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Solvent-extractable lipids in an acid andic forest soil; variations with depth and season

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Abstract

Total lipid extracts from an acid andic soil profile located on Madeira Island (Portugal) were analysed using gas chromatography (GC) and GC–mass spectrometry (GC/MS). The profile was covered mainly by grass. Bulk soil characteristics determined included soil pH (H_2O) ranging from 4.5 to 4.0 and TOC, ranging from 84 to 30 g kg^{-1}. A decrease of the contribution of lipids per TOC with depth was observed. The absence of typical bacterial markers might be an indication for reduced bacterial activity, most likely related to the low soil pH and the presence of Al and Fe (oxides). The distribution observed in the top layer with a dominant C_{26} *n*-alkanol, steroids and triterpenoids, reflected mainly an input by grass leaves. A strong decrease in both relative and absolute concentration of these leaf-derived compounds was observed when comparing the litter layer with the mineral soil. The presence of C_{22}–C_{32} *n*-alkanoic acids, C_{22}–C_{26} ω-hydroxy acids, C_{31} *n*-alkane and C_{22}–C_{32} *n*-alkanols observed in the sub-soil is indicative of an important contribution by (grass) roots. In summer, a signal most likely reflecting the leaching of microbially derived products from the litter and/or aerial vegetation at the surface was observed.

Keywords: Total lipid extracts; Depth profile; Seasonal influence; Steroids; *n*-Alkanes; *n*-Alkanols; *n*-Alkanoic acids

1. Introduction

Andisols cover between 0.76 and 0.84% of the world's land area (Leamy et al., 1980) and are typically associated with the weathering of recent volcanic ash deposits in humid, temperate environments (Parfitt and Kimble, 1989; Lowe, 1997). Despite their apparent small contribution to the total of soils in the world, they are extremely important for the global carbon cycle because of their capacity to stabilize large quantities of organic carbon for thousands of years (Torn et al., 1997).

Vascular plants are the main source of organic carbon to these and other soils through litterfall and roots (Oades, 1993). Relative inputs by these two pathways vary with plant type. For grasses, roots are more important than for trees (Oades, 1993). In general, it is assumed that up to half of the organic matter input to soils is through root systems of plants. In addition to being introduced by roots and leached from surface litter, fresh plant material is mixed into the soil by earthworms and other animals (Oades, 1993).

The characteristic vegetation of Mediterranean ecosystems is especially favourable for the accumulation of waxes, lipids and resins in soil (Almendros et al., 1996). Such accumulation is important in soils. For example, lipids on the surface of soil particles can cause such particles to become hydrophobic and thereby decrease the rate of organic matter degradation as a whole (Jambu et al., 1995).

Literature data concerning the molecular composition of the total free lipid fraction are scarce (Almendros et al., 1996; Bull et al., 1998). The total lipid fraction of soil organic matter (SOM) can be isolated readily from soils with no substantial alteration by means of solvent extraction. This fraction normally constitutes a small portion (2–50 g kg^{-1}) of the total SOM (Stevenson, 1982). However, as mentioned this fraction can be very important, for example when retarding the decomposition of SOM (Amblès et al., 1993). Moreover, lipids exhibit an inherent diagnostic value with respect to SOM, both endogenous and exogenous, and the pedological processes to which it is subjected (Bull et al., 2000a).

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Lipids are, by classical definition, organic compounds insoluble in water but soluble in common organic solvents. In addition to being present as free extractable compounds in soils, lipids are part of complex organic structures such as biopolyesters. Extraction of these bound lipids requires more vigorous extraction procedures than solvent extraction and therefore bound lipids are not considered in this paper.

Lipids include fatty acids, *n*-alkanols, hydroxy acids, ketones, steroids, terpenoids, acyl glycerols and hydrocarbons, as well as phospholipids and lipopolysaccharides (e.g. Dinel et al., 1990; Zelles et al., 1992; Stevenson, 1994). These compounds originate from both plants and animals as products of deposition, decomposition and exudation, as well as from various pedogenic sources, including fungi, bacteria and mesofauna (Bull et al., 2000a). However, as aforementioned, the main source of lipids in soils is normally the vegetation (Oades, 1993; van Bergen et al., 1997).

In this paper, total lipid extracts obtained from an andic soil profile on Madeira Island, covered mainly by grass, were analyzed using gas chromatography (GC) and GC–mass spectrometry (GC/MS). The results are discussed in terms of the relationship between the chemical composition of total lipid extracts (TLEs) and specific inorganic andic soil properties, i.e. soil pH and aluminum concentrations. In addition, the origin of compounds identified together with variations in their chemical composition with depth and season will be evaluated.

2. Materials and methods

2.1. Soil profiles

Madeira Island (Portugal) is located in a fully (Atlantic) oceanic domain between 32°38′ and 32°52′N and 16°39′ and 17°16′ W, approximately 600 km from the African coast, at the same latitude as Casablanca. Soil samples were taken from andic profiles located on this island that have been classified as either being Umbric or Haplic Andisols (FAO, 1998) depending on their color (Madeira et al., 1994).

The profile studied is situated on a hill (slope 30°W) near the village of Poiso (altitude 1175 m). The vegetation consisted mainly of grasses (55%), ferns (20%), deciduous trees, i.e. oak and birch (20%), and some mosses (5%). Tree- and some grass roots were found to a depth of about 80 cm, but most of the intensive 'root-activity' was found in the top 0–40 cm consisting mainly of grass fibrils. Three horizons were distinguished; a thin O-horizon (0–2 cm), an A-horizon (2–73 cm) and an E/B horizon (73– 90 cm), the latter being formed by weathered basalt bedrock material. The deeper layer, i.e. C-horizon, was formed by a non-friable, compact layer of weathered basalt, the parent material for this Andisol (Madeira et al., 1994) and therefore not encountered. The O-horizon consisted mainly of litter and vegetation, i.e. grass, moss, twigs, leaves, etc. The reddish A horizon (5YR4/6) was characterized by a non-friable structure, clay aggregates with a diameter up to 1 cm, and highly weathered basalt fragments up to a diameter of 10 cm. Many roots were found in this horizon, ranging from mainly very fine grass roots in the top 30 cm to coarser roots, up to a diameter of 2 cm, at greater depth, i.e. 30–70 cm.

2.2. Sampling, sample pre-treatment, total organic carbon and solvent extraction

In February 2001, about 500 g of sample was taken every 10 cm up to a depth of 70 cm (including the O-horizon). In June 2000, samples had already been taken from a depth of 15–50 cm. On Madeira, the samples were air dried in the dark and wrapped in aluminium foil. After 1 week, the samples were transported to the Netherlands and subsequently oven dried at 60 °C and sieved over a 2 mm and a 250 μm sieve to remove large roots and basalt fragments. Total organic carbon contents (TOC) of the dried and sieved (<250 μm) samples were measured using a Fisions Instruments NA 1500 NCS analyzer, with a cycle time of 180 s, a source temperature of 190 °C and an oxygen flow of ca. 30 l min^{-1}.

