MOLECULAR MARKER RECORDS OF LAND USE CHANGE.

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Abstract.

Evidence of a changing environment in the catchment area of a small lake (Gormire, Yorkshire, U.K.) over the past 3000 years is provided by the mean carbon number of *n*-alkanes and the ratio of lignin thermochemolysis products, together with pollen analysis and bulk organic geochemistry.

Periods of deforestation, which commenced at ~ 600 BC and AD 1200, display a significant decrease in organic carbon contents of the lake sediments, which probably reflects dilution by enhanced influx of clastic material. The mean carbon number of waxy ($C_{27} - C_{31}$) *n*-alkanes closely corresponds with the percentage of pollen derived from grass in the sediments. A higher-plant derived triterpenoid, tentatively identified from its mass spectrum as 28-carboxyursen-12-enol, appears exclusively in samples where tree and shrub-derived pollen is dominant. Thermochemolysis of lignin, confirms that there was a significant change in the nature of material deposited in the lake sediments from woody to grass dominated. Changes in both the sedimentary C/N ratios and yields of lignin-derived phenols suggest that deforestation events led to enhanced aquatic productivity, initially through the development of reed-swamp vegetation.

Keywords. Lake sediments, Land-use change, Deforestation, Biological markers, *n*-Alkanes, Thermochemolysis, Lignin

1. Introduction.

The dominant sources of organic matter (OM) to lacustrine sediments are from aquatic production (autochthonous OM; including primary and secondary producers and heterotrophic bacteria) and/or from the residues of biota from the surrounding catchment (allochthonous OM, mainly plants). A key goal in the reconstruction of lake and catchment history, is to discern the sources of OM to the lake and to assess any changing inputs that might be associated with environment change (*e.g.* change in trophic status of the lake; Schelske *et al.*, 1983; Schelske and Hodell, 1995; Ostrom *et al.*, 1998; change in vegetation of the land in the catchment, either natural or anthropogenic; Ho and Meyers, 1994).

Biological markers, particularly lipids, have often been employed to provide historical information of OM accumulation in lacustrine sediments (*eg.* Cranwell, 1977, 1978, 1981; Meyers *et al.*, 1984; Wünsche *et al.*, 1988; Ho and Meyers, 1994; Pratono and Wolff, 1998). Lipids only make up a small percentage of the bulk OM (Meyers, 1997). However, their structural diversity and different distributions in autochthonous and allochthonous OM inputs allow these sources to be distinguished (for reviews, see Meyers and Ishiwatari, 1993; Meyers and Lallier-Vergès, 1999), and changes in the aquatic primary producers, for example through enhanced inputs of macrophytes, to be assessed (Ficken *et al*., 1998; 2000). More subtle changes in OM inputs, for example from changes in catchment vegetation, have been less frequently observed, but potentially, differences in distributions of certain compound classes can be informative (*e.g. n*-alkanes; Cranwell *et al.*, 1987; van Bergen *et al.*, 1997). The coupling of biological marker distributions with information on their isotopic composition may also

be of value (Rieley *et al*., 1991), particularly where C_3 and C_4 plant biomass changes have occurred in the catchment (Ficken *et al*., 1998).

Since individual lipid biological markers are relatively minor components of OM, which have significantly different rates of degradation, the characterisation of the bulk, macromolecular components of allochthonous OM, particularly of the lignins, has also been attempted. Chemical oxidation (Hedges and Parker, 1976; Hedges and Mann, 1979 and thermochemolysis (Hatcher *et al*., 1995; Clifford *et al*., 1995) of lignins and subsequent analyses of the resultant phenols are useful methods, since they provide information on specific vascular plant sources (gymnosperm *vs.* angiosperm; woody *vs*. non-woody). Furthermore, quantification of the phenols allows an estimate of the amount of lignin present in (Ertel and Hedges, 1985), and potentially, therefore, of vascular plant fluxes to, the sediments.

The present study formed part of a larger project based in the UK (Land-Ocean Evolution Perspective Study; LOEPS), which was concerned with the relationship between sediment fluxes between the land and ocean and their influence from changes in sea level, climate, geomorphology and land use in the Holocene. Here, we consider whether the distributions of molecular markers in lake sediments reflect the sources of OM to the sediment and whether molecular parameters can act as a proxy for change in catchment vegetation. Gormire Lake (Yorkshire, U.K.) was chosen as the study site; its' catchment has undergone periods of land use change and deforestation during the Holocene period. Simple biomarker parameters, such as the mean carbon number of *n*-alkanes and the ratio of lignin pyrolysis products are

employed, together with classical pollen analysis and bulk organic geochemistry, to assess qualitative and semi-quantitative changes in OM inputs to the lake sediments.

2. Materials and Methods.

2.1 Sample site, sediment collection and preparation.

Gormire Lake (Ordinance Survey Grid Reference: SE 503832) is a small crescent-shaped closed basin with a maximum water depth of 6.5 m and a surface area of ~ 0.06 km^2 situated in the North Yorkshire Moors at the northern edge of the Vale of Mowbray (part of the Vale of York), just below the scarp of the Hambledon Hills at Whitestone cliffs. It has a steep, well-defined catchment (0.2 km^2) with a maximum relief of 150 m. The mean annual rainfall is 825 mm y^{-1}.

Sediments were collected from the lake in August 1995 using 1 and 3 m Mackareth corers (three cores are discussed in this paper; 1 m cores, GCA and GCB; 3 m core G3). The cores were packed in dry ice (-78°C) and transported to the laboratory where they were stored frozen (-20°C). Frozen sediment cores were defrosted slightly and sliced into sections (2 cm) using a heated saw. The samples were then re-frozen, prior to freeze-drying (-60°C; 10^{-2} Torr; 24 – 48 h). Lake sediment samples for lipid analysis (Section 2.7) were dry-sieved (> and < 63 μm) using a mechanically agitated all metal sieve system). Grassland and woodland soils and peats collected from the catchment and analysed by quantitative Curie-point pyrolysis-GC-MS (see Section 2.8; Table 4) were frozen and freeze dried as above.

