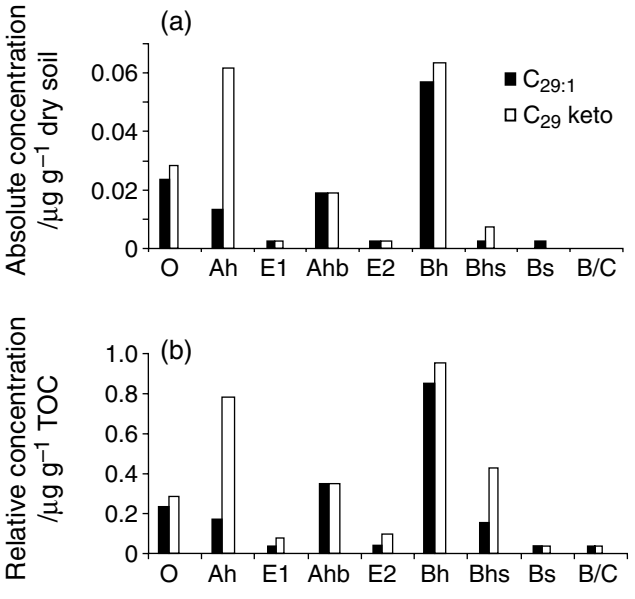

Figure 5 Concentration of β-sitosterol and C_{29} ketosteroid by horizon: (a) absolute concentration (μg g^{-1} dry soil), and (b) relative concentration (μg g^{-1} TOC).

Composition of the TLE from the Ah horizon. Both the relative (Figures 2b and 5b) and absolute (Figure 5a) amounts of β-sitosterol decrease from the litter to the Ah horizon. A decrease in sitosterol, as well as other steroids and terpenoids associated with aerial parts of vegetation (Killops & Frewin, 1994), was seen when lipids from forest soil litter layers or leaf extracts were compared with those from the underlying A horizon (Jambu *et al*., 1993; Amblès *et al*., 1994a,b; van Bergen *et al*., 1997; Bull *et al*., 2000a). It is suggested that these compounds are easily mineralized in the soil (van Bergen *et al*., 1997) or that their decrease in soluble lipid fractions can be caused by their condensation into more stable, insoluble moieties (Gobé *et al*., 2000). In addition, they may be chemically altered to form modified steroids and triterpenoids (van Bergen *et al*., 1997).

The significant increase in concentration of the C_{29} ketosteroid (Figure 5) and taraxerone from the O to the Ah horizon probably results from oxidation (van Bergen *et al*., 1997). In addition, taraxerone (T3), friedoolean-3-one (T8) and T9 triterpenoids have also been found in heather rootlets (van Smeerdijk & Boon, 1987; Nierop *et al*., 2001), reflecting a direct input. In general, more unsaturated compounds are more susceptible to (microbial) degradation. The total decrease of stigmasterol ($C_{29:2}$) compared with that of β-sitosterol ($C_{29:1}$) is therefore related to the degree of unsaturation. In addition, a source of β-sitosterol has been found in ester-linked lipids obtained from soil samples taken from a depth of 15–50 cm (Naafs & van Bergen, 2002).

Another group of lipids that decreases rapidly from the O to the Ah horizon is the *n*-alkanes (Figures 6c and 7c), as observed in acid soils (Marseille *et al*., 1999). This decrease in *n*-alkane concentration is accompanied by an increase in the

