PERGAMON

Organic Geochemistry 31 (2000) 389–408

Organic Geochemistry

www.elsevier.nl/locate/orggeochem

Organic geochemical studies of soils from the Rothamsted classical experiments—V. The fate of lipids in different long-term experiments

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Abstract

Lipid extracts from four long-term experiments (Broadbalk Wilderness, Geescroft Wilderness, Hoosfield Spring Barley and Park Grass) were analysed using a combination of gas chromatography, gas chromatography–mass spectrometry and gas chromatography–combustion–isotope ratio mass spectrometry. The lipid content of the primary organic inputs for each soil were also analysed in order to assess the early diagenetic fate of the various compound classes present. Soil pH was observed to, either directly or indirectly, have a significant effect on lipids with a relative increase in abundance of *n*-alkanes at higher pH (7.31) and a large relative increase in *n*-alkanoic and ω-hydroxy acids at low pH (3.74). Triacylglycerols exhibited severe losses irrespective of pH. In an arable soil, *n*-alkanoic acids showed a temporal decrease in concentration whilst levels of *n*-alkanols remained static, the difference was ascribed to a more rapid turnover and possible leachate migration of the *n*-alkanoic acids. The phytosterol, sitosterol, was observed to rapidly diminish in soils possibly as a result of assimilation by soil dwelling invertebrates. Analysis of 5β-stigmastanol (a faecal biomarker) showed that it remained at levels indicative of manuring even after 113 years. Furthermore, analysis of 5β-stanyl esters revealed a manuring signal even more persistent than that exhibited by the free stanols. Knowledge of the biogeochemical cycling of lipids in the soil environment will help facilitate understanding of the processes which underpin carbon cycling in soils.

Keywords: Grassland; Lipid; Soil pH; Stanol; Sitosterol; Woodland vegetation

1. Introduction

Soil organic matter is an important, dynamic reservoir in the global carbon cycle and other elemental cycles. It has been estimated that about 1760×10^{12} kg of carbon exists in the soil, over twice the amount residing in the atmosphere (O'Neill, 1993). Hence, soil plays a central role in the biogeochemical cycling of carbon. Soil is intimately involved in the regulation of atmospheric 'greenhouse' gases, for example, it provides anchorage and nutrition for the biosphere which is a major reservoir for CO_2 (Wayne, 1991). Furthermore, CH_4 another gas implicated in global warming is subject to bioremediation by methanotrophic bacteria found in the soil environment (Hanson and Hanson, 1996). The rate of organic matter degradation/mineralisation in soils is currently a key research issue, especially with regard to the factors influencing the eventual release of CO_2 back into the environment and the ability of soil to sustain increased floral growth under elevated levels of CO_2 (Cresser et al., 1993).

1.1. The origin and fate of lipids in soils

Lipids are generally defined as 'organic molecules insoluble in water and extractable by various organic

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0146-6380/00/$ - see front matter
PII: S0146-6380(00)00008-5

solvents'. The description is both ambiguous and over simplistic, seeking to singly classify an astonishingly diverse series of organic compounds. Lipids vary structurally from derivatives of complex organic components (phospholipids, sphingolipids and lipoproteins) to simpler functional classes such as hydrocarbons, aldehydes, ketones, alcohols and carboxylic acids which can combine to form other compounds such as wax esters, steryl esters, terpenyl esters, mono-, di- and triacylglycerides (Dinel et al., 1990). The very nature of extracted lipids will vary according to the polarity of the solvent system used for extraction. Globally, soil lipids constitute between 4 and 8% soil organic carbon although quantities of up to 42% have been observed to occur in a cultivated organic soil (Preston et al., 1987). Whilst they are admittedly a minor component in soils, lipids exhibit an inherent diagnostic value regarding organic matter both (endogenous and exogenous) and the pedological processes to which it is subjected. They occur in both flora and fauna as products of deposition, exudation and decomposition as well as other sources such as fungi and algae. The functional diversity of lipids confers a variable rate of degradation/transformation to this group of compounds, this may be exploited to obtain information on the action of different soil processes. Additionally, the composition, concentration and diagenetic fate of lipids can vary between soil environments due ultimately to differences in sources of organic matter and changes in inorganic mineral contents (Miller and Donahue, 1995). Soils exhibiting low microbiological activity have been shown to possess a relatively higher lipid content than those exhibiting high levels of activity whilst soils exhibiting a low pH also possess a higher concentration of lipid components (Fridland, 1976). Such preservation is the result of both physical conditions in the soil environment and the action of the endogenous fungal population along with cohabiting micro-, meso- and macrofauna (the populations of which are also determined by the soil environment). The complexity of this scenario is increased still further by the reciprocal effect that soil lipids can have on the physical and biological properties of a soil. Such 'feed-back' mechanisms are demonstrated by Dinel et al. (1992) who observed a correlation between increases in bound aliphatic compounds and the water stability of soil aggregates. Whilst a large body of research concerning lipids in marine and lacustrine sediments already exists, investigations concerning lipids in soils are more scarce making it a relatively poorly understood subject. Certainly investigations concerning sedimentary lipids would appear less problematic given the relatively greater homogeneity and chemical stability inherent to such depositional environments. In comparison, terrestrial soils are transient, heterogeneous assemblages subject to constantly fluctuating redox conditions, leaching and erosion (Hedges and Oades, 1997).

Over the last 5 years this laboratory (Bristol) has endeavoured to determine the fate of lipids in soils from a series of carefully maintained long-term experiments located on Rothamsted Experimental Station, Harpenden, UK. By using such soils, lipid contents may be assessed and their occurrence rationalised with a full knowledge of exogenous organic matter inputs over time whilst maintaining a valid ecological context for the soil system, cf. laboratory based studies. This communication both reviews previous work and presents a substantial amount of new data obtained from the Rothamsted Classical Experiments.

1.2. The Rothamsted classical experiment

During the period 1843 to 1856, nine long-term agricultural experiments were started at Rothamsted Experimental Station. In 1878 one of these experiments was discontinued, a second was stopped in 1990. However, the remaining seven have continued, with some modifications, to the present day (Anon, 1991). They consist of, amongst others, plots which have supported continuous growth of arable crops such as barley (Hoosfield Spring Barley) and wheat (Broadbalk Wilderness), as well as continuous grassland (Park Grass). Other areas have been allowed to revert back to natural woodland following many years arable cropping (Broadbalk Wilderness and Geescroft Wilderness). The bulk organic matter contents of soils from these sites, as well as other elemental compositions have already been extensively investigated (e.g. Warren and Johnston, 1964; Jenkinson, 1971; Jenkinson and Johnston, 1977; Jenkinson et al., 1992). As well as comprehensive records detailing the various inorganic and organic inputs on each experiment, there is also an extensive archive of soils, organic manures and crops sampled from the experiments over the course of time. Therefore, for some of the experiments, temporal changes in the lipid profiles of soils may be investigated. The aims of the studies reported on herein can be defined thus:

(i) characterisation of the lipid composition of soils with well-defined biological inputs,
(ii) assessment of the fate of specific lipid classes in soil profiles, and
(iii) determination of the factors which control the preservation of soil organic matter.

2. Experimental

2.1. Experimental considerations

Consistent methods of extraction and chemical separation have been adopted throughout these studies

thereby maintaining a valid basis for comparison of experimental data. This is a factor which should be critically examined when making inter-lab comparisons of data since differing experimental protocols may well render direct comparisons of data invalid. Whilst it is acknowledged that certain soil treatments may eventually change the actual soil type in an experiment, differently treated soils within any one experiment currently all adhere to the same soil classification. Therefore, direct comparisons between various intra-experimental plots may be made without undue concern about the occurrence of anomalous results derived from the chemical properties of different soil classes.

2.2. Sample preparation and solvent extraction

Fresh soil and vegetation samples were initially oven dried at 60°C. All soil samples were crushed with a pestle and mortar and subsequently sieved over a 2 mm and a 75 μm sieve, pH was measured in H_2O (soil:H_2O 1:2.5). Dried vegetation was crushed using the same method but with the addition of liquid nitrogen to facilitate the process. This was then sieved over 5 and 2 mm sieves.

All soil samples were Soxhlet extracted for 24 h using 200 ml dichloromethane (DCM):acetone (9:1 v/v) to obtain a total lipid extract (TLE). 2-Hexadecanol (**IS_1**), 10-nonadecanone (**IS_2**), 5β-pregnan-3-one (**IS_3**), 5β-pregnan-3α-ol (**IS_4**), 5α-cholestanol (**IS_5**) and hexadecyl-octadecanoate (**IS_6**) were added as internal standards. The same standards were added to the vegetation samples which were ultrasonically extracted (5×) with DCM: acetone (9:1 v/v); total volume 50 ml. Extracts were combined and solvent removed under reduced pressure. Redissolved residues were transferred with DCM: isopropanol (2:1 v/v) and filtered over defatted cotton wool. For total lipid analysis aliquots were filtered through a glass column packed with 2 g of activated silica gel 60 (160°C, > 24 h; Fluka) to remove highly polar compounds. Samples were trimethylsilylated (see Section 2.5) and redissolved in hexane prior to analysis by HTGC and HTGC–MS.

2.3. Acid/neutral fractionation

TLEs were separated into two fractions, 'acid' and 'neutral', using an extraction cartridge with a bonded aminopropyl solid-phase (500 mg sorbent, 2.8 ml eluent capacity; Varian). Extracts dissolved in DCM:isopropanol (2:1 v/v) were slowly flushed through a cartridge pre-eluted with hexane. After further elution with DCM:isopropanol (2:1 v/v, 8 ml) the collected 'neutral' fraction was removed and the cartridge slowly flushed with 2% acetic acid in diethylether (8 ml) thereby eluting the 'acid' fraction. Solvent was removed from both fractions under a gentle stream of nitrogen. Acid fractions were derivatized and analysed using GC and GC–MS.

2.4. Column chromatography of neutral lipids

Glass columns were packed with dried activated silica gel 60 (160°C, > 24 h; Fluka) and pre-eluted with hexane. Samples were applied to the column as a mixture of dissolved and finely suspended particulates in hexane. Gradient elution was performed under positive pressure supplied by a stream of nitrogen providing an elution rate of ~15 ml min^{-1}. The eluents used comprised five separate solvent systems: hexane, hexane:DCM (9:1 v/v), DCM, DCM:methanol (1:1 v/v) and methanol, applied in elutropic order to give five fractions: 'hydrocarbon', 'aromatic', 'ketone/wax-ester', 'alcohol' and 'polar', respectively. The relative volumes of solvents applied were determined by the ratio 2:1:3:2:2, following the above elutropic series, and the size of the column being used for a particular separation. Column fractions were collected and dried in an identical manner to fractions from the acid/neutral separation.