2.2 Radiometric analyses

Sediment cores (1 m) from Gormire lake were analysed for ^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am by direct gamma assay. Ortec HPGe GWL series well-type coaxial low background intrinsic germanium detectors were used (Appleby *et al*., 1986).

2.3 AMS radiocarbon dating

15 samples (macrofossil remains, sieved material and untreated bulk sediments) from the 3 m cores were dated using AMS radiocarbon dating. Samples were digested in HCl (6 M; 80°C, 10 h), rinsed with distilled water and digested in KOH (0.5 M; 80°C, 10 h) to extract and discard humic material. Solid residues were rinsed with distilled water and digested in HCl (2 M; 80°C; 10 h), before finally rinsing free of mineral acids with distilled water, drying and homogenising. The samples were converted to graphite at the NERC Radiocarbon Laboratory, East Kilbride. ^{14}C analysis was carried out at the University of Arizona NSF AMS Facility.

2.4 Pollen analyses

Pollen analyses were carried out on sub-samples from 1 and 3 m cores using standard preparation techniques (Oldfield *et al*., in press), including acetolysis and hydrofluoric acid treatment. A minimum pollen sum of 300 non-aquatic grains was used.

2.5 Total organic carbon and total nitrogen

Concentrations of total organic carbon (TOC) and total nitrogen (TN) were determined on the carbonate-free samples (1 M HCl; left to stand 1h; shaken 0.5 h) using a Carlo Erba 1106 CHN Elemental Analyser. The reproducibility of analysis for

TOC was better than 2% (RSD for replicate analyses; n=5) for a homogenised sediment sample.

2.6 Extraction, isolation, analysis and quantification of lipids.

All glassware and stainless steel items were soaked in a solution of hot detergent (Decon-90; 2%; BDH Chemicals Ltd; 6 h), rinsed with deionised water (18.2 MΩ cm^{-1} resistivity; MilliQ), wrapped in foil and dried in an oven. Finally, the glassware was heated in a muffle furnace (400 °C, 4 h). Cellulose thimbles (Whatman), glass wool, Pasteur pipettes and anti-bumping granules were pre-extracted in a Soxhlet apparatus with dichloromethane (DCM; 24 h).

Sieved sediment samples (< 63 μm and >63 μm; 0.5 – 20 g, depending on organic content) in a cellulose thimble were spiked with internal standards (2,21-dimethyldocosane, 200 μg; 5β(H)-cholanic acid, 200 μg) and were extracted in a soxhlet apparatus (10 % methanol in DCM; 24 h). The extracts were then evaporated *in vacuo* to a small volume (~1 mL) and treated with 6% KOH in methanol (w/v; 15 mL; 24 h). Water was added (15 mL) and the neutral fraction was extracted into hexane (3 x 30 mL). The aqueous layer was then acidified to pH 2 (6M HCl) and the acid fraction recovered as above. After evaporation *in vacuo* (to 0.5 mL), BF_3-methanol was added (15 mL) and the solution left to stand (24 h). Excess methylating reagent was destroyed with water (15 mL) and the methylated acid fraction was recovered by extraction (hexane, 3 x 30 mL).

Prior to analysis by gas chromatography-mass spectrometry, a quantification standard (5α(H)-cholestane; 20 μg) was added to the sample (~0.5 mg), which was then derivatized (*bis*-(trimethylsilyl)-trifluoroacetamide, 50 μL, 40°C, 40 min).

GC-MS analyses were performed on the derivatised extracts using a Hewlett Packard 5890-A Gas Chromatograph, fitted with an on-column injector, a fused high temperature silica column (30 m × 0.2 mm i.d.; 5% phenyl/95% methyl polycarborane siloxane, HT5), and helium was used as the carrier gas (ca. 1.5 - 2 mL min^{-1}). A retention gap of deactivated silica (1 m x 0.32 mm i.d.) was used at the front of the column. Typically, the oven temperature was programmed from 40°C to 320°C at 5°C min^{-1} after 1 minute, and held at 320°C for 20 minutes. The column was fed directly into the EI source of a VG TS-250 mass spectrometer. Typical GC-MS operating conditions were: ionisation potential 70 eV; source temperature 210°C; trap current 300 µA. The instrument was operated in Full Data Acquisition mode, at a mass resolution of 500, and cycled every 1 s (50-600 D). Data was collected on a VAX 3500 Workstation, and processed using VG OPUS software.

Lipid identifications were made by comparison of relative retention times and indices and mass spectra of the analytes with standard compounds where available and with literature data. Quantitative data were calculated by comparison of the peak areas in the total ion current (TIC) chromatogram with the peak areas of the internal standards. The precision of the analysis was better than 6.5% (RSD, n=4), whilst reproducibility of extraction and analysis measured for a homogenised sediment sample, was better than 10% for all analytes (RSD, n=4).

The mean carbon numbers of *n*-alkanes, *n*-alcohols and *n*-alkanoic acids in the Gormire samples were calculated according to Peltzer and Gagosian (1989).

$$\text{Mean Carbon Number (MC\#)} = \Sigma([C_i] \times C_i) / \Sigma C_i$$

where $[C_i]$ is the concentration of the *n*-alkane with carbon number i, over the range 27 – 31.

