

EARLY DEGRADATION OF PLANT LIPIDS IN SOILS : A FIELD EXPERIMENT USING ¹³C-LABELLED LEAVES

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Lipids are important components in soil organic matter (SOM) due to their hydrophobic nature and their high reactivity toward polyvalent cations. Indeed, they influence aggregate stability, water retention and fertility of soil. Moreover, lipidic molecules can constitute powerful biomarkers to trace the source and degradation pathways of SOM. Higher plants are among the major contributors of SOM. However, limited studies focused on the effects of degradation on plant lipids in soil. While plant lipids comprise a number of typical plant biomarkers, they also include several ubiquitous compounds that also occur in soil microorganisms such as fungi and bacteria. A crucial issue in studying the evolution of soil lipids is determining the exact origin (e.g. higher plant vs microbial) of such ubiquitous compounds. Therefore, to trace the original plant lipids, or their degradation products, and distinguish them from exogenous microbial molecules, we set up a degrading field experiment with ¹³C-labelled organic matter. ¹³C-Enriched leaves were obtained by growing young beech trees (*Fagus sylvatica*) under ¹³C-enriched CO₂ in controlled chamber for 6 months. ¹³C-labelled leaves and leaves of natural isotope composition were enclosed in litter bags and let to degrade in a forest soil (Breuil-Chenué experimental forest, Parc Naturel Régional du Morvan, France) for 18 months. This study is the first molecular investigation of ¹³C-enriched litter bags within a field experiment.

The litter bags were retrieved from the field after 5, 10, 15, 20, 30, 40, 52 and 79 weeks. After removal of extraneous material, leaf lipids were solvent extracted, fractionated on column chromatography and analysed by gas chromatography coupled to quadrupolar and isotope ratio mass spectrometry. Total leaf lipids accounted for 11.3 wt% of the dried senescent leaves and underwent a weight loss of 74% of the initial solvent extractable lipids after 18 months of degradation. The bulk weight of the leaves and their weight/area ratio undergone a total loss significantly lower than that of lipids (i.e. -44% and -18%, respectively) indicating that lipids, as a whole, were more degraded than the non-lipid constituents of beech leaves. Microscopic examination showed that the morphological aspect

of the leaves was strongly affected by degradation, with increasing herbivory holes and progressive invasion by animal faeces, fungi and bacteria, as degradation took place.

GC-MS analyses revealed that the total extract of beech leaves mainly corresponds to a complex mixture of classical components of Angiosperm lipids and their degradation products. They also contain a few typical fungal molecules. Quantification of the main components of the extracts showed that they were all affected by degradation but at different rates, and the following stability scale was established: *n*-alkanes > β -sitosterol > *n*-alcohols > *n*-aldehydes > phytadienes > *n*-acids > squalene > phytol esters > α -tocopherol. *n*-Alkanes are rather ubiquitous components and their apparent high stability may reflect either a good preservation of the original alkanes or a contribution from exogenous alkanes. Indeed, several fungi and bacteria can produce alkanes of chain length similar to that of higher plants.

Specific carbon isotope composition of *n*-alkanes was analysed to further investigate their fate through degradation. The *n*-alkanes extracted from natural leaves exhibited rather constant individual isotope composition (Fig. 1), which would lead to favour the hypothesis of good preservation of the original alkanes.

However, the *n*-alkanes extracted from the ^{13}C -enriched leaves showed a significant decrease in their individual isotope composition all along the degradation experiment (Fig. 1). This is interpreted as the result of increasing contribution from exogenous *n*-alkanes, probably originating from the microorganisms progressively colonizing the leaves. Therefore, the

apparent relative stability of *n*-alkanes may be an artefact due to contributions from microbial *n*-alkanes. These results emphasize the need for caution in using the occurrence and isotope composition of rather ubiquitous molecules such as *n*-alkanes for palaeoenvironmental purposes. Comparing the isotope composition of specific components of natural, and ^{13}C -labelled samples can thus provide detail insights into the fate of organic molecules through degradation. Specific isotope characterization of the other lipid components from degrading beech leaves are under progress and should help in better understanding the fate of plant lipids in soils.

