



## Early degradation of plant lipids in soil: A field experiment using $^{13}\text{C}$ -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  $^{13}\text{C}$ -labelled organic matter.  $^{13}\text{C}$ -Enriched leaves were obtained by growing young beech trees (*Fagus sylvatica* L.) under  $^{13}\text{C}$ -enriched  $\text{CO}_2$  in controlled chamber for 6 months.  $^{13}\text{C}$ -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  $^{13}\text{C}$ -enriched litter bags within a field experiment.

The litter bags were retrieved from the field after 5, 10, 15, 20, 30, 40, 52, 79 and 129 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 90% of the initial solvent extractable lipids after 2,5 years 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. -48% and -17%, 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:  $\beta$ -sitosterol > *n*-alkanes > *n*-alcohols > *n*-aldehydes > phytadienes > *n*-acids > squalene > phytol esters >  $\alpha$ -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, which would lead to favour the hypothesis of good preservation of the original alkanes. However, the *n*-alkanes extracted from the <sup>13</sup>C-enriched leaves showed a significant decrease in their individual isotope composition all along the degradation experiment. 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 <sup>13</sup>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.