Carbon Preference Index (CPI) for *n*-alkanes, *n*-alcohols and *n*-alkanoic acids were calculated according to the formulae:

$$CPI_{n\text{-alkanes}} = (\Sigma[\text{odd } C_{21}\text{-}C_{29}] + \Sigma[\text{odd } C_{23}\text{-}C_{31}])/ (\Sigma[\text{even } C_{20}\text{-}C_{28}] + \Sigma[\text{odd } C_{22}\text{-}C_{30}])$$

$$CPI_{n\text{-alcohols}} = (\Sigma[\text{even } C_{20}\text{-}C_{28}] + \Sigma[\text{even } C_{22}\text{-}C_{30}])/ (\Sigma[\text{odd } C_{19}\text{-}C_{27}] + \Sigma[\text{odd } C_{21}\text{-}C_{29}])$$

$$CPI_{n\text{-acids}} = (\Sigma[\text{even } C_{20}\text{-}C_{28}] + \Sigma[\text{even } C_{22}\text{-}C_{30}])/ (\Sigma[\text{odd } C_{19}\text{-}C_{27}] + \Sigma[\text{odd } C_{21}\text{-}C_{29}])$$

2.7 *Quantitative Curie-point Pyrolysis-GC-MS.*

Pyrolysis wires (Curie point temperature of 610°C) were cleaned by ultrasonication in nitric acid (2% v/v, 15 min), milli-Q water (15 min), redistilled acetone (15 min) and DCM (15 min) and oven-drying (60 °C)

Typically, an aliquot of homogenised sample (2 mg), quantification standard (poly-(*t*-tetrabutyl)-styrene; Sigma Chemicals Ltd, 20 µg in cyclohexane) and tetramethyl ammonium hydroxide (TMAH, 25% in methanol, 250 µL) were placed into a vial and agitated vigorously. The mixture was allowed to stand (20 min) and excess solvent was removed under a stream of nitrogen. The sample and wire were placed between two stainless steel discs and transferred to a hydraulic press (15 tonnes; 5 s). The wire was removed, suspended in the sample tube and transferred to the pyrolysis unit.

A Pi-rho Technology Curie-point Pyrolyser and Control Unit (Horizon Instruments Ltd) was used for all of the pyrolyses. The Pyrolysis Unit was interfaced with the split/splitless injector of a Hewlett Packard 5890A Gas Chromatograph, with an interface temperature of 250 °C. Typically, pyrolyses were carried out with a rise time of < 0.4 s, an equilibrium time of 2s and radio-frequency power of 160 W. The

GC-MS conditions were similar to those above except that the fused high temperature silica column was a non-polar methyl polycarborane siloxane (30 m × 0.2 mm i.d.; HT1) and there was no retention gap. The GC oven was programmed from –40 °C to 280 °C at a rate of 6 °C min^{-1} . Pyrolysis products were identified by their relative retention indices and by comparison of their mass spectra with those found in the literature.

The reproducibility of pyrolysis for 8 analytes: 1,2-dimethoxybenzene; 3,4-dimethoxybenzaldehyde (vanillin); 3,4-dimethoxyacetophenone (acetoguaicone); 3,4,5-trimethoxybenzaldehyde (syringaldehyde); 3,4-dimethoxycinnamic acid methyl ester (ferulic acid); *n*-eicosanoic acid methyl ester; 1-nonacosene, selected randomly, were better than 7% (RSD; n=4).

3. Results and Discussion

3.1 Chronology, pollen record, total organic carbon and total nitrogen.

The two 1 m cores (Cores GCA and GCB), both with an undisturbed sediment-water interface, were dated using ^{210}Pb, ^{137}Cs and ^{241}Am radionuclides. In both cores, ^{210}Pb activity below ~ 10 cm declines more or less uniformly with depth (Fig. 1; only GCB is shown). Chronologies were calculated using the CRS dating model (Appleby and Oldfield, 1978), together with the levels corresponding to 1963, suggested by artificial radionuclide fallout records. The transition at 10 cm is dated to the early 1960s, with fairly steady accumulation before this date (0.013 ± 0.002 and 0.018 ± 0.001 g cm^{-2} y^{-1}). Above 10 cm, accumulation rates are significantly higher (0.032 ± 0.007 and 0.031 ± 0.006 g cm^{-2} y^{-1}).

The radiometric chronologies for Cores GCA and GCB indicate that the 8 - 10 cm missing from the top of Core G3 is roughly the equivalent of the period since AD 1970. Both the magnetic (Oldfield et al., submitted) and pollen comparisons support a correlation between 13 cm in Core G3 and 21 cm in the dated mini-cores. The calculated ages for sediments at this depth are AD 1853±18 years for GCA and AD 1890±30 years for GCB, giving a mean age of ca. AD1870. Moving down core, the next horizon identifiable for correlation is marked by both a peak in *Myriophyllum alterniflorum* and the first increase in *Pinus* pollen. This occurs at ca. 32cm in Core GCA and 24cm in G3. Taking the mean age of the 21cm horizon and the mean of the earliest ^{210}Pb-based sedimentation rates for the mini-cores, we derive an age of ca. AD 1770 for this feature, one that is compatible with the strong probability that, as elsewhere in the north of England, the *Pinus* pollen increase reflects the beginning of amenity plantations in country estates from the second half of the 18th century onwards.

AMS ^{14}C dates were obtained on 15 samples. The initial strategy was to select terrestrial macrofossils as well as other organic components, so that both a secure chronology and some estimate of the error associated with different organic fractions could be obtained (Oldfield et al. 1997). It proved impossible to achieve this goal as only one depth yielded enough terrestrial material for a date, albeit with a large standard error, and most of the initial set of picked plant macro-fossils and sieved material that was submitted for AMS ^{14}C dating proved to have too little carbon for the system used. It was necessary to date a mixture of sieved material and untreated bulk sediments. The results are shown in Table 1 and Figure 2. The combined chronology for Core G3 is discussed briefy below.

