



Organic geochemical studies of soils from the Rothamsted Classical Experiments—I. Total lipid extracts, solvent insoluble residues and humic acids from Broadbalk Wilderness

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Abstract—Total lipid extracts and insoluble organic matter, i.e. solvent insoluble matter and humic acids, were studied from soil samples taken from the three adjacent plots comprising the Broadbalk Wilderness at Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K. Analyses involved high-temperature gas chromatography (HT-GC) and HT-GC-mass spectrometry (HT-GC-MS) to investigate trimethylsilylated total lipid extracts and Curie-point pyrolysis-GC (Py-GC) and Py-GC-MS to investigate solvent insoluble fractions. The plots were chosen specifically for their different types of vegetation cover. Samples of the vegetation were examined in parallel with the underlying soils in an effort to follow the fate of the major plant components in soil. The application of HT-GC and HT-GC-MS allowed changes in high molecular weight lipids, particularly intact acyl lipids, such as triacylglycerols, wax esters, steryl and triterpenyl esters, to be studied in leaf and soil extracts. The total lipid extracts of the soil samples from the wooded area were dominated by the input from leaf-derived lipids. The lipid extracts of soils from the grazed and stubbed areas were markedly different from those from the wooded area, and reflected the mixed vegetation cover dominated by grass species. In marked contrast, the pyrolysis data from the insoluble organic matter and humic fractions of the soils did not reflect the composition of the lignin comprising the overlying vegetation, but rather showed evidence of amino acid moieties probably present as polypeptides. The absence of the lignin signal is possibly due to rapid diagenetic changes presumed to be influenced by the slightly alkaline pH of the soil. The ability to recover recognizable chemical signals from soil lipids has important implications for archaeological investigations aimed at revealing temporal changes in vegetation cover and/or differences in land use at specific site locations. © 1997 Elsevier Science Ltd

Key words—soil organic matter, humic acids, total lipid extracts, Rothamsted Classical Experiment, Curie-point pyrolysis

INTRODUCTION

One major pool of carbon in the biosphere is represented by soil organic matter (SOM; Post *et al.*, 1982). Hence, understanding the changes in the composition of SOM due to alterations in vegetation, land use or management are potentially of great importance (cf. Kinchesh *et al.*, 1995). Although the effects of cultivation and land clearance on SOM levels (measured as total organic carbon) are well known (e.g. Skjemstad *et al.*, 1986; Schulten and Hempfling, 1992; Golchin *et al.*, 1994; Guggenberger *et al.*, 1995), relatively little is known about the effects caused by these changes at the molecular level.

Numerous studies have examined the bulk of SOM [the complex high molecular weight fractions (HMW), i.e. humic acids, humin] using various different techniques, such as spectroscopy (solid state ¹³C NMR; e.g. Skjemstad *et al.*, 1986;

deMontigny *et al.*, 1993; del Rio *et al.*, 1994; Guggenberger *et al.*, 1995; Kinchesh *et al.*, 1995), chemolysis (CuO oxidation, e.g. Kögel-Knabner *et al.*, 1988; deMontigny *et al.*, 1993; Guggenberger *et al.*, 1994; BCl₃, e.g. Richnow *et al.*, 1994) and pyrolysis (e.g. Martin *et al.*, 1979, 1994; Martin and Gonzalez-Vila, 1983; Saiz-Jimenez and de Leeuw, 1986b, 1987a,b; Schulten and Hempfling, 1992; Hatcher and Clifford, 1994; del Rio *et al.*, 1994). From these studies it has become apparent that lignin-cellulose, derived from higher plants, is a major contributor to HMW together with tannins, highly aliphatic macromolecules and/or microbially derived material.

To date, relatively few studies have considered the changes in HMW composition related to changes in vegetation or land use (e.g. Skjemstad *et al.*, 1986; Schulten and Hempfling, 1992; Schulten and Leinweber, 1993; Guggenberger *et al.*, 1994, 1995; Kinchesh *et al.*, 1995; Saiz-Jimenez *et al.*, 1996). The results so far are inconclusive and seem to show little significant molecular variation of the

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HMW fractions with respect to the different vegetation or agricultural practices; rather, they reveal differences related to particle size or total amounts of SOM. It should, however, be noted that many of these studies used ^{13}C NMR data which provides information on the carbon environments rather than detailed molecular information which can be obtained from pyrolysis and chemolysis techniques. Pyrolysis techniques have been shown to be of particular value in rapidly providing detailed molecular information on the insoluble organic matter fractions of soils (e.g. Schulten and Hempling, 1992; Schulten and Leinweber, 1993; Saiz-Jimenez, 1994; del Rio *et al.*, 1994; Saiz-Jimenez *et al.*, 1996).

In contrast to the numerous studies on the HMW fraction, rather less attention has been given to the low molecular weight components, such as lipids (e.g. Moucawi *et al.*, 1981; Bridson, 1985; Amblès *et al.*, 1989, 1993, 1994; Jambu *et al.*, 1991, 1993, 1995). The main source of lipids in the majority of soils is the vegetation growing at the surface. Hence, the resistant chemical constituents of plants will have the greatest expression within the soil profile. While the proportion of lipids deriving from the *in situ* activities of microorganisms will be comparatively smaller (Fridland, 1982), the presence of certain classes of microorganisms can be revealed through the use of specific biomarkers, e.g. hopanoids and branched short-chain monoacids are ascribed to bacterial activity (Ries-Kautt and Albrecht, 1989; Amblès *et al.*, 1994), whereas substantial amounts of C_{28} sterols are indicative of fungal activity (Weete, 1976).

The majority of research performed on soil lipids relates to the input of vegetation-derived components to the soil and/or the subsequent products of diagenesis (e.g. Moucawi *et al.*, 1981; Amblès *et al.*, 1989, 1993, 1994; Jambu *et al.*, 1991, 1993, 1995). The results obtained to date show that a wide range of chemical processes, including oxidation, reduction, hydrolysis and transesterification, will affect the composition of soil lipids. The nature and rate of these changes are directly affected by soil pH, moisture, clay content, plant types, microbial biomass, etc.

In order to study specific variations occurring in both the low and high molecular weight fractions due to changes in vegetation and/or land use, well-defined samples preferably from locations with well-recorded histories of use are of crucial importance. One of the oldest and best documented modern-day sites which can provide such samples is Rothamsted Experimental Station, Hertfordshire, U.K. Long-term experiments have been in progress at this site for more than 150 years and detailed records are available. This site is an ideal locality at which to study the effects of changing agricultural practices and vegetation on solvent soluble and insoluble soil

organic matter and so improve our understanding of the organic geochemistry of soils.

Our studies are prompted largely by an interest in archaeology. Most specifically in the application of organic geochemical methods to improve our understanding of the use of particular areas of the landscape, enclosures or features revealed during excavations or survey. Such methods are potentially highly complementary to other techniques used for studying ancient landscapes and sites. Most successful studies performed to date in this area have shown 5β -stanols to be useful biomarkers of faecal inputs into archaeological sediments. For example, the presence of faecal material was confirmed in sediments from a Roman ditch (Knights *et al.*, 1983) and excavations in Paris (Pepe and Diazabo, 1990). Recent work has identified cess pits from Roman and Late Saxon/medieval sites (Bethell *et al.*, 1994). In addition, the complementary use of 5β -stanols with bile acids as multi-molecular biomarkers has been shown to have the potential to enhance identifications of the origins of faecal inputs (Evershed and Bethell, 1996). We have also shown that 5β -stanols, such as 5β -stigmastanol, have potential value as indicators of manuring episodes in archaeological soils (Evershed *et al.*, 1997).

From an archaeological view point, obtaining insights into the changes in vegetation or land use are of special importance. Knowledge of the processes once affecting the soil within the sites vicinity would provide valuable new information into the activities practised by ancient man. In particular, changes in cultivated crops or general vegetation changes are an important aspect. Indeed, it is not unreasonable to expect the environment surrounding an archaeological site to have changed radically over time. Fortunately, the very act of a change in vegetation provides a potential opportunity to utilize geochemical techniques to monitor the impact of different vegetation through its effect on the soil organic matter constituents.

In this paper, total lipid extracts and insoluble organic matter from soils of three adjacent plots of the Broadbalk Wilderness at Rothamsted possessing different types of vegetation were studied, together with samples of the vegetation itself. High-temperature gas chromatography (HT-GC), HT-GC-mass spectrometry (HT-GC-MS), Curie-point pyrolysis-gas chromatography (Py-GC) and Py-GC-MS, were used to establish whether or not characteristic signals for the vegetation are retained in the soil. The results obtained are discussed with regard to their implications for archaeological investigations.

SAMPLE DESCRIPTION

Rothamsted Experimental Station

The soil samples studied were taken from three plots of the Broadbalk Wilderness (Fig. 1) which is

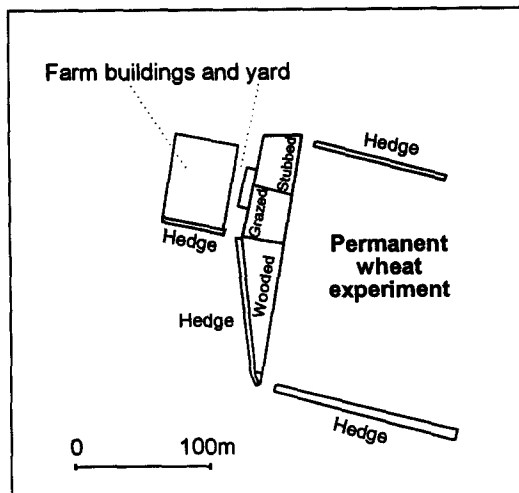


Fig. 1. Schematic outline of Broadbalk Wilderness showing wooded, grazed and stubbed area (modified after Kerr *et al.*, 1996).

part of the long-term Broadbalk Classical Experiment at Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K., in May 1995. Soils were sampled using a 2 cm diameter auger to a depth of 23 cm. The soil at the site is a Stagnogleyic paleo-argillic brown earth, with a loamy surface layer overlying clay-with-flint (Jenkinson *et al.*, 1992) classified as Chromic Luvisol (FAO, 1990) or Aquic Palendolf (USDA, 1992). The mechanical composition of the soil at the three plots is similar with approximately 21% sand ($60\ \mu\text{m}$ – $2\ \text{mm}$), 53% silt (2 – $60\ \mu\text{m}$) and 26% clay ($<2\ \mu\text{m}$) (Avery and Catt, 1995). The clay fraction is composed mainly of interstratified expanding-layer silicates containing smectite and other layers, with subsidiary mica and kaolinite and small amounts of feldspar, chlorite and crystalline (goethite) or amorphous ferric oxides (Avery and Catt, 1995).

