

Tracing the origin and fate of lipids in soils from the Rothamsted Classical Experiment

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Soil lipids originate from both plants and animals as products of deposition, exudation, and decomposition as well as various other sources, such as fungi, bacteria and mesofauna. The composition, concentration and diagenetic fate of soil lipids varies between environments due to differences in sources of organic matter and changes in inorganic mineral components (Miller and Donahue, 1995). For example, it has been shown that a relatively greater lipid content is found in soils with a low microbiological activity, whilst soil pH also affects lipids with strongly acidic environments exhibiting higher lipid contents (Fridland, 1976). Lipids can exist in a number of microenvironments within the soil being either free entities in the soil matrix, chemically bound components of humic material or physically adsorbed to clay particles (Jambu *et al.*, 1978). In this paper we will consider the lipid compositions of soils sampled from the Rothamsted Classical Experiment. The research employed a combination of analytical techniques to investigate both free, solvent extractable components, and those associated with insoluble residues of the vegetation and soils in order to assess the fate of the various lipid classes present in the original biological inputs.

The Rothamsted Classical Experiment. This site is the oldest and best-documented agricultural site in the world. Long-term experiments have been in progress for more than 150-years and records detailing histories of use are available. It consists of plots which have supported continuous growth of arable crops such as barley (Hoosfield Spring Barley) and wheat (Broadbalk), as well as continuous grassland (Park Grass Experiment) and other areas which have been allowed to revert back to natural woodland following cultivation (Broadbalk Wilderness and Geescroft Wilderness). Thus, this site provides a unique opportunity to study the effects of changing vegetation and agricultural practices on solvent

soluble organic matter and thereby improve our understanding of the fate of soil lipids. The work focused on: (i) characterising the lipid composition of soils with well-defined biological inputs, (ii) assessing the fate of specific lipid classes in a soil profile relative to biomacromolecules, and (iii) determining the factors which control the preservation of soil organic matter.

Lipid composition of soils with known recent histories of use. Broadbalk Wilderness is composed of three distinct sub-plots each of which exhibits a different type of overlying vegetation (i.e. woodland, grassland and mixed herb- and grassland). Analyses were made on total lipid extracts using high temperature gas chromatography (HT-GC) and HT-GC mass spectrometry (HT-GC/MS). Samples of vegetation were examined in parallel with underlying soils in an effort to follow the fate of major plant components. A number of different compound classes were identified including, *n*-alkanes, *n*-alkanols, (hydroxy-) *n*-alkanoic acids, steroids, triterpenoids, wax esters, mono-, di- and triacylglycerols. Lipids from the woodland are dominated by inputs from leaf-derived components. In contrast, the lipid extracts of soils from the grassland and mixed vegetation area are markedly different reflecting the mixed vegetation cover dominated by *grass species*. Particularly important classes of compounds are the *n*-alkanols and wax esters, i.e. a very strong predominance exists of the C₂₆ alkanol (and wax esters based on this alkanol) in the soils with grass input whereas the alkanols and wax esters of the woodland are dominated by the C₂₄ homologue. These data quite clearly indicate that molecular signals derived from the standing vegetation are reflected in the soils.

Fate of specific lipids in a soil profile. The main soil horizons from another reverted woodland (Geescroft Wilderness) were studied to determine the fate of

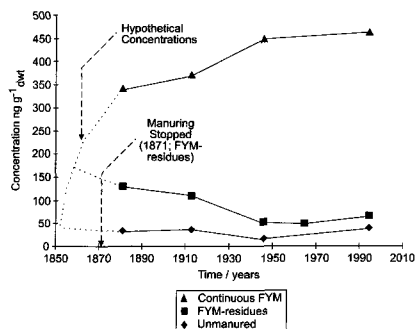


FIG. 1. Plots depicting the absolute concentration of 24-ethyl-5 β -cholestan-3 β -ol (5 β -stigmastanol) in the Unmanured, FYM-residues and Continuous FYM soils from the Rothamsted Classical Experiment. The results confirm that a biomarker signal for manuring still persists 124 years after cessation of manuring in 1871.

