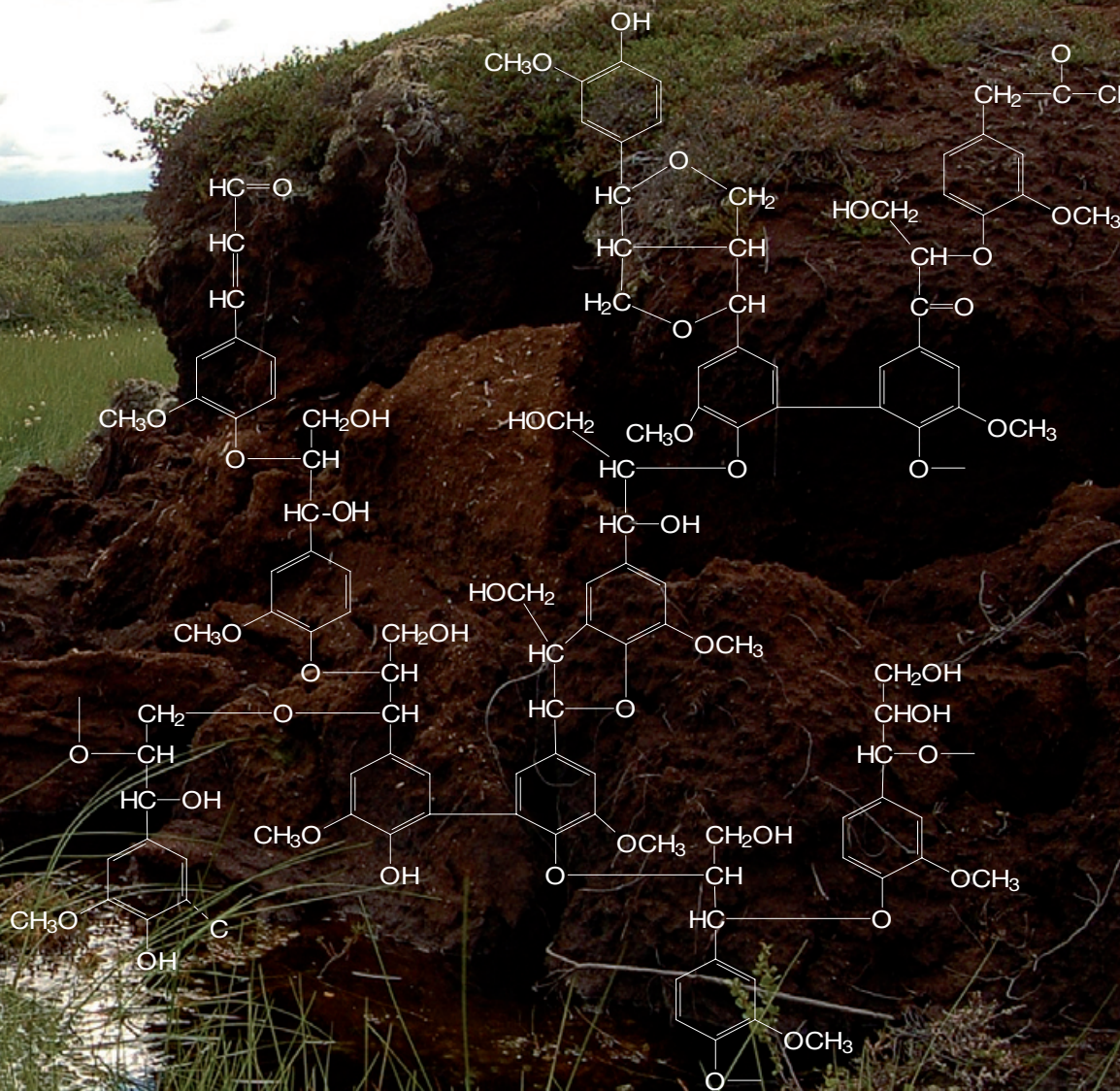


**NATURAL MOLECULAR STRUCTURES
AS DRIVERS AND TRACERS OF
TERRESTRIAL C FLUXES (MOLTER)**

Standing Committee for Life, Earth and Environmental Sciences (LESC)



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- Nuclear Physics
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Introduction