Many studies have shown that even in soft water lakes like Gormire, both bulk lake sediments and fractions with an aquatic component tend to give ^{14}C dates that are too old (Oldfield et al., 1997). In the Gormire samples, the mean calibrated age of the single date on terrestrial macrofossil material is some 300 years younger than >125µm sieved material from the same depth. Dates on material between 270 and 274 cm show that at that depth, the ^{14}C date of >125µm sieved material is between 200 and 450 years younger than those for the bulk samples. Dates based on bulk sediment from the transitions to local pollen zones (LPZ2; 178 - 206cm; see below) and LPZ4 (66 - 68cm) are older than dates from the preceding LPZ1 (220 - 224cm) and 3 (100 - 106cm). The transitions are marked by changes in pollen assemblage (see below), magnetic properties and element concentrations (Oldfield et al., in press) that are interpreted as indicative of deforestation and erosive input, including terrigenous organic matter, to the sediments. This would be consistent with some additional dilution of ^{14}C activity by in-wash of old soil carbon (cf. O'Sullivan et al., 1973).

These observations suggest that radiometric data alone fail to provide a secure basis for a chronology for most of the core and especially between 25cm and 220cm. The additional dated points used in Figure 2 have been established by comparing changing Pb concentrations with the dated curves presented by Renberg et al. (2000). Using results from widespread sites in western and northern Europe, they show that there were two periods of high Pb concentrations prior to the most recent increase. These have been dated to 200BC - AD100, the period of Roman lead production, and AD1000 - 1200, a period of increased lead pollution during Medieval times. The onset of each of these episodes of increased lead concentrations can be clearly identified in the G3 record, the terminations are less confidently placed. The age/depth curve obtained using these dates is conformable with the dates above and

below and diverges from a linear interpolation between the Œwood[1] date at 220 - 224cm and the AD1770 horizon at 25cm by no more than 100 years at any point. According to this chronology, the dates based on bulk sediments deposited during periods of deforestation and erosion are too old by some 700 to 1200 years. By contrast, the mean age of the set of dates from100 - 104cm, a depth indicative of closed forest cover and minimum erosive input, appears to be old by no more than 200 years. An age of 1450 BC is obtained by extrapolation for the base of the core. Overall, it would be unrealistic to claim better than century scale accuracy at best for the chronology used below ca. 30cm.

Pollen analyses of samples indicate that there are four distinct zones in the 3m sediment core (summarised in Table 2; Fig. 3). These appear to be associated with periods of change in the vegetation cover of the catchment (Oldfield *et al*., in press): *Local Pollen Zone 1 (LPZ1; 275 – 200 cm; ca 1100 BC – 600 BC)* has high relative amounts of pollen from trees and shrubs, especially in the upper part of the zone, where *Quercus* (Oak) and *Ulmus* (Elm) reach maximum levels. The catchment appears to have been stable at this time and no signs of forest clearance are evident. *Local Pollen Zone 2 (LPZ2; 200 – 110 cm; ca. 600 BC – AD 800)* shows a rapid decline in tree and shrub pollen. Simultaneously, there is an increase in grass, cereal and other non-tree pollen. This suggests that deforestation occurred during the time of deposition of these sediments. Aquatic taxa, *e.g. Myriophyllum alterniflorum* and *Sparganium*-type and finally *Equisetum* also increased in relative abundance during this period.

Local Pollen Zone 3 (LPZ3; 110 – 75 cm; AD 800 – AD 1200), shows trees and shrubs apparently making almost a complete recovery, with the exception of *Ulmus*. The decline in grassland also suggests a period of woodland recovery. Aquatics

established in LPZ2, cereals, *Calluna* (ling) and *Pteridium* (bracken) are also present in low relative abundance.

Local Pollen Zone 4 (LPZ4; 75 – 0 cm; AD 1200 – AD 1970) again shows a decline in trees and shrubs, whereas pollens not derived from trees, such as those from aquatic plants, ferns, cereals and grasses increase in relative abundance. In the uppermost section of the sediment record, however, there is an increase in *Pinus* (pine) and *Betula* (birch), indicating some forest regeneration.

The down-core profile of TOC concentrations co-varies closely with that of the % pollen derived from trees and shrubs (Fig. 3). Hence, highest TOC values (~37 %) are found in LPZ1, but these fall to ~ 10 % in LPZ2, where there the pollen record indicates forest clearance. This is interpreted as reflecting dilution by inorganic material, which was washed in to the Gormire sediments as a result of increased erosion on deforestation. Apparently, there is a significant increase in the organic carbon flux to the lake during LPZ2 (Fig. 4). Fluxes have been calculated on the basis of a mean sedimentation rate throughout the period covered by core G3, of 0.016 g dry sed cm^{-2} y^{-1}, except between depths of 178 and 140 cm, where, from the preferred age/depth line, the sedimentation rate is estimated to have been 0.051 g dry sed cm^{-2} y^{-1} (Fig. 2). It should be noted that the dips in flux at either side of the peak values in LPZ2 are almost certainly artefacts of the uncertain chronology. On the other hand the changes in mean sedimentation rates of organic carbon, between the inflexion points on the age/depth curve may reflect enhanced in wash of soil material. In LPZ3, where pollen analyses suggest some recovery of woodland, TOCs again increase, but then decrease dramatically in the uppermost section of the core. This decline again probably reflects the enhanced influx of lithogenic material to the uppermost sediments.