Approximately 10 g of the sieved (<250 μm) samples was Soxhlet extracted using dichloromethane/methanol (DCM/MeOH) (9:1 v/v) for 24 h. The collected and combined DCM/MeOH phase was rotary evaporated to complete dryness. The dry extract was dissolved in DCM/isopropanol (2:1 v/v) and filtered using a Pasteur pipette packed with defatted wool, 0.5 cm Na_2SO_4 and 2 cm SiO_2 and dried using N_2. Free hydroxyl and carboxylic acid groups present in an aliquot were derivatized to their corresponding trimethylsilyl (TMS) ethers and esters respectively, using BSTFA (*N*,*O*-bis(trimethylsilyl) trifluoroacetamide, containing 1% trimethylchlorosilane and heated for 1 h at 70 °C). The derivatized aliquots were dried using N_2 and dissolved in hexane. An aliquot of a standard solution containing 0.15 μg μl^{-1} 10-nonadecanone was added and the total extract analyzed by GC and GC/MS. The residue was air-dried.

2.3. Gas chromatography (GC)

GC analyses were performed using a Hewlett–Packard 6890 series gas chromatograph equipped with a CP-sil 5CB silica column (50 m × 0.32 mm, film thickness 0.12 μm). Derivatized extracts (1.0 μl) in hexane were injected on-column. The oven temperature was programmed from 70 to 130 °C at 20 °C min^{-1} and from 130 °C to 320 °C (isothermal for 20 min) at 4 °C min^{-1}. Compounds were detected using a flame ionisation detector at 325 °C. Helium was used as carrier gas.

Table 1
Organic bulk soil characteristics and pH (H_2O)

Depth (cm)	TOC (g kg^{-1})	N (g kg^{-1})	C/N molar ratio	pH (H_2O)
0	84	7	13.4	4.5
0–10	79	7	13.6	4.1
10–20	71	7	12.4	4.0
20–30	67	6	13.0	4.2
30–40	61	6	11.8	4.1
40–50	56	5	12.2	4.1
50–60	44	4	12.9	4.2
60–70	30	5	7.5	4.1

2.4. Gas chromatography–mass spectrometry (GC/MS)

Gas chromatography–mass spectrometry analyses were performed using 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 with a cycling time of 0.65 s. The capillary column and temperature programme were as described for the GC analyses. Compound identification was based on published data (e.g. Holloway, 1982; Walton, 1990; Killops and Frewin, 1994; van Bergen et al., 1997).

3. Results

Total organic carbon, total nitrogen, C/N molar ratios and pH (H_2O) values are summarized in Table 1. TOC, expressed as g kg^{-1} of the total sieved (<250 μm) soil mass, ranged from 84 in the top-soil to 30 in the hard basaltic E/B-horizon. Total nitrogen, also expressed as weight percentage of the total sieved (<250 μm) soil mass, showed a decrease from 7 to 4 g kg^{-1}. C/N molar ratios revealed a relatively minor variation from 13.4 to 11.8. In addition, for all samples an acid soil pH (H_2O) ranging from 4.5 to 4.0 was measured.

Mass spectral characteristics of steroidal and triterpenoid constituents mentioned in the text are listed in Table 2. Steroids identified included camposterol ($C_{28:1}$), stigmasterol ($C_{29:2}$) and β-sitosterol ($C_{29:1}$) (Figs. 1 and 2), the latter component being the most abundant steroid identified. Triterpenoids include β-amyrin, lupeol, lupa-2,20(29)-diene and two triterpenyl acids (Table 2). Except for β-sitosterol, steroids and triterpenoids were only identified in the winter sample taken from the first 5 cm (Fig. 1a). β-Sitosterol together with C_{26} *n*-alkanol dominated the total lipid extract obtained from this layer. Other compounds identified in this sample included long-chain (>C_{25}) *n*-alkanes with a strong odd over even predominance, even *n*-alkanols ranging from C_{20} to C_{28}, and even *n*-alkanoic acids ranging from C_{16} to C_{26}. In addition, C_{22} and C_{24} ω-hydroxy acids were identified.

In the winter, from the litter layer to the top of the mineral horizon (10–20 cm), long-chain (>C_{20}) fatty acids, ranging from C_{16} to C_{32} maximizing at C_{22}, C_{24}, C_{26} and C_{28} with a strong even predominance, together with C_{22} and C_{24} ω-hydroxy acids became relatively more abundant (Fig. 1b). In addition, C_{23}–C_{33} *n*-alkanes, maximizing at C_{31} with a strong odd over even predominance together with even dominated C_{20}–C_{32} *n*-alkanols, still maximizing at C_{26}, were identified.

Even deeper in the profile, i.e. 20–30 cm, C_{22} and C_{24} ω-hydroxy acids became the most abundant compounds identified in this winter sample, together with C_{20}–C_{32} *n*-alkanoic acids (Fig. 1c). Other compounds identified in relatively minor concentrations included C_{20}–C_{32} *n*-alkanols with a strong even over odd predominance maximizing at C_{26} and C_{23}–C_{33} *n*-alkanes, maximizing at C_{31} with a strong odd over even predominance.

Monoacids identified in the summer extract (Fig. 2) were dominated by a bimodal distribution. Short-chain (<C_{20}) components were characterized by C_{16} acid, other dominant short-chain components identified included C_{14}, C_{18}, $C_{16:1}$

Table 2
Characteristic mass fragments of steroidal and known and unknown triterpenoid components (analyzed as their TMS derivatives)

Component (in order of elution)	Characteristic fragment ions (*m/z*)	$[M]^+$
24-Methylcholest-5-en-3β-ol[a] (Camposterol) *(S-$C_{28:1}$)*	75, 129, 213, 255, 261, 343, 367, 382, 457	472
24-Ethylcholest-5,22-dien-3β-ol[a,b] (Stigmasterol) *(S-$C_{29:2}$)*	83, 129, 255, 351, 379, 394, 469	484
Olean-12-en-3β-ol[a,b,c] (β-Amyrin) *(T1)*	73, 189, 190, 203, 218, 279, 408	498
24-Ethylcholest-5-en-3β-ol[a], 3 (β-sitosterol) *(S-$C_{29:1}$)*	129, 255, 275, 357, 381, 396, 471	486
Lupeol[a,b] *(T2)*	73, 189, 190, 203, 218, 369, 393, 408, 483	498
Unknown titerpenoid *(T3)*	55, 124, 203, 218, 257, 271, 288, 355, 413, 483	520?
Lupa-2, 20(29)-diene[a] *(T4)*	121, 135, 147, 161, 189, 203, 241, 297, 339, 365, 393	408
4,4,14α-Trimethyl-9β,19-cyclo-5α-ergost-24-en-3β-ol[b,c] (24-Methylenecycloartenol)	95, 135, 175, 300, 353, 379, 407, 422	512
Unknown titerpenoid *(T5)*	73, 203, 320, 393, 410, 428, 513	528?
C_{30} Triterpenyl acid[b] *(Ta1)*	73, 133, 189, 203, 279, 320, 483, 585	600
C_{31} Triterpenyl acid[b] *(Ta2)*	73, 133, 189, 263, 320, 483, 585	?