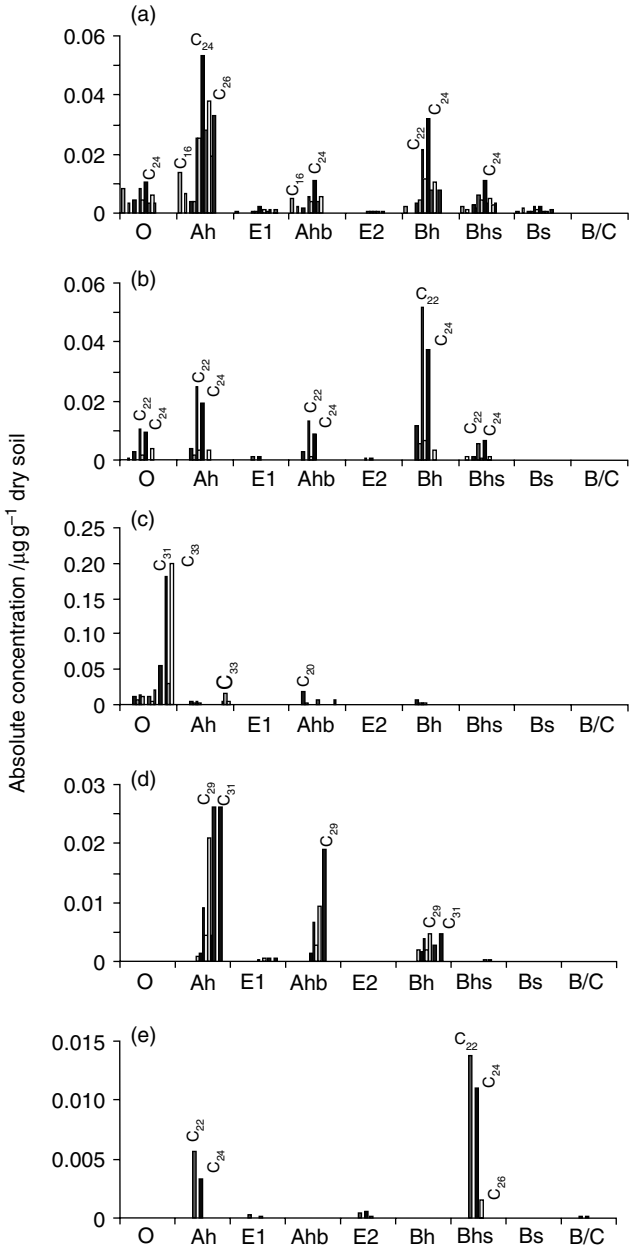

Figure 6 Absolute concentration ($\mu g\,g^{-1}$ dry soil) of C_{16}–C_{33} lipid compounds by horizon: (a) *n*-alkanoic acids, (b) *n*-alkanols, (c) *n*-alkanes, (d) ketones, and (e) ω-hydroxy acids.