C/N values for the Gormire sediments vary significantly, from ~ 10 in surficial sediments (LPZ4) to ~ 17 in the deepest sediment (LPZ1) and they tend to increase with increasing sediment depth (Fig. 4). This is contrary to the change in C/N that is normally observed in lacustrine sediments, where C/N values decrease through preferential utilisation of carbon and bacterial immobilisation of nitrogen (Meyers and Ishiwatari, 1993). For example Bourbinniere (1979) found that the C/N ratio of living spruce was 46, whereas that of spruce buried in lake sediments for 10,000 years was 20. Hence, the changes observed in the Gormire sediment may rather reflect changes in the source of organic material to the sediment. The decrease in C/N ratios in the topmost part of the core (35 – 0 cm), most likely reflects a gradual increase in the proportion of autochthonous material in the sediments. Algal material, unlike higher-plant derived OM, contains little cellulose and therefore tends to have a lower C/N (Meyers and Ishiwatari, 1993). Furthermore, algal OM is rich in labile nitrogen, which is degraded relatively rapidly and would lead to an increase in C/N with increasing sediment depth.

The sediments deposited in LPZ3 and 1 have relatively stable C/N ratios, whereas in LPZ4 and 2, which were apparently influenced by deforestation, significant decreases in the C/N ratios are apparent. This may reflect a change in the inputs of catchment material. However, it could also arise from a change in the aquatic ecosystem, involving increased productivity by phytoplankton and reed-swamp development, both of which could lead to lowering of C/N ratios (Meyers and Ishiwatari, 1993). Indeed, comparison of TN values in LPZ 1 and 3, shows consistently higher concentrations in the shallower sediments, which may reflect an increased contribution of aquatic flora (Fig. 3; Table 2). Pollen data reinforce this hypothesis, since there is an increase in the abundance of such plants (*e.g.*

Myriophyllum alterniflora) above 200 cm. The enhanced influx of catchment material through increased rates of erosion (Oldfield *et al*., submitted), may have enriched the sediments and overlying waters with nutrients, for example soil associated phosphorous (Schelske *et al*., 1983), and also have provided shallow marginal environments, which allowed reed-swamp to take hold.

3.2 Response of molecular markers to environment change in the Gormire catchment.

3.2.1 Lipids Gormire core sediments were sieved to separate fine (< 63 μm) from coarse (> 63 μm) fractions for analysis of molecular markers. The > 63 μm sediment fraction might be expect to reflect change in catchment vegetation, which is more likely to be represented in the coarse fractions during sediment deposition, whereas the fine fraction most likely contains reworked/aged soil-derived material as well as autochthonous algal and bacterial organic matter; Hedges and Oades, 1997; see Section 3.1). Nevertheless, the lipid distributions of both fine and coarse fractions were very similar and typical of lacustrine sediments, being complex mixtures of *n*-alkanes and *n*-alcohols, methyl ketones, sterols, triterpenoids, *n*-alkanoic and *n*-alkenoic acids, hydroxy acids, branched acids and diacids (*e.g.* Figs. 5a & b; > 63 μm fractions not shown; Table 3). The relative abundance of each group varied little and absolute concentrations of most of the compound classes did not vary systematically with increasing sediment depth. The exceptions were the *n*-alkenoic and branched acids, which decreased in concentration with increasing sediment depth (Fig. 6a & b; > 63 μm not shown), both in fine and coarse fractions. These compounds derive largely from autochthonous algae and bacteria, respectively (*e.g.* Oliver and Colwell,

1973; Morris and Culkin, 1976; Volkman and Johns, 1977; Perry *et al.*, 1979; Volkman *et al.*, 1980; Parkes and Taylor, 1983; Fredrickson *et al.*, 1986; Cranwell *et al.*, 1987). They are present in relatively higher concentrations in the fine fractions of the sediments (Table 3) and their concentration profiles in the sediment cores probably reflect the relative lability of the autochthonous OM. Hence, the unsaturated acids may be relatively rapidly degraded, whilst decreasing concentrations of the bacterial markers may reflect a decreasing active population of microorganisms with increasing sediment depth, which in turn reflects the increasingly refractory nature of the aging sediment OM. Alternatively, the concentration profiles could also reflect recent, gradually increasing lake productivity (Pratono and Wolff; 1997).

The concentration profile of one compound, tentatively identified from its mass spectrum (Fig. 7) as 28-carboxyursen-12-enol (**1**), a higher plant-derived triterpenoid, does show some relationship with the apparent changes that have occurred in the Gormire catchment that are evident from the pollen analyses. Hence, this compound only occurs in the sediments of LPZ1 and 3 (Fig. 8; > 63 μm fraction), where tree-shrub derived pollen is dominant, implying a specific origin from tree-derived material. In LPZ1, **1** is present in relatively high amounts (3.4 – 34 μg g^{-1} dry sed; ; > 63 μm fraction). An unidentified compound with a mass spectrum similar to that in Fig. 7 has been found in woodland soil samples containing leaf litter from *Quercus* (Oak), *Fraximus* (Ash) and *Hedera* (Ivy), (van Bergen et al., 1997; although only the major ions of the spectrum were reported), but was absent in grazed soils and grass samples from the same location.