2.5. Derivatisation

Analytes were derivatized to their respective trimethylsilyl (TMS) ethers and/or esters by adding 30 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Sigma), containing 1% trimethylchlorosilane (TMCS), to sample aliquots and heating for 30 min at 70°C. Excess derivatisation agent was removed under a gentle stream of nitrogen and samples redissolved in hexane.

2.6. Gas chromatography (GC) and high temperature–gas chromatography (HT–GC)

Derivatized fractions were analysed using a Hewlett–Packard 5890 series II gas chromatograph equipped with a fused-silica capillary column (Chrompack CPSil-5CB, 50 m length × 0.32 mm i.d. × 0.12 μm film thickness). Derivatized fractions in hexane were injected (1.0 μl) on-column. The temperature was programmed from 40°C (1 min isotherm) to 200°C at a rate of 10°C min^{-1} and finally to 300°C (20 min isotherm) at 3°C min^{-1}. HT–GC analyses of TLEs were made using an alternative column capable of performing at elevated temperature (DB1, 15 m length × 0.32 i.d. × 0.1 μm film thickness). The temperature was programmed from 50°C (2 min isotherm) to 350°C at a rate of 10°C min^{-1} (10 min isotherm). The detector temperature was kept at 320°C (GC)/350°C (HTGC). Hydrogen was used as carrier gas (10 psi head pressure).

2.7. Gas chromatography–mass spectrometry (GC–MS) and high temperature-gas chromatography–mass spectrometry (HTGC–MS)

GC–MS analyses were made using a Carlo Erba 5160. GC separation was achieved using a fused silica capillary

column (Chrompack CPSil-5CB, 50 m length × 0.32 mm i.d. × 0.12 μm film thickness) and the temperature was programmed from 40°C (1 min isotherm) to 200°C at a rate of 10°C min^{-1} and finally to 300°C (20 min isotherm) at 3°C min^{-1}. HTGC–MS analyses of TLEs were made using an alternative column capable of performing at elevated temperature (DB1, 15 m length × 0.32 i.d. × 0.1 μm film thickness). The temperature was programmed from 50°C (2 min isotherm) to 350°C at a rate of 10°C min^{-1} (10 min isotherm). Helium was used as carrier gas (10 psi head pressure) equipped with on-column injection coupled, via a transfer line heated at 320°C (GC)/350°C (HTGC), to a Finnigan MAT 4500 quadrupole mass spectrometer scanning in the range of m/z 50–650 with a cycle time of 1.0 s. The current was maintained at 300 μA with an ion source temperature of 190°C and an electron voltage of 70 eV.

2.8. Gas chromatography combustion–isotope ratio mass spectrometry (GCC–IRMS).

Stable carbon isotope ratios were measured on a Varian 3400 GC (SPI-type injector tracking oven temperature) [separation was achieved using a fused silica capillary column (Chrompack CPSil-5CB, 50 m length × 0.32 mm i.d. × 0.12 μm film thickness) and the temperature was programmed from 40°C (1 min isotherm) to 200°C at a rate of 10°C min^{-1} and finally to 300°C (20 min isotherm) at 3°C min^{-1}; helium was used as carrier gas (15 psi head pressure)] coupled to a Finnigan MAT Delta S isotope mass spectrometer. The combustion oven was held at 850°C. $\delta^{13}C$ values were calculated relative to the PeeDee Belemnite standard. Calculations to correct for the three exogenous carbon atoms present in the trimethylsilyl group were performed after $\delta^{13}C_{tms}$ was determined from a cholesterol standard of known isotopic composition (after Jones et al., 1991).

3. Results and discussion

3.1. Lipid compositions of soils with known recent histories of use

Broadbalk Wilderness is a small site occupying about 0.2 ha of land. The land has long been in cultivation with maps dating it as such from as early as 1623 (Jenkinson, 1971). From 1843 to the early 1880s the site was in continuous wheat whereupon, in 1882, it was divided into two subsections. One area was allowed to revert back to natural woodland occupied by species of tree indigenous to the local area, i.e. *Crataegus monogyna* (hawthorn), *Acer pseudoplatanus* (sycamore), *Fraxinus excelsior* (ash) and *Quercus robur* (common oak), as well as a primary ground cover of *Hedera helix* (ivy; wooded area). The other section had all woody shrubs cut out at ground level annually (stubbed). This latter area was subdivided again in 1957 to give a grazed area and a now smaller stubbed area. The primary vegetation covering the grazed area was *Lolium perenne* (rye grass). The stubbed area flora consisted of mixed herbs and grass with a significant cover of *Rubus sp.* (bramble) later on in the growing season. Due to heavy chalking in the 18th or early 19th century the pH of the site is still relatively high; 7.3 in 1991 (Kinchesh et al., 1995). Coring revealed no discernible horizonation in the wooded soils (0–23 cm depth). For more details regarding this site and the samples taken refer to van Bergen et al. (1997).

Fig. 1a and b are partial gas chromatograms of the total lipid extracts of rye grass and mixed herbs, respectively, both obtained from the grazed area. Each sample exhibits peaks corresponding to phytol and an identical range (C_{23}–C_{33}) of *n*-alkanes, at low abundance, with a maximum at C_{31}. As expected the grass extract is dominated by *n*-hexacosanol (C_{26}) as are the mixed herbs albeit at a slightly lower relative abundance (e.g. Tulloch, 1976; Walton, 1990). Peripheral *n*-alkanol homologues are also observed to occur in both samples (C_{22}–C_{28}, rye grass; C_{22}–C_{30}, mixed herbs) as are a range of fatty acid-phytol esters although the mixed herbs lack any components with C_{14} and C_{20} acid moieties. 24-ethylcholest-5-en-3β-ol (sitosterol) can be observed in both vegetation extracts whilst that of the mixed herbs also contains a relatively lower proportion of 24-ethylcholest-5,22-dien-3β-ol (stigmasterol) both compounds being common components of terrestrial vascular plants (Goad, 1991). At later retention time a series of wax esters can be observed in both extracts each of which exhibits a maximum at C_{44} (C_{38}–C_{48}, grass; C_{38}–C_{50}, mixed herbs). In both cases wax esters contain a predominance of the C_{26} *n*-alkanol moiety thereby agreeing with previous findings (Kolattukudy, 1975). Two latter envelopes of triacylglycerols (TAGs; C_{52} and C_{54}) can also be observed (dominant fatty acid moieties: $C_{16:0}$ and/or $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) as well as two unidentified steryl esters.

The lipid components observed in the TLE of the soil (Fig. 1c) from the grazed area are also dominated by hexacosanol (C_{26}) with a wider range of peripheral *n*-alkanol homologues (C_{18}–C_{34}). Thus, the predominant component observed in the overlying vegetation is quite clearly retained in the associated soil. The *n*-alkane components have a slightly wider range (C_{23}–C_{35}) with a maximum at C_{31}, again paralleling the input of grass and mixed herbs. The phytosterol sitosterol is also present in the grazed soil although its abundance is much lower relative to the TLE when compared with the two vegetation TLEs. Processes which may be responsible for this loss are: (i) complete mineralisation, (ii) chemical alteration to modified sterols, or (iii) condensation of steroid moieties to form involatile constituents (van Bergen et

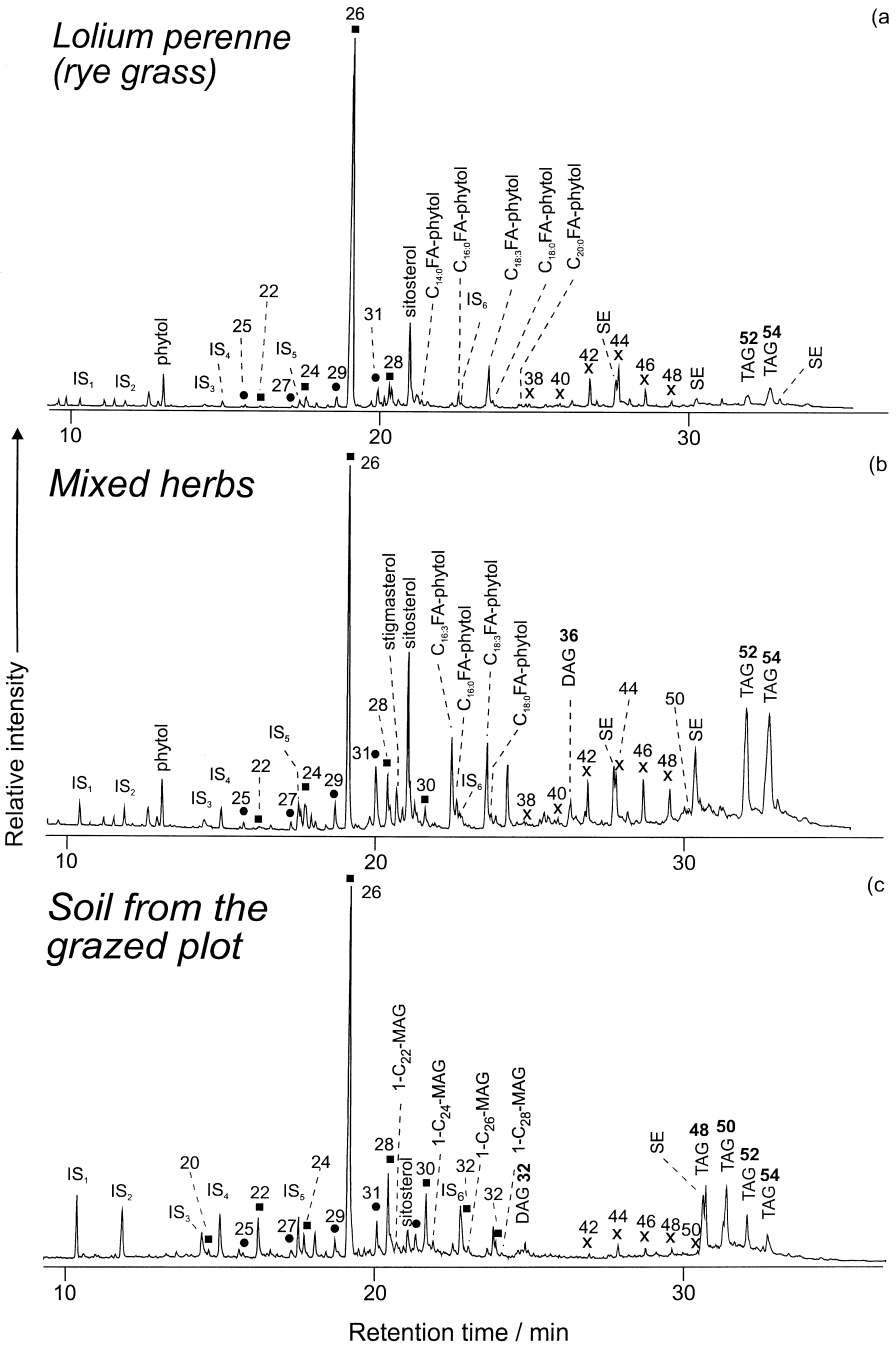

Fig. 1. Partial gas chromatograms of the total lipid extracts of samples from the Broadbalk Wilderness: (a) *Lolium perenne* (rye grass), (b) mixed herbs and (c) soil from the grazed plot. Key: IS_x = internal standards; ● = *n*-alkanes; ■ = *n*-alkanols; *x* = wax esters; SE = steryl esters; DAG = diacylglycerols; TAG = triacylglycerols; C_{xx} refers to total carbon numbers; numbers in bold indicate total

al., 1997). An overall loss of wax esters is seen in the TLE of the grazed soil, the loss presumably due to a combination of hydrolysis and β-oxidation. No fatty acid-phytol esters are observed although a range of wax esters is present at low abundance (C_{42}–C_{50}; C_{44} maximum) exhibiting a relative shift in abundance to higher homologues thereby correlating with previous reports of selective degradation of shorter-chain compounds (e.g.