Indications as used in Fig. 3 are given between brackets in *italics*.
[a] Killops and Frewin, 1994.
[b] van Bergen et al., 1997.
[c] Grandmougin-Ferjani et al., 1999.

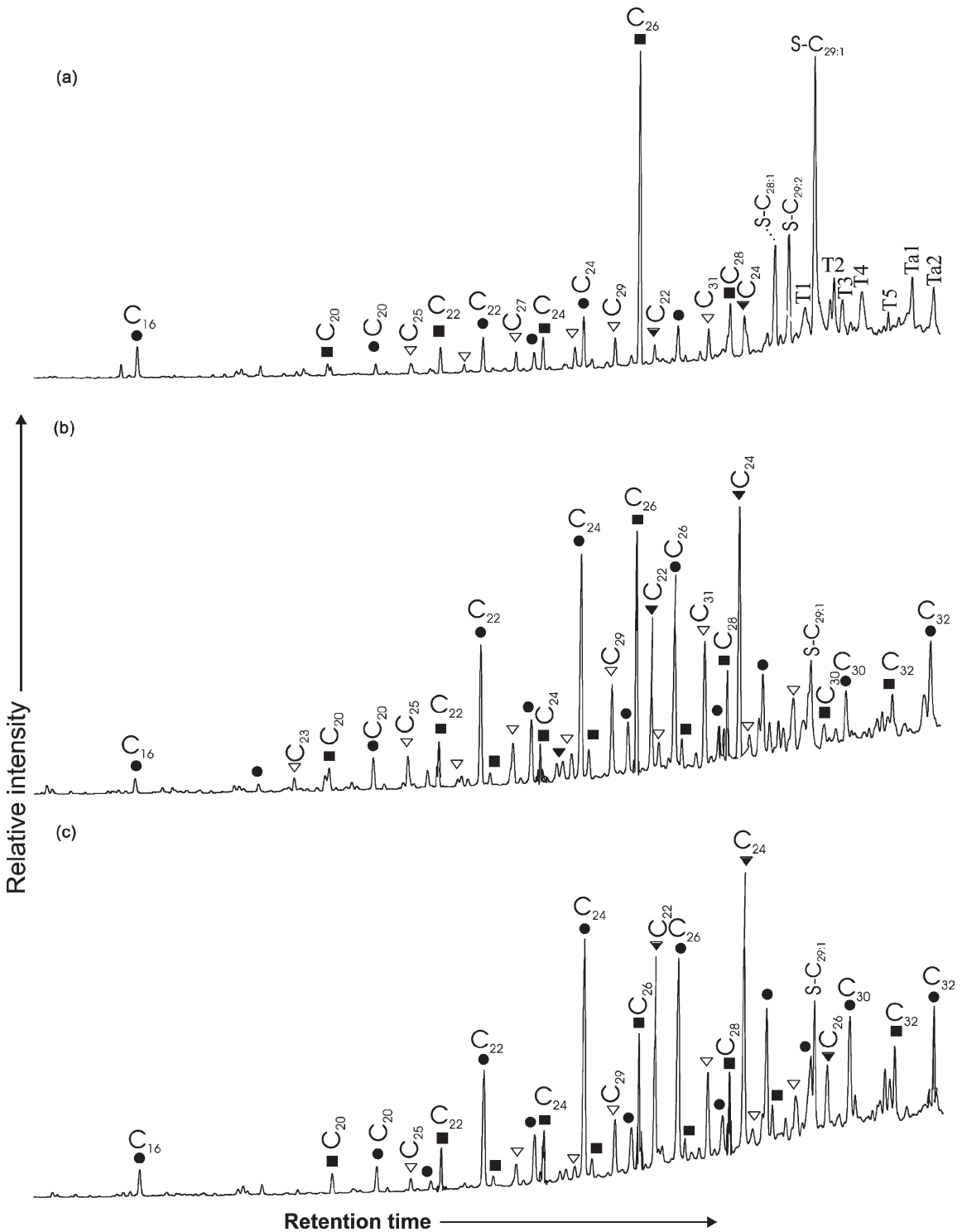

Fig. 1. Gas chromatograms of total lipid extracts (TLEs) obtained in the winter from the grass location at depths of: (a) 0–5 cm (O-horizon), (b) 10–20 cm (top Ah horizon) and (c) 20–30 cm (Ah horizon). ■: *n*-alkanols, ●: *n*-alkanoic acids, ▽ : *n*-alkenes, ▼ : ω-hydroxy acids, ○: ketones, ▲: α,ω-alkanedioic acids. C_x above the peaks refers to the total number of carbon atoms. S–C_x refers to steroids with *x* referring to the total number of carbon atoms. Number after colon refers to the total number of double bonds. Tx and Tax refers to triterpenoids and triterpenoic acids, respectively, with *x* referring to their number as assigned in the text and Table 2.

and $C_{18:1}$ fatty acids, the double bond located at position 9. Long-chain ($>C_{20}$) fatty acids were dominated by C_{22}–C_{28} members. In addition to short-chain monoacids, short-chain ($<C_{20}$) *n*-alkanols including C_{14}, C_{16}, $C_{18:1}$ and C_{18} were found in considerable relative amounts in the summer extract. Long-chain *n*-alkanols, like the *n*-alkanes, resembled those identified in the winter extract (Fig. 1b and c). Other compounds identified in relatively minor