Broadbalk Wilderness samples

The Broadbalk Wilderness site is located in a small area of land (0.2 ha) that had been used as arable land growing wheat continually from 1843 until the early 1880s, after which it was fenced off in 1882. One half was allowed to revert back to natural woodland (wooded area, see Fig. 1 and Table 1) whereas the other half was stubbed every year, i.e. woody shrubs cut out at ground level. This latter area was subdivided into stubbed area, furthest away from the wooded area, and grazed area in 1957 (Fig. 1). The Broadbalk site was heavily chalked during the 18th or early 19th century, resulting in the soil of the wooded part still having a pH of 7.7 in 1985 (Jenkinson *et al.*, 1992) and slightly less alkaline, 7.3 in 1991 (Kinchesh *et al.*, 1995). Two replicate soil samples (denoted soil a and b) were taken from each of the three plots, wooded, stubbed and grazed area, respectively (Fig. 1 and Table 1).

In addition to the soils, the main types of vegetation present on each of the three different plots were sampled (Table 1). A detailed list of the vegetation of these plots was given by Jenkinson (1971) and Kerr *et al.* (1996). Briefly, at the sampled spots, the wooded area was dominated by trees, *Crataegus monogyna* (hawthorn), *Acer pseudoplatanus* (sycamore), *Fraxinus excelsior* (ash) and *Quercus robur* (common oak). In addition, *Hedera helix* (ivy) was the primary ground cover. Grass, mainly *Lolium perenne* (rye grass), was the dominant type of plant on the grazed plot. A small contribution of mixed herbs was also noted. The main plants constituting the stubbed area were mixed herbs, including substantial amounts of grass. However, later on during the growing season *Rubus* sp. (bramble) becomes an important element of this vegetation. The bulk vegetation samples from the grazed and stubbed areas were mixtures of whole herbaceous plants sampled in May. At the same time, leaves from *Rubus* sp. were collected. The vegetation samples from the wooded area were senescent leaves from the main plant species, i.e. *Acer*, *Quercus*,

Table 1. Samples studied from Broadbalk Wilderness

	Samples	TLE*	Residue†	Humic acids
Wooded area	Soil a	✓	✓	✓
	Soil b	✓	✓	—
	<i>Hedera helix</i> leaves	✓	✓	—
	<i>Quercus robur</i> leaves	✓	✓	—
	<i>Fraxinus excelsior</i> leaves	✓	✓	—
	<i>Acer pseudoplatanus</i> leaves	✓	✓	—
Grazed area	Soil a	✓	✓	✓
	Soil b	✓	✓	—
	Grass	✓	✓	—
Stubbed area	Soil a	✓	✓	✓
	Soil b	✓	✓	—
	Mixed herbs	✓	—	—
	<i>Rubus</i> sp. leaves	✓	—	—

*Total lipid extract analyzed using HT-GC and HT-GC-MS.

†Residue after solvent extraction analyzed using Py-GC and Py-GC-MS.

Fraxinus, *Hedera*, as it has been suggested that leaf-fall in this Wilderness constitutes about two-thirds of the carbon input to the top soil (Jenkinson *et al.*, 1992). These samples were taken in November 1995. For further information on the Broadbalk Wilderness site the reader is referred to Jenkinson (1971), Jenkinson *et al.* (1992) and Kerr *et al.* (1996).

EXPERIMENTAL

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. Dried vegetation samples were crushed using the same method but liquid nitrogen was added to facilitate the process.

All soil samples, approximately 10 g, were Soxhlet extracted for 24 h using DCM/acetone (9:1 v/v). A mixture of internal standards, i.e. 2-hexadecanol (IS₁), 10-nonadecanone (IS₂), 5β-pregnan-3α-ol (IS₃), 5β-pregnan-3-one (IS₄), 5α-cholestane (IS₅), hexadecyloctadecanoate (IS₆) was added to the solvent mixture. All vegetation samples were ultrasonically extracted (6×) with DCM/acetone (9:1 v/v) and the same internal standard mixture was added to these samples. Extracts were collected and rotary evaporated. The residues were vacuum dried.

The collected extracts were transferred with DCM/isopropanol (2:1 v/v) and filtered over defatted cotton wool. Aliquots were taken and filtered using a Pasteur pipette packed with activated SiO₂ (2 g) to remove highly polar compounds. Subsequently, the extracts were trimethylsilylated (see "Derivatization"). Samples were dissolved in hexane (ca. 4 µg µl⁻¹) and analyzed using high-temperature gas chromatography (HT-GC) and HT-GC-mass spectrometry. The residues after solvent extraction were analyzed using Curie-point pyrolysis-gas chromatography (Py-GC) and Py-GC-mass spectrometry (Py-GC-MS).

Humic acid extraction

Approximately 15 g of dried extracted soil was weighed into a polypropylene flask, 200 ml Na₄P₂O₇·10 H₂O was added and the air was displaced by N₂. The solution was stirred for 24 h at ambient temperature. The suspension was centrifuged (7000 rpm; 30 min), the supernatant recovered and stored under N₂ in a fridge. This extraction was repeated three to four times until the supernatant was colourless. The final residue was washed (2×) with double distilled H₂O (centrifuged 7000 rpm; 30 min) and subsequently freeze dried. The extracts were combined, acidified with 6 M HCl to pH 1 and left for 24 h at ambient temperature to allow humic acids (HA) to precipitate. The suspension was centrifuged (9000 rpm; 30 min) and the

supernatant (fulvic acids; FA) decanted from the HA. The HA were washed (2×) with double distilled H₂O (centrifuged 9000 rpm; 30 min) and then freeze dried. The HA were analyzed using Py-GC.

Derivatization

Free hydroxyl and carboxylic acid groups were derivatized to their corresponding trimethylsilyl (TMS) ethers and esters, respectively, by adding 30 µl of *N,O*-bis(trimethylsilyl)trifluoroacetamide, containing 1% trimethylchlorosilane, to sample aliquots and heating for 1 h at 70°C.

High-temperature gas chromatography (HT-GC) and high-temperature gas chromatography-mass spectrometry (HT-GC-MS)

HT-GC was performed using a Hewlett-Packard 5890 series II gas chromatograph equipped with a fused-silica capillary column (15 m × 0.32 mm) coated with DB-1 (film thickness 0.1 µm). Derivatized total lipid extracts (1.0 µl) in hexane were injected on-column. The temperature was programmed from 50°C (2 min) to 350°C (10 min) at a rate of 10°C min⁻¹. The detector temperature was 350°C. Hydrogen was used as carrier gas (head pressure 10 psi). The HT-GC-MS analyses were performed using a Carlo Erba 5160 mega series gas chromatograph connected to a Finnigan 4500 mass spectrometer operating at 70 eV scanning the range *m/z* 50–850 in a cycle time of 1.5 s. The interface temperature was 360°C. The capillary column and temperature programme were as described for the HT-GC analyses. Compound identification was based on mass spectral data and retention time comparisons with reference samples.

Curie-point pyrolysis-gas chromatography (Py-GC) and Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

Py-GC analyses were performed using a Horizon Instruments Curie-point pyrolyser attached to a Carlo Erba 4160 GC. The samples were pressed onto a ferromagnetic wire with a Curie temperature of 610°C. The interface temperature of the pyrolysis unit was set at 250°C and the pyrolysis time was 5 s. The GC oven was programmed from 30°C (5 min) to 320°C (10 min) at a rate of 4°C min⁻¹. Separation was achieved using a fused-silica capillary column (25 m × 0.32 mm) coated with CPSil-5 CB (film thickness 0.4 µm). Helium was used as the carrier gas. Py-GC-MS analyses were performed using the same GC-MS configuration as described for the HT-GC-MS analyses. The pyrolysis conditions, capillary column and temperature programme were as described for the Py-GC analyses. Compound identification was based on mass spectral data and retention time comparisons with reference samples.

Table 2. Carbon number ranges and distributions of characteristic compounds detected in the TLEs of the samples from Broadbalk Wilderness

	Soil							Vegetation														
	Wooded area	Grazed area	Stubbed area	Quercus leaves	Acer leaves	Fraxinus leaves	Hedera leaves	Grass	Herbs	Rubus leaves	Wooded area	Grazed area	Stubbed area	Quercus leaves	Acer leaves	Fraxinus leaves	Hedera leaves	Grass	Herbs	Rubus leaves		
Alkanes*	C ₂₁ -C ₃₃ (C ₂₉)	C ₂₃ -C ₃₅ (C ₃₁)	C ₂₃ -C ₃₅ (C ₃₁)	C ₂₃ -C ₃₁ (C ₂₇)	C ₂₃ -C ₃₁ (C ₂₉)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₂₉)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₂₉)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₁ (C ₂₉)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₂₉)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	C ₂₃ -C ₃₃ (C ₃₁)	
Alkanols*	C ₂₀ -C ₃₄ (C ₂₄)	C ₁₈ -C ₃₄ (C ₂₆)	C ₁₈ -C ₃₄ (C ₂₆)	C ₂₂ -C ₃₂ (C ₂₄)	C ₂₀ -C ₃₂ (C ₂₄)	C ₂₂ -C ₃₂ (C ₂₄)	C ₂₀ -C ₃₂ (C ₃₀)	C ₂₂ -C ₃₂ (C ₂₆)	C ₂₂ -C ₃₂ (C ₂₆)	C ₂₂ -C ₃₂ (C ₂₆)	C ₂₀ -C ₃₂ (C ₃₀)	C ₂₂ -C ₃₂ (C ₃₀)	C ₂₂ -C ₃₂ (C ₂₆)	C ₂₂ -C ₃₀ (C ₂₆)	C ₂₂ -C ₃₀ (C ₂₆)	C ₂₂ -C ₃₀ (C ₂₆)	C ₂₀ -C ₃₂ (C ₃₀)	C ₂₂ -C ₃₀ (C ₂₆)	C ₂₂ -C ₃₀ (C ₂₆)	C ₂₂ -C ₃₀ (C ₂₆)	C ₂₂ -C ₃₀ (C ₂₆)	
Wax esters*	C ₃₈ -C ₅₂ (C ₄₄)	C ₄₂ -C ₅₀ (C ₄₆)	C ₄₀ -C ₅₀ (C ₄₆)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₀)	C ₄₂ -C ₅₀ (C ₄₆)	C ₃₈ -C ₅₀ (C ₄₆)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₆)	C ₃₈ -C ₅₀ (C ₄₀)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₆)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₀ (C ₄₄)	C ₃₈ -C ₅₂ (C ₄₄)	
Aldehydes	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₄ , C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈	C ₂₆ , C ₂₈	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	C ₂₆ , C ₂₈ , C ₃₀	
Fatty acids	C ₁₆ , C ₁₈ , C ₂₆ , C ₂₈ , C ₃₀	C ₁₆ -C ₃₄	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	C ₁₆ -C ₂₈	
MAG†	C ₁₈ -C ₂₂	C ₁₆ -C ₂₈	C ₁₆ -C ₂₆	C ₁₆ -C ₂₆	C ₁₈	C ₁₈	C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	C ₁₆ , C ₁₈	
DAG†	C ₄₈ , C ₅₀ , C ₅₂ , C ₅₄	C ₃₂ , C ₃₄ , C ₃₆	C ₃₂ , C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	C ₃₄ , C ₃₆	
TAG†	C ₄₈ , C ₅₀ , C ₅₂ , C ₅₄	C ₄₈ , C ₅₀ , C ₅₂ , C ₅₄	C ₄₈ , C ₅₀ , C ₅₁ , C ₅₂ , C ₅₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	C ₃₀ , C ₃₂ , C ₃₄	
Sterols‡	(1), (4)	(1), (4)	(1), (4)	(4)	(1), (4), (7)	(4)	(4)	(1), (4)	(1), (4)	(1), (4)	(4)	(1), (4)	(1), (4)	(1), (4)	(1), (4)	(1), (4)	(1), (4)	(1), (4)	(1), (4)	(1), (4)	(4)	
Triterpenols†	—	—	—	(2), (3), (5)	(3), (6), (9-12)	—	(5), (8)	(3)	(3)	(3)	—	(5), (8)	(3)	(3)	(3)	(3)	(5), (8)	(3)	(3)	(3)	—	
Phytol esters†	—	—	—	C _{16:3} , C _{16:0} , C _{18:3} , C _{18:0} , C ₂₀ , C ₂₂	C _{18:3}	C _{18:3} , C _{18:0} , C ₂₀ , C ₂₂	C _{18:3} , C _{18:0} , C ₂₀ , C ₂₂	C ₃₀ , C ₂₂	C ₈ , C ₁₂ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂ , C ₂₄	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C _{18:3} , C _{18:0} , C ₂₀ , C ₂₂	C ₃₀ , C ₂₂	C ₈ , C ₁₂ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂ , C ₂₄	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	C ₈ , C ₁₄ , C ₁₆ , C ₁₈ , C ₂₀ , C ₂₂	
Steryl esters§	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Triterpenyl esters	—	—	—	Triterpenyl acids (TA1), phytol, tocopherols	Phytol	Triterpenyl acids (TA1), phytol, tocopherols	Triterpenyl acids (TA1), phytol, tocopherols	Triterpenyl acids (TA2), phytol, tocopherols	Phytol	Phytol, tocopherols	Triterpenyl acids (TA1), phytol, tocopherols	Triterpenyl acids (TA2), phytol, tocopherols	Phytol	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols	Phytol, tocopherols
Additional compounds	Triterpenyl acids (TA1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