Amblès et al., 1989, 1994 a,b; Jambu et al., 1991, 1993). The series of TAG envelopes observed in the soils is relatively lower and there is a shift to a dominance of lower molecular weight homologues (cf. wax esters). This is most likely due to the greater degree of unsaturation in the longer C_{18} fatty acid moieties giving rise to complex hydroperoxide mixtures which are easily decomposed (Frankel, 1998). Losses via bioesterification are also inferred by the occurrence of a C_{51} TAG envelope with an abundant $C_{17:0}$ acyl moiety which is most likely derived from soil bacteria (van Bergen et al., 1997). Certainly the loss of these compounds is not surprising given their high energy value for soil microorganisms leading to rapid hydrolysis and β-oxidation mediated by the various lipases inherent to soil microfauna (Hita et al., 1990). Fig. 2a and b depicts partial gas chromatograms of TLEs obtained from macerated, senescent leaves of common oak and sycamore, respectively. The most obvious difference between these extracts and those obtained from the rye grass and mixed herbs is the dominance of a series of sterols, triterpenols and related acetate derivatives and the lack of a dominant hexacosanol (C_{26}) component. The TLE of common oak leaves exhibits a maxima identified as sitosterol with less abundant peripheral peaks corresponding to taraxerol, β-amyrin and lupeol. Similarly, the TLE obtained from the sycamore leaves is also dominated by sitosterol with stigmasterol, β-amyrin, β-amyrin acetate, 24-methylenecycloartenol and 24-methylenecycloartenol acetate residing at lower abundance. Homologous series of *n*-alkanes (C_{23}–C_{31}, C_{27} maximum, common oak; C_{23}–C_{31}, C_{29} maximum, sycamore) and *n*-alkanols (C_{22}–C_{32}, C_{24} maximum, common oak; C_{20}–C_{32}, C_{24} maximum, sycamore) are observed to occur in both extracts the distributions agreeing with those of previous studies (Rieley et al., 1991). At late retention time, ranges of wax esters can also be seen (C_{38}–C_{50}, C_{44} maximum, common oak; C_{38}–C_{50}, C_{40} maximum, sycamore) all predominantly comprising C_{22} and C_{24} *n*-alkanol moieties. A restricted distribution of relatively significant TAG envelopes elute in both samples at late retention time and range from C_{50} to C_{54} with a dominant C_{52} peak. A number of other compounds are also present at low abundance in the oak (phytol, fatty acid-phytol esters, a terpenyl ester and a steryl ester) and sycamore (phytol, a fatty acid-phytol ester and a diacylglycerol) leaf extracts.

Fig. 2c depicts the TLE obtained from soil beneath the woodland. Whilst a number of the trends between the soil and overlying vegetation, e.g. wax ester and TAG distributions, are more subtle and directly parallel those observed for the grazed plot samples there are some very striking differences. Compared with the leaf extracts there has been a substantial loss in the sterol component(s) with only sitosterol, at intermediate abundance, being identified with any certainty. The soil lipid profile is dominated by a homologous series of *n*-alkanes (C_{21}–C_{33}, C_{29} maximum) and *n*-alkanols (C_{20}–C_{34}, C_{24} maximum). However, whilst the *n*-alkanol components still have a maximum at C_{24} (cf. leaf extracts) there is no great prominence of this component relative to the peripheral homologues, presumably this a result of other inputs of overlying vegetation, e.g. ivy, which possesses a different weight range of *n*-alkanol components (van Bergen et al., 1997). As stated above, the loss of sterol components is very large. Since these compounds are relatively more resistant to degradation than other lipids, e.g. *n*-alkanols (Cranwell, 1981), and there are no detectable free or bound transformation products it is difficult to rationalise the loss of these compounds via the three pathways of physical loss listed above (van Bergen et al., 1997). One possible explanation is direct assimilation rather than just degradation and/or transformation. With but a few known exceptions an exogenous source of dietary sterol is necessary to support the normal growth, development and reproduction of all insects. Although they are unable to synthesise sterols *de novo* many arthropods can produce cholesterol from higher sterol homologues, e.g. sitosterol, by a dealkylation at the C-24 position (e.g. Svoboda and Thompson, 1985; Nes et al., 1997). Soil arthropods are an important factor in the uptake and cycling of organic matter in the soil environment. For example, *collembola* (one genus of arthropoda) have been found to exist at densities in excess of 10^5 m^{-2} in an English moorland soil (Cragg, 1961). Such a massive reworking of the soil organic matter corrolates with the substantial loss observed for phytosterols and highlights the fact that the biological utility of a molecule is at least as important as chemical lability in determining the preservation of lipids in soils.

3.2. Fate of specific lipids in a soil profile

Like the Broadbalk Wilderness, the Geescroft Wilderness site is located in a small area of previously arable land. The site was part of an experimental field growing beans from 1847 to 1878, with frequent breaks towards the end of this period. It was subsequently fenced off and allowed to revert to natural woodland from 1883 (Jenkinson et al., 1992). Unlike Broadbalk, the site has never been chalked (except for the receipt of a little lime on occasions prior to reversion) which is reflected in the low soil pH of 4.2 measured in 1991 (Kinchesh et al., 1995). Samples were collected from Geescroft Wilderness in May 1995. Three distinct horizons were sampled: (i) a surface leaf litter consisting of dried and partially decomposed leaves of *Quercus robur* (common oak), (ii) a humic rich upper horizon (0–5 cm), and (iii) a mineral horizon (5–18 cm). Replicate samples of the soil horizons were taken at a position approximately 5 m from the first sampling location. All soils were sampled using

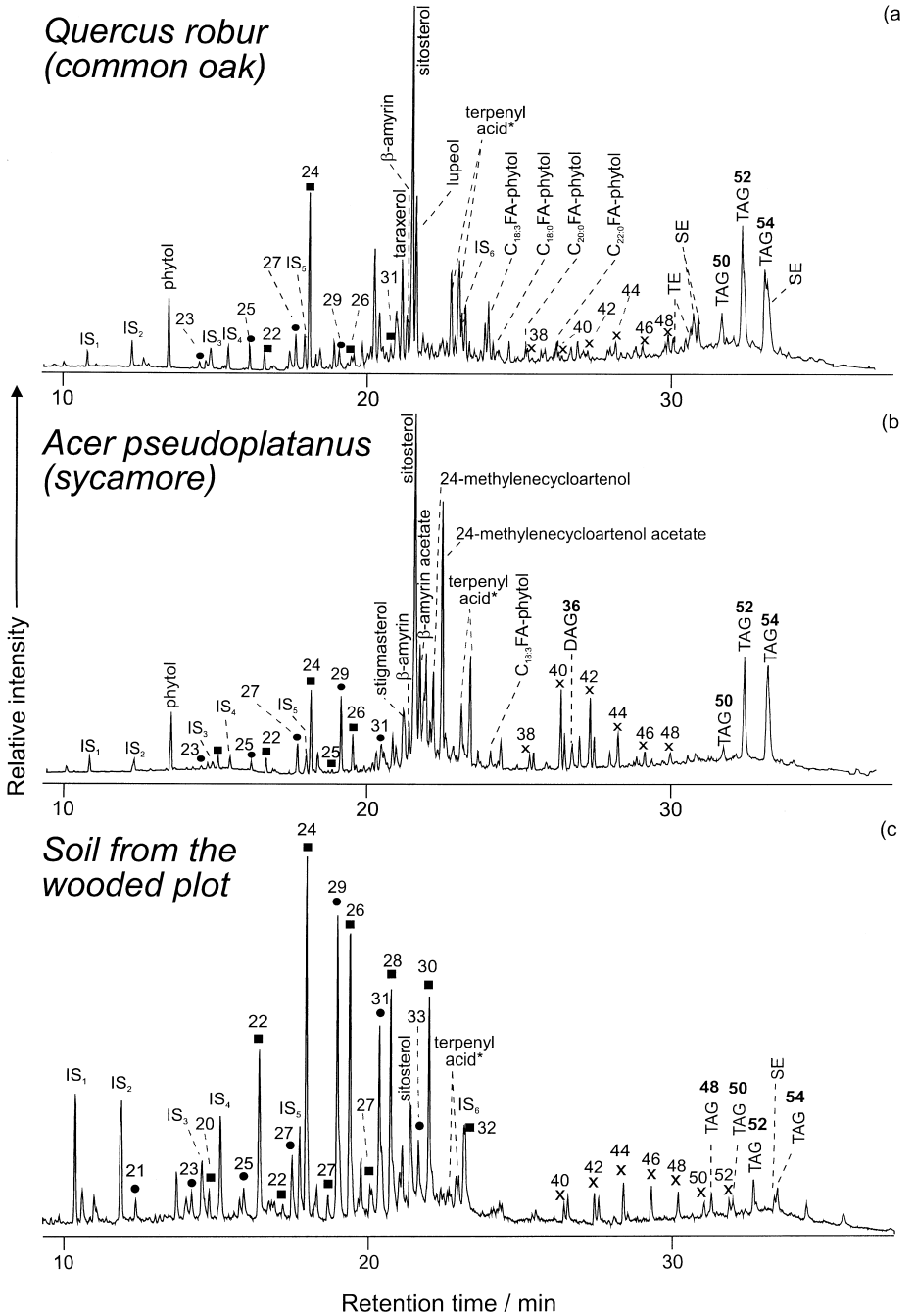

Fig. 2. Partial gas chromatograms of the total lipid extracts of samples from the Broadbalk Wilderness: (a) *Quercus robur* (common oak) leaves, (b) *Acer pseudoplatanus* (sycamore) leaves and (c) soil from the wooded plot. Key: IS_x = internal standards; ● = *n*-alkanes; ■ = *n*-alkanols; *x* = wax esters; SE = steryl esters; TE = terpenyl esters; DAG = diacylglycerols; TAG = triacylglycerols; C_{xx} refers to total carbon numbers; numbers in bold indicate total acyl carbons. Modified from van Bergen et al. (1997). *Terpenyl acids—MS characteristics, *m*/*z* (% abundance): 73(63), 133(47), 189(30), 203(100), 320(50), 482(8), 586(4).

a 2 cm diameter auger to a depth of 23 cm. The horizonation evident in the soil lends itself to a more classical organic geochemical approach of studying the effect of early diagenesis on a known input to a soil system.