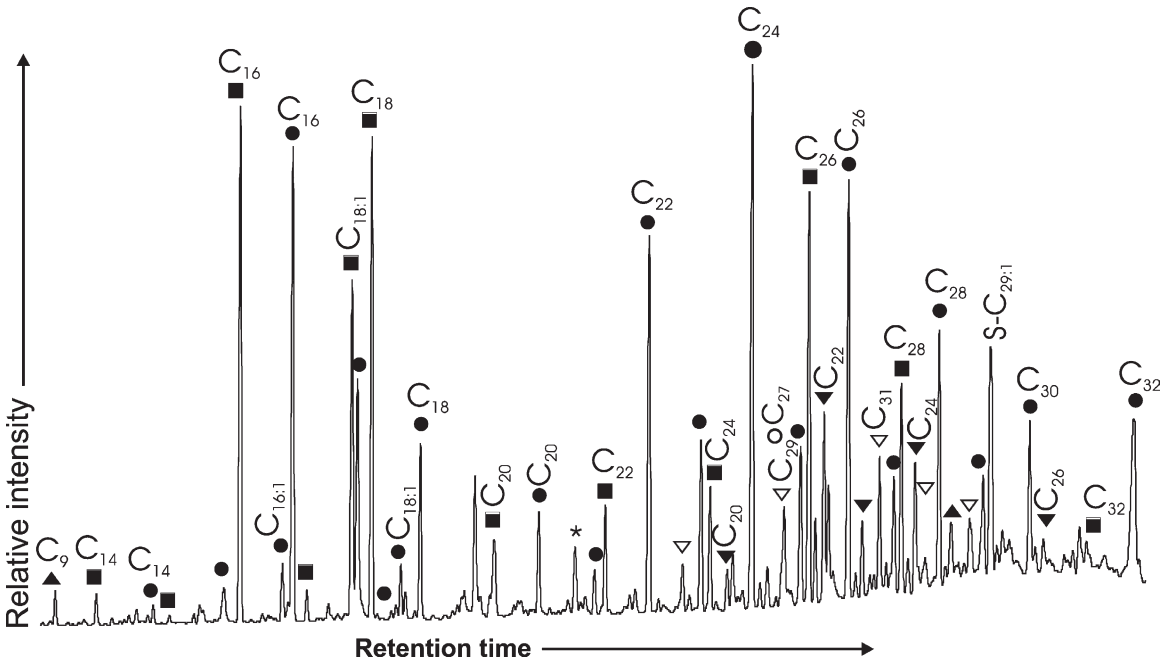

Fig. 2. Gas chromatograms of total lipid extract (TLEs) obtained from the grass location sampled in summer at a depth of 15–50 cm. ■: *n*-alkanols, ●: *n*-alkanoic acids, ▽: *n*-alkenes, ▼: ω-hydroxy acids, ○: ketones, ▲: α,ω-alkanedioic acids. C_x above the peaks refers to the total number of carbon atoms. S–C_x refers to steroids with *x* referring to the total number of carbon atoms. Number after colon refers to the total number of double bonds.

amounts in the summer extract included C_9 α,ω-alkanedioc acid and the C_{27} methylketone.

4. Discussion

4.1. Total organic carbon vs depth and the contribution of lipids to TOC

Total organic carbon decreases with depth, (Fig. 3). From the litter layer to the top of the A-horizon around a depth of 10 cm, and from the A to the E/B-horizon around 45 cm a relatively stronger decrease in TOC with depth was observed (Table 1 and Fig. 3). The first transition is caused by the decomposition of litter (e.g. Cortez et al., 1996) and mixing with the inorganic matrix, whereas the latter is caused by a strong decrease in the number of roots observed in the hard basaltic E/B-horizon.

The decrease in the contribution of free lipids to TOC with depth (Fig. 4), has been observed by others (e.g. Dinel et al., 1990; Jambu et al., 1991, 1993; Amblès et al., 1993, 1998). This phenomenon is suggested to be mainly caused by (I) a substantially lower input of lipids by roots deeper in the soil profile compared with the litter layer (most important in first 20 cm), together with (II) selective biodegradation of lipid molecules and possibly (III) a simultaneous (partial) incorporation of lipids and their microbial degradation products into macromolecular entities (Amblès et al., 1991). In addition, clays have been shown to bind lipids to their organo-mineral matrix (Amblès et al., 1989). Considering the very high complexing capacity known for all allophanic soils (e.g. Dahlgren et al., 1993), the transfer of some free lipids to organo-mineral complexes might also have contributed to the decrease in free lipids with depth observed.

4.2. C/N molar ratios and factors influencing the preservation of lipids in acid andic soils

Acid soils are known to have a low bacterially activity (Wardle, 1992; Motavalli et al., 1995; Andersson and Nilsson, 2001). In general, the amount of nitrogen relative to soil organic carbon increases with depth due to microbial activity and selective degradation of N-poor moieties (e.g. van Bergen et al., 1998b; Marseille et al., 1999). However, C/N ratios decreased only slightly with increasing depth (Table 1) suggesting, together with TOC data obtained (Table 1 and Fig. 3), that SOM was not strongly decomposed with increasing depth in the andic profile. This phenomenon is most probably related to the high protective properties against biodegradation of all types of organic matter in andic soils. These protective capacities are strongly associated with the presence of both high levels of free aluminum together with sorption to allophane, imogolite and ferrihydrite (Boudot et al., 1986; Shoji et al., 1993; Parfitt et al., 1997; Torn et al., 1997). In addition to aluminum toxicity and complexation, the preservation of lipids is favoured by a low soil pH (e.g. Jambu et al., 1985; Dinel et al., 1990).

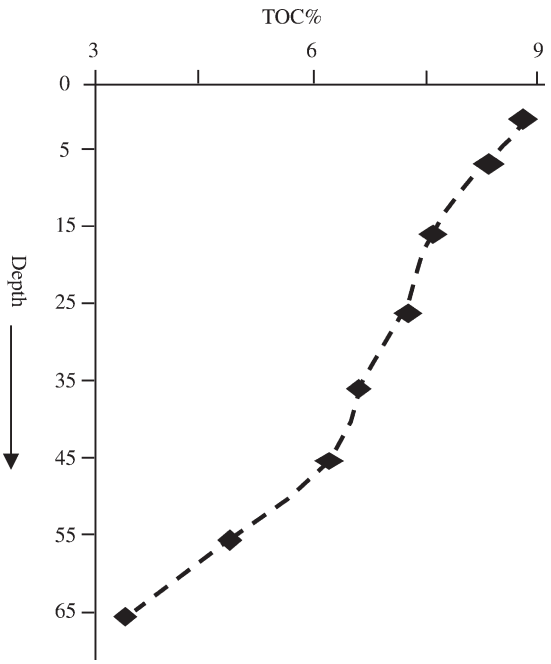

Fig. 3. Total organic carbon (TOC%) plotted vs depth (cm).

4.3. Origin of compounds identified

4.3.1. Steroids and triterpenoids

Steroids and triterpenoids identified, dominated by β-sitosterol (Fig. 5) have frequently been identified in soil and leaf/litter extracts (e.g. Killops and Frewin, 1994; van Bergen et al., 1997; Bull et al., 1998, 2000a). In addition to an input from vascular plants, steroids are known to be derived from fungi (Weete, 1974, 1976; Grandmougin-Ferjani et al., 1999).