Soil organic matter (SOM) represents the largest terrestrial carbon (C) reservoir. This C is stored in the form of a highly complex mixture of organic molecules. A wide range of plant molecules enters the soil system, from soluble amino acids to structural lignin polymers, from labile starch to recalcitrant tannin structures, from hydrophilic sugars to hydrophobic alkanes. Depending on their properties, such as size, structure and functional group content, as well as soil and climatic conditions, these molecular structures undergo (i) abiotic reactions, (ii) are directly adsorbed on mineral surfaces, or (iii) assimilated by soil microbes. New chemical structures are produced, such as microbial amino sugars and phospholipid fatty acids. The molecular nature of these various compounds is therefore a determining factor for C stabilisation in soils. Certain compounds might be strongly adsorbed on soil mineral surfaces and become directly stabilised. Other compounds will be quickly assimilated by the microbial biomass. The growth yield efficiency of soil microbes,

i.e. their ability to maximise C assimilation versus CO₂ losses, also depends on the molecular structure of the assimilated compounds. Multiple non-biotic reactions can further alter the molecular composition of soil organic matter.


Molecular tracers are records of the origin and history of chemical transformations of soil organic compounds. Small variations in the chemical composition of plant molecules can yield important clues about the plant species and organs from which they originate. The ¹³C isotopic signature of plant molecular families is characteristic of the type of photosynthetic pathway used by the vegetation. In soils under vegetation-type transitions, the resulting isotopic contrast at the molecular level provides us with a powerful tool to investigate the turnover rate of these compounds in the soil environment. Microbial activities affecting these compounds can also be traced with molecular methods. The alteration degree of certain molecules tells us about the environment in which they have evolved. MOLTER promotes the investigation of the molecular mechanisms controlling C fluxes in terrestrial ecosystems.

The story of C stabilisation in soils is one of complex molecular transformations. However, up to now, the wealth of information on carbon cycling contained in the chemical structure of the soil organic matter remained little exploited for lack of appropriate methodology. This is now changing rapidly due to the development of environmental isotopic and organic chemistry techniques. Examples of these techniques are ¹³C and ¹⁵N nuclear magnetic resonance, chemolysis and multiple pyrolysis systems associated with gas chromatography and compound specific isotope ratio mass spectrometry (IRMS), as well as thermal analyses coupled to mass spectrometers (MS) and IRMS.

MOLTER focuses on five major research themes in order to stimulate and develop knowledge on natural molecular structures and their role as drivers and tracers of terrestrial C fluxes:

1. Molecular composition and turnover time of soil organic matter
2. Plant molecular structures as drivers of C stabilisation in soils
3. Fire transformations of plant and soil molecular structures
4. Molecular markers in soils
5. Dissolved organic molecules in soils: origin, functionality and transport

The running period of the ESF MOLTER Research Networking Programme is five years from March 2008 to February 2013.



What is MOLTER?

Natural Molecular Structures as Drivers and Tracers of Terrestrial C Fluxes (MOLTER) is an ESF Research Networking Programme aimed at stimulating European research on isotopic and organic chemistry exploration of carbon stabilisation and biogeochemistry in terrestrial ecosystems and soils in particular.

Our understanding of the formation, stabilisation and decomposition of complex organic compounds in the environment (e.g. organic matter in plants, litter, soils and water) is currently being revolutionised by advanced techniques in identification, quantification, and origin tracing of functional groups and individual molecules.

MOLTER promotes exchanges of ideas on emerging concepts and paradigms in this field of research. In particular, MOLTER recognises the need to further promote research on:

1. the continued development of new analytical techniques,
2. the inter-comparison of results from different techniques,
3. the creation of molecular databases on studied ecosystems,
4. the development of molecular modelling from soil to ecosystem levels.

Research Themes

Theme 1: Molecular composition and turnover time of soil organic matter

Molecularly uncharacterised organic matter comprises most organic C in soils and other terrestrial systems. Contrasting molecular structures display contrasting properties that ultimately govern their fate in natural environments and also control major soil functions. SOM molecular properties that relate to soil functions are not only the concentration of organic matter, but also its hydrophobic or hydrophilic character that controls the soil water-holding capacity. The affinity of SOM to the soil mineral matrix is decisive for the formation of soil structure. The origins, reactions and fates of these ubiquitous organic materials are relatively obscure, in large part because the rich vein of chemical information that typically derives from detailed structural and stereochemical analysis is yet to be tapped.