Fig. 3a–c are partial gas chromatograms of the TLEs from partially decomposed oak leaves, the humic horizon soil and the mineral horizon soil, respectively. The *n*-alkanes of the oak leaves have a distribution

(C_{21}–C_{33}, C_{27} maximum) characteristic of higher plant waxes and are similar to those reported previously (Prasad and Gülz, 1990; Prasad et al., 1990). In the underlying horizons there is a significant decrease in abundance of *n*-alkanes and a shift in the distribution to higher molecular weight (C_{21}–C_{33}, C_{29} maximum, humic and mineral horizons) although the strong odd-over-even predominance, indicative of higher plant input, is retained (Peters and Moldowan, 1993). This change in the distribution could be due to relatively decreased degradation associated with increasing chain length (Moucawi et al., 1981; Amblès et al., 1989). However, it is probably the result of an input from another source (e.g. fungi) to the soil, this is further supported by stable carbon isotope data obtained for the major *n*-alkanes (Nott, van Bergen, Bull and Evershed, unpublished data). Whilst the lower pH of this site, compared with the almost neutral Broadbalk Wilderness, may not directly affect the relative accumulation/degradation of lipid components at Geescroft the more acidic environment will affect biological and physical soil attributes. Changes in microbial, meso- and macrofaunal populations, sorption affinities, mineral structure etc. will have an observable affect on the rate of loss of different lipid components. From the litter layer to the humic horizon and the humic horizon to the mineral horizon there is an increase in the *n*-alkanoic acids, relative to other lipid components, describing a biomodal distribution ranging from C_{10} to C_{32} and maximising at C_{16} and C_{28}. The distribution is weighted towards the more hydrophobic high molecular weight components. It should be stressed at this point that the *n*-alkanoic acids still degrade albeit at a lower rate relative to other lipids. The abundance of *n*-alkanoic acids in the soil extracts is significant in that the orientation of these polar compounds 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). This may account for the greater recalcitrance (>100 years) of lipids from any vegetation present before the current overlying species predominated at this site (see below). However, this physical effect certainly does not affect all compound classes. TAGs present in the senescent leaves are totally lacking in the TLEs of the humic and mineral soils. The loss of these valuable 'high energy' compounds is evidently even greater at lower pH (cf. Broadbalk Wilderness).

Fig. 4a–c depicts quantitatively the abundance of *n*-alkanol components, as determined by GC, for the isolated *n*-alkanol fractions of the senescent oak leaves, humic horizon soil and mineral horizon soil respectively. An even-over-odd predominance, characteristic of higher plants is clearly defined (Jambu et al., 1993). The distribution observed in the oak leaf litter fraction ranges from C_{22} to C_{31} with a pronounced maximum at C_{24}. This distribution is essentially maintained in the humic horizon although it is slightly wider C_{18}–C_{32} and the homologues peripheral to the maximum at C_{24} are relatively higher than in the litter (an exact mechanism explaining the relatively greater loss of the C_{24} component is currently not clear although a possible explanation could be the existence of a bacterial population which can preferentially utilise the more abundant C_{24} component). Hence, the *n*-alkanol fraction of the humic horizon demonstrates a clear input of oak leaf litter. However evidence for this input is not apparent in the distribution of the mineral horizon. In this case the homologous series ranges from C_{16} to C_{32} with a less pronounced maximum at C_{26}. This probably represents a contribution from a different source since frequently in terrestrial vascular plant waxes the predominant *n*-alkanol is C_{26} or C_{28} (Walton, 1990). Comparison of the *n*-alkanol distributions in Fig. 4b and c with the *n*-alkanoic acid distributions in Fig. 3b and c clearly demonstrates little correlation between the two compound classes. Hence, the incorporation of previous plant material is the most likely explanation for the shift observed in the *n*-alkanol distribution. Sterol/triterpenol fractions were isolated from each of the Geescroft horizons and analysed by GC, Fig. 5a–c depicts partial gas chromatograms obtained from the litter, humic and mineral horizon extracts, respectively. As expected, 24-ethylcholest-5-en-3β-ol (sitosterol) is the most abundant compound in the fraction obtained from senescent leaves (Goad, 1991). The peripheral sterols: cholest-5-en-3β-ol (cholesterol), 24-methylcholest-5,22-dien-3β-ol (epibrassicasterol), 24-methylcholest-5-en-3β-ol (campesterol) and 24-ethyl-5β-cholestan-3β-ol (5β-stigmastanol) are present as minor components as is the oxidation product 24-ethylcholest-4-en-3-one. The triterpenol taraxerol is present at an intermediate concentration along with lower quantities of β-amyrin, α-amyrin and lupeol. The most significant difference seen in the humic horizon fraction, and subsequently that of the mineral horizon, is the overwhelming loss of sitosterol, whilst the triterpenol components become the predominant compounds. This loss of sitosterol can be explained by a possible combination of assimilation by soil insects, to produce cholesterol (e.g. Svoboda and Thompson, 1985; Nes et al., 1997) as above, and rapid oxidative degradation. The action of an oxidative pathway is supported by the occurrence of 24-ethylcholest-4-en-3-one in all samples. This compound is an intermediate in both the oxidation and reduction of sitosterol and fills a role analogous to that of cholest-4-en-3-one in the oxidation and reduction of cholesterol (Arima et al., 1969; Nagasawa et al., 1969; Eyssen et al., 1973, Ren et al., 1996). However, since there is no concomitantly large increase in the relative abundance of 5α- (or 5β) stigmastanol the majority of sitosterol lost via this intermediate must have been degraded oxidatively, eventually forming a series of non-steroidal compounds before total mineralisation. However,

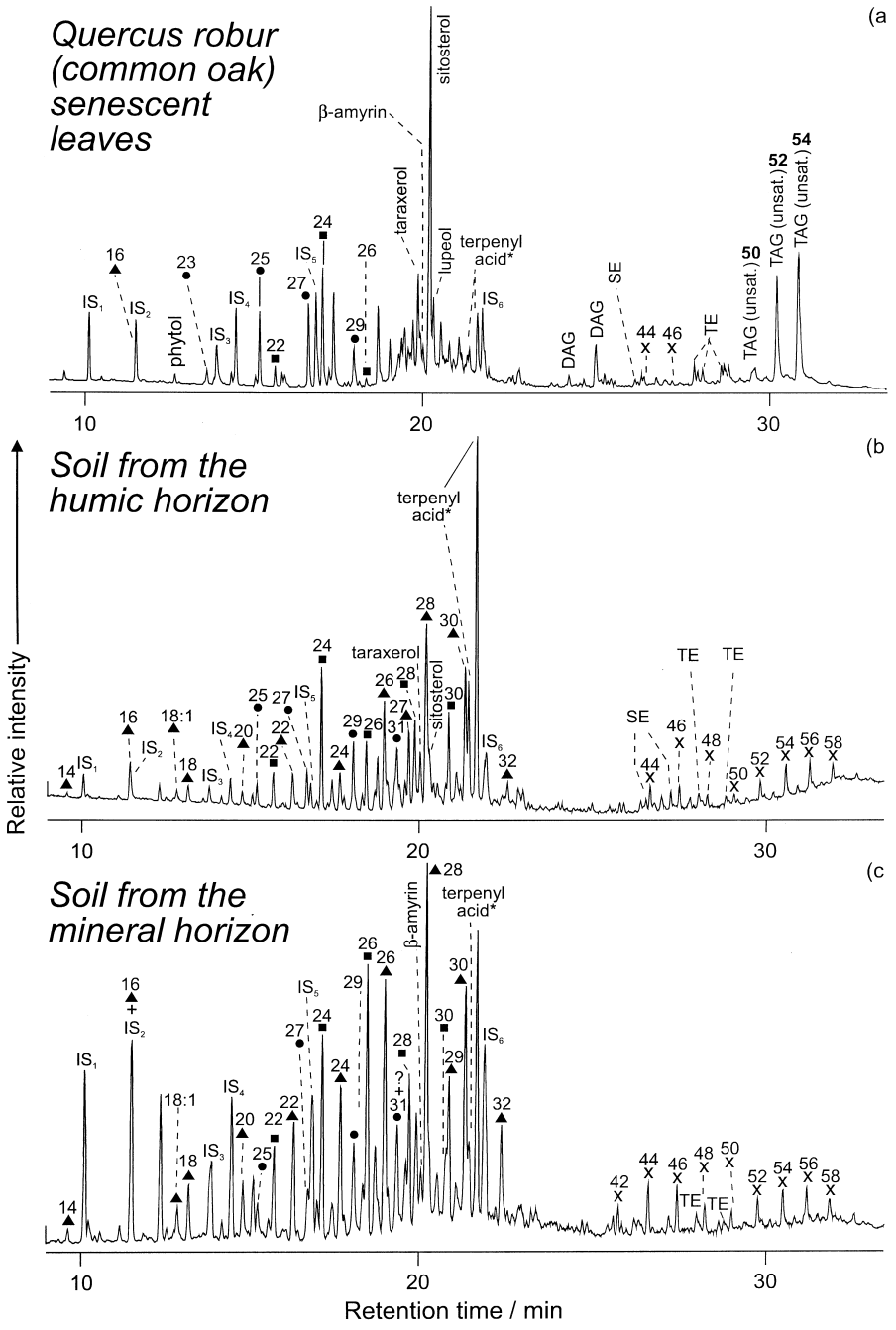

Fig. 3. Partial gas chromatograms of the total lipid extracts of samples from the Geescroft Wilderness: (a) *Quercus robur* (common oak) senescent leaves, (b) soil from the humic horizon and (c) soil from the mineral horizon. Key: IS_x = internal standards; ● = *n*-alkanes; ■ = *n*-alkanols; ▲ = *n*-alkanoic acids; *x* = wax esters; SE = steryl esters; TE = terpenyl esters; DAG = diacylglycerols; TAG = triacylglycerols; C_{xx} refers to total carbon numbers; numbers in bold indicate total acyl carbons. *Terpenyl acids—MS characteristics, *m*/*z* (% abundance): 73(63), 133(47), 189(30), 203(100), 320(50), 482(8), 586(4).

oxidative degradation by micro-organisms does not explain why sitosterol is lost to a much greater extent than apparently more labile lipids, e.g. *n*-alkanols and *n*-alkanoic acids (Cranwell, 1981). Hence, the former route involving assimilation by soil arthropods is most likely an important route leading to the major loss of sitosterol. Triterpenol components are observed to be comparatively more recalcitrant.