β-sitosterol is by far the most abundant sterol identified in arbuscular mycorrhizal fungi (Grandmougin-Ferjani, 1999), although substantial amounts of C_{28} sterols have also been found to be indicative of fungal activity (Weete, 1976). Other steroids identified in association with fungi in smaller or trace amounts include cholesterol, camposterol, stigmasterol, 24-methylene-cycloartenol, α-amyrin and β-amyrin, their amounts probably depending on production by the plant partner (Grandmougin-Ferjani, 1999). The steroid distribution (Fig. 1a) may therefore, in addition to

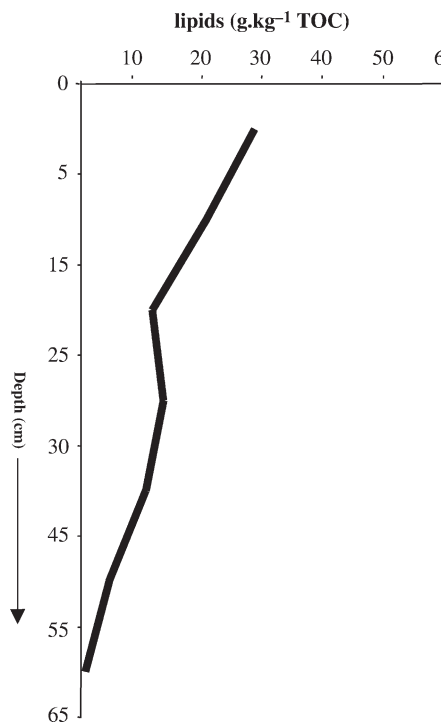

Fig. 4. Contribution of free extractable lipids to the total organic carbon content (TOC) vs depth (cm).

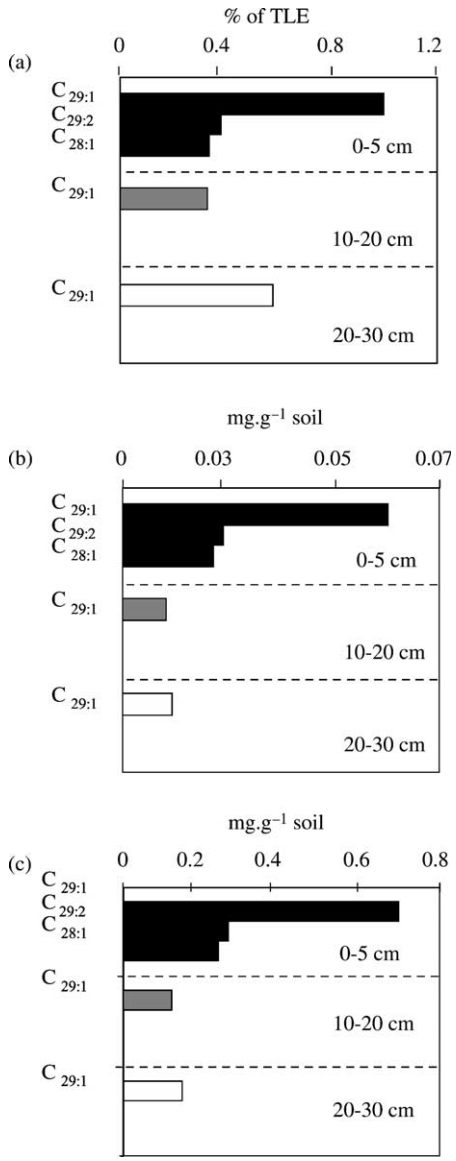

Fig. 5. Diagrams reflecting changes in: (a) relative concentration (as % of TLE) (b) absolute concentrations (mg g^{-1} soil) and (c) changes normalized to TOC (mg g^{-1} TOC) with depth of β-sitosterol ($C_{29:1}$), stigmasterol ($C_{29:2}$) and camposterol ($C_{28:1}$).

a contribution from vascular plants, be derived from fungi in the litter layer.

4.3.2. n-Alkanes

The ($>C_{25}$) odd dominated alkane pattern observed (Figs. 1 and 2) is traditionally ascribed to epicuticular waxes and protective layers on vascular plants and commonly observed in lipid extracts from soils and/or plant leaves (e.g. Jambu et al., 1991; Kolattukudy, 1980b; Amblès et al., 1989; van Bergen et al., 1997, 1998a; Marseille et al., 1999). These compounds can, however, also be produced by certain fungal populations (Weete, 1972, 1976; Marseille et al., 1999). Comparing lipid extracts from mineral soils covered by Quercus with extracts from Quercus leaves revealed enhanced contributions of odd long-chain C_{29}, C_{31} and C_{33} *n*-alkanes not directly related to the leave lipids (Nott, 1996 BSc thesis). Moreover these latter alkanes, mainly C_{31} and C_{33}, were significantly ^{13}C enriched (up to 4 per mill) in the soil compared with those from the leaves. A fungal contribution of ^{13}C enriched odd long-chain alkanes could explain such data (van Bergen, Bull, Nott, Poulton and Evershed, unpublished results).

The dominance of C_{31}, most strongly seen in the samples taken from the mineral horizon (Figs. 1b, c and 6a), has been observed before in grass roots and rhizomes (Marseille et al., 1999). The relatively low abundance of alkanes in the top-soil when compared with *n*-C_{26} alkanol and β-sitosterol (Fig. 1a) also typically reflects the input by grasses (van Bergen et al., 1997, 1998a).

4.3.3. n-Alkanols

The distribution found in the top layer (Figs. 1a and 6b) with a very dominant contribution from C_{26} alcohol, typically reflects an input by grasses (van Bergen et al., 1997. 1998a; Bull et al., 2000a; Nierop et al., 2001). From the litter layer to the mineral horizon, *n*-alkanols other than C_{26}, ranging from C_{20} to C_{32}, become relatively more abundant (Fig. 6b). *n*-Alkanols identified in grass root thermochemolysis-GC traces (Nierop et al., 2001) included C_{26}, C_{28} and C_{30} members in similar relative concentrations as found for the 20–30 cm sample (Figs. 1c and 6b), strongly suggesting a grass root dominated input in the mineral horizon. Moreover, *n*-alkanols ranging from C_{15} to C_{32} without a predominant C_{26} member have been identified in base-hydrolysates from this profile (Naafs and van Bergen, 2002a).

C_{14}–C_{20} compounds, including a C_{18} *n*-alkenol, were only identified in the summer extract (Fig. 2). These compounds are most likely derived from lower plants, fungi, spore waxes or hydrolysis products of esters by microbial action (Weete, 1976; Colina-Tejada et al., 1996; Amblès et al., 1998).