*Carbon numbers in parentheses indicate the most abundant homologue.
 †The carbon numbers indicated refer to the total number of fatty acyl carbons.
 ‡Numbers in parentheses refer to the steroid and triterpenoid compounds listed in Table 3.
 § + = present; + + = abundant.

RESULTS

Influences of different vegetation inputs on the composition of soluble and insoluble soil organic matter fractions were studied in order to obtain insights into the effects of changes in the vegetation cover. Total lipid extracts were analyzed using HT-GC and HT-GC-MS, whereas the solvent insoluble fractions were investigated using Py-GC and Py-GC-MS to provide "fingerprints" for the vegetation and soils.

Soil extracts from the Broadbalk Wilderness

The composition of the total lipid extracts (TLEs) of the duplicate samples (denoted soil a and b) from each of the three plots were very similar. In contrast, the compositions of the extracts from the different plots revealed substantial differences. The compositions of the most characteristic lipids, i.e. compound type and carbon number distributions, present in the TLEs of the soils and vegetation studied are summarized in Table 2. Mass spectral characteristics of steroidal and triterpenoid constituents mentioned in the text are listed in Table 3.

The extracts of the wooded area were dominated by homologous series of *n*-alkanols and *n*-alkanes, with the former being the more abundant [Fig. 2(a)]. In addition, abundant wax esters were present, eluting later in the chromatograms. The alkanols ranged from C₂₀ to C₃₄, maximizing at C₂₄, with their distribution profiles showing a strong even over odd predominance. The alkanes, which showed a strong odd over even predominance, ranged from C₂₁ to C₃₃ (maximizing at C₂₉). The wax esters ranged from C₃₈ to C₅₂ with a maximum at C₄₄. The main alkanol moieties of the wax esters were saturated C₂₂ and C₂₄. Other characteristic compounds, present in relatively low concentrations (not all annotated), included saturated aldehydes, fatty acids, 1-monoacylglycerols (MAG), 24-ethylcholest-5,22-diene-3 β -ol (1), 24-ethylcholest-5-en-3 β -ol (4), two C₃₀ triterpenyl acids (TA1), triacylglycerols (TAG; containing 48, 50, 52 and 54 acyl carbons) and a homologous series of unknown compounds, the mass spectra of which only showed two character-

istic fragment ions of *m/z* 177 and 192. The principal fatty acyl moieties present in the TAGs were C₁₆ (C_{16:0} and C_{16:1}) and/or C₁₈ (C_{18:1} and C_{18:2}).

In sharp contrast, the TLEs of the grazed area were dominated by *n*-hexacosanol [Fig. 2(b)] while other *n*-alkanols, unsaturated TAGs, *n*-alkanes and wax esters were present in relatively low concentrations. The distribution of *n*-alkanols showed an even over odd predominance and carbon numbers ranged from C₁₈ to C₃₄ with a maximum at C₂₆. It is interesting to note that the C₂₂ homologue is more abundant than the C₂₄ homologue. The alkanes ranged from C₂₃ to C₃₅ (maximum at C₃₁), whereas the wax esters ranged from C₄₂ to C₅₀ with a maximum at C₄₄. The main *n*-alkanol moiety of the wax esters was *n*-hexacosanol. The TAGs detected were similar to those identified in the wooded area TLEs. However, the distribution was different, showing relatively higher concentrations of TAGs with C_{16:1} fatty acyl moieties (containing 48 and 50 acyl carbons). Other compounds present (not all annotated) included saturated aldehydes, fatty acids, 24-ethylcholest-5,22-diene-3 β -ol (1), 24-ethylcholest-5-en-3 β -ol (4), MAGs, unsaturated diacylglycerols (DAG), triterpenyl fatty acyl esters (β -amyirin) and steryl fatty acyl esters (C₂₉ Δ^5 sterol).

The total lipid extracts of the stubbed area were dominated by a homologous series of *n*-alkanols and substantial amounts of unsaturated TAGs [Fig. 2(c)]. Wax esters, 24-ethylcholest-5-en-3 β -ol (4) and a homologous series of *n*-alkanes also contributed to these extracts. The *n*-alkanols and *n*-alkanes showed the same ranges and distribution patterns as in the extract of the grazed plot. However, the C₂₆ alkanol was relatively less abundant in the TLEs when compared with those of the grazed area. The wax esters ranged from C₄₀ to C₅₀ with a maximum at C₄₆. As with the extracts of the grazed area, the wax esters with a C₂₆ alkanol moiety dominated. Although the TAGs detected were similar to those observed in the wooded and grazed area TLEs their distribution pattern differed, showing relative higher amounts of TAGs with C_{18:1} and

Table 3. Mass spectral characteristics of steroidal and triterpenoid constituents (analyzed as TMS derivatives)

Compound	Characteristic fragment ions (<i>m/z</i>)	M ⁺
(1) 24-ethylcholest-5,22-dien-3 β -ol	83, 129, 255, 351, 379, 394, 469	484
(2) Taraxerol	121, 135, 189, 204, 269, 284, 359, 374, 393, 408, 483	498
(3) β -Amyrin	73, 189, 190, 203, 218, 279, 408	498
(4) 24-Ethylcholest-5-en-3 β -ol	129, 255, 275, 375, 381, 396, 471	486
(5) Lupeol	73, 189, 190, 203, 369, 393, 408	498
(6) β -Amyrin acetate	95, 135, 189, 203, 218, 408, 453	468
(7) C _{29:1} sterol	55, 147, 213, 229, 255, 381, 396, 471	486
(8) Unknown triterpenol	73, 189, 203, 263, 320, 497, 599	614
(9) 24-Methylenecycloartenol	95, 135, 175, 300, 353, 379, 407, 422	512
(10) 24-Methylenecycloartenol acetate	55, 95, 175, 203, 300, 379, 407, 422, 467	482
(11) Unknown triterpenol	73, 175, 203, 227, 297, 391, 409, 424	500
(12) Unknown triterpenol	73, 143, 203, 297, 325, 359, 363, 391, 406, 453, 481	496
C ₃₀ Triterpenyl acid (TA1)	73, 133, 189, 203, 279, 320, 483, 585	600
C ₃₁ Triterpenyl acid (TA2)	73, 133, 189, 263, 320, 389, 497, 524, 599	614