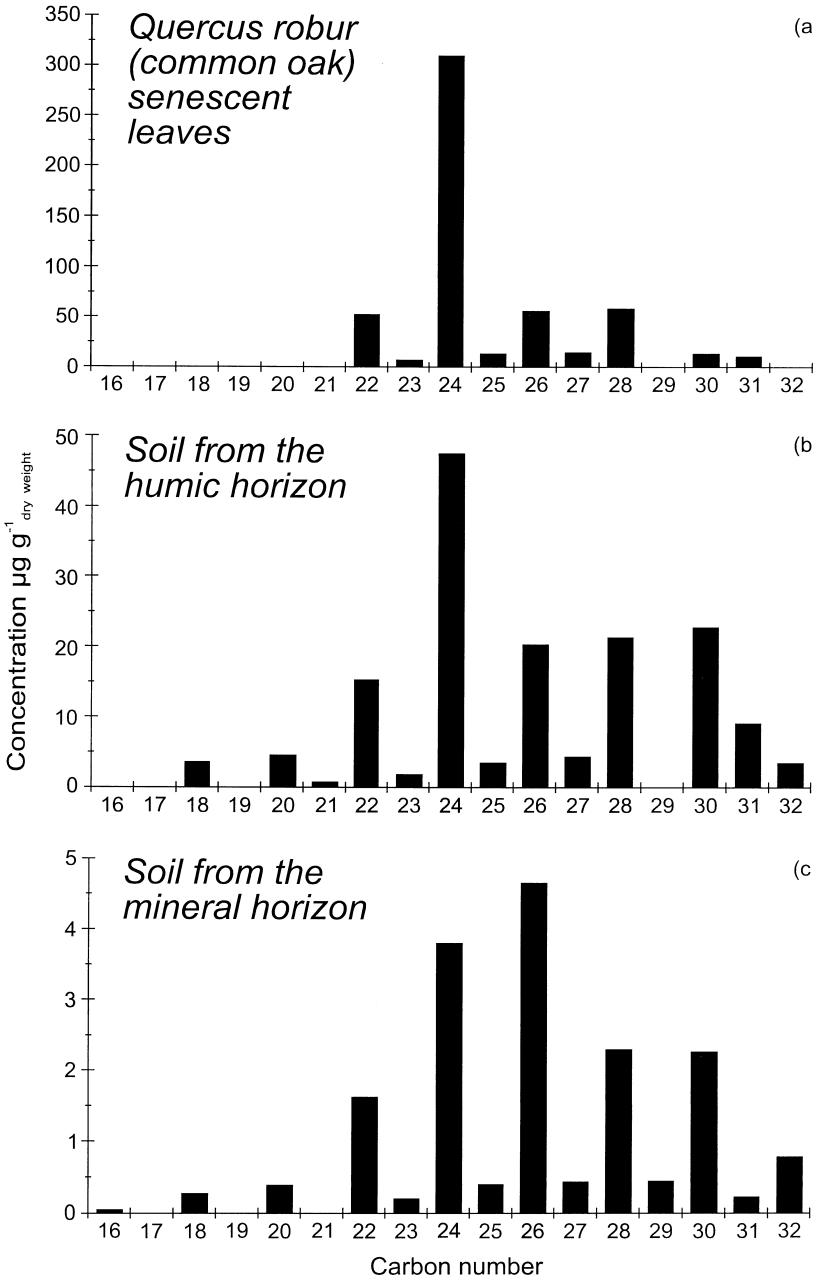

Fig. 4. *n*-Alkanol distributions in samples from the Geescroft Wilderness: (a) *Quercus robur* (common oak) senescent leaves, (b) soil from the humic horizon and (c) soil from the mineral horizon. These were determined using purified *n*-alkanol fractions and not using the TLEs.

The lower pH of this site makes it an interesting comparison for the Broadbalk Wilderness site. Certainly there is a significant effect on organic matter preservation both on a bulk (formation of a humic horizon) and molecular scale (e.g. *n*-alkanoic acids). Despite these differences, certain processes of loss are still fully operational, e.g. TAG and sitosterol degradation, indicating them to be routes of loss apparently independent of pH changes in the soil environment. However, since both examples likely arise from the action of micro-/mesofauna the same processes may actually be effected by different biological species at the two sites.

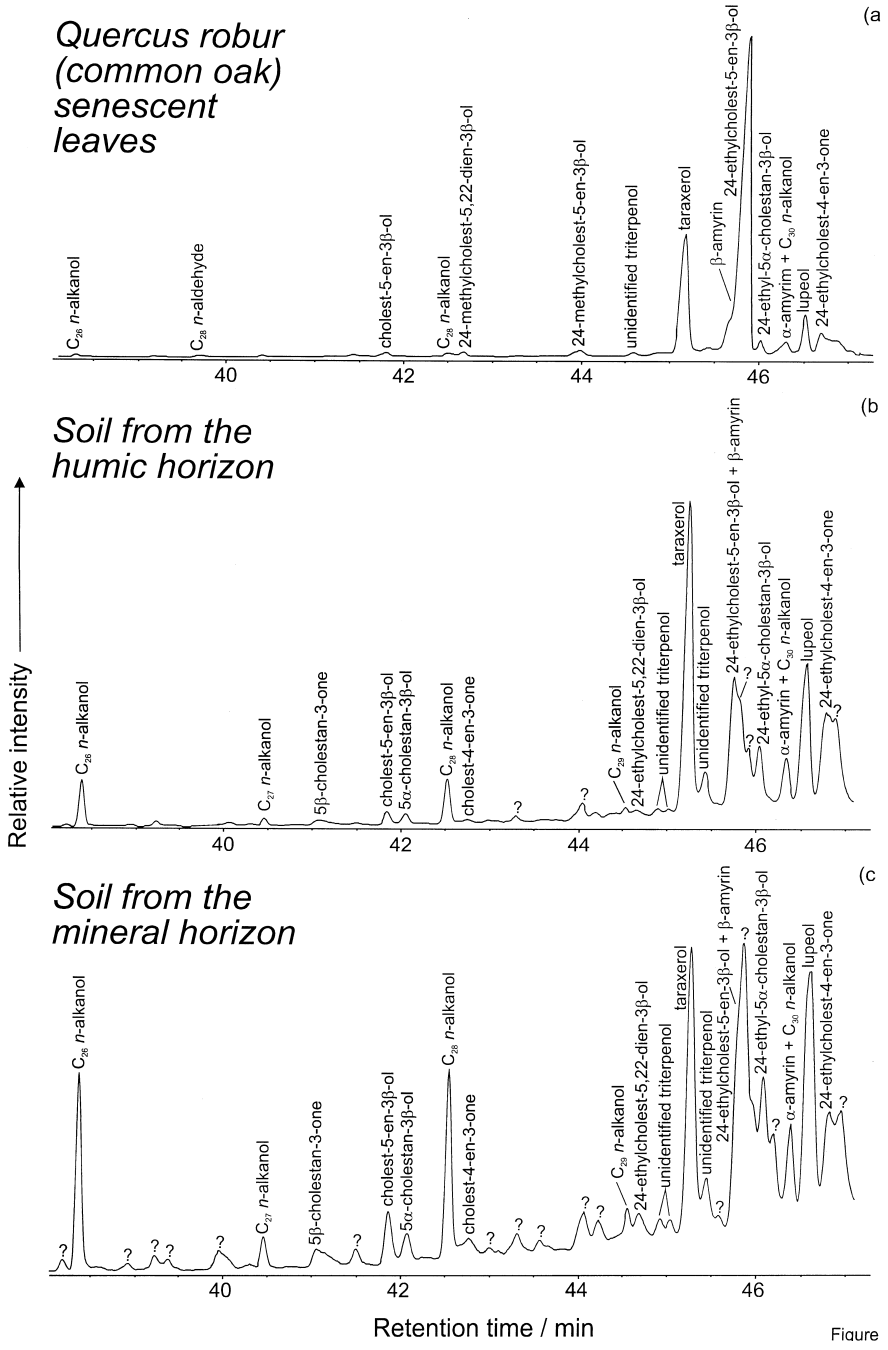

Fig. 5. Partial gas chromatograms of sterols in samples from the Geescroft Wilderness: (a) *Quercus robur* (common oak) senescent leaves, (b) soil from the humic horizon and (c) soil from the mineral horizon.

3.3. Temporal changes in the distribution and abundance of lipids

The Hoosfield Spring Barley Experiment was started in 1852 making it the longest running cereal experiment in the world with the exception of the adjacent Broadbalk Wheat Experiment. It consists of a series of large unreplicated strips receiving different combinations of phosphorus, potassium and magnesium. Each strip was originally divided at right angles to test various forms of nitrogen fertiliser, this test was discontinued in 1966. Additional plots included a farmyard manure (FYM)

treatment which was divided after 20 years to test the effect of FYM residues. Since 1968 each main plot has been divided to test 4 rates of addition of inorganic nitrogen. Fuller details may be obtained from Warren and Johnston (1967) and Jenkinson and Johnston (1977). This study concerns soils from three experimental sub-plots: one has received no FYM since at least 1852 (unmanured), another received FYM (35 t ha^{-1} yr^{-1}) for 20 years from 1852 to 1871 then none since (FYM residues) and the final soil has received FYM each year (35 t ha^{-1} yr^{-1}) since 1852 (continuous FYM). Spring barley has been grown each year and the stubble ploughed back into the soil. Fresh soils were sampled in May 1995 to a depth of 23 cm using a 2 cm diameter auger. Additionally, archived soils from 1882, 1913, 1946 and 1965 were obtained as well as modern samples of the two exogenous inputs, namely FYM and spring barley vegetation. For a more detailed discussion of sample history and treatment refer to Bull (1997) and Bull et al. (1998).