4.3.4. Fatty acids (n-alkanoic acids)

A distribution of long-chain ($>C_{20}$) fatty acids, ranging from C_{16} to C_{32} and maximizing at C_{22}, C_{24}, C_{26} and C_{28} with a strong even predominance (Figs. 1, 2, 6c), is

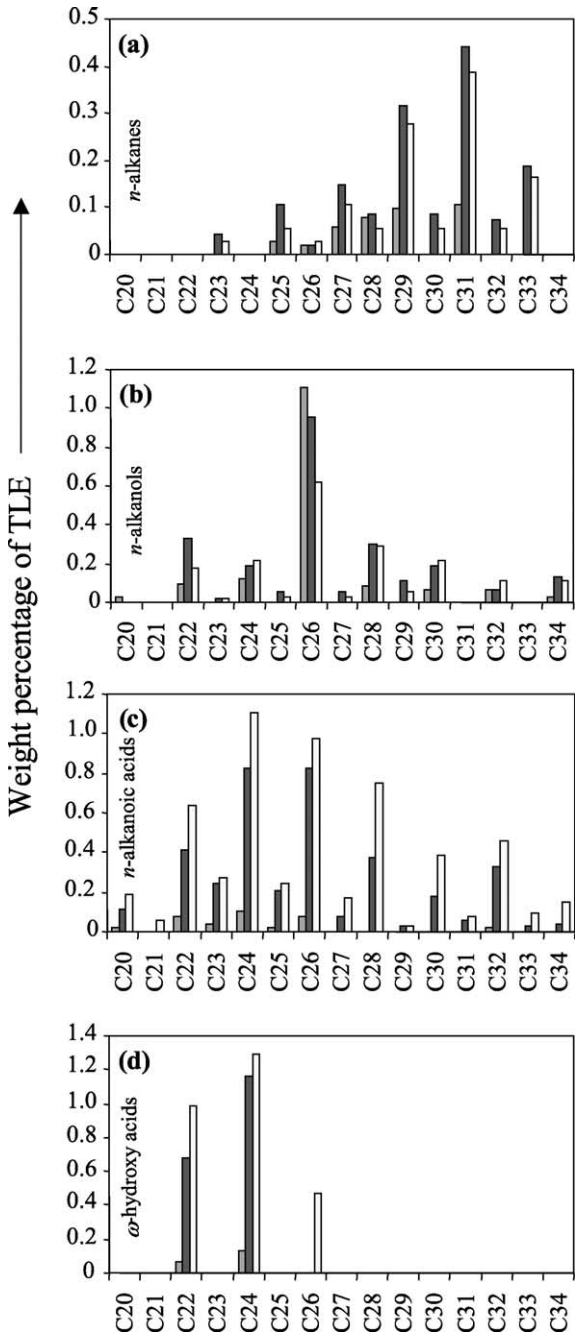

Fig. 6. Diagrams reflecting changes in the relative concentration (as % of TLE) with depth of: (a) *n*-alkanes, (b) *n*-alkanols, (c) *n*-alkanoic acids and (d) ω-hydroxy acids. ▩: litter layer, ■: 10–20 cm, □: 20–30 cm.

commonly observed in extracts from acid soils covered by higher plant vegetations (e.g. Almendros et al., 1996; van Bergen et al., 1998a), such as grasses (Bull et al., 2000b).

Thermally assisted hydrolysis and methylation (THM) of grass roots (Nierop et al., 2001) revealed a very similar distribution of fatty acids as observed in the A-horizon samples (Figs. 1b, c and 6c). Moreover, base hydrolysis of solvent insoluble soil residues of this profile revealed the presence of C_{12}–C_{30} fatty acids (Naafs and van Bergen,

2002a). These ester-linked compounds, mainly derived from root biopolyesters, i.e. suberins (e.g. Riederer et al., 1993), could be released into the soil upon (microbial) hydrolysis. In addition, straight-chain components of fungal origin could be a source of *n*-alkanoic acids but they only range from C_{10} to C_{24} (Weete, 1974). Based on their distribution, a contribution from the oxidation of *n*-alkanes or *n*-alkanols (Amblès et al., 1994), is unlikely.

Short-chain ($<C_{20}$) monoacids identified in the summer extract (Fig. 2) have been found to be mainly of microbial origin (Bridson, 1985; Parlanti et al., 1994; Marseille et al., 1999), although C_{16}, C_{18}, $C_{18:1}$ and $C_{16:1}$ fatty acids have also been identified in arbuscular fungi (Madan et al., 2002; Ruess et al., 2002). Hydrolysis of primary esters, including triglycerides, could also release short-chain ($<C_{20}$) acids into the soil (Bridson, 1985; Marseille et al., 1999).

4.3.5. ω-Hydroxy acids

One obvious precursor for the C_{22}, C_{24} and C_{26} ω-hydroxy acids identified mainly in the samples from the mineral soil A-horizon (Fig. 1b and c), is the biopolyester suberin (van Bergen et al., 1998a, 2000b). This root biopolyester has been reported to contain substantial amounts of either the C_{22} and C_{24} homologues (e.g. Kolattukudy, 1980a; Walton, 1990; Matzke and Riederer, 1991, 1993; Nierop, 1998).

Base-hydrolysis of solvent insoluble residues from these soils as well as solvent extracted (grass) root residues (Bull et al., 2000b) released ω-hydroxy acids ranging from C_8 to C_{28}, including C_{22}, C_{24} and C_{26} ω-hydroxy acids (Naafs and van Bergen, 2002a,b). The presence of C_{22} and C_{24} ω-hydroxy acids in THM data of grass leaves (Nierop et al., 2001), suggests a grass leaf derived input, in addition to roots, of these ω-hydroxy acids in the litter layer. Considering the resemblance between the distribution of free fatty acids and ω-hydroxy acids, i.e. dominant C_{22}, C_{24} and C_{26} components, oxidation of free fatty acids as a source for ω-hydroxy acids cannot be excluded.

4.3.6. α,ω-alkanedioic acids

The presence of a C_9 α,ω-alkanedioic acid in the summer extract (Fig. 2) is remarkable considering that these compounds are unusual constituents of extractable lipids. The short chain length suggests microbial β-oxidation of unsaturated alkanoic acids and/or mid-chain hydroxy acids (Regert et al., 1998; Nierop et al., 2003), which are common cutin and suberin monomers (e.g. Kolattukudy, 1980a; Holloway, 1982). Upon such oxidation, unsaturated alkanoic acids and/or dihydroxy fatty acids with mid-chain hydroxyl groups in either the C_7, C_8, C_9 or C_{10} position (del Río and Hatcher, 1998) can produce short-chain alkanedioic acids.