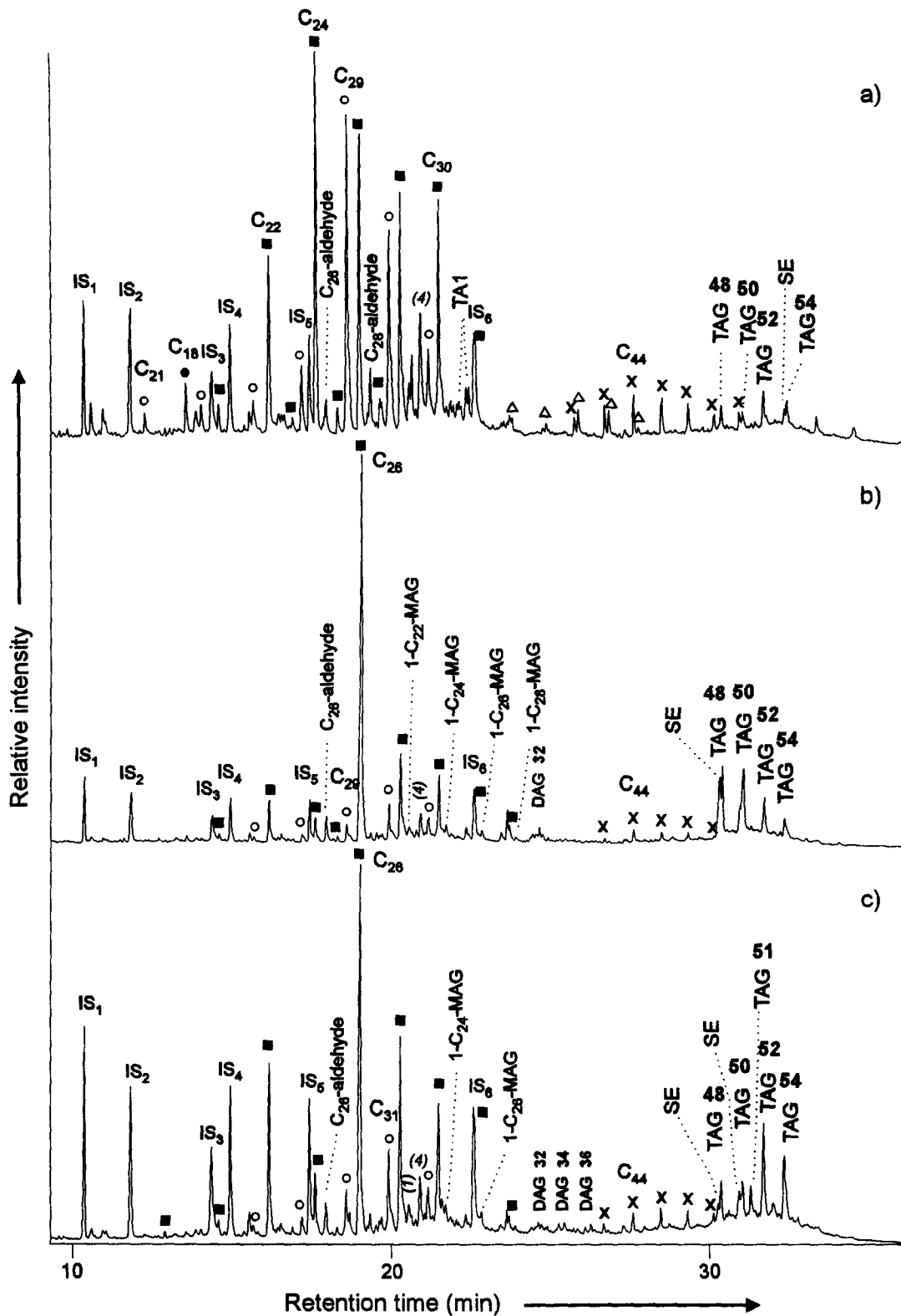


Fig. 2. Partial gas chromatograms of the total lipid extracts from: (a) wooded area soil b; (b) grazed area soil a; and (c) stubbed area soil b. Key: IS_x = internal standards; ● = fatty acids (FATMS); ○ = n-alkanes; ■ = n-alkanols; x = wax esters; MAG = monoacylglycerols; DAG = diacylglycerols; TAG = triacylglycerols; TA1 = C₃₀ triterpenyl acids; △ = compounds with mass fragment ions 177, 192; C_{xx} refers to total carbon numbers; numbers in bold indicate total acyl carbons; numbers in parentheses and italics refer to sterols and triterpenols listed in Tables 2 and 3. For additional information and source of the material see text and Table 1.

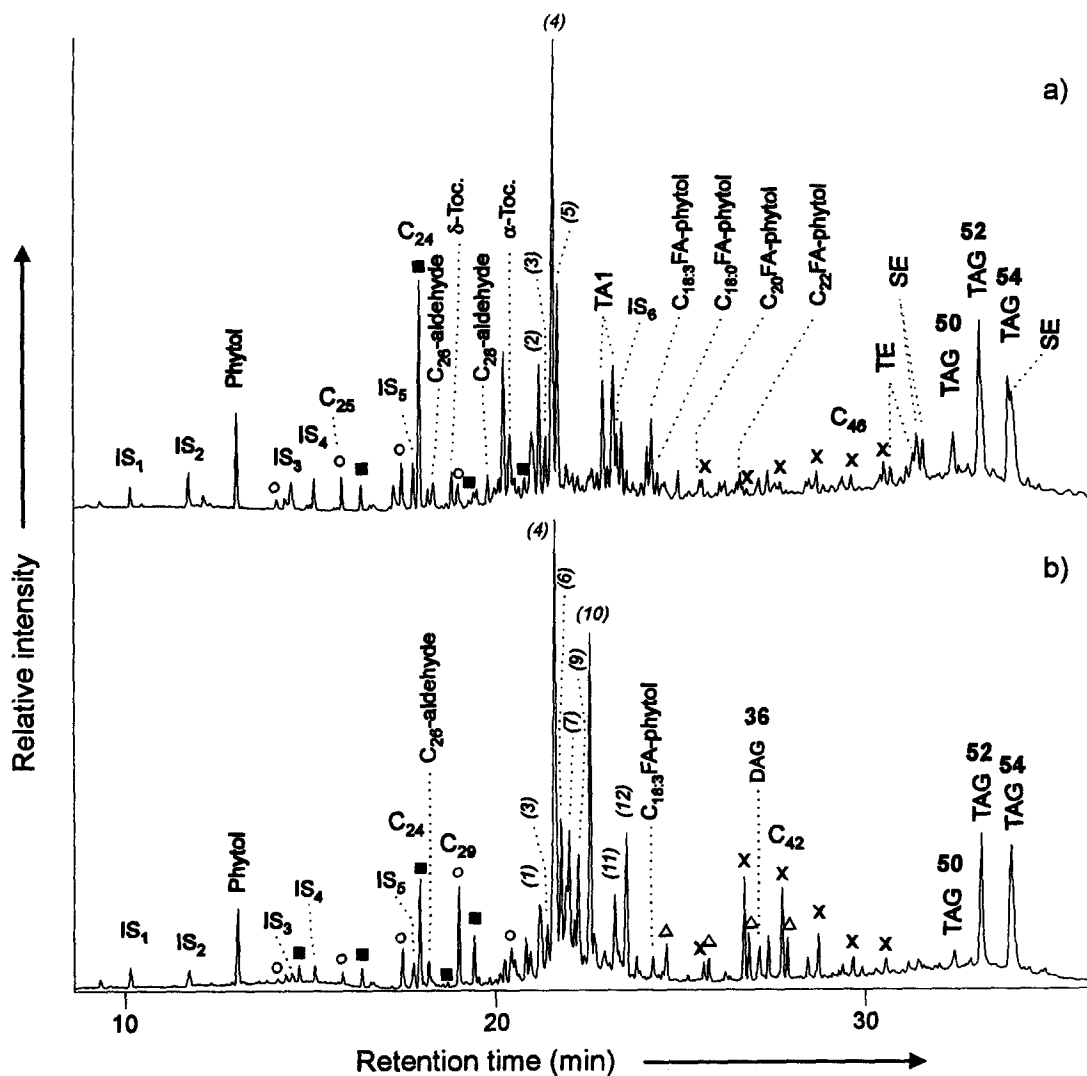


Fig. 3. Partial gas chromatograms of the total lipid extracts of leaves of (a) *Quercus*, (b) *Acer*. Key: IS_x = internal standards; ○ = *n*-alkanes; ■ = *n*-alkanols; × = wax esters; DAG = diacylglycerols; TAG = triacylglycerols; SE = steryl esters; TE = triterpenyl esters; TA1 = C₃₀ triterpenyl acids; C₁₆FA-phytol = C₁₆ fatty acyl phytol ester; Toc. = tocopherol; △ = compounds with mass fragment ions 177, 192; C_{xx} refers to total carbon numbers; numbers in bold indicate total acyl carbons; numbers in parentheses and italics refer to sterols and triterpenols listed in Tables 2 and 3. For additional information and source of the material see text and Table 1.

C_{18:2} fatty acyl moieties (containing 52 and 54 acyl carbons). Interestingly, one of the TAG peaks corresponded to a component(s) bearing 51 acyl carbons with an abundant C_{17:0} acyl moiety. Other (minor) compounds identified included saturated aldehydes, MAGs and unsaturated DAGs.

Vegetation extracts

Wooded area. The total lipid extract of senescent *Quercus* leaves was dominated by 24-ethylcholest-5-en-3 β -ol [(4), Fig. 3(a)]. In addition, *n*-tetracosanol, triterpenoids [including taraxerol (2), β -amyrin (3) and lupeol (5)], C₃₀ triterpenyl acids (TA1), as well as two peaks of unsaturated TAGs were present in reasonable abundance. Homologous series of *n*-

alkanes and *n*-alkanols were present but only in low abundance. The alkanols ranged from C₂₂ to C₃₂ (maximum at C₂₄) with an even over odd predominance, while the alkanes ranged from C₂₃ to C₃₁ (maximum at C₂₅ and C₂₇) with the expected odd over even predominance. Wax esters ranging from C₃₈ to C₅₀ (maximum at C₄₄) were only present in low concentrations. Their main alkanol moiety was C₂₄. In contrast to the soil extracts, only three TAG envelopes were observed containing 50, 52 and 54 acyl carbons. The two dominant TAG envelopes (52 and 54 acyl carbons) contained C_{16:0} and C_{18:1} and/or C_{18:2} fatty acyl moieties. Other components (not all annotated) included phytol, saturated aldehydes, tocopherols, unsaturated

DAGs, phytol-, steryl-(C₂₉ sterol) and triterpenyl fatty acyl esters. The triterpenyl acids detected were the same as those identified in the soil extracts of the wooded area. The principal compounds identified and their distribution patterns are in concordance with previous studies of *Quercus* leaf lipids (Prasad and Gülz, 1990a; Prasad *et al.*, 1990).

The TLE of the senescent *Acer* leaves [Fig. 3(b)] was dominated by 24-ethylcholest-5-en-3 β -ol [(4), Fig. 3(b)] and 24-methylenecycloartenol acetate (10). In addition, phytol, *n*-alkanols, *n*-alkanes, C₂₉ sterols (1, 7) and triterpenoids [including β -amyrin (3), β -amyrin acetate (6), 24-methylenecycloartenol (9) and two unknown triterpenoids (11, 12)], wax esters as well as two peaks of unsaturated TAGs

were present in reasonable abundance. The alkanols ranged from C₂₀ to C₃₂ (maximum at C₂₄) with an even over odd predominance, while the alkanes ranged from C₂₃ to C₃₁ (maximum at C₂₉) with an odd over even predominance. The wax esters, which ranged from C₃₈ to C₅₀ showing no obvious predominance of specific alkanol moieties, were dominated by the C₄₀ and C₄₂ homologues. Three TAG envelopes were detected (containing 50, 52 and 54 acyl carbons) with the two dominant TAG envelopes (containing 52 and 54 acyl carbons) bearing C_{16:0} and C_{18:1} and/or C_{18:2} fatty acyl moieties. Other components detected (not all annotated) included saturated aldehydes, unsaturated DAGs, phytol esters and a homologous series of unknown