Higher plant triterpenoids, such as the functionalised compounds with the ursane or oleanane skeleton, are highly susceptible to diagenetic degradation under both oxic and anoxic conditions (*e.g.* Trendel et al. 1987; Wolff et al., 1989; ten

Haven et al., 1992; Rulkötter et al., 1994; Poinsot et al., 1995; Pratono and Wolff, 1998), hence their use as robust proxies of changing sedimentary inputs to sub-aquatic sediments maybe suspect. Hydrocarbons, particularly the waxy, high molecular weight *n*-alkanes are less likely to undergo post-depostional changes than compounds containing functional groups (Meyers and Ishiwatari, 1993, Westerhausen et al., 1993). The *n*-alkane distributions in Gormire are consistent with a dominant input from vascular plants (Table 3), having a high CPI and MC#. It has been suggested that a predominance of *n*-untriacontane (*n*-C_{31}) in lake sediments from the Lake District, UK could be attributed to an input from grasses, whereas *n*-heptacosane (*n*-C_{27}) and *n*-nonacosane (*n*-C_{27}) are more prevalent in sediments where deciduous trees are dominant (Cranwell, 1973; Cranwell *et al*., 1987). Hence, it might be expected that shifts in the MC# (Pelzer and Gagosian, 1989; see *2.9*) of the *n*-alkanes would reflect such changes in vegetation. Comparison of the MC# profile with that of the % pollen derived from trees and shrubs (Fig. 9) shows an extremely close fit. Hence, samples from LPZ1 and 3, which according to pollen analyses reflect periods when the catchment was forested, have a significantly lower MC# (28 – 28.5) than samples collected in LPZ2 and 4 (MC# 29 – 29.5), which are associated with periods of deforestation. Similar observations have been made in lake sediments (Ho and Meyers, 1994) and soils (van Bergen *et al*., 1997), where subtle changes in *n*-alkane distributions have been ascribed to changing land plant cover. The exceptionally close relationship between pollen and the *n*-alkane MC# suggests that the latter parameter is a very sensitive indicator that mirrors rather subtle changes in the pollen abundance (Fig. 9, see LPZ2) and may be a valuable tool in palaeoenvironmental studies in similar high latitude, humid environements.

3.2.2 Macromolecular components Flash pyrolysis with *in situ* methylation (thermochemolysis) using tetramethylammonium hydroxide (TMAH) of selected Gormire sediment samples was carried out in order to assess the influence of changing catchment characteristics on the macromolecular OM, particularly the lignin. It should be noted that the yields of phenol derivatives released by thermochemolyis are similar to the release of phenols by CuO oxidation of plant tissues (Hatcher et al., 1995). Total yields of phenol derivatives (Λ, concentration normalised per 100 mg TOC; Hatcher *et al*., 1995) from catchment peats wood and grassland soils analysed in the present study were ~ 3.5, 2.2 and ~ 3.9, respectively, which are of the same order as those determined for degraded wood samples by Hatcher *et al.* (1995). Maximum values of Λ for the lake sediments were ~20. These values are clearly not absolute, because they were determined with respect to the pyrolysis of an internal standard polymer (*t*-butyl polystyrene), which yields *t*-butylstyrene, as it's dominant (>95%) pyrolysis product *via* C-C bond cleavage. The lignin phenol derivatives are, on the other hand, produced *via* cleavage of ether linkages in lignin (Clifford *et al*., 1995), hence, relative response factors for the lignin phenols are unlikely to have been 1 (as assumed; see 2.8). Nevertheless, the reproducibility of the pyrolyses was satisfactory (see 2.8), so the relative changes between samples are likely to reflect real changes in lignin composition and concentration.

The yields of lignin products (relative to TOC) varied between zones (Fig. 10; Table 4), most notably their levels in LPZ2 were higher than in the other zones (Λ up to ~ 20). The relative concentrations of the cinnamyl (C), vanillyl (V) and syringyl (S) phenols decrease in proportion to the extent of dilution of vascular plant remains by other types of non-lignified remains (Hedges and Mann, 1979). Hence, the decrease in

the overall proportion of total phenol methylated derivatives in LPZ 3 and 4 may reflect the increase in the proportion of autochthonous material in these zones (see 3.1 and 3.2.1). The relative proportions of C phenol derivatives were highest in the LPZ 2 samples, which is consistent with the apparent increase in grasses in the catchment area during this period. This is also evident from the C/V profile (Fig 11; high values reflect a relatively high input of non-woody *vs*. woody OM; Hedges and Mann, 1979; Saiz-Jimenez and de Leeuw, 1985), which shows elevated values in LPZ 2 (deforested catchment) when compared to LPZ 1. However, C/V values remain high in LPZ 3 (reforestation of catchment) and decrease again in LPZ 4 (deforestation of catchment). As non-lignin OM does not influence this parameter, it is possible that the difference in C/V values between LPZ 1 and 3, when the catchment was dominated by woodland, reflects the larger contribution of OM derived from emergent reed swamp plants to the sediments in LPZ 3.

4. Conclusions

The organic geochemistry of the Gormire Lake sediments reflects its changing environment over the past 3000 years. The integration of data from detailed analysis of the organic matter of the lake with that obtained by classical palaeolimnology, leads to convincing evidence of a changing environment in the catchment area.

1. Comparison with the pollen inputs shows that during periods of deforestation, which commenced at ~ 600 BC and AD 1200, there was a significant decrease in organic carbon contents of the lake sediments, which probably reflects dilution by enhanced influx of clastic material.

2. The mean carbon number of *n*-alkanes, which are dominated by high molecular weight ($>C_{25}$) homologues *vs* sediment depth, shows an extremely close correspondence with the percentage of pollen derived from trees and shrubs in the sediments. This reflects the more waxy nature of *n*-alkanes associated with the epicuticular waxes of grasses when compared to trees. A higher-plant derived triterpenoid, tentatively identified from its mass spectrum as 28-carboxyursen-12-enol, appears exclusively in samples where tree and shrub-derived pollen is dominant, and it appears to be a specific marker for organic material derived from these plants.
3. Thermochemolysis of lignin, which allows source specific analysis of the bulk component of the lignin in the sediments, confirms that there was a significant change in the nature of material deposited in the lake sediments from woody to grass dominated, which is contemporaneous with the deforestation event at ~ 600 BC.
4. There is evidence from both the pollen record, sedimentary C/N ratios and yields of cinnamyl and vanillyl phenols derived from lignin pyrolysis, to suggest that the deforestation events have led to enhanced aquatic productivity, beginning with the development of reed-swamp vegetation. This may reflect an enhanced influx of soil-associated nutrient to the lake.

