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## Resistant ultralaminae in soils

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**Abstract-** Observation of a chemically resistant organic residue from maize crop soils by transmission electron microscopy reveals the occurrence of thin laminae with 10-50 nm thickness, similar to resistant, highly aliphatic biopolymers found in kerogens and microbes. This discovery shows evidence for a novel and alternative mode of formation of soil organic matter by *selective preservation* of resistant biopolymers from soil microbes, in addition to the classical *recondensation* pathway proposed by the French chemist Maillard in 1917.

*Key words* - soil biopolymer, transmission electron microscopy.

### INTRODUCTION

Soil organic matter is one of the major pool of carbon on Earth. It has a key position in the global carbon cycle, for instance as a sink of atmospheric CO<sub>2</sub> and a source of sedimentary debris. The main part of soil organic matter is composed of complex, partly macromolecular, brown-yellow components called humic substances (Schnitzer, 1991). The precise biological origin, mode of formation and transformation of humic substances are so far poorly understood. As early as 1917, when he observed similar elemental compositions of natural humic substances and of condensation products of sugars and amino acids, the French chemist Maillard postulated that natural humic substances could be formed by recondensation of small molecules. More recently, numerous investigations in sediments have shown the existence of an alternative pathway of formation of sedimentary organic matter *via* selective preservation of resistant biopolymers (Tegelaar *et al.*, 1989, Largeau *et al.*, 1990, Derenne *et al.*, 1991). In a study of forest profiles, Kögel-Knabner *et al.* (1992) further suggested that aliphatic biopolymers appear to be selectively preserved with increasing depth. Moreover, we have shown recently on isotopic grounds that the bulk of crop organic matter must be formed *in situ*, either by selective preservation of resistant biopolymers from soil microbes or by recondensation of small molecules from exudates and degradation products (Lichtfouse *et al.*, 1995). Here, we provide microscopic evidence for the occurrence of resistant biopolymers in soil.

## RESULTS AND DISCUSSION

A soil sample (290 g, 0.97% of organic C) cropped 23 years with maize was thoroughly extracted with  $\text{CHCl}_3$ -MeOH 3/1 v/v (3 x) to remove free lipids, then with NaOH 0.1 M to remove humic and fulvic acids. The residue (humins and minerals) was then treated for classical kerogen extraction : 24 h. with 1050 ml HF (48%)/700 ml HCl (36%), 24 h. with 700 ml HF, 24 h. with 700 ml HCl, and then washed with  $\text{H}_2\text{O}$  to pH 6 to yield 3.9 g of residue. The removal of main soil silicates and Fe minerals by acid hydrolysis was checked by the very low abundance of Si and Fe relative to C and O by X-Ray diffraction. Elemental analysis gave 31% of C, 3 % of H, 2.5 % of N and 40% of uncombusted matter. Several attempts to reduce the uncombusted matter content by further drastic acid hydrolysis (HCl 36%, reflux 48 h.) resulted in the total degradation/solubilization of organic C, as observed by Schulten and Schnitzer for humic acids (1993). An other attempt to perform a strong alkaline hydrolysis (KOH-MeOH 5 M, reflux 24 h.) gave similar results. The residue obtained by our procedure thus represents the most chemically resistant carbon pool that can be isolated in substantial amounts from a soil. The residue was embedded in epoxy resin, sliced in ultrathin sections, stained with  $\text{KMnO}_4$  then observed by transmission electron microscopy (TEM).

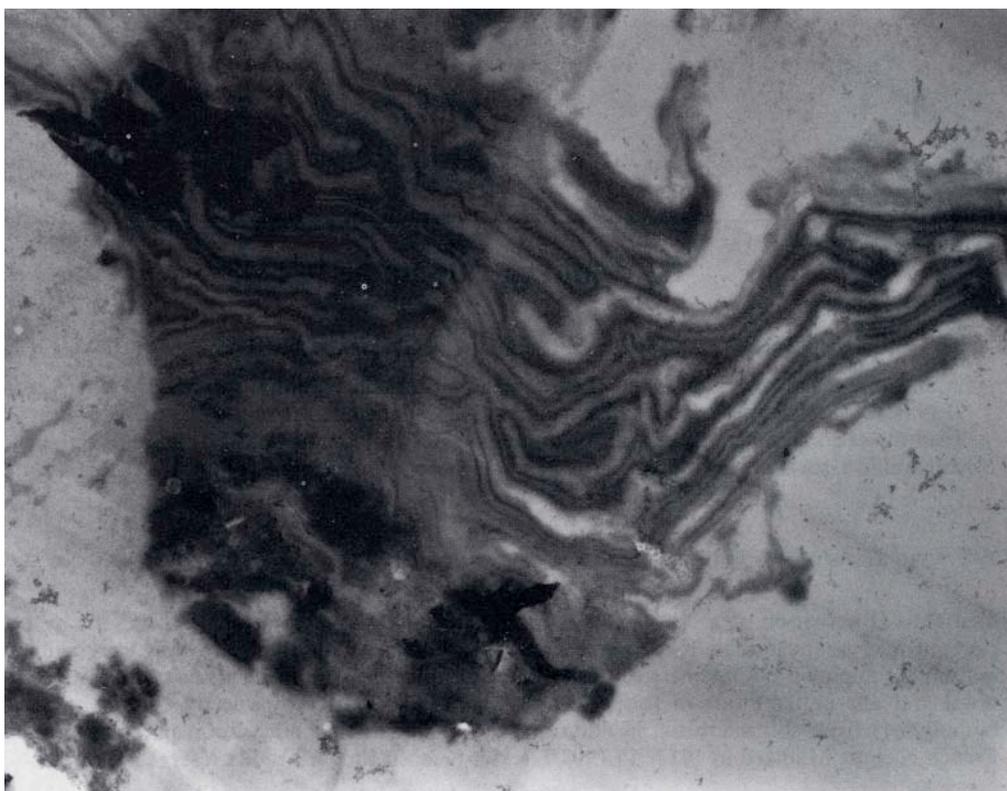


Figure 1. Transmission electron microscopy of a resistant residue, 'humins' or 'kerogen', from a maize crop soil, showing the occurrence of a resistant soil ultralaminae with thicknesses ranging from 10 to 50 nm (x 17 000).

Observation of the soil residue by TEM showed a large contribution of amorphous organic matter, estimated to more than 60%, along with recognizable wall debris either from plants or fungi (0.2-2  $\mu\text{m}$  thickness). In addition, very thin laminae were observed as shown on Figure 1. Laminae were 10 to 50 nm thick and few  $\mu\text{m}$  long. They were composed of dark and light alternate zones, strongly then weakly

KMnO<sub>4</sub>-stained, respectively. Since KMnO<sub>4</sub> reacts rather specifically with unsaturated molecules such as aromatic rings from lignin debris (Ruel *et al.*, 1984), the light zones are enriched in saturated carbon. A major part of this soil ultralaminae is therefore composed of saturated carbon.

These morphological features are very similar to very thin resistant walls that have been recently discovered in various microbes and sediments, and termed 'ultralaminae' (Largeau *et al.*, 1990, Derenne *et al.*, 1991). Recognition of resistant ultralaminae in soils imply that the formation and subsequent stabilization of soil organic carbon is not only performed by the classical recondensation of small molecules such as amino acids and carbohydrates (Maillard, 1917), but also by selective preservation of resistant biopolymers from soil microorganisms. Further structural elucidation studies of these novel components are in progress.

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