4.4. Variations with depth

4.4.1. Lipid signal in top- vs sub-soil

The most striking change in the chemical composition of total lipid extracts with depth is the sharp decrease in both relative and absolute concentrations of steroids (Fig. 5) and triterpenoids going from the litter to the top of the A-horizon. Such a decrease in steroid and terpenoids was observed previously 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 has been suggested that these compounds can be easily mineralized in the soil (Almendros et al., 1996; van Bergen et al., 1997). Assimilation by arthropods (Nes et al., 1997; Bull et al., 2000a) may also contribute considering their significant abundance in this soil profile (Naafs et al., unpublished pyrolysis data). In addition, their absence in soluble lipid fractions can be caused by their condensation into more stable, insoluble structures (Amblès et al., 1991, 1996; van Bergen et al, 1997) or may result from chemically alteration to form modified steroids and triterpenoids. Evidence for the latter two processes has, however, not yet been found (Naafs et al., unpublished pyrolysis data).

The total decrease of stigmasterol ($C_{29:2}$) compared with that of β-sitosterol ($C_{29:1}$) (Fig. 5) is probably related to the higher susceptibility towards oxidation of di-unsaturated steroids (Dragun, 1988). In addition, β-sitosterol has been found in base-hydrolysates obtained from soil samples taken from a depth of 15–50 cm (Naafs and van Bergen, 2002a). Thus, hydrolysis of these ester-linked steroids could be an extra source of free β-sitosterol in the sub-soil.

Considering that epicuticular waxes present on and in the protective layers on vascular plants are the most likely source for *n*-alkanes in these soils (see Section 4.3.2), we suggest that these layers are still relatively intact in the litter layer. Only after mixing with the acid mineral soil and with time, the *n*-alkanes are released from these layers into the top of the mineral soil (10–20 cm), thereby increasing their absolute (Figs. 7a and 8a) and relative concentrations (Fig. 6a). In addition, the fungal contribution could increase.

The decrease in both relative and absolute concentration of C_{26} *n*-alkanol at greater depth (Figs. 6b, 7c and 8c) might be the result of (I) a reduced input at greater depth, (II) limited transport of this compound in the profile, (III) microbial degradation, and/or (III) a simple kinetic factor, the more abundant component decreasing faster than the other (Marseille et al., 1999). The other *n*-alkanols seem to have either a slower degradation, or an additional input at depth.

The *n*-alkanoic acids clearly show a significant increase in both relative (Fig. 6c) and absolute concentration (Figs. 7c and 8c) with depth, most likely indicating that these compounds, unlike for example C_{26} *n*-alkanol, have their major input in the mineral soil. The accumulation of C_{22}, C_{24} and C_{26} ω-hydroxy acids (Figs. 6d, 7d and 8d) has been observed to parallel the accumulation of *n*-alkanoic acids

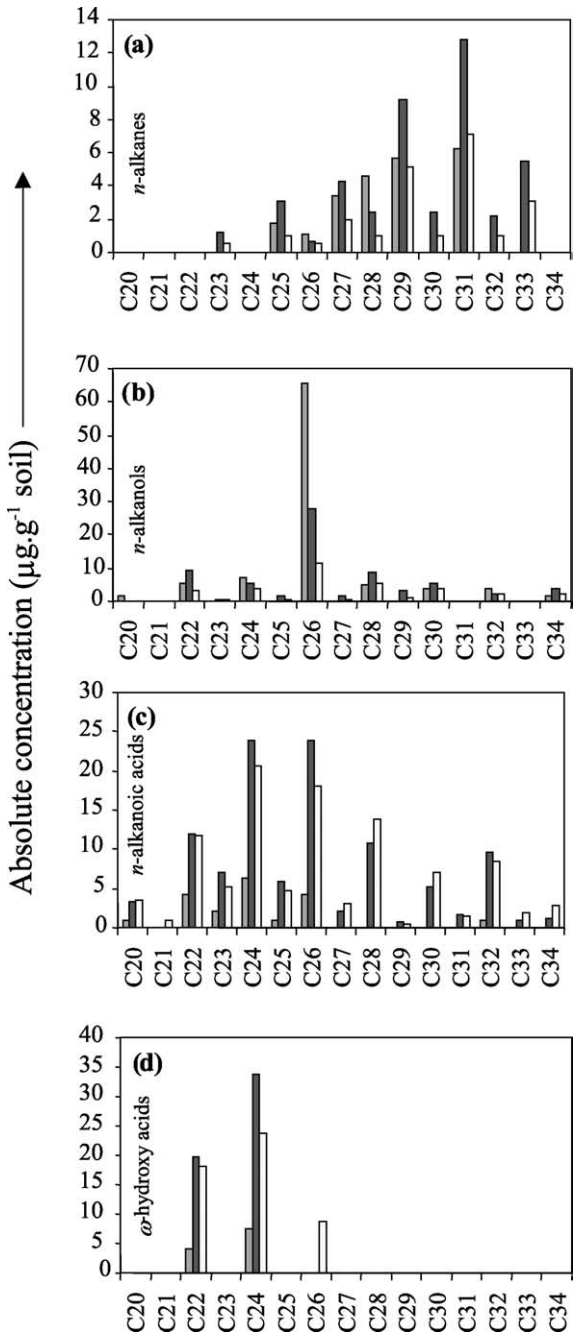

Fig. 7. Diagrams reflecting changes normalized to absolute concentrations (as µg g^{-1} soil) with depth of: (a) *n*-alkanes, (b) *n*-alkanols, (c) *n*-alkanoic acids and (d) ω-hydroxy acids. ▒: litter layer, ■: 10–20 cm, □: 20–30 cm.

