

**NATURAL ORGANIC MATTER IN THE GLOBAL CARBON CYCLE:
STRUCTURES AND ORGINS AS REVEALED BY MODERN NMR
SPECTROSCOPY**

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Natural Organic Matter (NOM) in soils and water represent the largest reserves of carbon on earth. The global soil carbon pool is 3.3 times the size of the atmospheric pool and 4.5 times that of the biotic pool and it is considered that at least 50% of this soil carbon can be categorized as the chemically resistant component known as humic substances (HS). Conversely, dissolved organic matter (DOM) represents the largest pool of mobile carbon on the Earth and is a fundamental link between terrestrial and aquatic environments. This communication utilizes the latest technologies and novel 1-dimensional and multi-dimensional NMR spectroscopy to assess the composition and sources of NOM. As a culmination of years of NMR-based research, we attempt to define the major structural components in organic matter from soils and water, explain why the key structural components in the aquatic and terrestrial NOM are so different, and assess the key contributions from microbial biomass, which in many soil samples, is underestimated by orders of magnitude.

HS in soils have largely remained uncharacterized at the molecular level and have necessarily been defined operationally in terms of the methods used to extract or isolate them. Formation processes of HS have been debated for decades. It has traditionally been thought that extractable HS consist of novel categories of structures formed through varying biotransformation processes. Today, the predicted future and modelling of the soil carbon stock relies heavily on the temperature sensitivity of this carbon component. In this study, advanced Nuclear Magnetic Resonance (NMR) approaches were used to look at major components (proteins, carbohydrates, aliphatic biopolymers and lignin) that are known to be present in HS, and identify their fingerprints in humic mixtures. Theoretically, once all known components have been identified the remaining signals should be from materials with novel structures, themselves forming a distinct chemical category of humic materials. Surprisingly, nearly all of the NMR signals in traditional HS fractions could be assigned to intact and degraded biopolymers. We therefore suggest that the vast majority of operationally defined

humic material in soils is a very complex mixture of microbial and plant biopolymers and their degradation products but not a distinct chemical category.

Furthermore, microbial contributions to soil organic biomass, appears in many cases to be significantly underestimated. Generally it is accepted that <5% of soils organic matter is comprised of microbial biomass (Alef 1993; Dalal 1998). However comparisons of cultured microbial biomass to soil organic matter indicate that in many cases $\geq 50\%$ of the humic and humin (non extractable component) is actually derived from microbial cells. Much of this material that is highly enriched in proteinaceous material is likely present due to the release of microbial cell contents during lysis by the NaOH extraction, which is employed in the standard isolation procedure of humic substances. Much of this cellular material that in many cases contributes more than 50% by weight to the operationally defined "recalcitrant humic fraction", may be chemically labile and present inside living cells.

Similarly, aquatic DOM contains a range of interesting and novel structures very different from those derived from parent plant and microbial biopolymers in soils. Building on exceptional work by Hertkorn et al. (2006) and utilizing novel long range multiple bond correlations, we confirm that carboxyl-rich alicyclic molecules (CRAM), heteropolysaccharides (HPS) and open chain aliphatic polycarboxylic acids (OCAP) are the major components in aquatic DOM (Hertkorn et al. 2006). Using long range correlations the structure of the OCAP can be further refined and many of the carboxyl groups are found to be attached at methyl substituted carbons. 2-dimensional NMR identifies large concentrations of linear terpenoids present in Lake Ontario DOM that contain conjugated unsaturations with methylated branches analogous to those found in carotene structures. It appears these linear structures are the most likely the precursors of the OCAP material in the aquatic DOM whereas CRAM is likely derived from cyclic terpenoids. Implications of these findings in terms of the global carbon cycle will be considered and a range of novel NMR approaches designed for the study of extremely complex environmental mixtures will be introduced.

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