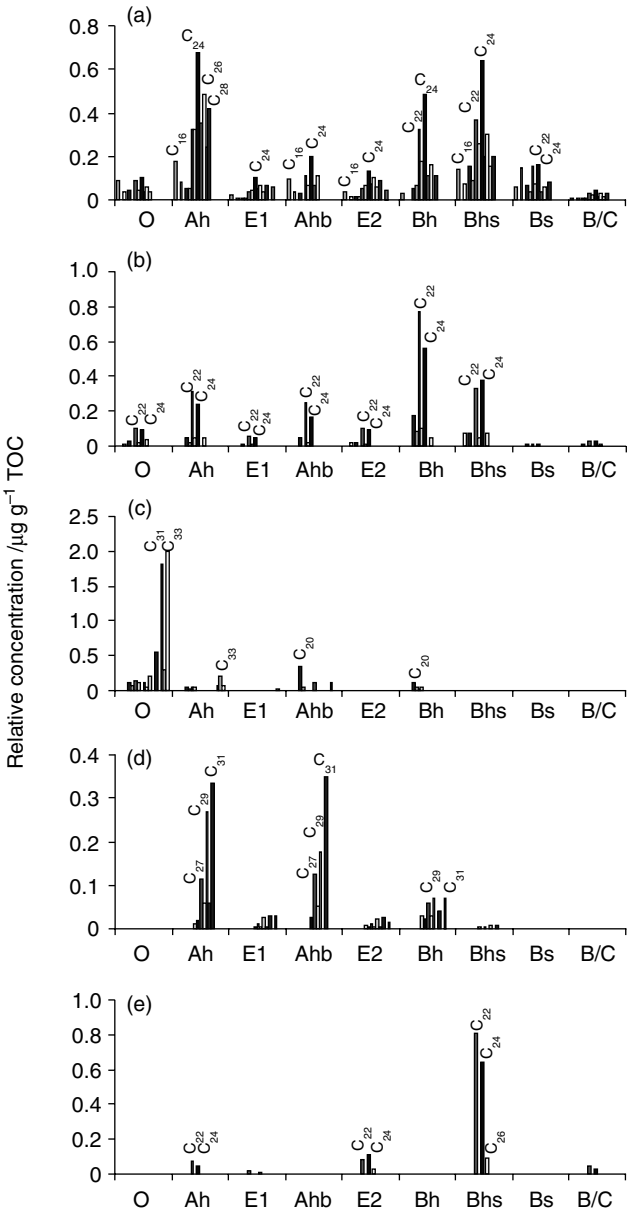

Figure 7 Relative concentration ($\mu g\,g^{-1}$ TOC) of C_{16}–C_{33} lipid compounds by horizon: (a) *n*-alkanoic acids, (b) *n*-alkanols, (c) *n*-alkanes, (d) ketones, and (e) ω-hydroxy acids.

concentration of methylketones (Figures 2b, 6d and 7d), which are not known as primary plant products. Considering that *n*-alkanes are a likely substrate for *in situ* microbial β-oxidation based on similarities found in the distribution of ketones and *n*-alkanes (Amblès *et al*., 1993), and that such similarities are found in the podzol profile (Figure 2a), we assume that methylketones in the Ah horizon are derived from such oxidation. The relatively small contribution of methylketones to the total amount of lipids in the litter layer (Figure 6d) is expected because methylketones are usually detected in such layers only in trace amounts (Amblès *et al*., 1993). Furthermore, because of their biodegradation, the amount of methylketones determined in soil is much less than the amounts produced from *n*-alkanes (Amblès *et al*., 1993).

The increase in absolute (Figure 6a) and relative (Figure 7a) concentrations of long-chain ($> C_{20}$) *n*-alkanoic acids (cf. Amblès *et al*., 1994b), together with the increase in the total amount of lipids (Figure 3), indicates that these compounds are produced in the Ah horizon. Hydrolysis of wax esters (van Bergen *et al*., 1998) and an input from roots (Bull *et al*., 2000b) are the most likely processes involved. The presence of

hexadecanoic acid and C_{18} *n*-alkenoic acid could be related to an input from mosses (Nierop *et al.*, 2001), but the acid has also been observed in root- and rhizosphere-derived total lipid extracts (Bull *et al.*, 2000a,b). Moreover, short-chain ($< C_{20}$) acids, including C_{14}, C_{16}, $C_{18:1}$ and C_{18} fatty acids, were found to be the dominant acids leached from the litter and Ah horizon during an experimental study of podzol soil lipids (Amblès *et al.*, 1998).

The absolute (Figure 6b) and relative (Figure 7b) increase in the concentration of *n*-alkanols is most likely to be the result of an input both from heather roots, considering their relatively large abundance in heather root pyrolysates (van Smeerdijk & Boon, 1987; Nierop, 1998; Nierop *et al.*, 2001), and from the hydrolysis of wax esters (Jambu *et al.*, 1993). The latter process is enhanced by the low soil pH (Wolfe *et al.*, 1989). The detection of C_{22} and C_{24} ω-hydroxy acids (Figures 6e and 7e) in this horizon reveals an input derived possibly both from aerial vegetation as well as from roots (Bull *et al.*, 2000b).

Composition of the TLE from the E1 horizon. As mentioned, the composition of the total lipid extract from the E1 horizon (Figure 2c) resembles to a great extent that from the overlying Ah horizon (Figure 2b). If we take into account the large C content (2%) for a leached horizon (Figure 3), this strongly suggests to us that the process of formation of this E1 horizon is not yet complete, although both absolute and relative amounts of lipids are very small (Figures 6 and 7). Normalized to the total C% there is, apart from the decrease in *n*-alkanoic acids and *n*-alkanols, a strong decrease in the concentration of ketones (Figure 7d) caused by their relatively rapid biodegradation as intermediates in the *n*-alkane degradation pathway (Amblès *et al.*, 1993).