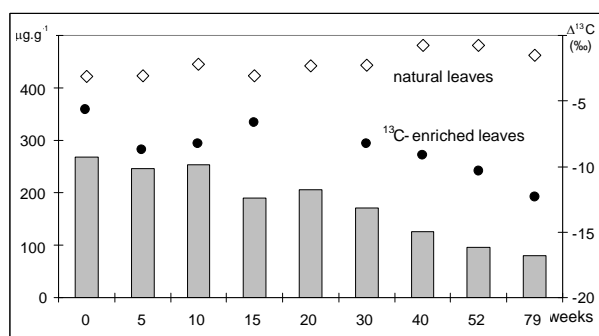


Figure 1. Evolution of concentration (bars) and specific ^{13}C composition of *n*-heptacosane (◇, ●) extracted from degrading beech leaves.

$$\Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{heptacosane}} - \delta^{13}\text{C}_{\text{bulk}}$$

TURNOVER OF CUTIN AND SUBERIN IN A CROPLAND SOIL

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Plant residues and microorganisms are the main sources of organic matter in cropland soils. However the contrasting effects of humification and degradation processes turning the fresh plant material into stabilised soil organic matter (SOM) remain unclear. One major question concerns the contribution of aboveground versus belowground plant residues to SOM. To address this question, we analysed some biomarkers originating from biopolyesters which are specific for these tissues in non-woody plants. Indeed, cutin and suberin are supposed to be present respectively in the aboveground and the belowground tissues of higher plants. Cutin is an amorphous polyester usually constituted of carboxylic acids with an aliphatic chain length of 16 and 18 carbon atoms, bearing epoxy and hydroxy groups. Suberin is a structured polyester mostly composed of carboxylic acids, ω -hydroxy carboxylic acids and diacids. The particularity of the suberin is the large amount of monomers with a carbon chain length higher than 20 carbon atoms. The monomeric composition and the quantification of these macromolecules can be obtained through saponification and GC/MS analysis of the silylated derivatives.

In the present study we quantified these biopolyesters in several parts (leaves, stems and roots) of two plants, wheat and maize. These plants were chosen because of their different biosynthetic pathway (C3 and C4, respectively), hence different ^{13}C contents. Incorporation of these monomers in soil can therefore be followed by measuring the ^{13}C content of the same monomers in a soil on which a maize cropping replaced a wheat cropping. The chronosequence studied here is that of the Closeaux experimental field, onto which the change in cropping occurred up to 12 years ago.

Six classes of compounds were released upon saponification of the leaves, stems and roots of wheat and maize samples: carboxylic acids, alcohols, ω -hydroxy carboxylic acids, α -hydroxy carboxylic acids, diacids and mid chain hydroxylated compounds. As expected, mid chain (C_{16} and C_{18}) hydroxylated compounds dominated the monomers from leaves and stems. However differences in their distribution are noted between the two parts of the plant and also between wheat and maize. In wheat and maize roots, the ω -hydroxy carboxylic acids contributed for ca.50% of the total monomers with a significant contribution of the C_{20} to C_{28}

monomers, characteristic for suberin. Diacids from C₁₆ to C₂₂ were also released, but in lower amount than ω -hydroxy carboxylic acids (ca.18%). However diacids are characteristic for roots, since they were not detected in the other parts of the plants. In soils, the most abundant monomers released were the hexadecanoic acid (C₁₆), followed by the mixture of the 16,x-dihydroxy hexadecanoic acid isomers (Di-OH C₁₆), the octadecenoic acid, the ω -hydroxy docosanoic acid (ω C₂₂), the ω -hydroxy tetracosanoic acid (ω C₂₄), the octadecanoic acid (C₁₈) and the 9-hydroxy hexadecanedioic acid (9-OH DC₁₆). Carboxylic acids are not specific enough to be used to distinguish between the aboveground or the belowground sources. On the contrary, the Di-OH C₁₆ and 9-OH DC₁₆ monomers can be assigned to cutin polyester whereas the ω C₂₂ and the ω C₂₄ monomers are characteristic for root suberised tissues. Based only on the monomer concentrations in soils, the contribution of the two biopolymers into SOM can be assessed. However, to estimate their incorporation into SOM, the natural ¹³C labeling of the monomers is needed.

In wheat plant the $\delta^{13}\text{C}$ values ranged from -24‰ to -37‰ depending on the monomer. For maize, the $\delta^{13}\text{C}$ of the monomers ranged from -14‰ to -23‰. Carboxylic acids with C₁₆ and C₁₈ carbon atoms were enriched in ¹³C compared to mid chain carboxylic acids. However, no tendency in the ¹³C content was observed to distinguish between the monomers present in the three parts of the plant. In soils, an enrichment in ¹³C with years of maize cropping was observed due to maize incorporation. Nevertheless these changes depend on the considered monomer. For example, the $\delta^{13}\text{C}$ value of the carboxylic acid C₁₆ was -26‰ in soil under wheat and reached -23‰ after 12 years of maize cropping whereas no changes were detected in Di-OH C₁₆ $\delta^{13}\text{C}$ values. An increase of ¹³C was generally observed for other (mono/poly)-hydroxy carboxylic acids such as ω C₂₂, ω C₂₄ or 9-OH DC₁₆ monomers.