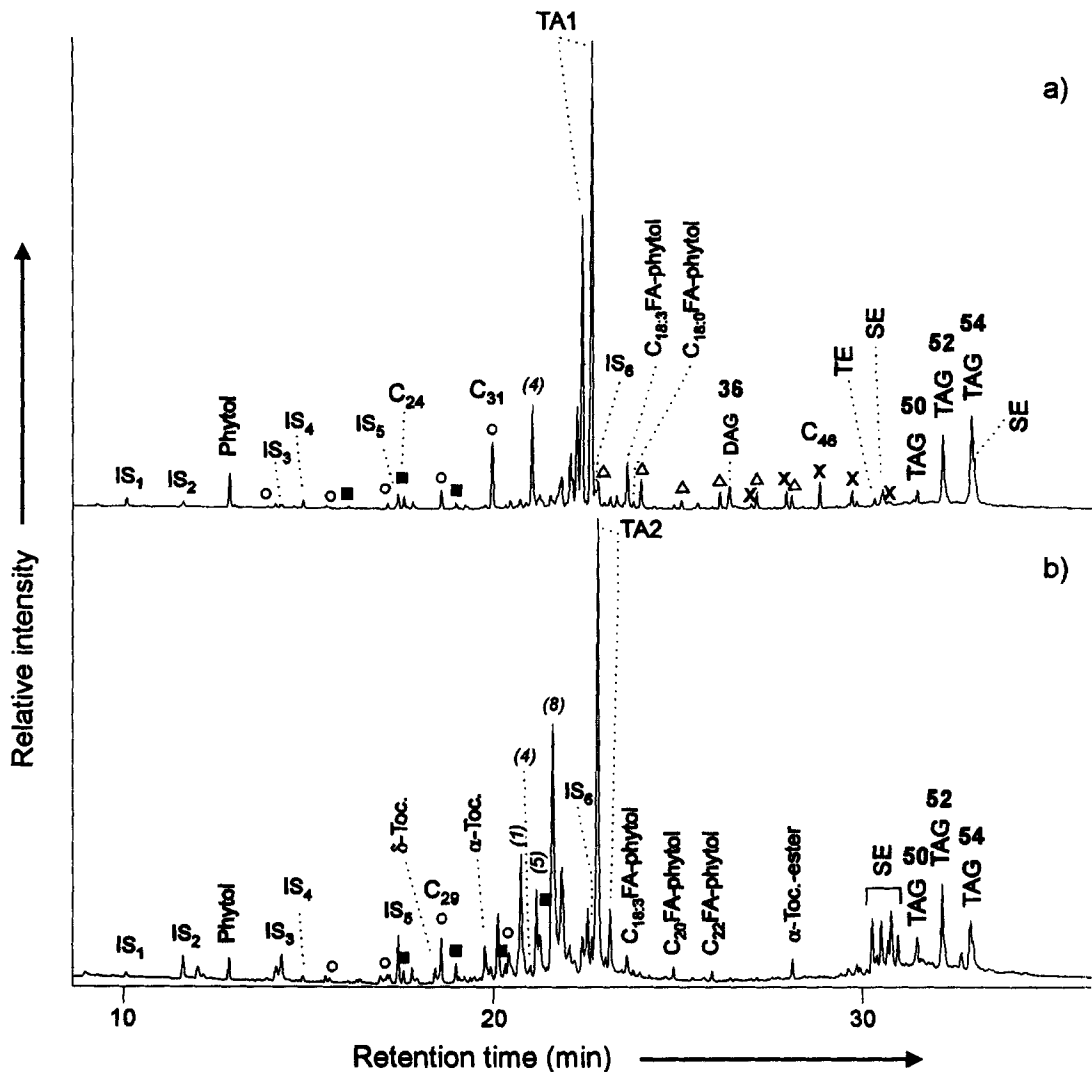


Fig. 4. Partial gas chromatograms of the total lipid extracts of leaves of (a) *Fraxinus*, (b) *Hedera*. Key: IS_x = internal standards; ○ = *n*-alkanes; ■ = *n*-alkanols; × = wax esters; DAG = diacylglycerols; TAG = triacylglycerols; SE = steryl esters; TE = triterpenyl esters; TA1 = C₃₀ triterpenyl acids; TA2 = C₃₁ triterpenyl acids; C₁₆FA-phytol = C₁₆ fatty acyl phytol ester; Toc. = tocopherol; Δ = compounds with mass fragment ions 177, 192; C_{xx} refers to total carbon numbers; numbers in bold indicate total acyl carbons; numbers in parentheses and italics refer to sterols and triterpenoids listed in Tables 2 and 3. For additional information and source of the material see text and Table 1.

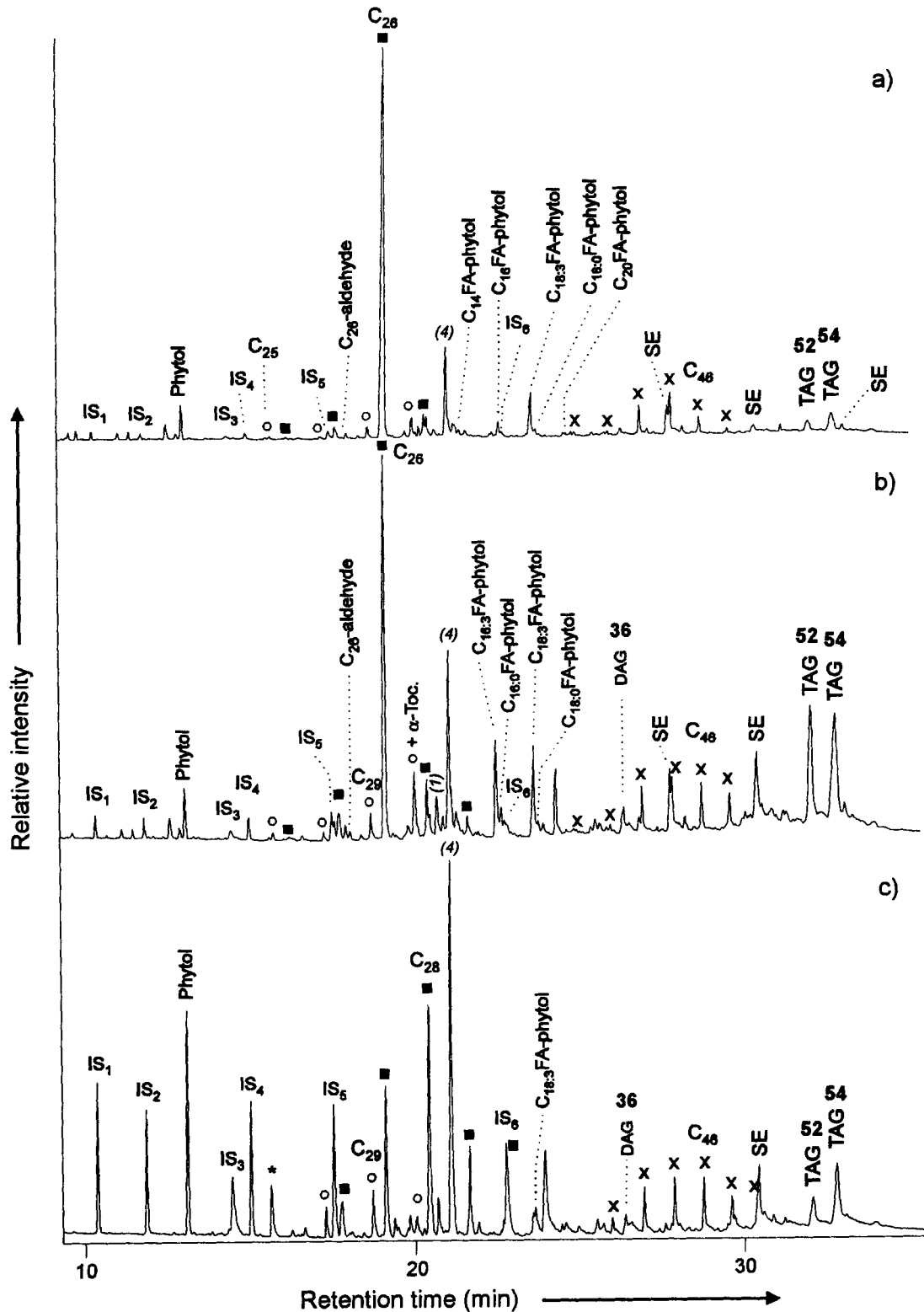


Fig. 5. Partial gas chromatograms of the total lipid extracts of (a) grass, (b) mixed herbs and (c) *Rubus* leaves. Key: IS_x = internal standards; \circ = *n*-alkanes; \blacksquare = *n*-alkanols; \times = wax esters; DAG = diacylglycerols; TAG = triacylglycerols; SE = steryl esters; C_{16} FA-phytol = C_{16} fatty acyl phytol ester; Toc. = tocopherol; C_{xx} refers to total carbon numbers; numbers in bold indicate total acyl carbons; numbers in parentheses and italics refer to sterols and triterpenols listed in Tables 2 and 3. For additional information and source of the material see text and Table 1.

compounds (m/z 177, 192) identical to those observed in the extract of the soil from the wooded area. The main components identified and their distribution patterns are in concordance with previous results on *Acer* leaf lipids (Prasad and Gülz, 1990b).

The total lipid extract of the senescent *Fraxinus* leaves [Fig. 4(a)] was dominated by the same two C_{30} triterpenyl acids (TA1) observed in the *Quercus* leaf extract. Other relatively abundant components included 24-ethylcholest-5-en-3 β -ol (4) and two peaks of unsaturated TAGs. *n*-Alkanols, *n*-alkanes and wax esters were present in low concentrations. The alkanols ranged from C_{22} to C_{26} (maximum at C_{24}), while the alkanes ranged from C_{23} to C_{33} (maximum at C_{31}) with the usual odd over even predominance. The wax esters ranged from C_{42} to C_{50} (maximum at C_{46}) with those based on C_{22} and C_{24} alkanol moieties predominating. Three TAG envelopes were detected (containing 50, 52 and 54 acyl carbons) with the two dominant TAG envelopes (containing 52 and 54 acyl carbons) comprising components bearing $C_{16:0}$ and $C_{18:1}$ and/or $C_{18:2}$ fatty acyl moieties. Other characteristic components (not all annotated) included phytol, unsaturated DAGs, phytol esters and a homologous series of unknown compounds (m/z 177, 192) identical to those found in the soil extract of the wooded area and the *Acer* TLE.

The *Hedera* total lipid leaf extract [Fig. 4(b)] was dominated by a C_{31} triterpenoid acid (TA2). In addition various other triterpenoids (5,8), 24-ethylcholest-5,22-diene-3 β -ol (1) and two peaks of unsaturated TAGs were relatively abundant. *n*-Alkanes and *n*-alkanols were present at low relative abundance. The *n*-alkanols showed an even over odd predominance and ranged in chain length from C_{20} to C_{32} (maximum at C_{30}). The homologous series of *n*-alkanes ranged from C_{23} to C_{33} with the distribution maximizing at C_{29} . The two most dominant TAG peaks (containing 52 and 54 acyl carbons) corresponded to TAGs bearing mainly mono- and diunsaturated C_{18} fatty acyl moieties. Other components (not all annotated) identified included phytol, tocopherols, phytol fatty acyl esters, one α -tocopheryl ester and steryl (mainly C_{29} $\Delta^{5,22}$ sterol) esters.