Composition of the TLE from the Ahb horizon. The Ahb extract again shows a signal dominated by steroids and triterpenoids (Figure 2d), with abundant contributions from β-sitosterol (see also Figure 5), C_{29} ketosteroid, and friedoolean-2-ene (T1), T2, friedoolean-3-one (T8) and T9 triterpenoids. All these compounds have been identified in heather rootlets (van Smeerdijk & Boon, 1987). Relative to the total C%, β-sitosterol (Figure 5b), *n*-alkanols (Figure 7b) and ketones (Figure 7d) show a significant increase. These compounds are enriched in the buried A horizon either as remnants of the buried vegetation, or as the result of an input by roots (Nierop *et al.*, 2001). A contribution from above-ground litter lipids through transport to Ah horizons in sandy soils, on the other hand, has been found to be negligible. Possible evidence for a contribution from previous vegetation could be the increase in both absolute (Figure 6d) and relative (Figure 7d) methylketone concentrations most probably derived from the oxidation of *n*-alkanes (Amblès *et al.*, 1993; van Bergen *et al.*, 1997).

Composition of the TLE from the E2 horizon. C_{22} and C_{24} ω-hydroxy acids, in combination with long-chain ($> C_{20}$) acids and C_{22} and C_{24} *n*-alkanols, are the lipids that remain in the E2 horizon. In addition to these root-derived compounds (Nierop, 1998; Naafs *et al.*, 2004), C_8 and C_9 ω-hydroxy acids were detected, probably as a result of the oxidation (Regert *et al.*, 1998) of unsaturated suberin building blocks (Nierop *et al.*, 2003). Note that the distributions of both *n*-alkanoic acids and ω-hydroxy acids resemble those observed in samples of rhizosphere and mineral horizons (Bull *et al.*, 2000a,b).

Methylketones also remain in this horizon as oxidation products of alkanes (Amblès *et al.*, 1993). Because of this intensive leaching or degradation, absolute amounts of all compounds identified in this horizon are very small (Figure 6). Normalized to the total C%, however, it is shown that the decrease in the contribution of lipids to the total amount of C is caused mainly by a decrease in steroids (Figure 5b), triterpenoids and ketones (Figure 7d). Because of the labile nature of these compounds (van Bergen *et al.*, 1997) and their hydrophobicity, (microbial) degradation of these compounds seems the most likely explanation for this decrease. The contributions of *n*-alkanoic acids (Figure 7a), *n*-alkanols (Figure 7b) and ω-hydroxy acids (Figure 7e), by contrast, remain similar, or even increase. These compounds therefore reflect a root-derived input that is hard to degrade or leach. No evidence of accumulation of lipids derived from the soil surface, such as long-chain ($> C_{20}$) *n*-alkanes, has been observed in the E2 horizon. Thus, the E2 signal could be considered to be derived from an input by roots.

Composition of the TLE from the Bh horizon. Just as observed for the O and Ahb horizons, the organic matter rich Bh horizon is characterized by β-sitosterol and C_{29} ketosteroid, a series of triterpenoids, and C_{22} and C_{24} *n*-alkanols (Figure 2f). Comparison with the large total C content (Figure 3), and both absolute and relative amounts of lipid compounds identified, clearly indicates that organic matter, including lipids, accumulates in this horizon. Podzol B horizons might contain more root-derived than illuviated organic matter (van Breemen & Buurman, 1998).

The contribution of litter-derived organic matter to the subsoil in sandy soils was found to be negligible (Nierop *et al.*, 2001). Moreover, roots were found to develop preferentially in podzol Bh horizons (De Coninck, 1980). Thus, the significant increase in the contribution of steroids (Figure 5), triterpenoids and C_{22} and C_{24} *n*-alkanols (Figures 6b and 7b), all known to be derived from heather roots (van Smeerdijk & Boon, 1987; Nierop & Buurman, 1999; Nierop *et al.*, 2001), can be interpreted as resulting from the accumulation of root-derived lipids produced *in situ*. In addition to this root-derived signal, a bacterial contribution is again reflected in the presence of short-chain alkanes and methylketones. Note that, like the observations by Nierop & Buurman (1999) with respect to the insoluble organic matter fraction, we found no evidence for a contribution from lipid compounds transported from the top of the profile.