The primary aim of this particular study was to examine the pedological persistence of FYM and vegetation derived lipids in some soils which had received unchanging treatments and had been sampled over the entire course of the experiment. The use of archived soils enables temporal changes in the soil lipid profiles to be monitored rather than analysing a single modern-day soil, the lipid profile of which is a net result of all inputs and pedological processes over time (e.g. Broadbalk and Geescroft Wildernesses). We have estimated that ca. 28, 44 and 84 t_{carbon} ha^{-1} from crop stubble has been ploughed back into the unmanured, FYM-residues and continuous FYM plots, respectively. Additionally, FYM treatments account for an extra ca. 56 t_{carbon} ha^{-1} to the FYM-residues plot and ca. 400 t_{carbon} ha^{-1} to the continuous FYM plot. Hence, in the lipid profile of the latter plot there is likely to be a large bias towards an input of FYM.

Fig. 6 depicts the quantified *n*-alkanol profiles for each soil plot from 1882 and 1995 as well as those for FYM and spring barley. Inspection of these data quite clearly demonstrates that annual ploughing of the stubble results in the concentration of *n*-alkanol components being maintained over time. The relatively high amount of *n*-alkanol homologues observed to occur in the unmanured soil implies that stubble is the primary source for these components in each plot although there will be an input associated with straw in the FYM for the continuous manure plot (resulting in an increased abundance of the C_{26} component). This is not surprising given the high concentration of *n*-alkanols in spring barley vegetation and the fact that the overall abundance in any one year correlates with the actual order of recorded crop yields, i.e. unmanured < FYM-residues < continuous FYM (Warren and Johnston, 1967). However, the soil samples do exhibit a number of peripheral *n*-alkanol homologues which do not occur in the two inputs, i.e. C_{18}, C_{20}, C_{32} and C_{34}. Potential sources of these components are microbial reduction of long chain *n*-alkanoic acids as described by Jambu et al. (1993) and/or homologues released *via* hydrolysis of wax esters, i.e. old carbon. The observed loss of the C_{26} homologue, relative to peripheral components, may well result from preferential utilisation by a specific microbial population although such a hypothesis has yet to be tested.

Fig. 7 depicts the quantified *n*-alkanoic acid profile for the soil from each plot from 1882 and 1995 as well as those for the FYM and spring barley. The concentration of *n*-alkanoic acids in the two inputs is, in this case, much closer, cf. *n*-alkanols. Whilst the dominant C_{16} homologue in the spring barley is clearly paralleled in the 1882 soils only the unmanured soil retains this dominance 113 years later. The most striking temporal difference is the loss of *n*-alkanoic acids in the unmanured (~83%) and FYM-residues (~75%) plots. This demonstrates a rapid turnover combined with possible migration of these compounds in the soil leachate leading to a loss which cannot be compensated for by annual ploughing of stubble. However, the annual application of manure does compensate for losses of *n*-alkanoic acids in the continuous FYM plot leading to a small net increase in overall *n*-alkanoic acid abundance after 113 years (~9%). The accumulation of FYM derived lipids is further demonstrated by the overwhelming dominance of long-chain *n*-alkanoic acids centred about a C_{26} maximum, paralleling the distribution obtained for FYM.

As well as assessing the general fate of soil lipids, the input of FYM to two of the experimental soils enables an appraisal of the fate of lipids diagnostic of the manure input to be made. The use of 5β-stanols as biomarkers of faecal deposition has solicited significant attention (Grimalt et al., 1990; Bethell et al., 1994; Evershed and Bethell, 1996; Evershed et al., 1997; Simpson et al., 1998, 1999; Bull et al., 1999a,b). Whilst saturated sterols are relatively more stable than their unsaturated analogues during early diagenesis (Mackenzie et al., 1982) the longevity of 5β-stanols demands assessment especially in the light of the rapid loss of Δ^5-sterol components observed in the soils of the two wildernesses. Additionally, at least one species of insect, the Mexican bean beetle, has been shown to assimilate saturated 5β-stanols adding still further weight to this demand (Svoboda and Thompson, 1985). Fig. 8 is a temporal plot of 24-ethyl-5β-cholestan-3β-ol (5β-stigmastanol; the major 5β-stanol in FYM) absolute concentration for the three treatments studied. Values obtained for the continuous FYM soils continue to increase over time corroborating an annual input of FYM whilst values from the unmanured plot act effectively as a background of 5β-stanols in the soil. It can be

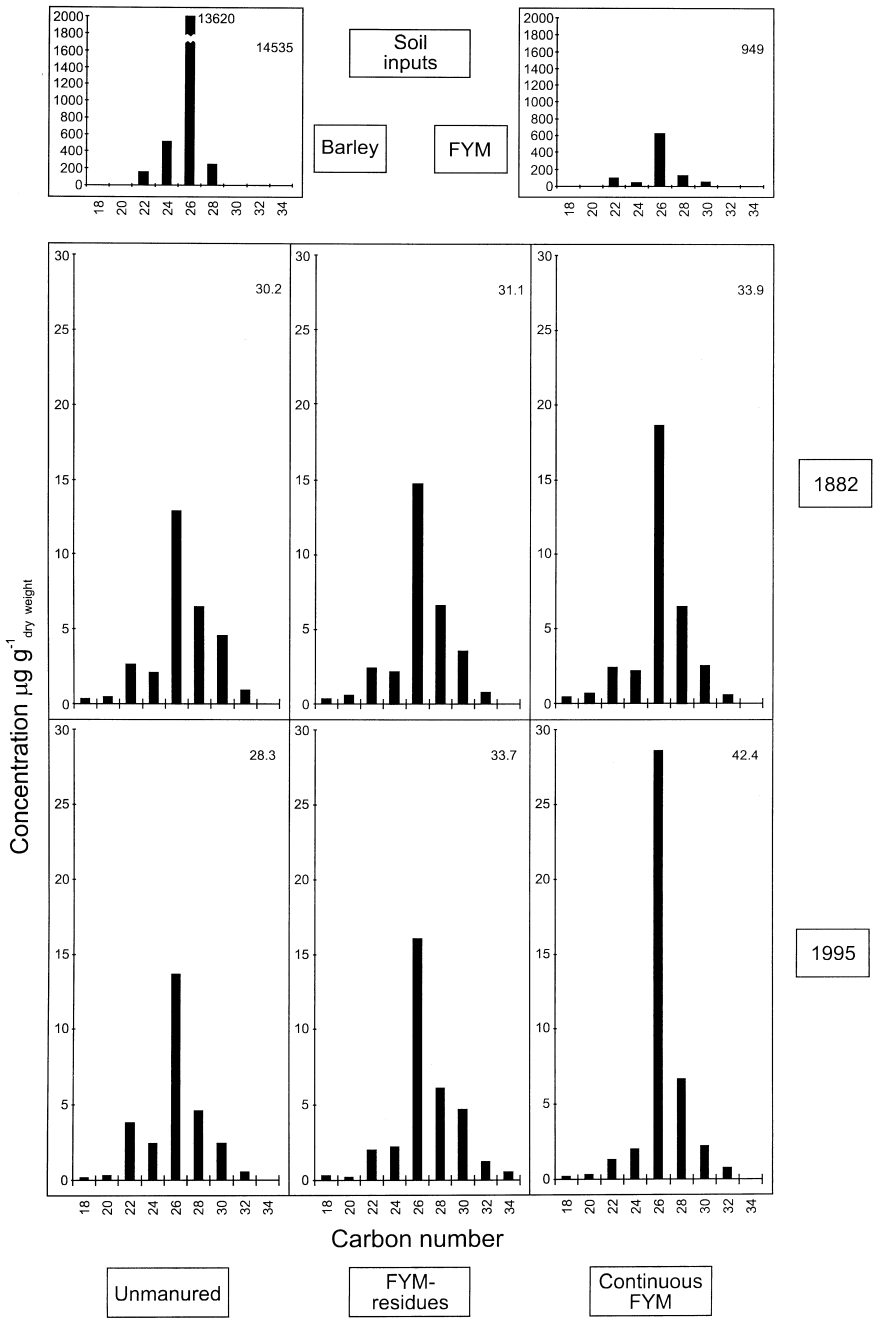

Fig. 6. Carbon number distributions for the major *n*-alkanol components detected in fresh and archived soils from the Hoosfield Spring Barley experiment. Each column relates to a different series whilst each row is concerned with a specific year. The value below each figure letter denotes total abundance (µg $g^{-1}_{soil\ dwt}$). Modified from Bull et al. (1998).

clearly seen that after an initial drop in concentration, on the cessation of manuring, the abundance of 5β-stigmastanol in the FYM-residues soil remains above that of the background till the modern-day. The degradation of 5β-stigmastanol is analogous to that of total organic carbon (TOC) indicating the possible use of this compound, in this case, as a proxy for TOC. This result thereby validates the use of 5β-stanols as molecular markers of faecal deposition in terrestrial soils.