These results evidenced 1) the different behaviors of cutin and suberin in soils during the degradation and/or preservation processes; 2) a selective incorporation of some monomers for a same source of monomers (cutin or suberin) These contrasted behaviours of the monomers can be related to their chemical functions and their position into the polymeric structure.

BIOGEOCHEMICAL PARAMETERS SURROGATED TO THE RECOVERY OF SOILS AFTER FOREST FIRE EVENTS

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Forest fires are considered as the main disturbance in the Mediterranean basin (Naveh, 1990) and exerts both immediate and lasting ecological and environmental impacts. This is in part caused by a depth transformation of soil physical, chemical and biological characteristics associated to qualitative and quantitative changes in the organic matter (OM) (González-Pérez *et al.*, 2004) which is reflected in the structure of the whole system including microbial populations (Pastor and Post, 1986; Pietikäinen *et al.*, 2000). This negatively affects soil health and quality favouring the occurrence of erosive processes and the lost of a non-renewable natural resource such is soil.

However, the effects of fire on soil colloidal properties and on the microbial community are reversible. After the passage of fire soil properties may naturally revert to a situation close to that before fire; erodibility is reduced and soil functionality is again achieved (Cerdá, 2004). This is especially evident in the so-called cold fires including controlled burnings and fires in open forest at semiarid environments with pyrophytic vegetation (Mutch, 1970; Pyne, 1996). The starting hypothesis is that if reversible effects do occur, then we may be able to find out biogeochemical surrogated indicators informing on the recovery of soil quality. An early detection of the impact of fire in soil and the possibilities for soil recovery is relevant for decision making and planning of environmental restoration actions.

The objective of this work was the identification of proxies for the recovery potential of soils affected by forest fires. This was approached by a transdisciplinary study of fire affected soils at different time scale (cronosequences) chosen in a variety of scenarios from Andalusia (Southern Spain). Included were different soil types affected by fires during the past 10 years under different vegetation, located close to control soils with no recent history of forest fires.

Changes in soil chemical properties and in different forms of OM (free lipid fractions, fulvic acids and humic acids and other highly resilient forms of C that may be incorporated

like charred biomass) were studied by different techniques including molecular-level analytical techniques (chromatographic, spectroscopic and degradation methods). The effect of fire on soil microbiota was approached using standard techniques for biodiversity estimation (viable counts in adequate culture media, microbial total count by fluorescence microscopy), microbial activity (soil respiration and SIR) as well as by the use of specific molecular techniques (DGGE, FISH and cloning 16S rRNA) and by establishing physiological profiles (enzyme activities) (Arias et al., 2005).

Among the main results obtained is the detection of markers to monitor the recovery of soils short time after fire events by the analysis of thermoevaporation products, mainly furan derivatives, released from whole soil samples using sub-pyrolysis temperatures. Shifts in fatty acid patterns and other families of pyrolysis products from soil were observed by conventional Py-GC/MS. Extracted soil lipids were also a valid proxy and differences were found in the distribution pattern of certain aliphatic series and terpenes. Main results obtained on the biodiversity analysis through conventional, biochemical and molecular techniques allow to discriminate different patterns of microbial populations between fire affected soils and unburned soils

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TRACKING SOIL ORGANIC CARBON TRANSPORT TO CONTINENTAL MARGIN SEDIMENTS USING SOIL SPECIFIC BACTERIOHOPANEPOLYOL BIOMARKERS

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The transport and subsequent deposition of terrestrially derived organic matter into the ocean is an important but poorly constrained aspect of the modern global carbon cycle. In regions associated with large river systems it is likely that the terrestrial input of organic carbon is much more complex than commonly considered and very difficult to trace based on established geochemical proxies. It is therefore important to develop proxies that target the movement and fate of this terrestrial organic material.

The identification of bacteriohopanepolyol (BHP) biomarkers unique to soil derived organic carbon (SOC) has enabled the transport of SOC into aquatic sediments to be traced. The extreme recalcitrance of BHPs enables these source specific compounds to be used on recent and ancient sediments to identify periods of high and low SOC input into sediments.

BHPs are bacterial membrane compounds with a high degree of structural variability. They are analogous to steroids in eukaryotes and have been identified in over half of all bacteria studied for their presence. BHPs have a wide range of over 40 functional groups on the side chain, with up to 6 functional groups in each structure, and with methylation and unsaturation over 100 total structures have been identified¹.