Composition of the TLE from the transition from the Bh to the Bs horizon. In the sample from the transition layer between the Bh and Bs horizons (Bhs; Figure 2g), the total lipid signal again changes dramatically into a signal dominated by ω-hydroxy acids (Figures 6e and 7e) and *n*-alkanoic acids, as observed for the E2 horizon (Figure 2e). The sharp decrease in total C content (Figure 3) indicates that no accumulation of organic matter took place in this horizon, again strongly suggesting that the signal observed is characteristic for a degraded root-derived input. The microbial signal in this horizon comprises short-chain alkanes and methylketones. It has been suggested that complexed organic matter is degraded in this horizon to yield Al and Fe which then precipitate in the underlying Bs horizon (Lundström *et al*., 1995). We suggest that this degradation may also affect the biopolyester suberin, resulting in the release of relatively large amounts of long-chain ($> C_{20}$) ω-hydroxy acids (Figures 5e and 6e) characteristic of an input by roots to the lipid signal in soils (Bull *et al*., 2000b; Naafs *et al*., 2004).

Composition of the TLE from the Bs and B/C horizons. Both the total organic carbon contents (Figure 3) and absolute amounts of lipids (Figures 3 and 6) are very small in the Bs horizon (Figure 2h). The signal observed most probably reflects the very small remnant of lipids or a minor contribution from lipids that penetrate into this hard layer from the overlying Bhs horizon (Figure 2g). Again, most root-derived steroids and triterpenoids have been degraded.

The total lipid signal from the B/C horizon (Figure 2i) again resembles that of the other horizons with a small total C content, i.e. the E2, Bhs and Bs horizons (Figure 3). The lipid signal consists of compounds derived from degraded remnants of old roots that were present in this horizon before it was cut off from the rest of the profile by the formation of the hard, cemented Bs horizon (De Coninck, 1980).

Implications of lipid data for the podzolization process. The lipids in the Bh horizon are derived mainly from a fresh input by roots that accumulate in this horizon. The limited penetration of lipids derived from the surface vegetation into the profile indicates that the influence of vertical transport of lipids is negligible compared with (bacterial) degradation. The detection of lipid degradation products such as ketones, and bacterial markers such as short-chain alkanes and fatty acids throughout the profile, further indicates that (bacterial) degradation of lipids is an important process in this podzol profile. Because both steroids and triterpenoids have been shown to degrade rapidly within soils (van Bergen *et al*., 1997), this is a likely explanation for their small concentration in podzol E horizons. After degradation of most of these steroids and triterpenoids, a signal derived from bacteria and root-derived long-chain fatty acids and ω-hydroxy acids remains in the E horizon. These latter compounds reflect *in situ* lipid input produced by the degradation of remnants of root suberins and remain after degradation of the rest of the root-derived lipids.

Conclusions

The composition of total lipid extracts from nine characteristic horizons in a podzol from the Veluwe area (The Netherlands) has been analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS). In addition, soil pH (H_2O) and ammonium oxalate- and sodium pyrophosphate-extractable aluminium and iron were determined. From these analyses we conclude the following.

1 Lipids accumulate in horizons with large organic matter contents, but there is no increase relative to other soil organic matter.

2 An *n*-alkane, steroid and triterpenoid signal related to the aerial parts, i.e. leaves and flowers, of *Calluna* is observed only in the O horizon. This signal is quickly lost in the underlying Ah horizon as a result of (bacterial) oxidation.

3 All total lipid extracts, except that from the O horizon, are dominated by root-derived lipids.

4 In subsoil horizons with large organic matter contents, i.e. the Ahb and Bh horizons, root-derived friedooleanan and steroid compounds dominate the total lipid signal.

5 C_{22} and C_{24} ω-hydroxy acids, long-chain ($> C_{20}$) *n*-alkanoic acids with a strong even-over-odd dominance and C_{22} and C_{24} *n*-alkanols, characteristic of an input from roots, dominate the horizons with small contents of organic matter, i.e. the E2, Bhs, Bs and B/C horizons. Steroid- and root-derived triterpenoids with a friedoolean structure, once present in these horizons, have been degraded.

6 Based on total C content and molecular lipid composition, the formation of an E1 horizon has started, but is not yet complete.

7 In the Ahb horizon, a contribution from buried vegetation to the total lipid signal is still present, although degradation and an input by roots have significantly altered the original signal.

8 Overall, lipid data indicate that degradation of lipids (mainly oxidation) is an important process that should be taken into account, in addition to leaching, when describing the so-called 'podzolization process' in soils.

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