5β-Stanols are also observed to occur in manure as constituents of stanyl esters. Fig. 9 depicts the wax ester distribution derived from FYM. Amongst the wax ester

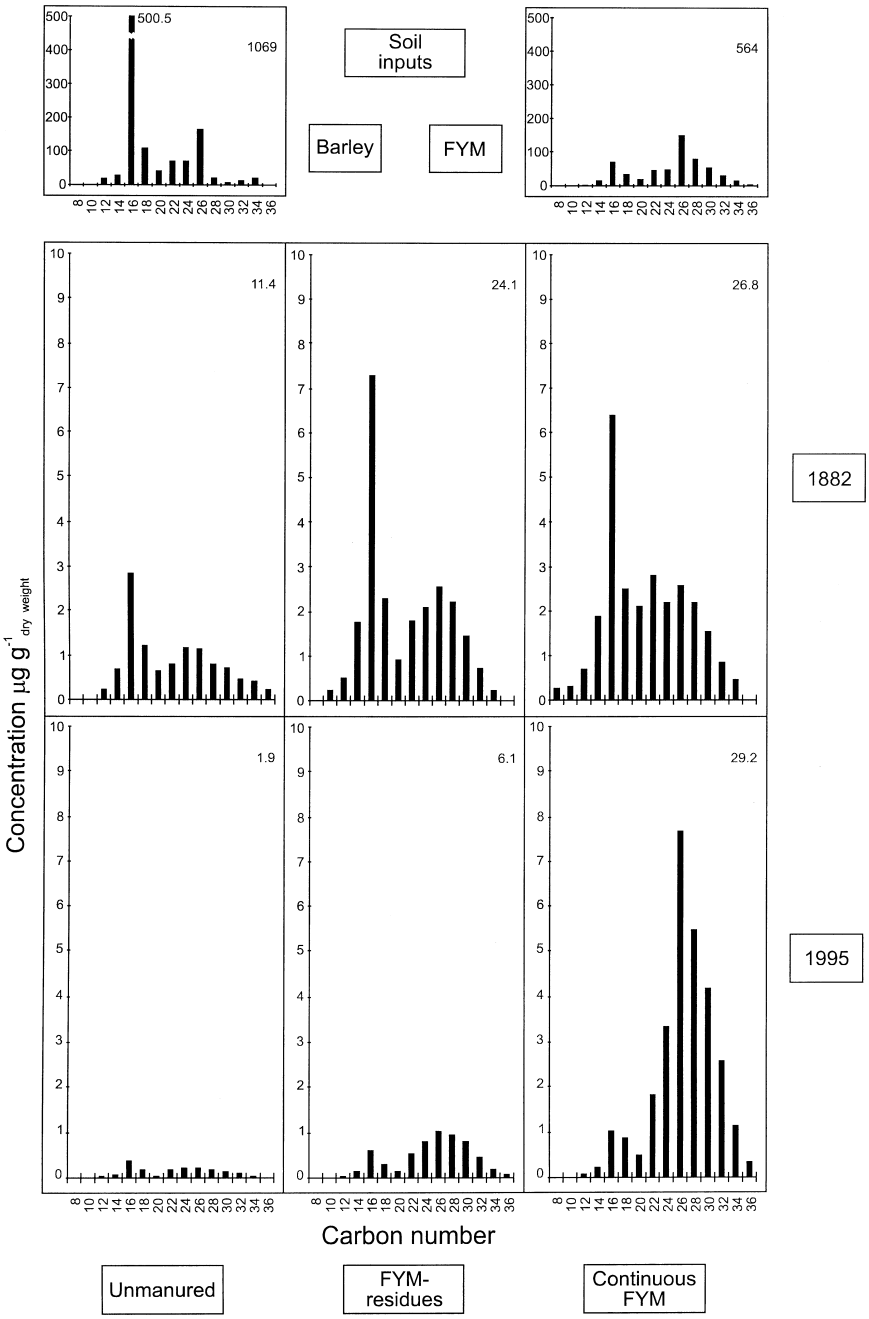

Fig. 7. Carbon number distributions for the major *n*-alkanoic acid components detected in fresh and archived soils from the Hoosfield Spring Barley experiment. Each column relates to a different series whilst each row is concerned with a specific year. The value below each figure letter denotes total abundance (µg $g^{-1}_{soil\ dwt}$). Modified from Bull et al. (1998).

homologues 24-ethyl-5β-cholestanyl palmitate is observed to occur as are its C_{27} and C_{28} congeners, albeit at lower abundances. The absolute concentration of 24-ethyl-5β-cholestanyl palmitate in FYM and each of the soils sampled in 1995 is also included. It may be observed that the concentration of the compound in FYM is much higher than levels determined for the three soil samples. However, the effect of manuring can still be clearly seen by comparing the levels determined for the unmanured and continuous FYM soils; the former being an order of magnitude lower than the latter. Furthermore, the value determined for the FYM-residues soil is also significantly

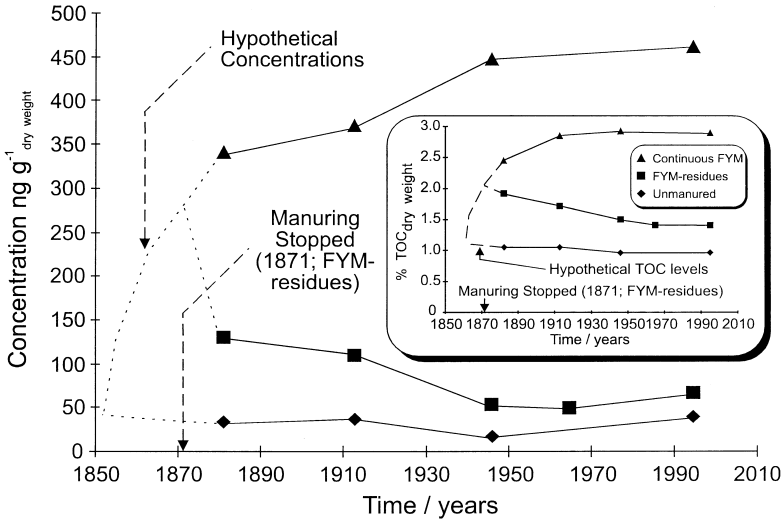

Fig. 8. Plots depicting the temporal change in absolute concentration of 24-ethyl-5β-cholestan-3β-ol in the three plots sampled from the Hoosfield Spring Barley experiment (the broken line represents a hypothetical predication of previous 24-ethyl-5β-cholestan-3β-ol levels). Insert: the total organic carbon levels (TOC) of the Unmanured, FYM-residues and Continuous FYM soils (the broken line represents a hypothetical predication of previous TOC levels). Modified from Bull et al. (1998).

higher than that of the unmanured sample (~400% higher) giving a clearer signal of previous manuring than is obtained from the free 5β-stanols. This provides compelling evidence for the use of esterified 5β-stanols, which demonstrate better preservation of the manuring signal, in future studies involving the detection of faecal deposition.

3.4. Effect of soil pH on the preservation of various compound classes

The Park Grass experiment was started in 1856, the field on which it is located having been under pasture for at least a century before this (Tilman et al., 1994). At the beginning of the experiment the site had received no regular dressings of calcium carbonate and the pH was in the region of 5.7. A test of liming began in 1903, was modified in 1965, and the plots now range in pH from 7.3 down to 3.5 depending upon treatment (Johnston et al., 1986). Soils were sampled in May 1995 and 1996 to a depth of 23 cm using a 2 cm diameter auger. Single samples were collected from four plots of varying pH, identified as: 10d (pH 3.8), 11/1a (pH 6.3), 11/1d (pH 3.7) and 18/2 (pH 7.3). The soil samples from the more acidic plots, i.e. 10d and 11/1d, were subdivided into a dark brown humic horizon (ca. 0–5 cm) and a deeper mineral horizon (ca. 5–18 cm). The overlying vegetation for the two acidic soils consists of a single grass species on each: *Anthoxanthum odoratum* (10d) and *Holcus lanatus* (11/1d). The other two plots (11/1a and 18/2) have a mixed sward containing various grasses, legumes and forbs (Thurston et al., 1976; Tilman et al., 1994). For more details concerning sample treatments refer to van Bergen et al. (1998).

This study was made with the primary intent of assessing the effect of pH on the preservation/degradation of plant derived biomolecules in the soil environment. The different species of vegetation in each subplot are a result of the different liming and fertiliser treatments. Whilst a single species of vegetation for each plot might be desirable for our purposes this is neither feasible or ecologically valid. In soils exhibiting a more alkaline pH (7.31) *n*-alkanes (not shown) are present at higher relative abundances. Since the quantity of *n*-alkanes in each of the vegetation samples is about equal a high pH may well help to preserve vegetation derived *n*-alkanes relative to other classes of lipid (van Bergen et al., 1998). However, an input from endogenous soil organisms such as fungi cannot be wholly excluded (Weete, 1976). Conversely, *n*-alkanoic acids (not shown) are observed to accumulate relative to other lipids in the soils with a low pH, e.g. plots 10d and 11/1d. This agrees with the similar relative accumulation observed in the soils from the Geescroft Wilderness. Although *n*-alkanoic acids can derive directly from vegetation we would contend that the majority of these components arise from the oxidation of other lipids such as *n*-alkanes and *n*-alkanols (Moucawi et. al., 1981; Amblès et al., 1994a,b). There is also a relative accumulation of *n*-alkanoic acids in the soil from plot 11/1a. This can be explained by the fact that until fairly recently this plot also had a low pH, the pH in 1970 being 4.8. Thus, the presence of the organic acids does not substantially affect the current soil pH. It also demonstrates the profound

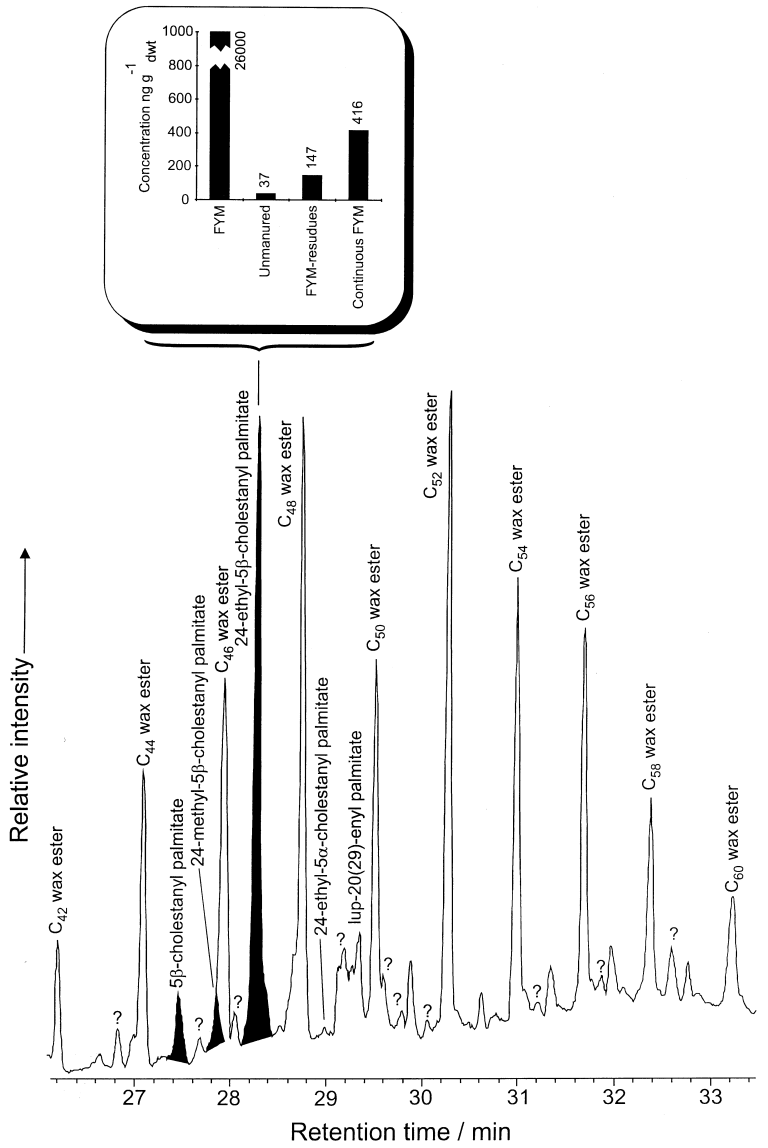

Fig. 9. A partial gas chromatogram of the wax ester fraction derived from FYM. Insert: A quantitative summary of the 5β-stanyl palmitate concentration in each of the plots sampled from the Hoosfield Spring Barley experiment in May 1995.

differences by which present-day lipidic preservation can be enhanced by previous treatments of soils.