Grazed area. The total lipid extract of the grass sample was, as expected (e.g. Tulloch, 1976; Walton, 1990), dominated by hexacosanol [Fig. 5(a)]. In addition, phytol, 24-ethylcholest-5-en-3 β -ol (4), a phytol $C_{18:3}$ fatty acyl ester and wax esters were relatively abundant. The wax esters ranged from C_{38} to C_{48} (maximum at C_{44}) all of which had the C_{26} alkanol as the dominant *n*-alkanol moiety. The C_{42} , C_{44} and C_{46} components were significantly more abundant than the other members of the series. In addition to hexacosanol, other alkanols were detected in the range C_{22} – C_{28} . *n*-Alkanes were

present in relatively low abundance and ranged from C_{23} to C_{33} (maximum at C_{31}). Other components identified (not all annotated) included hexacosanol, MAGs, 24-ethylcholest-5,22-diene-3 β -ol (1), phytol fatty acyl esters and two unsaturated TAG envelopes (containing 52 and 54 acyl carbons). The dominant fatty acyl moieties of these latter components were $C_{16:0}$ and/or $C_{18:1}$, $C_{18:2}$, $C_{18:3}$.

Stubbed area. The total lipid extract of the mixed herbs from the stubbed plot, which contained substantial amounts of grass, was dominated by hexacosanol [Fig. 5(b)]. Phytol, 24-ethylcholest-5-en-3 β -ol (4), wax esters, two unsaturated fatty acyl phytol esters and two peaks of unsaturated TAGs were relatively abundant. *n*-Alkanes and *n*-alkanols were also present and showed the same carbon number ranges and distribution patterns as those seen in the extract of the grass, although the C_{26} alkanol was slightly less dominant in the sample from the mixed herbs. The wax esters ranged from C_{38} to C_{50} (maximum at C_{44}) with the C_{42} , C_{44} and C_{46} members predominating. As with the wax esters identified in the grass extract, those in the mixed herbs all contained largely the C_{26} *n*-alkanol moiety. The TAGs (containing 52 and 54 acyl carbons) were analogous to those observed in the grass total lipid extract. Other components (not all annotated) included hexacosanol, MAGs, 24-ethylcholest-5,22-diene-3 β -ol (1), β -amyirin, phytol esters, unsaturated DAGs and steryl esters (containing mainly C_{29} Δ^5 sterol).

The total lipid extract of the *Rubus* leaves was dominated by phytol, a homologous series of *n*-alkanols, 24-ethylcholest-5-en-3 β -ol (4) and a mixture of wax esters [Fig. 5(c)]. In addition, *n*-alkanes, TAGs, steryl esters (C_{29} Δ^5 sterol) and one unidentified component were present in reasonable abundance. The *n*-alkanols ranged from C_{22} to C_{34} with a maximum at C_{28} . The *n*-alkanes ranged from C_{25} to C_{33} (maximum at C_{29}). As with all other extracts, the alkanes showed a strong odd over even predominance, whereas the alkanols were dominated by even carbon number homologues. The wax esters ranged from C_{38} to C_{52} with the C_{44} , C_{46} and C_{48} homologues predominating. There was no obvious predominance of any alkanol moiety amongst the wax esters. Only two distinct envelopes of TAGs (containing 52 and 54 acyl carbons) were detected containing $C_{16:0}$ and/or $C_{18:2}$, $C_{18:3}$ fatty acyl moieties. Other components detected (not all annotated) included MAGs, unsaturated DAGs and triterpenyl fatty acyl esters.

Insoluble organic matter in the soils and vegetation from Broadbalk Wilderness

The three pyrolysates [cf. Fig. 6(a)] of the residues of the senescent tree leaves were all dominated by methoxyphenols [both 2-methoxy- and 2,6-dimethoxyphenols, (G) and (S), respectively] and

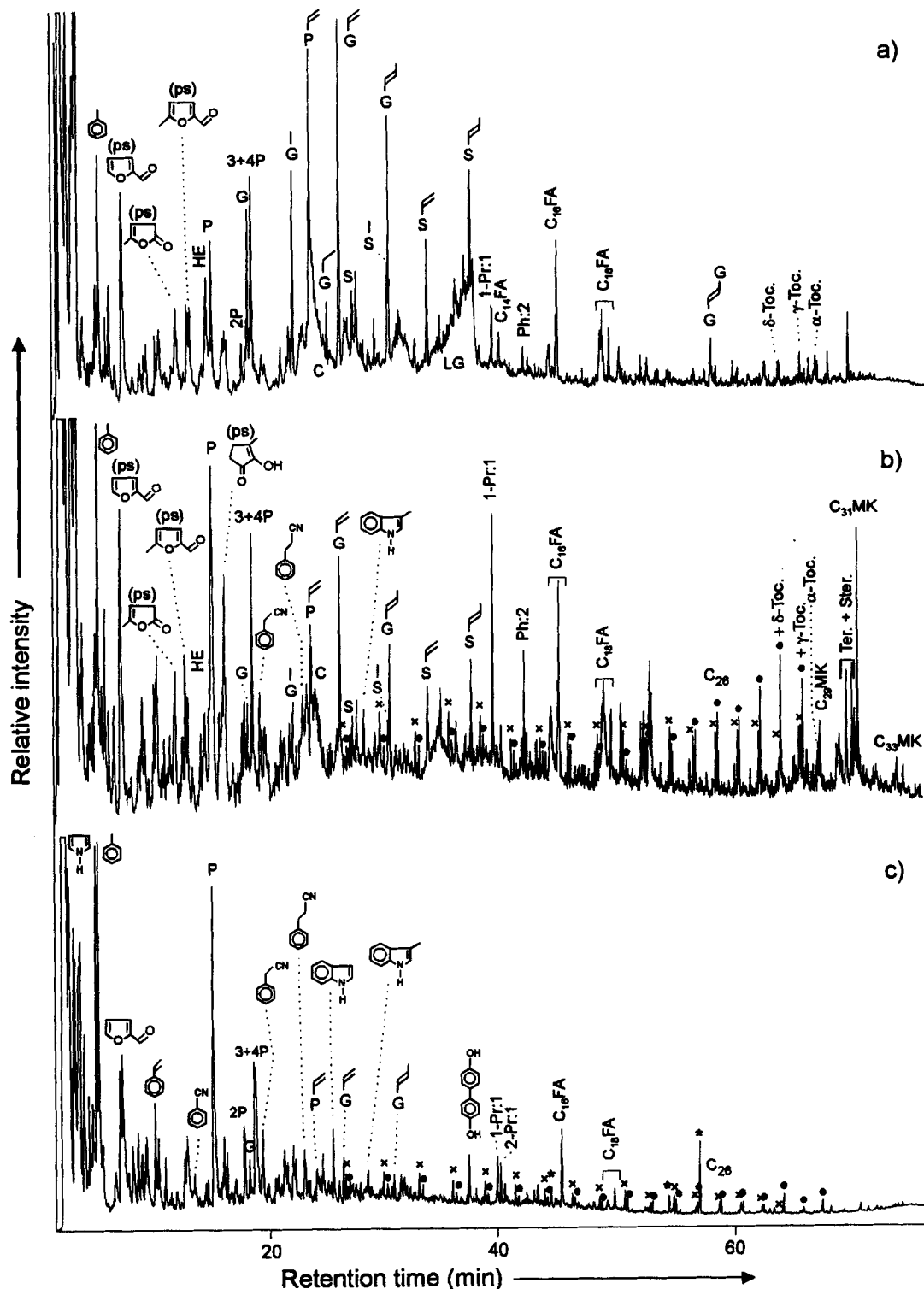


Fig. 6. Gas chromatograms of the pyrolysates (Curie-temperature 610°C) of the residues of (a) *Quercus* leaves, (b) *Hedera* leaves and (c) soil b from wooded area. Key: PS = polysaccharide pyrolysis product; P = phenol; 2P = 2-methylphenol; 3 + 4P = co-eluting 3- and 4-methylphenol; C = 1,2-benzenediol (catechol); C₁₆FA = hexadecanoic acid; 1-Pr:1 = prist-1-ene; 2-Pr:1 = prist-2-ene; Ph:2 = phytadiene; HE = hemicellulose marker; LG = levoglucosan; Toc. = tocopherol; MK = methylketone; Ter. = terpenoids; Ster. = steroids; C₂₆ indicates hexacos-1-ene and hexacosane; * = contaminants. Side chains (attached at positions 4) of phenol-(P), 2-methoxyphenol-(guaiacyl; G) and 2,6-dimethoxyphenol-(syringyl-; S) components are indicated. For additional information and source of the material see text and Table 1.