Figure 1. Soil in a burned *Nothofagus-Araucaria* ecosystem in southern Chile

But molecular investigations have recently played a key role in the understanding of SOM by providing molecular-level details about its composition. New advanced techniques in organic and isotopic chemistry, both spectrometric and spectroscopic, are now giving us the capacity to probe for specific molecular structures of specific origin in soils and other natural environments. For example, ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy provides unsurpassed detail on the distribution of molecular functional groups within soils.

Pyrolysis (Py) coupled to mass spectrometry (MS) and isotope mass spectrometry (IRMS) allow us to analyse the distribution and turnover time of molecular structures and moieties within soil samples. Thermogravimetry and differential scanning calorimetry (TG/DSC) coupled to IRMS allow us to explore the turnover time of SOM across operationally defined fractions.

Research Theme 1 supports activities aimed at unravelling the molecular composition and respective turnover times of soil organic matter components.

Theme 2. Plant molecular structures as drivers of C stabilisation in soils

Plant molecular structures from above-ground residues and root activities represent the largest flux of C to the soil system. Only a small fraction of this large C input is eventually stabilised in soils. Understanding the molecular origin of this stabilised C is crucial in helping maximise energy output from plant residues while increasing C storage in soils. For a long time, the lack of appropriate in situ methods meant that the fate of C derived from specific molecular structures was probed through laboratory incubations.

These studies have heavily emphasised short-term mineralisation rather than long-term C stabilisation, while the latter gives the relevant information in terms of C storage in the context of global change studies. This overemphasis on short-term mineralisation has led to misinterpretations. For example, lignin has long been considered as a major contributor to stable soil organic C because of its short-term chemical recalcitrance to mineralisation, while recent studies suggest that its long-term stabilisation is not especially high. These recent studies are based on combined chemical (MS) and isotopic (IRMS) determination of molecular structures, which are isolated from soils either by wet chemistry or by pyrolysis. Models of the molecular-C flux on the field scale are being adapted to these emerging datasets. As a result, a new paradigm for the stabilisation of plant C derived from specific molecular structures is currently emerging. Recent investigations have addressed, among others things, macromolecules such as lignin, polysaccharides, tannins, cutins and suberins, and simpler molecules such as sugars and amino acids.

Research Theme 2 supports activities aimed at deriving a comprehensive picture of molecular C fluxes in the plant-soil continuum, from frontier methods in organic chemistry as well as from modelling and database development.

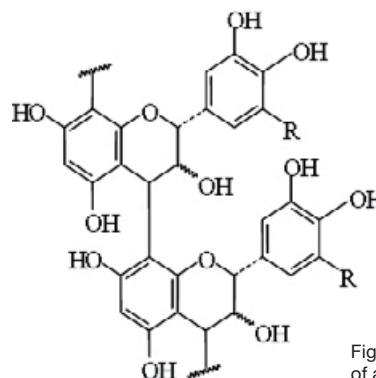


Figure 2. Structure of a condensed tannin

Theme 3. Fire transformations of plant and soil molecular structures

Wildfires have a substantial bearing on the long-term sequestration of C and N in forest soils. Some fire-affected ecosystems may act as sinks for atmospheric C, because fire events transfer C from high-turnover vegetation pools to refractory C pools displaying long-term geological turnover in soils. Most studies probing the formation of refractory C have focused on thermal degradation of lignocellulosic material.

Laboratory experiments have proved that forest fires or controlled burnings of crop residues induce a series of inter- and intra-molecular reactions leading to condensed materials with chaotic structure and a close resemblance to humic or kerogen-like matter. In addition, other biomolecules, such as peptides or lipids, may experience major alterations during heat exposure.

The simultaneous occurrence of different processes during forest fires makes it extremely difficult to accurately describe SOM transformations in burned areas.

These may range from the total loss of SOM in the top soil or the surface of swamps and peat deposits, to an increase in the passive SOM pool due to the incorporation of necromass from fire-affected vegetation, but also partially and heavily charred plant remains (highly refractory organic matter collectively referred to as 'black carbon') on the ground.



Figure 3. Wildfire destroying vegetation

Nevertheless, laboratory simulation experiments have unambiguously documented the selective destruction of humus fractions, the chemical alteration in SOM properties, and external inputs of charcoal and charred ligno-cellulose from the burned vegetation.

Research Theme 3 supports activities aimed at studying refractory molecular structures formed during fire events and the contribution of these structures to long-term soil C storage.