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7. Tables

Table 1. ^{14}C dates for samples taken from core G3.

Depth (cm)	Sample	Date years BP (+/- 1σ)
66-68	Bulk sediment	1,500 ± 45
100-102	Bulk sediment	1,300 ± 45
104-106	Bulk sediment	1,480 ±50
130-132	Bulk sediment	2140 ± 50
160-162	Bulk sediment	2,610 ± 55
178-180	Bulk sediment	2,970 ± 50
184-186	Bulk sediment	2,880 ± 55
204-206	Bulk sediment	2,925 ± 60
220-224	Wood fragments	2,510 ±210
220-224	>125 µm organic matter	2,735 ± 45
234-236	>125 µm organic matter	2,905 ± 45
270-272	Bulk sediment	3370 ± 50
272-274	>125 µm organic matter	3155 ± 45
272-274	Bulk sediment	3,550 ± 60

Table 2. Summary pollen data for selected representative samples from the identified local pollen zones (LPZ 1 – 4). Data are quoted as percentages of the total non-aquatic pollen.

Sample	Trees and Shrubs	Miscellaneous herbs	Cultivated	Grassland	Marsh	Ferns	Forest plants	Ruderals	Aquatics
LPZ4 (17 cm depth)	36.73	6.64	1.99	38.05	0.66	15.27	0.00	0.66	14.60
LPZ3 (89 cm depth)	72.81	2.46	0.00	17.90	0.82	5.33	0.00	0.68	6.01
LPZ2 (139 cm depth)	21.40	6.98	0.23	60.00	1.63	7.21	0.70	1.86	10.70
LPZ1 (263 cm depth)	93.11	0.39	0.00	4.42	0.00	1.82	0.13	0.13	0.39

Table 3 Typical concentrations and distributions of major lipid compound classes in the Gormire Lake sediments (Core G3); data are presented for coarse (>63 μm) and fine (< 63 μm; in parentheses) fractions of horizons representative of each local pollen zone (LPZ 1 – 4; the same horizons analysed for pollen, where possible, or the closest horizon if not).

Compound class	LPZ1 (261 cm depth)	LPZ2 (137 cm depth)	LPZ3 (91 cm depth)	LPZ4 (17 cm depth)
***n*-alcohols**				
C_{13} - C_{32}[a]	252 (388)	230 (171)	172 (618)	58 (227)
CPI	7.9 (8.1)	9.0 (23.6)	12.8 (14.0)	9.3 (24.1)
Cmax	C-22 (C-22)	C-22 (C-22)	C-22 (C-22)	C-22 (C-22)
MC#	24.6 (24.6)	25.5 (25.2)	24.8 (24.3)	24.8 (23.9)
***n*-alkanes**				
C_{13} - C_{33}[a]	95 (152)	59 (28)	45 (22)	16 (24)
CPI	28.9 (32.8)	15.1 (16.2)	30.5 (14.6)	9.3 (8.1)
Cmax	C-27 (C-27)	C-31 (C-31)	C-29 (C-29)	C-25/C-29 (C-29)
MC#	28.4 (28.3)	29.5 (29.5)	28.4 (28.7)	28.8 (28.8)
***n*-alkanoic acids**				
C_{12} - C_{34}[a]	1008 (1160)	504 (556)	1172 (1824)	430 (532)
CPI	15.9 (15.5)	19.2 (19.3)	14.0 (12.3)	9.0 (10.2)
Cmax	C-26/C-16 (C-26/C-16)	C-26/C-16 (C-26/C-16)	C-26/C-16 (C-28/C-16)	C-26/C-16 (C-26/C-16)
MC#	25.6 (25.7)	25.7 (26.1)	25.7 (25.5)	25.3 (26.0)
alkenoic acids[a,b]				
$C_{16:1}$	0 (3)	1 (1)	3 (2)	3 (17)
$C_{18:2}$	0 (0)	0 (1)	0 (0.5)	0.2 (1.5)
$C_{18:1}$	20 (13)	18 (38)	31 (5)	3 (13)
phytol[a]	44 (77)	20 (14)	65 (15)	14 (38)
sterols[a,c]				
$C_{27}\Delta^{5,22}$	0 (0)	0 (0)	7 (0)	1 (0)
$C_{27}\Delta^{5}$	29 (21)	7 (12)	64 (18)	8 (4)
$C_{27}\Delta^{0}$	10 (5)	3 (7)	38 (12)	4 (2)
$C_{28}\Delta^{5,22}$	0 (8)	0.5 (7)	9 (5)	0 (0)
$C_{28}\Delta^{5}$	10 (17)	2 (5)	15 (5)	3 (2)
$C_{28}\Delta^{0}$	0 (5)	0 (0)	14 (12)	0 (1)
$C_{29}\Delta^{5,22}$	9 (7)	0 (7)	27 (8)	3 (0.5)
$C_{29}\Delta^{5}$	62 (33)	14 (19)	112 (28)	16 (8)
$C_{29}\Delta^{0}$	49 (22)	6 (12)	55 (13)	11 (6)

For calculation of CPI and MC#, see Section 2.7.

[a] Concentrations quoted in μg g^{-1} of dry sediment

[b] Shorthand nomenclature for alkenoic acids: C-x:y, where x = carbon number and y = number of double bonds. Note that only the concentrations of the major compounds are listed.