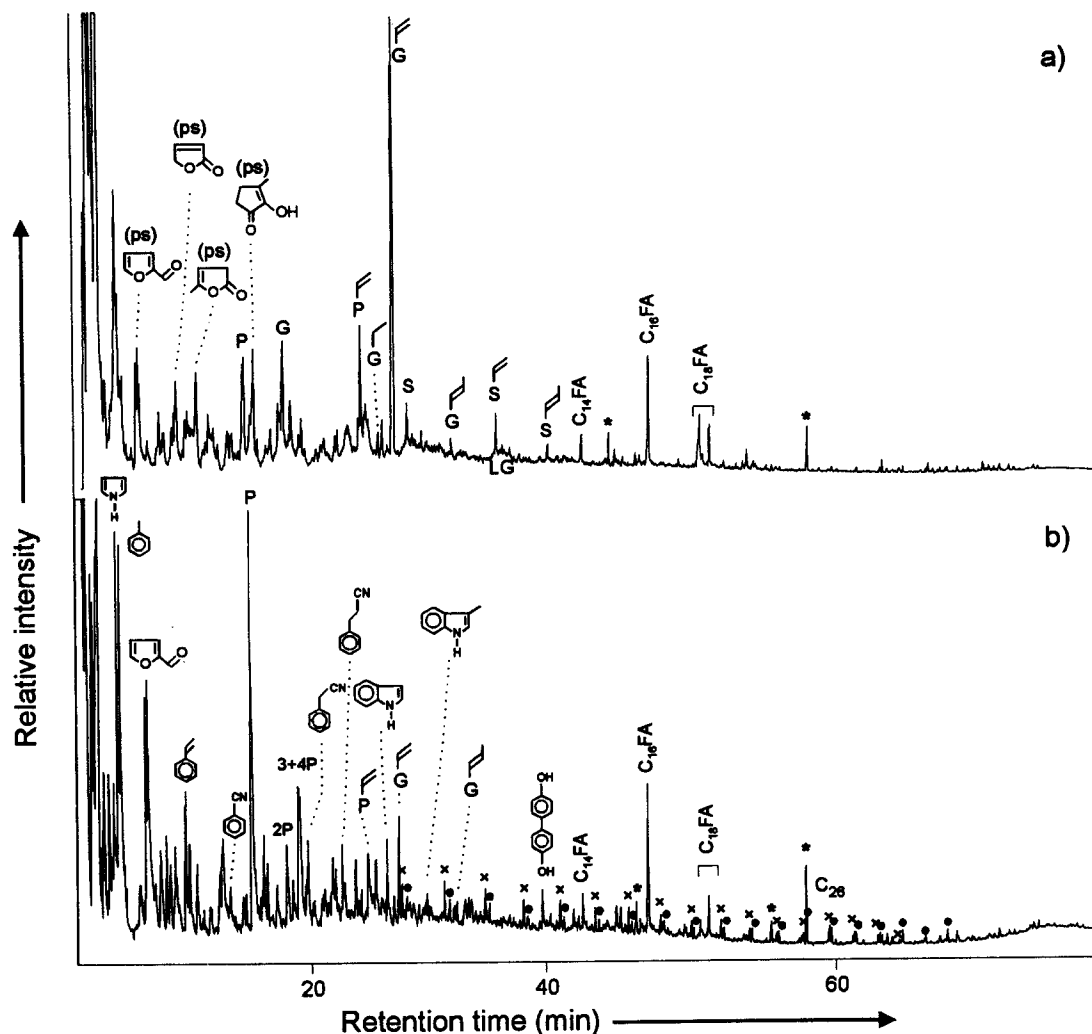


Fig. 7. Gas chromatograms of the pyrolysates (Curie-temperature 610°C) of the residues of (a) grass and (b) soil b from grazed area. Key: PS = polysaccharide pyrolysis product; P = phenol; 2P = 2-methylphenol; 3 + 4P = co-eluting 3- and 4-methylphenol; C₁₆FA = hexadecanoic acid; LG = levoglucosan; C₂₆ indicates hexacos-1-ene and hexacosane; * = contaminants. Side chains (attached at positions 4) of phenol-(P), 2-methoxyphenol-(guaiacyl; G) and 2,6-dimethoxyphenol-(syringyl-; S) components are indicated. For additional information and source of the material see text and Table 1.

polysaccharide pyrolysis products, such as 2-furaldehyde, 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one (HE) and levoglucosan (LG). In addition, phenols and, in particular, 4-ethenylphenol were relatively abundant in all three pyrolysates. Other components that were detected in all three leaf samples (albeit in varying abundance) included fatty acids (FA), N-containing compounds, prist-1-ene, phytadienes and tocopherols. Although, the pyrolysate of the solvent insoluble residue of the senescent *Hedera* leaves contained abundant polysaccharide pyrolysis products and methoxyphenols, the pyrolysate was dominated by toluene, phenol, prist-1-ene and hentriacontan-2-one and homologous series of *n*-alkanes and *n*-alk-1-enes, ranging from C₇ to C₃₂ [Fig. 6(b)]. Other distinct components included fatty

acids, phytadienes, tocopherols and N-containing pyrolysis products, such as pyrrole, alkylphenyl nitriles, indole and one methylindole. The pyrolysate of the extracted grass residue was dominated by 4-ethenyl-2-methoxyphenol and 4-ethenylphenol [Fig. 7(a)]. Other significant components included polysaccharide pyrolysis products, 2-methoxy- and 2,6-dimethoxyphenols and the same fatty acids as were observed in the pyrolysates of the senescent tree leaves.

In sharp contrast to the plant tissues, the pyrolysates of the whole soil residues of the three different plots were largely analogous, being dominated by pyrrole, toluene, phenol and C₁-alkylated phenols [cf. Figs 6(c) and 7(b)]. Other distinct pyrolysis products were 2-furaldehyde, alkylphenyl nitriles, indole

and a methylindole, in addition to homologous series of alkanes and alk-1-enes ranging from C₇ to C₃₂. Methoxyphenols, which were abundant components of the pyrolysates of the vegetation [Figs 6(a) and (b), and 7(a)], were detected in only relatively low abundance in the soils. The similarity seen in the pyrolysates of the whole soil residues of the different plots was also mirrored in the purified humic acids. While the pyrolysates of the humic acids were also dominated by phenols, 2-methoxy- and 2,6-dimethoxyphenols were slightly more abundant than in the soils. The distribution patterns of the methoxyphenols in the humic acid pyrolysates differed markedly from those obtained from the residues of the vegetation covers.

DISCUSSION

Soil organic matter at the Broadbalk Wilderness site

The compositional data obtained from the replicate analyses of the total lipid extracts and insoluble fractions of the soils (soil a and b) of each of the three plots are more or less identical, indicating that a degree of homogeneity in the distribution of soil organic matter exists over the individual plots. The characteristic distributions of *n*-alkanes, *n*-alkanols and wax esters observed in the soil extracts, is consistent with higher plants as the main contributors to the soil lipid fraction. Microbial biomass which is considered to make an important contribution to soil organic matter, being an active but small component (Jenkinson *et al.*, 1992), seems to make only a limited contribution to the soil lipids. This conclusion is based on the fact that no abundant specific bacterial or fungal biomarkers were detected. In addition, there is no obvious evidence of a lipid input from soil fauna to the soil lipid profiles.

With respect to the LMW compounds, substantial differences are noted between the TLEs of the three plots (Fig. 2). Comparison of the TLEs of the main vegetation (Figs 3–5) with those of the soils (Fig. 2) shows that compounds derived from different types of the vegetation (grass vs. woodland) can be ascribed as major contributors to the soluble fractions for each of the plots. In the case of the woody area, it has been suggested that leaf-fall, i.e. senescent autumn leaves, constitute about two-thirds of the carbon input to the top soil (Jenkinson *et al.*, 1992). Hence, it can be readily assumed that lipids from leaves such as those from *Quercus*, *Acer*, *Fraxinus* and *Hedera* are important input sources. The data obtained from the soil TLEs, in particular, the *n*-alkanol and *n*-alkane distributions [Fig. 2(a) vs. Figs 3 and 4] clearly reflect this mixed input. For example, the C₂₄ alkanol appears to derive from *Quercus*, *Acer* and *Fraxinus* leaves [Fig. 2(a)], whereas the presence of the longer chain homologues (C₂₆, C₂₈, C₃₀) is consistent with a con-

tribution from the *Hedera* leaves [Fig. 3(a)]. Furthermore, the dominance of the C₂₉ and C₃₁ alkanes appears to indicate that lipid contributions from *Fraxinus*, *Acer* and *Hedera* predominate over that from *Quercus*. Significantly, the two C₃₀ triterpenyl acids which occur only in the leaf extracts of *Quercus* and *Fraxinus* persist in the soil, and hence represent more specific indicators of inputs. However, since triterpenyl acids are widespread in the plant world (e.g. Walton, 1990; Mahato *et al.*, 1992) and the fact that distribution patterns of alkanes and alkanols similar to those in the soil from the wooded area are reported from other leaves, a contribution from leaf lipids from other species, or species that previously grew at this site, cannot be discounted. However, as *n*-octacosanol is not the sole dominant compound in the TLE of the soil from the wooded area, we have to conclude that the lipid input from earlier vegetation cultivated, i.e. wheat, the epicuticular wax of which is dominated by the C₂₈ alkanol (Tulloch, 1976), on this plot prior to the reversal in 1882 has been overprinted by the vegetation currently growing on the plot.

Some important differences have been noted between the compositions of the TLE of the leaves and that of the soil from the wooded area [Fig. 2(a) vs. Figs 3 and 4]. In particular, the decreases in relative abundances of the sterols, triterpenoids and TAGs are noted. A significant decrease in sterols and terpenoids was observed previously when lipids from a forest-litter layer (L layer) were compared with those from the underlying A1 soil horizon (Jambu *et al.*, 1993; Ambles *et al.*, 1994). The observations suggest that one or several of the following diagenetic processes are occurring: (i) complete mineralization; (ii) chemical alteration to form modified sterols and triterpenoids; or (iii) condensation of steroid or triterpenoid moieties to form involatile constituents that are intractable to GC analyses [cf. (bio)transesterification suggested by Jambu *et al.*, 1993]. Minor chemical modifications to give slightly altered structures, e.g. reduction to stanols or oxidation to give keto- or hydroxylated products, are a highly unlikely explanation since no such products are evident in any of the soil TLEs. The condensation of sterols or triterpenols with other organic components of the soil cannot be ruled out. However, simple transesterifications with *n*-alkanoic acid or *n*-alkanols can be excluded since steryl and triterpenyl esters are readily amenable to GC analysis under the conditions employed in this work (cf. Figs 2–5). Incorporation of sterols and triterpenols into soil macromolecules, e.g. humic substances, is feasible (Michaelis *et al.*, 1989). Interestingly, however, no evidence has been obtained from the Curie-point pyrolysis-GC and Py-GC-MS analyses of the extracted soils or humic acid fractions for the presence of abundant sterol or triterpenoid moieties.

Ester-linked sterols and triterpenoids are recognizable in pyrolysates as sterene and triterpene products. The data presented herein and that provided by earlier workers (e.g. Soliman and Radwan, 1981; Jambu *et al.*, 1993) do not, as yet, provide a clear explanation for the diagenetic fate of sterols and triterpenoids in soils.

The decreases of the proportion of TAGs in the soil is most likely due to their susceptibility to enzymatic hydrolysis and the subsequent assimilation by microorganisms of the released fatty acids via β -oxidation (e.g. Hita *et al.*, 1995). Preferential degradation of the TAGs bearing unsaturated fatty acyl moieties is presumed to be due to their relatively greater susceptibility to oxidation (i.e. singlet oxygen attack). The presence of a peak reflecting C_{48} TAGs with $C_{16:1}$ fatty acyl moieties, which was not observed in the extracts of the vegetation, would be in concordance with transesterification processes occurring in the soil (Amblès *et al.*, 1994). The loss of phytol and phytol fatty acyl esters from the soil during humification is believed to be due to the combined effects of hydrolysis and β -oxidation leading to mineralization.

In contrast to the extracts from the wooded area, the TLEs from the soils of the grazed and stubbed areas [Fig. 2(b) and (c)] are less ambiguous with regard to their main input source. The abundance of the C_{26} alkanol in conjunction with the wax esters, which are all based on C_{26} alkanol moieties, clearly reflect the dominance of grass input (e.g. Tulloch, 1976). However, the predominance of *n*-hexacosanol is much more obvious in the TLE of the grass itself [Fig. 5(a)] implying that lipids from other sources must also be considered. This concurs with the observation of small proportions of other, "non-grass", herbs in the plot. Alternatively, this compound, together with other long-chain alkanols, may have been selectively degraded or have been transesterified in the top soil (cf. Amblès *et al.*, 1994). Oxidation and subsequent transesterification of those alkanols could provide an explanation for the origin of the monoacylglycerols with long-chain (C_{22} , C_{24} , C_{26} and C_{28}) fatty acyl moieties. The wax esters in this soil sample appear to have decreased in relative abundance when compared with the grass. Moreover, it should be noted that the wax esters in the TLE of the grass are dominated by the C_{42} and C_{44} homologous, whereas the C_{44} and C_{46} members dominate the soil sample. This shift in wax ester distribution could be due to an input from plants, other than grasses. However, the selective degradation of shorter-chain compounds leading to a relative enrichment of compounds with both longer alkanol and alkanolic moieties appears to be the most likely explanation (cf. Amblès *et al.*, 1989, 1994; Jambu *et al.*, 1991, 1993). As with the extracts from the tree leaves (see above), the sterols showed a significant decrease in abundance.