Theme 4: Molecular markers in soils

Biomarkers are a powerful tool to unravel the processes that govern organic matter transformations in soils. Among these, microbially driven processes are central. Knowing which microorganisms are active is essential for biogeochemical research. Since most microorganisms cannot be cultured (easily) in the laboratory, alternative molecular techniques have been developed, based on observations that specific biomarker molecules, characteristic of a specific type of microorganism present in an ecosystem, can be isolated and quantified. Linking these molecular biomarker techniques with compound-specific stable isotope techniques (e.g. GC-c-IRMS and LC-c-IRMS) provide additional advantages.

Labelled substrates can be introduced in an ecosystem via stable isotopes probing (SIP), e.g. via ($^{13}\text{C}_2$) pulse labelling of the vegetation. Transfer of ^{13}C via root exudates to active microorganisms makes it possible to identify metabolically active microorganisms according to the isotopic composition of their respective biomarkers. Because of the great specificity and structural diversity of the phospholipid fatty acid (PLFA) building blocks of cell membranes and because of their instability after necrosis, PLFAs are an ideal proxy for the microbial biomass.

Cell walls of fungi, bacteria and actinomycetes are partially constructed from amino sugars (AS) which can be used as biomarkers for microbial necromass. The relative and absolute amounts of AS in soil, together with their individual $\delta^{13}\text{C}$ values can be used to determine turnover times and contribution to C-sequestration of microbial soil communities. These state-of-the-art approaches in isotope and environmental ecology are essential to link microbiological ecology to biogeochemistry.

Biomarkers are also used to probe the origin and alteration degree of soil organic matter. For example, the biomarker potential of root- and shoot-derived aliphatic macromolecules (suberin and cutin) is currently under investigation in order to trace the origin of soil organic carbon. The acid-to-aldehyde ratio of soil lignin structures is a function of the lignin alteration degree, which can be soil formation specific and thereby help determine the origin of SOM, e.g. in sedimentary systems.

Research Theme 4 supports activities on soil biomarkers aimed at unravelling the microbial transformations and decomposition processes affecting soil organic matter, as well as tracing its origin in terms of plant species and organ type.

Theme 5: Dissolved organic molecules in soils: origin, functionality and transport

Dissolved organic molecules in the soil solution comprise a wide variety of compounds relating to their molecular mass or their hydrophilic/hydrophobic character. Some compounds, such as complex-forming organic ligands and siderophores, probably carry significant physiological and ecosystemic functions.

High-molecular mass molecules, operationally defined as humic acids, have been suggested to be supramolecular associations. The sources of dissolved organic molecules are mainly litterfall, root litter, root exudates and microbial production. Their sinks are adsorption and precipitation in the soil, mineralisation to CO₂ and leaching out of the soil. Investigating molecular structures helps us evaluate interactions in the plant-mycorrhiza-bacteria-soil system, and their impact on the ecosystem C balance. By using molecular techniques such as Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) or Inductively Coupled Plasma Mass Spectrometry (LC-ICP-MS) for studying complexation with metals, molecules of significance can be determined. Also near-edge structure spectroscopy (XANES) is used to study complexation with sulphur-containing molecules, and NMR techniques are used for identification of functional groups. By these and other techniques, the fate of dissolved organic molecules in the plant-soil-water continuum can be followed, which helps us understand its significance for terrestrial ecosystem functioning.

Research Theme 5 supports activities aimed at identifying, quantifying and fate-tracing of dissolved organic molecular structures in terrestrial environments.



Figure 4. Runoff from a Norwegian agricultural field

MOLTER will organise and/or support several activities addressing one or more of the above research themes:

- **Conferences.** For the five-year period two international science conferences are planned.
- **Summer schools.** One MOLTER summer school will be held, providing training to PhD students in relevant fields of research.
- **Scientific workshops.** Five workshops are planned for the five-year period, focusing on specific MOLTER research topics.
- **Exchange grants (long- and short-term),** to visit institutions in other participating countries in order to foster the exchange of knowledge at the European level.

Calls for selected activities will be opened twice a year, with application deadlines on 31 March and 30 September of each year. For more and up-to-date information please visit the MOLTER webpage: www.molter.no

All scientists actively focusing on any of the MOLTER Research Themes are encouraged to apply. Applications will be selected according to scientific excellence and in agreement with ESF priority rules (see “Guidelines for Management of ESF Research Networking Programmes”, www.esf.org/RNP-guidelines).

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For the latest information on this
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consult the MOLTER websites:

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