During the BHP analysis of a wide range of soils from around the world consistently high levels adenosyl hopane (m/z 788; Fig. 1), known to originate from purple non-sulphur, nitrogen fixing and ammonia oxidising bacteria and 2-methyl adenosyl hopane (m/z 802; Fig. 1)², from nitrogen fixing bacteria were identified. Only 2 lacustrine sediments with large SOC supply from their catchments areas have been found to contain these markers in a survey of over 40 different recent marine and non-marine settings.

The Rhone River is the major freshwater input into the Mediterranean with sediment accumulation rates of 20 cm^y⁻¹ and an annual discharge of 7-10 10⁶ tonnes³. First analysis of surface samples taken from a core transect across the shelf confirms the presence of both adenosyl and 2-methyl adenosyl hopane in the sediment. The concentrations of both BHPs decrease with distance from the coast, suggesting progressive dilution of the terrestrial

organic signal with marine sources. These first results from the Gulf of Lions are supported by recent results on non-isoprenoidal glycerol dialkyl glycerol tetraethers (GDGTs) in the same area, more specifically the branched and isoprenoid (BIT) index³.

Recent studies on Late Quaternary sediments from the Congo deep sea fan (OPD site 1075, ~2 km water depth) provide a strong case to expect markers for SOC⁴. An initial analysis of the core samples confirms the presence of soil specific BHP markers in each sample analyzed down to 89 m depth in addition to the presence of common sediment associated BHPs (see Fig. 1). Concentrations of soil markers are high in the upper sediment section down to about 49 m supporting the case for these molecular markers as novel proxies for SOC supply and burial. Distinct peaks of adenosyl and 2 methyl adenosyl hopane at about 200, 300 and 550 kyrs tentatively imply that the rate of terrestrial organic matter discharge from tropical Africa significantly increased at these times, possibly associated with periods of reduced soil stability in the Congo catchment.

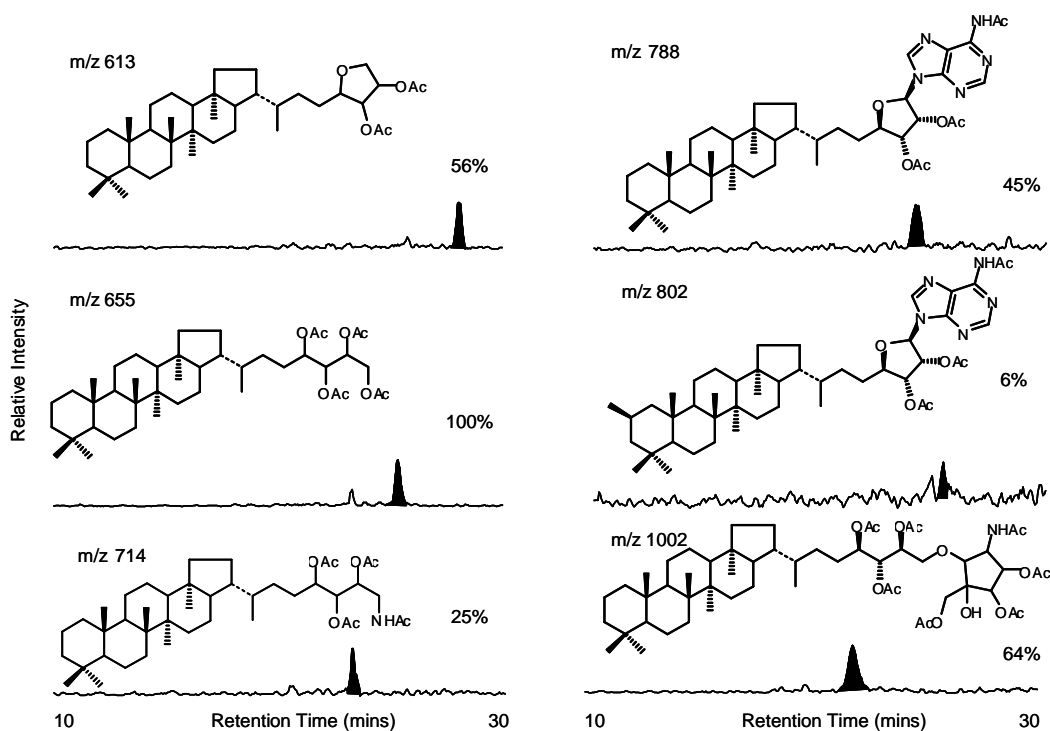


Figure 1. APCI mass chromatograms and relative intensity of the major BHPs found in Congo fan sediment age approximately 300 kyrs. (BHPs analysed as peracetate derivatives; Ac = COCH₃)

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