As mentioned above, the TLEs of the stubbed soil also reveal a clear, but less pronounced, grass signal. This is to be expected since the mixed herb assemblage is dominated by grasses. The trends in the changes in the distributions of the lipid components largely mirror those observed for the other two plots.

A substantial organic matter input from leaves, in the case of the woody area, appears to be in concordance with suggestions of leaf-litter being the main carbon source input in this plot (Jenkinson *et al.*, 1992). However, this contribution from leaves to the soil organic matter, as reflected in the composition of the TLEs, appears to be contradicted by the pyrolysis data of the HMW fractions [Fig. 6(a) and (c)]. Based on the presence of 2-methoxyphenols, 2,6-dimethoxyphenols and polysaccharide pyrolysis products in the pyrolysate, the insoluble bulk of the tree leaves [Fig. 6(a)] analysed is composed of native dicotyledonous lignin-cellulose (e.g. Saiz-Jimenez and de Leeuw, 1986a). In addition, the pyrolysates also reveal evidence of various other macromolecular complexes such as a cutan-type highly aliphatic macromolecule, based on the presence of homologous series of *n*-alkane and *n*-alk-1-ene and long-chain methylketones (Tegelaar *et al.*, 1993), proteins, as revealed by toluene, probably phenol and N-containing compounds (e.g. Tsuge and Matsubara, 1985; Bracewell and Robertson, 1984), tannins, as reflected by the abundance of 1,2-benzenediol (e.g. Galletti and Reeves, 1992) and chlorophyll, as revealed by phytadienes and pristenes (van der Meent *et al.*, 1980a,b; Ishiwatari *et al.*, 1991). Alternative sources for these latter compounds are tocopherols (Goossens *et al.*, 1984), which are also present as pyrolysis products [Fig. 6(b)], or tocopheryl moieties (van Bergen *et al.*, 1994). Such compounds are relatively abundant in chloroplasts (Peulvé *et al.*, 1996), and hence their presence in the leaves is not unexpected. The contribution of the various macromolecular or polymeric compounds vary substantially amongst the various samples. For example, the pyrograms of the *Quercus* leaves were dominated by lignin-cellulose products, whereas the pyrolysis products of the *Hedera* leaves appear to indicate a greater contribution from other macromolecules, e.g. cutan.

In sharp contrast to the results from the leaves, the pyrolysis data obtained from the insoluble organic matter in the whole soil residue and the humic acid of the wooded area [Fig. 6(b)] shows little evidence of a lignin-cellulose signature. Instead, the pyrolysate is dominated by toluene, pyrrole and (alkyl)phenols, indicating amino acid moieties probably present as polypeptides (Bracewell and Robertson, 1984). These marked differences between the pyrolysates of the vegetation and soil are also observed in the other plots (Fig. 7). For example, the pyrolysate of the grass residue

[Fig. 7(a)] clearly indicates the presence of so-called "grass-lignin" with an abundant contribution of *p*-coumaric acid [3-(4-hydroxyphenyl)-2-*E*-propenoic acid] and ferulic acid [3-(4-hydroxy-3-methoxyphenyl)-2-*E*-propenoic acid] derived pyrolysis products (e.g. Tegelaar *et al.*, 1989a; Ralph and Hatfield, 1991; Galletti *et al.*, 1991), whereas the pyrolysate of grazed soil sample [Fig. 7(b)] is analogous to that of the residue of the soil from the wooded area [Fig. 6(c)].

Lignin-cellulose has been shown to be one of the main contributors to soil organic matter (e.g. Martin and Gonzalez-Vila, 1983; Saiz-Jimenez and de Leeuw, 1986b; Guggenberger *et al.*, 1994, Guggenberger *et al.*, 1995; Saiz-Jimenez *et al.*, 1996). It is, therefore, somewhat surprising to find little evidence of lignin in the top soil at the Broadbalk Wilderness site. Work in progress, on soil samples from the Geescroft Wilderness site, also from Rothamsted, shows an abundant lignin-cellulose contribution to the insoluble soil organic matter in the top 5 cm. A significant difference between the Broadbalk and Geescroft Wildernesses is the pH (7.3 and 4.2, respectively) which leads us to suggest that the slightly alkaline nature of the soil at the Broadbalk site may be closely linked to the absence of lignin-cellulose. Variations in pH, rather than difference in land use, could also account for the differences observed by Guggenberger *et al.* (1994, 1995), who reported an abundant lignin contribution in soils from under a spruce forest (pH 3), deciduous forest (pH 4.1) and permanent grassland (pH 4.9), whereas lignin was much less evident in the soil from plots under arable rotation (pH 6.4). These observations suggest that differences in soil pH may have a profound effect on the composition of SOM. While it is known that soil pH and bulk organic matter composition are linked, to date, evidence with regard to the precise relationship between soil pH and organic matter decomposition have been conflicting (Motavalli *et al.*, 1995). An alternative explanation for the virtual absence of lignin-cellulose could relate to larger amounts of microbial biomass, leading to more extensive and, possibly, more rapid biodegradation of the HMW fraction, at the Broadbalk site when compared with the Geescroft Wilderness (Jenkinson *et al.*, 1992). It should, however, be borne in mind that although soil pH affects microbial activity (Moucawi *et al.*, 1981), we have no clear indication about the relative importance of biological compared with abiological processes on SOM diagenesis, particular in relation to the fate of the lignin-cellulose components.

The major pyrolysis products seen in the residues of the whole soil are toluene, pyrrole and (alkyl)phenols. Alkylphenols have been shown to be derived from modified lignin (e.g. Hatcher *et al.*, 1994), (modified) tannins (e.g. van der Heijden,

1994) or the insoluble organic residues of fungi (Ewbank *et al.*, 1993) all of which will contribute to various degrees to SOM. However, Bracewell and Robertson (1984) suggested that the presence of phenol and methylphenols together with toluene, alkylphenylnitriles and pyrrole in pyrolysates of soil humic acids can be solely ascribed to polypeptides. These polypeptides may have their origin in organic matter derived from the vegetation, since the same amino acid pyrolysis products were observed in the pyrolysates of the modern senescent leaves [Fig. 6(b)]. However, recent stable carbon isotope data (Lichtfouse *et al.*, 1995) has shown that rather than resulting from selective preservation of lignin, SOM is most likely produced by condensation of small plant-derived molecules or is the result of selective preservation of resistant macromolecules present in soil microorganisms (Lichtfouse *et al.*, 1995). In particular, condensation of amino acids, most likely derived from the soil biomass, could react with carbohydrate monomers to form condensation products the so-called melanoidin complexes (e.g. Eglinton and Logan, 1991; Lichtfouse *et al.*, 1995). With respect to the selective preservation of resistant molecules, the aliphatic signal, which is present in the pyrolysate of the whole soil residues, could be either derived from highly aliphatic macromolecules present in the cuticle of leaves (cutan) or from bacteria (e.g. Tegelaar *et al.*, 1989b; de Leeuw and Largeau, 1993; Flaviano *et al.*, 1994).

Overall, comparisons of the soluble and insoluble organic matter from the soil of the three different plots at the Broadbalk Wilderness show that the soluble low molecular weight fractions record the most distinct signal for the growing surface vegetation. In contrast, the pyrolysis data obtained for the high molecular weight fractions reveal no direct evidence of the differences in surface vegetation. This and other work appears to suggest a relationship between soil pH and the retention of recognizable lignin derived humic materials.

Implications for archaeological investigations

As mentioned in the introduction, understanding the changes in vegetation and/or land use are of particular importance for archaeological studies. From the work presented here it is clear that there is great potential for the use of molecular information from soils, obtained by organic geochemical techniques, in order to trace the actual vegetation growing at a certain site. In particular, the soluble low molecular weight fraction of the soil organic matter reveals a clear signal derived from the corresponding vegetation cover. With regard to the high molecular weight fraction of the soil organic matter, which, in principal, also varies significantly amongst plant groups (i.e. different types of lignin), the data seem less conclusive. However, the degree to which the composition of the HMW fraction is influenced

by specific physical characteristics, e.g. soil pH, needs to be more fully investigated, before the use of such components in palaeoenvironmental reconstructions can be fully assessed.

CONCLUSIONS

In this investigation we undertook parallel studies of the total lipid extracts (TLEs) and solvent insoluble organic matter from soils of the three adjacent plots of the Broadbalk Wilderness, which was originally part of one of the Rothamsted Classical Experiments. The three plots selected for study possessed different types of vegetation including woodland, grassland and mixed herbs and shrub areas, all of which have developed and been maintained since 1882 following the use of the area for the cultivation of wheat for nearly 40 years (1843–1882). The vegetation growing on the plots were studied to provide a basis for assessing the diagenetic changes occurring in the molecular components of the plant tissues in order to determine whether or not a molecular signal for the vegetation is preserved amongst the constituents of the soil organic matter. The principal findings of the investigation can be summarized as follows:

(1) The compositions of TLEs of the soils, particularly the *n*-alkanol and wax ester constituents, sampled from the various areas reflect the contributions from the current surface vegetation.

(2) Profound differences were seen between the composition and concentrations of the steroidal and triterpoid constituents of the vegetation and underlying soils, which most likely reflect gross structural alterations or degradation, possibly involving condensation or oxidation reactions, or complete mineralization.

(3) Comparison of the Curie-point pyrolysis data from the solvent insoluble organic matter fractions of the soils and plant tissues show that a simple signal ("fingerprint") for the lignin component of the vegetation is not readily detectable in the underlying soil. The absence of a signal from lignin is believed to relate to the slightly alkaline pH of the soil associated with the Broadbalk Wilderness.

(4) The pyrolysate of the soils contain a high proportion of products derived from amino acids which are presumed to be present as polypeptides or possibly melanoidin-type complexes.

To our knowledge these data constitute the first direct molecular information for the lipid components of the soils from Rothamsted Experimental Station. These data, together with that we are accumulating from similar studies of other plots, are providing us with the essential background organic geochemical information required to underpin stu-

dies aimed at retrieving molecular information from soils that will reflect earlier vegetation cover and/or alterations in land use in the past.

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