

irm-LC/MS: $\delta^{13}\text{C}$ Analysis of Dissolved Organic Matter in Natural Waters

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Introduction

Dissolved organic matter (DOM) is now recognized as a key factor in hydrologic systems and aquatic environments¹. Beside its central role in microbial loops in aquatic food webs, DOM is involved in various biogeochemical processes such as alteration of minerals and rocks, mineral precipitation, trace metals and organic pollutants binding and transport or fixation, redox and acidification processes and is a small but quickly evolving pool of global carbon fluxes and reservoirs.

Quantitation of DOM concentrations in natural waters can now be routinely performed by automatic dissolved organic carbon (DOC) analyzers which convert DOC into CO_2 either by high temperature catalytic combustion or by wet oxidation. Stable isotope analysis of DOC can be used to characterize biogeochemical processes and carbon sources in the environment². Studying seasonal changes in lakes and rivers or tracing underground water circulation requires large numbers of water samples. As an illustration we present a field work mapping soil water geochemistry at the plot scale in a farmland area, focusing on pore-waters in soils and sediments.



Figure 1: Farmland plot "Le Pré Neuf" (pasture) near La Châtre (Région Centre, France)

The isotopic composition of DOM and DOC can now be measured by the Thermo Scientific LC IsoLink, the on-line coupling of a liquid chromatograph with an isotope ratio mass spectrometer. With the LC IsoLink™ interface, two approaches are possible: DOC $\delta^{13}\text{C}$ can be measured either by direct flow injection of water samples (as well as of fractions isolated off-line from larger volumes of water) or, alternatively, for on-line separation of DOM fractions size exclusion chromatography (SEC) for instance can be applied.

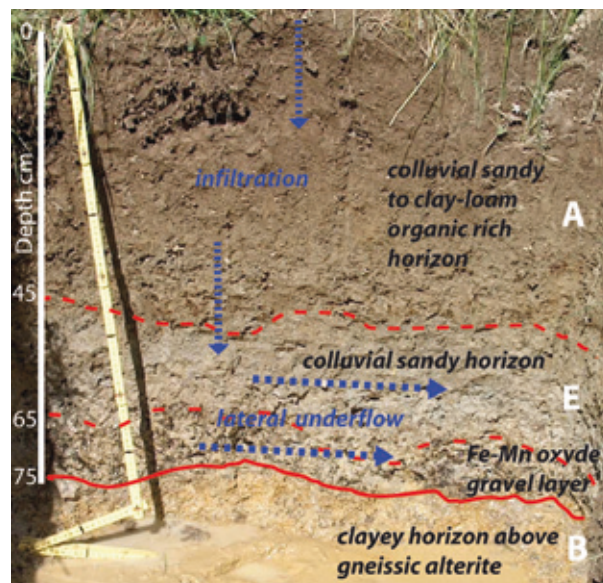


Figure 2: Typical soil profile in the plot. Seasonal lateral underflow of water is enhanced above the clayey B horizon of Planosols

Field Description

Landscapes marked by hedgerows (bocage landscape) are typically non-uniform areas where the 3D distribution of soil horizons and anthropogenic structures (e.g. hedges, ditches or wheel ruts) affect the composition and circulation of water. The plot selected for the study is located on a hillside used as pasture (Figure 1). Soils are Planosols developed on altered gneisses (Figure 2).

The plot is subdivided into four geochemical sectors (Figure 3)³. Each sector may differently contribute to the chemical composition of the stream draining the plot.



Figure 3: Separated geochemical sectors in the plot

Factors influencing DOC in soils are complex and numerous, e.g. the C/N ratio of substrate quality, decomposer community, adsorption in mineral soil horizons, pH, ionic strength, temperature, soil moisture, water fluxes and redox conditions⁴. In the plot, DOC was found spatially correlated with shallow water levels (sector C) enhancing contact time between soil solution and humic top soil horizons.

Considering the variation of the isotope composition of organic carbon in soil profiles, several factors are still discussed, among them selective sorption and preferential preservation of isotopically and chemically heterogeneous complex DOM^{5,6}. Mapping DOC $\delta^{13}\text{C}$ in soil water at the plot scale in geochemically contrasted conditions may be a complementary approach to decomposition or sorption experiments.

Experimental Section

Sample Preparation

Soil waters sampled in auger-drilled holes were filtered (0.45 μm) and acidified to pH 1 (either with concentrated nitric or phosphoric acid, but not HCl).

Separation of different fractions of DOM was made on larger volumes of water sampled in the stream draining the plot. Hydrophobic (HPO) and transphilic (TPH) acids were recovered by tandem XAD8/XAD4 adsorption chromatography⁷. HPOacid and TPHacid fractions are adsorbed at acidic pH and then eluted in a small volume of basic solution respectively from the XAD8 and XAD4 resins. TPHacids are more hydrophilic than HPOacids. The most hydrophilic compounds (HPI) are on the contrary lost in the water passing through the tandem columns. Analytical separation using only a small XAD8 column gave 50% HPOacid and 30% HPI+TPH (Figure 4)⁸. No differences were noticed between bulk filtered (GF/F filter) and ultrafiltered samples (10 and 1 kDa tangential plates).