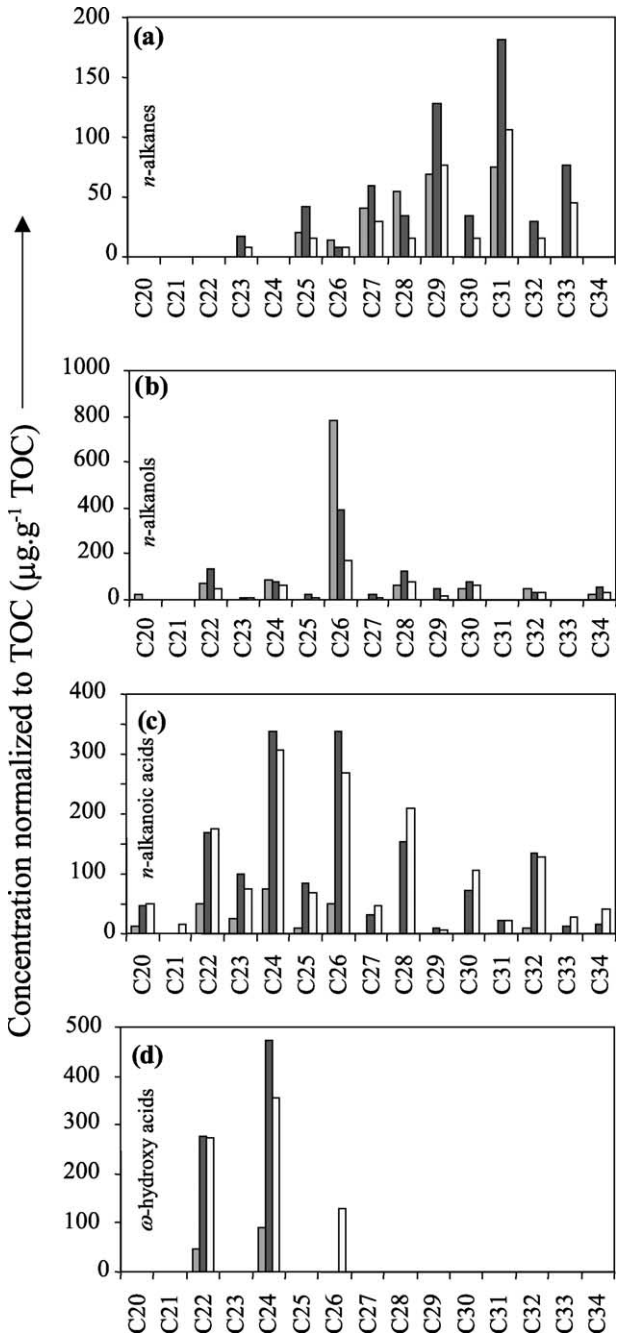

Fig. 8. Diagrams reflecting changes normalized to TOC (µg g^{-1} TOC) with depth of: (a) *n*-alkanes, (b) *n*-alkanols, (c) *n*-alkanoic acids and (d) ω-hydroxy acids. ▒: little layer, ■: 10–20 cm, □: 20–30 cm.

(Figs. 6c, d, 7c, d, 8c and d) in acid soils before (Bull et al., 2000a), suggesting that both ω-hydroxy and *n*-alkanoic acids have a similar source, i.e. roots.

4.4.2. Microbial activity

Indications for a limited bacterial activity in the andic profile could be the absence of specific lipid bacterial markers such as short-chain ($<C_{20}$) *n*-alkanes (Jambu et al., 1991), short-chain ($<C_{20}$) *n*-alkanols (Jambu et al., 1993), short-chain ($<C_{20}$) *n*-alkanoic acids and *iso* and *anteiso*-fatty acids (Boon et al., 1977; Lichtfouse et al., 1995; Perry et al., 1979), C_{30+} hopanoids (Rohmer et al., 1980), and methylketones, i.e. microbial oxidation products of *n*-alkanes (Allen et al., 1971; Amblès et al., 1993). Moreover, the strong odd over even predominance observed for *n*-alkanes and the strong even over odd predominance for both *n*-alkanols and *n*-alkanoic acids are constant with depth (Figs. 6–8). If microbial activity would be affecting these compounds, a decrease in these predominances is expected.

The detection of steradienes, i.e. $C_{28:2}$ and $C_{29:2}$, and a triterpadiene, i.e. lupa-2,20(29)-diene also seems to indicate a slow (microbial) transformation of these diagenetic intermediates (Killops and Frewin, 1994).

A low soil pH is known to increase the fungal contribution (Bumpus, 1993). Therefore, in the acid soil profile studied, the populations of filamentous fungi and actinomycetes are probably more abundant (as reflected in e.g. the *n*-alkane distribution), whereas in neutral and slightly alkaline soils, bacteria and other types of fungi are favoured (Dinel et al., 1990). Moreover, significant contributions from arbuscular mycorrhizal fungi to soil organic matter carbon pools in andic soils have been reported (Rillig et al., 2001).

4.4.3. Transport of compounds through the andic profile

During an experimental leaching study of soil lipids, Amblès et al. (1998) showed that hydrocarbons could be leached as soluble components from the litter layer into the soil profile. Despite their low solubility in water lipids can be present in true solution, colloidal dispersion and micelles (Nierop and Buurman, 1998; Piccolo et al., 1996). The strong decrease in the steroid, triterpenoid and C_{26} alkanol signal from the top of the profile (0–5 cm) to the deeper layers (Figs. 5 and 7b) may, in addition to a quick mineralization or assimilation, indicate a limited transport of these compounds through the profile. Considering the availability of enough precipitation to transport these compounds (Madeira et al., 1994), a very low mobility of organic compounds observed in andic profiles (Aran et al., 2001) is most likely caused by the strong sorption of organic compounds. Such sorption phenomena are well-known for (andic) soils containing large amounts of reactive mineral phases, such as Al and Fe hydrous oxides (Ulrich and Stumm, 1987; Ochs et al., 1994; Parfitt et al., 1999), especially near their pK_a, i.e. pH 4–5 (Hingston et al., 1967).

4.5. Seasonal influence

Differences observed between the chemical composition and distribution of both fatty acids and *n*-alkanols in the summer (Fig. 2) and winter extract (Fig. 1b) very closely resemble the differences found in the distribution of these compounds in summer and winter leachates (Colina-Tejada et al., 1996) and lipid extracts (Amblès et al., 1994a). A microbial origin for all short-chain ($<C_{20}$) compounds in these leachates and extracts has been suggested. A microbial origin becomes even more likely considering that $C_{16:1}$ and $C_{18:1}$ fatty acids identified together with C_9 diacid and a C_{27} methylketone (Fig. 2) are intermediate components in biodegradation processes (Parlanti et al., 1994; Amblès et al., 1993).

5. Conclusions

Total lipid extracts obtained from an andic soil profile on Madeira Island, covered mainly by grass, have been analyzed using gas chromatography (GC) and GC–mass spectrometry (GC/MS). The decrease in the contribution of free lipids to the TOC on going from the litter to the mineral A horizon is most likely indicative of a substantially lower input of lipids by roots compared with the litter layer.

The lipid distribution observed in the top layer, characterized by a very dominant contribution from C_{26} alcohol together with steroids and triterpenoids, typically reflects an input from grass leaves. The signal from long-chain *n*-alkanoic acids (C_{22}–C_{32}), ω-hydroxy acids (C_{22}–C_{26}), C_{31} *n*-alkane and *n*-alkanols (C_{22}–C_{32}), observed in the samples taken from the mineral A horizon, is indicative of an important contribution by (grass) roots. This contribution clearly increased with increasing depth, while a strong decrease was observed in the C_{26} alcohol, steroid and triterpenoids concentrations.

The absence of typical bacterial lipid markers could be indicative for limited bacterial activity. Possible molecular evidence of fungal activity, especially in the litter layer, has been found in the distributions of steroids, *n*-alkanes and *n*-alkanoic acids. In summer, a signal reflecting the leaching of microbially derived products from the litter and/or aerial vegetation at the surface is observed.

Overall, the lipid data obtained provide new detailed molecular insights into the origin and fate of SOM and further our understanding of the processes affecting andic soils.

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