[c] Sterol nomenclature: $C_{27}\Delta^{5,22}$ cholesta-5,22-dien-3β-ol; $C_{27}\Delta^{5}$ cholest-5-en-3β-ol; $C_{27}\Delta^{0}$ 5α(H)-cholestan-3β-ol; $C_{28}\Delta^{5,22}$ 24-methylcholesta-5,22-dien-3β-ol; $C_{28}\Delta^{5}$ 24-methylcholest-5-en-3β-ol; $C_{28}\Delta^{0}$ 5α(H)-24-methylcholestan-3β-ol; $C_{29}\Delta^{5,22}$ 24-ethylcholesta-5,22-dien-3β-ol; $C_{29}\Delta^{5}$ 24-ethylcholest-5-en-3β-ol; $C_{29}\Delta^{0}$ 5α(H)-24-ethylcholestan-3β-ol. Note that only the concentrations of the major sterols are listed.

Table 4. Typical concentrations (mg/g TOC) of selected pyrolysis product for Gormire Lake sediments (Core G3) and catchment materials.

Compound class[1]	Peat[2]	Woodland Soil[3]	Grassland Soil[4]	LPZ1 (261 cm depth)	LPZ2 (137 cm depth)	LPZ3 (91 cm depth)	LPZ4 (17 cm depth)
Syringyl phenols							
Syringic acid	3.09	1.11	2.99	8.24	3.10	5.82	1.88
Syringaldehyde	7.27	0.18	1.07	2.15	4.38	1.36	0.17
Acetosyringone	0.13	0.02	0.44	2.56	9.06	2.41	0.02
Other[5]	0	0	0	16.08	19.64	17.68	19.41
Vanillyl phenols							
Vanillin	5.89	4.0	2.0	4.05	1.52	2.94	1.27
Vanillic acid	7.87	9.33	4.72	2.36	0.65	1.54	0.57
Other[6]	2.09	2.68	2.69	13.86	12.11	12.87	19.41
Cinnamyl phenols							
p-Coumaric acid	2.39	1.40	7.80	1.15	1.71	3.56	0.51
Ferulic acid	3.94	2.16	16.86	2.82	1.33	3.36	0.99
Other[7]	2.52	1.47	0.87	6.86	27.34	15.36	14.09
TOTAL Phenols	**34.69**	**22.35**	**39.43**	**60.13**	**61.2**	**66.9**	**58.32**

[1] Compounds were determined as their methylated derivatives. Only the named compounds in the Table were included in the syringyl (S), vanillyl (V) and cinnamyl (C) pools.

[2] **Taken at the south side of the lake above the escarpment (see Section 2.1)**.

[3] A surface sample taken from below mixed deciduous woodland at a site on the south west lake.

[4] A light brown sandy soil, collected from improved grassland at the top of a small slope at the south end of the lake.

[5] Other compounds include: 1,2,3-trimethoxybenzene and 1,2,3,trimethoxy-5-(2-propenyl)benzene.

[6] Other compounds include: 1,2-dimethoxybenzene, 3,4-dimethoxystyrene, 3,4-dimethoxytoluene, acetoguaicone and isoeugenol.

[7] Other compounds include: methoxybenzene, 4-methoxystyrene, benzoic acid, 3-methoxybenzoic acid, 4-methoxybenzoic acid, 4-methoxybenzaldehyde, 4-methoxybenzophenone, 4-methoxytoluene and 1methoxy-4-(1-propenyl)benzene. Acids were present as their methyl esters.

8. Figure Legends.

Figure 1. Total supported and unsupported ^{210}Pb activity versus depth in core GCB from Gormire Lake.

Figure 2. Reconstructed chronology for core G3. Key: mag – magnetic properties; NAP, non-aquatic pollen; K, elemental composition; n-alk, *n*-alkane carbon number. For details, see Section 3.1.

Figure 3. Pollen derived from trees and shrubs compared with TOC and TN concentrations for core G3 from Gormire Lake. Key: Shaded area - % tree and shrub pollen. Unfilled triangles – %TN. Filled diamonds – %TOC.

Figure 4. C/N (wt) ratio and reconstructed organic carbon flux for core G3. Key: Rectangles – C/N ratio; Filled circles – reconstructed carbon flux. For details, see Section 3.1.

Figure 5. Representative total ion current (TIC) chromatograms of Gormire lake sediment samples < 63 μm fractions (Core G3; depth 17 cm; LPZ 4; > 63 μm fractions were very similar and are not shown). a. Neutral fraction. b. Acid fraction. For GCMS conditions see Section 2.6.

Figure 6. Down-core profile of total unsaturated acids and *iso* and *anteiso* acids (< 63 μm ;Gormire core G3; Distributions of unsaturated acids are dominated by $C_{16:1}$ and $C_{18:1}$, with minor contributions from $C_{18:2}$ and $C_{17:1}$; see Table 3) (< 63 μm; Gormire core G3). Filled triangles – total *iso*- and *anteiso*- acids. Filled circles – total alkenoic acids.

Figure 7. Mass spectrum of 28-carboxyurs-12-enol (*bis*-TMS derivative).

Figure 8. Comparison of % pollen from trees and shrubs with the concentration of 28-carboxyurs-12-enol (> 63 μm sediment fraction, Gormire Core G3).

Figure 9. Down-core profile of mean carbon number (MC#) for *n*-alkanes plotted against % pollen derived from grass (> 63 μm sediment fraction, Gormire core G3).

Figure 10. Down-core profile of syringyl, guaiacyl and cinnamyl phenols (Table 4) in Gormire lake sediments.

Figure 11. Down-core profile of C/V (Table 4) ratio in Gormire core G3.