various lipid classes in a soil profile. In this case the main input was oak (*Quercus robur*) leaf litter (Jenkinson *et al.*, 1992). Thus, studying both the leaf litter and the underlying soil horizons would provide information about the long-term survival and mixing of lipids from the earlier and current vegetation. HT-GC shows the composition of the humic horizon to be dominated by components that can be related to the most recent input, namely the standing vegetation which is dominated by *Quercus*. Major changes that occur in the lipids between the leaf litter and the humic horizon correspond to losses of sterols and triacylglycerols, presumed to be largely as a result of their utilisation/degradation by soil fauna and micro-organisms. This results in a dominance of the triterpenoid components which are present in the original litter and appear to be highly resistant to degradation compared with other compound classes. The lipid components of the humic layer and mineral soil appear to represent an input of *Quercus* derived organic matter combined with extant lipids possibly derived from vegetation which grew previously at the site. Fractionation of the total lipid extract and GC analysis of the hydrocarbons, alcohols and *n*-alkanoic acids reveals the most dramatic changes in their distributions to occur between the decomposing leaf litter and the humic rich layer. Methyl ketones are abundant in the humic layer, most likely arising from oxidation of their *n*-alkane analogues (Amblès *et al.*, 1993). These data indicate that while molecular signals from the standing vegetation are apparent in the mineral soil, mixing was far from complete, such that signals from the earlier vegetation are still apparent 118 years after reversion.

Temporal changes in lipid distributions and concentrations. The pedological persistence of vegetation and manure derived lipids was assessed using soils which had been intensively cultivated (Hoosfield Spring Barley Experiment). Total lipid extracts were obtained from soil samples taken in 1882, 1913, 1946, 1963 and 1995 from three treatments. One had received farm-yard manure (FYM) continuously since the experiment started in 1852, one was initially manured from 1852-1871 and the third treatment had never received FYM since the start of the experiment. The total lipid extracts were fractionated and molecular analyses performed using GC and GC/MS. In addition to the soil samples (contemporary and archived), the two primary organic inputs, barley and FYM, were also studied. The major aliphatic soil lipid components exhibit variable dominance with respect to the expression of barley and FYM derived lipids. Wax ester distributions are of low abundance and too strongly affected by degradation and transesterification processes to identify a dominant input whilst the composition of soil *n*-alkanols is largely determined by FYM with a minor pedogenic input. *n*-Alkanoic acids increase in overall abundance in soils with a continual FYM input and show appreciable degradation in soils receiving no manure. Measurements of absolute concentrations of 5 β -stanols, biomarkers characteristic of manuring, reveal that a manuring signal persists for over 120 years within the soil which had been intensively cultivated and had received no manure since 1871 (Fig. 1).

Effect of soil pH on the preservation of various compound classes. Total lipid extracts and solvent insoluble organic matter from soils at Park Grass, Rothamsted Classical Experiment, UK were studied to determine the effect of pH on the preservation/degradation of plant derived biomolecules in soil organic matter. The plots selected for study possessed different pH values ranging from 3.7 to 7.1, with soils of pH<5 being composed of two distinct soil horizons (i.e. humic rich top layer and mineral soil). The total lipid extracts of the soil samples with low pH reveal higher relative abundances of organic acids. These are believed to derive largely from oxidation of plant derived lipids of previous vegetation. The vegetation signature in the low molecular weight fraction is only retained in the humic rich top layer. The signal in the mineral layer is believed to derive primarily from previous vegetation. Compound specific stable carbon isotope ($\delta^{13}\text{C}$) measurements of long-chain alkanols demonstrate that differences in the rate of incorporation of vegetation lipids into the humic top layer are related to the pH and the grass species dominating the standing vegetation.