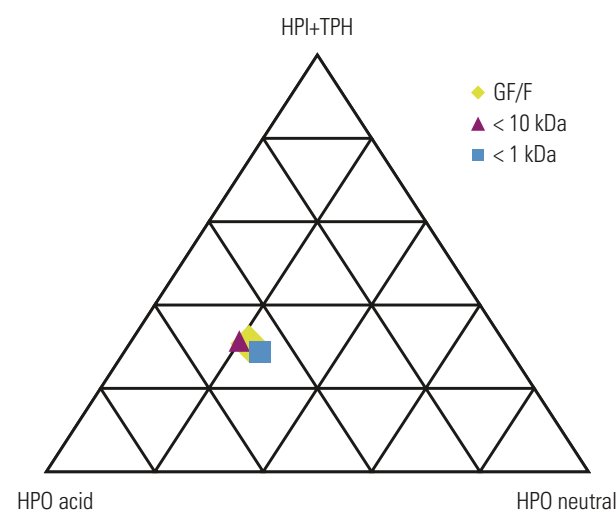


Figure 4: Proportions of HPO and TPH fractions in the stream water DOM (HPOacid 50%, HPI+TPH 30%) irm-LC/MS analysis

irm-LC/MS Analysis

FIA Mode

The LC IsoLink interface was used in flow injection analysis (FIA) mode for bulk analysis of ^{13}C -DOC (without column), a technique commonly referred to as “ μ -EA”. A volume up to 100 μL was routinely used to lower the detection to 0.5 mgL^{-1} DOC. Samples were gas stripped (for CO_2 elimination) with a stream of He prior to injection. $\delta^{13}\text{C}$ standardization was done using IAEA and USGS simple molecule standards and complex aquatic fulvic acids (Figure 5).

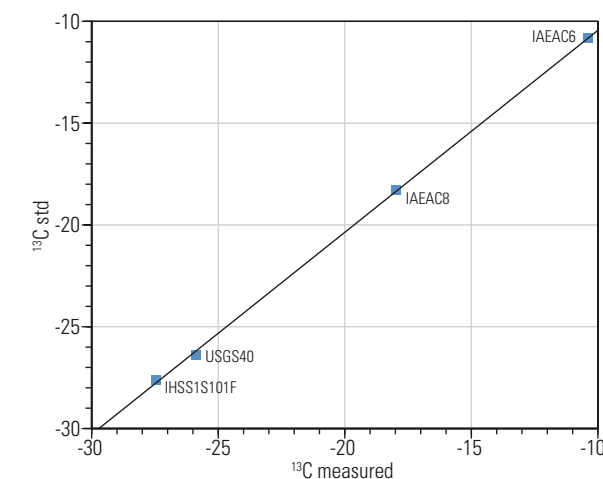


Figure 5: Correction curve based on international standards

For DOM concentrated fractions (HPOacid, TPHacid), 10 μL of sample diluted ten-fold with water were used (Figure 6).

Direct Flow Injection - Partial Loop Injection of 10 μL
Sample preparation: Filtration, acidification, gas stripping
HPOacids; $\delta^{13}\text{C}$ Bulk: $-30.02\text{‰} \pm 0.01\text{‰}$; Area Bulk: 144 Vs ± 0.7 Vs
RSD = 0.5%

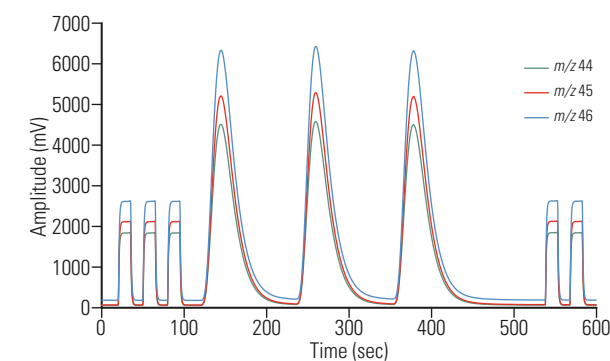


Figure 6: μ -EA mode: flow injection analysis of ^{13}C -DOC

HPLC Mode

Fractions isolated by the XAD resins were analyzed by high pressure size exclusion chromatography (HPSEC). Thermo Scientific BioBasic SEC120 and SEC60 30 cm columns were used with KH_2PO_4 0.05 M (pH 4.5) as mobile phase (300 $\mu\text{L}/\text{min}$).