Fig. 10a–c depicts partial gas chromatograms of the *n*-alkanol fractions obtained from the grass (*Anthoxanthum odoratum*), humic soil horizon and mineral soil horizon of plot 10d respectively. The *n*-alkanol distribution obtained for the grass ranges from C_{26} to C_{30} with a dominant C_{28} maximum. In comparison the distribution obtained from the mineral soil is slightly wider (C_{22}–C_{30}) and maximises at C_{26}. Distributionally, the homologous series of *n*-alkanols observed in the humic horizon is much closer to that of the mineral soil indicating that the *n*-alkanol components are dominated by an input most likely derived from previous standing vegetation. Interestingly, this conclusion is also corroborated by stable carbon isotope measurements of the major *n*-alkanol components. Whilst the grass derived *n*-alkanols give $\delta^{13}C$ values of −38.3‰, *n*-alkanols in both the humic and mineral horizon both produce values of ~36.5‰. This is concordant with a slower incorporation of *Anthoxanthum odoratum* derived organic matter into the soil organic matter and thus the presence of more 'old' carbon in the humic layer of plot 10d. Plot 11/1d (not shown), exhibits greater incorporation of the standing vegetation (*Holcus lanatus*). Both plots 10d and 11/1d receive N, P, Mg and Na fertilisers,

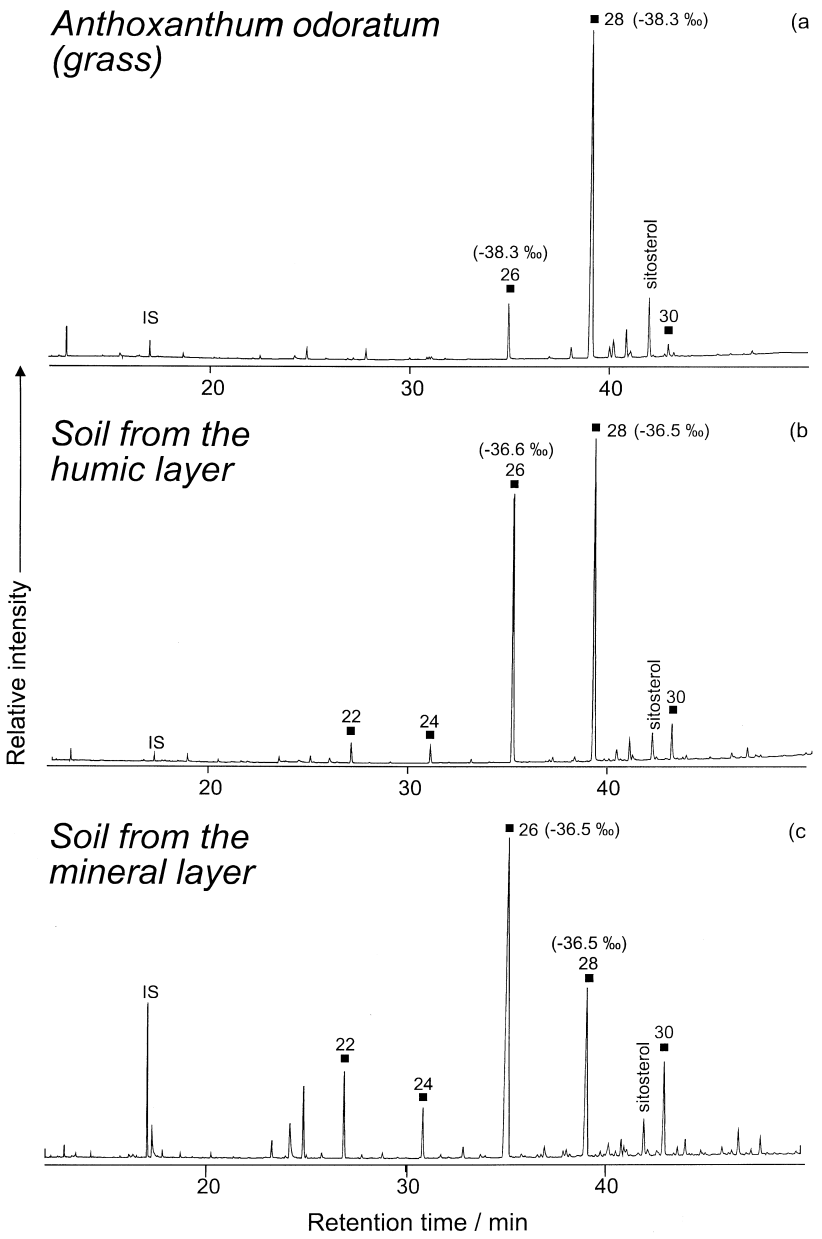

Fig. 10. Partial gas chromatograms of *n*-alkanols in samples from plot 10d of the Park Grass experiment: (a) *Anthoxanthum odoratum* (grass), (b) soil from the humic horizon and (c) soil from the mineral horizon. $\delta^{13}C$ values determined for the major components are included.

11/1d also receives K fertiliser but 10d does not. However, whether the difference in *n*-alkanol preservation is determined directly by this or indirectly by the growth of a different species of plant is currently unresolved.

Whilst ω-hydroxy acids are not detectable in soil from plot 18/2, plots 10d and 11/1d both exhibit similar distributions of even components (C_{22}–C_{26}). The distribution derived from 10d has an unpronounced maximum at C_{22} (180 μg g_{TOC}^{-1}) whilst the more abundant homologues derived from the 11/1d soil maximise about C_{24} (490 μg g_{TOC}^{-1}). The origin of the ω-hydroxy acids is not completely clear. Base hydrolysis of *Holcus lanatus* root material has been shown to yield C_{22} and C_{24} ω-hydroxy acids (Bull, Nott, van Bergen and Evershed, unpublished results), the obvious inference being that the ω-hydroxy acids are derived from root biopolyesters, i.e. suberin which has been reported to contain substantial amounts of either the C_{22} or C_{24} homologue (Walton,

1990; Matzke and Riederer, 1991; Riederer et al., 1993; Nierop, 1998). Interestingly these compounds also accumulate relative to the TLE in soils of low pH, paralleling the accumulation of *n*-alkanoic acids.

Overall, the Park Grass soils provide a sound experimental basis which confirms and reinforces observations made, concerning the preservation of lipids, on results obtained for the other three study sites, especially conclusions drawn from the Broadbalk and Geescroft Wildernesses.

4. Conclusions

The overall aim of this study has been to investigate and assess the lipid composition of a series of controlled experimental soils and thereby derive information about the fate of lipids during early diagenesis. Soils from four experiments, each subjected to a variation in organic input or exhibiting a different pH, were analysed and the findings discussed in relation to each other. Of the various results obtained the most significant are as follows.

1. Soil pH was observed to have a significant effect on the preservation of certain lipids. *n*-Alkane preservation was enhanced under more alkaline conditions whilst *n*-alkanoic and ω-hydroxy acids accumulated, relative to other components, in more acidic soils. Accumulated *n*-alkanoic acids were found to persist in soils even after a change to more alkaline conditions.
2. Severe losses in triacylglycerols were observed in soils irrespective of physical conditions this most likely being the result of preferential biological utility.
3. The effects of functional diversity were observed by comparing the preservation of *n*-alkanol and *n*-alkanoic acid components in an arable soil. The abundance of *n*-alkanols was seen to be preserved over time whilst, despite annual inputs, *n*-alkanoic acid abundance continually decreased. Only in a soil supplemented annually with FYM was this decline abated. Losses were ascribed to rapid turnover and possible loss of the more polar *n*-alkanoic acids in the soil leachate.
4. Sitosterol was observed to diminish in concentration at a greater rate than chemically more labile lipids in the same environment. This phenomenon was ascribed at least in part to its essential assimilation by soil arthropoda.
5. In light of point (3) the stability of 5β-stigmastanol, a chemical biomarker of ruminant faeces, was investigated. A viable manuring signal was observed to persist in a soil that had received no manure applications for 113 years thereby validating, for studies with appropriate controls, the use of 5β-stanols as biomarkers of faecal deposition.
6. The survival of a manure signal based on 5β-stanyl esters was also assessed and found to retain a more persistent biomarker signal of manuring than that exhibited by the free 5β-stanol components; presumably because the esterified component is more resistant to degradation.

Whilst this investigation has generated a number of interesting and useful results, there are still a number of ways in which the study of the early diagenetic fate of lipids in soils can be improved. As with any comparative study any inferences drawn by making comparisons are statistical probabilities albeit sometimes very persuasive ones. A route by which such probabilities could become certainties would be through the use of isotopes. Various isotopically enriched (or depleted) substrates (e.g. C_4 litter on C_3 soil, $^{13}CH_3$, $^{13}CO_2$) could be used in a multitude of soil incubation/degradation studies and the generation and/or interconversion of lipids followed isotopically (e.g. Lichtfouse, 1997; Bull et al., 1999b). The use of stable isotopes is particularly attractive since it enables natural in situ studies to be performed thereby leading to ecologically sound findings directly applicable to the natural environment. Recent advances in instrumentation have facilitated the routine trapping of single molecular species from biological extracts thus enabling single lipid components to be collected to levels where ^{14}C dating is a viable proposition (Eglinton et al., 1996, 1997). Radiocarbon dating of specific molecular components would prove invaluable in ascertaining lipid-carbon flows and soil residence times, adding a further dimension to soil lipid analysis (Bol et al., 1996; Huang et al., 1996). Only through such studies can we hope to fully understand the complex processes occurring in a pool which is fundamental to the global carbon cycle.

Acknowledgements

This project was undertaken whilst the authors were in receipt of a NERC Grant GR3/9578 to RPE. J. Carter and A. Gledhill are thanked for their help with the GC/MS and GCC/IRMS analyses. The use of the NERC Mass Spectrometry Facilities (Grants GR3/2951, GR3/3758, FG6/36/01) is gratefully acknowledged. IACR receives grant-aided support from the BBSRC.

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