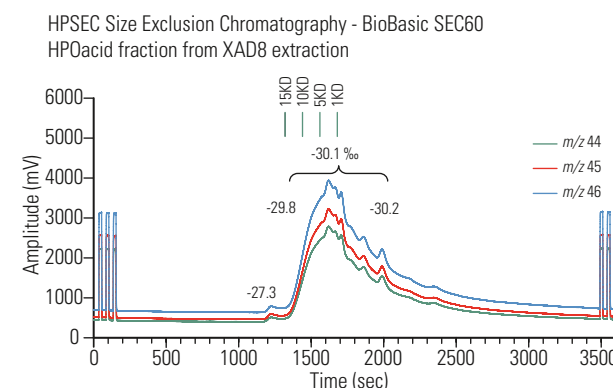


Figure 7: HPSEC of the stream water HPO fraction

Results and Discussion

Mapping $\delta^{13}\text{C}$ of DOC in soil water

Results are shown in Figure 8. Values fall in a narrow range, between -27.4 and -29.9‰ , with a mean of $-28.7 \pm 0.6\text{‰}$ (1σ , $n=70$). More negative values are grouped into a sector overlapping the “DOC” sector C (Figure 3), where higher DOC amounts are thought to be linked to upper levels of the water table and an enhanced contact between soil water and organic top soil layers.³

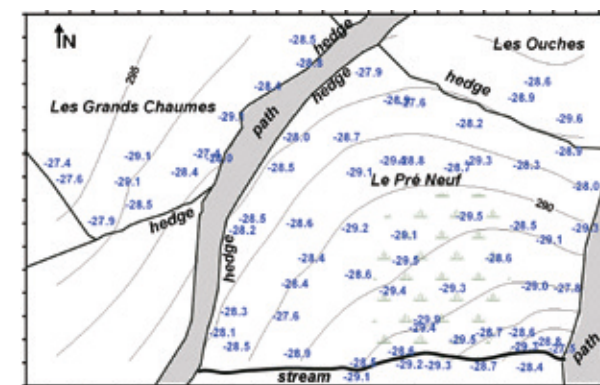


Figure 8: DOC $\delta^{13}\text{C}$ in soil water. More negative values ($< -29\text{‰}$) overlapped sector “DOC”

Comparison with the stream water DOM

The $\delta^{13}\text{C}$ of stream DOC ($-28.8 \pm 0.4\text{‰}$, $n=3$) as well as the TPH fraction (XAD4) extracted from the stream waters (-28.5‰) match the average soil water value ($-28.7 \pm 0.6\text{‰}$). The more hydrophobic HPO fraction (recovered from XAD8 resin) is slightly more negative (-30.0‰), closer to the set of values found in sector “DOC”.

SEC columns with small pore sizes (i.e. 60 or 120 \AA) were chosen to separate small size macromolecules making DOM. SEC60 offered the best operating range. The two fractions (HPO and TPH) had the same molecular weight range between 1 and 15 kDaltons (Figure 7). The small peak of the largest molecules were the heaviest (about -27‰) and there is a trend to more negative $\delta^{13}\text{C}$ at the end of the chromatogram (smaller molecules) (Figure 7, Table 1). The same small difference between HPO and TPH integrated peaks was observed, with the more hydrophobic DOC fraction about 1.5 ‰ more negative, somewhat less than found by others in forest soils.⁶

| | $\delta^{13}\text{C}$ (‰ vs V-PDB) |
|-------------------------|------------------------------------|
| Soil water DOC, mean | -28.7 ± 0.6 |
| Soil water DOC, maximum | -27.4 |
| Soil water DOC, minimum | -29.9 |
| Bulk stream DOC | -28.8 ± 0.4 |
| Bulk TPHacid stream | -28.5 |
| Bulk HPOacid stream | -30.0 |
| SEC60 TPH | -27.6; -28.5; -28.8 ; -30.3 |
| SEC60 HPO | -27.3; -29.8; -30.1 ; -30.2 |

Table 1: Bold: mean value of the main integrated peak

A comparison between TPH and HPO fractions of the stream and the spatial repartition of DOC $\delta^{13}\text{C}$ of the soil waters suggests that the different areas in the plot contribute differently to the stream DOC $\delta^{13}\text{C}$ composition. Well-aerated and drained hedgerow banks will contribute more hydrophilic and transphilic organic fractions (less negative $\delta^{13}\text{C}$ values) whereas more poorly drained downslope places will contribute a more hydrophobic fraction (more negative values). This will agree with the higher ionic strength and oxic conditions in the proximity of hedgerows, enhancing coagulation and sorption processes of humic substances comparatively to the downslope water logged zone.

Conclusions

The LC IsoLink interface is the first high sensitivity interface connecting Liquid Chromatography (LC) with Isotope Ratio MS (IRMS) for the reproducible and accurate on-line determination of $^{13}\text{C}/^{12}\text{C}$ isotope ratios.

The interface can be used in either of two operational modes, one for bulk analysis of DOC samples (Flow injection mode) and one for compound or fraction specific isotope analysis (*irm*-HPLC/MS mode) of DOM samples. The technique allows field studies to be made using filtered water samples instead of requiring the use of concentrated DOM fractions. On-line chromatographic separation of fractions of DOM is promising, with hydrophobic and ionic-based interactions more important than size separation.

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Patrick Albéric is a geochemist interested in the use of stable isotopes of Carbon to investigate the reactivity of dissolved organic matter in natural waters. Current research interests deal with coagulation, sorption and degradation processes at oxic-anoxic boundary in lake and in soil and river waters. Currently he is Maitre de Conférences (Associate professor) at the Observatoire des Sciences de l'Univers en Région Centre, France.

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