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## **Isotope evidence for soil organic carbon pools with distinct turnover rates. II. Humic substances<sup>#</sup>**

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**Abstract-** Two experiments using <sup>13</sup>C-enriched substrates have been undertaken to evaluate the relative turnover rates of the main pools of soil organic carbon namely bulk organic carbon, humin and humic acids. Firstly, soil organic matter was labelled naturally during a 5-year field experiment by cultivating *Zea mays*, a C<sub>4</sub> plant, on a soil that had previously grown isotopically distinct C<sub>3</sub> plants, e.g. wheat. Secondly, soil organic matter was labelled artificially with either D-glucose or <sup>13</sup>C-enriched D-glucose during a 21-day laboratory experiment. Isotopic variations observed during both experiments demonstrated the existence of soil carbon pools of decreasing turnover rates: humic acids > bulk organic carbon > humin.

*Key words* - humic acids, humin, carbon-13 labelled soil organic matter

### **INTRODUCTION**

Humic substances are naturally occurring, heterogeneous organic substances that can be characterized generally as yellow to black in color, of high molecular weight, and refractory (Maillard, 1916, 1917, Schnitzer, 1978, 1991, Ertel and Hedges, 1984, Stevenson, 1985, Aiken *et al.*, 1985, Rice and MacCarthy, 1988, Hayes *et al.*, 1989a, and refs. therein): they are ubiquitous, and are found where organic matter is decomposing in soils, sediments, and waters. Whatever their biological origin, the processes leading to the generation and transformation of humic substances are speculative (Hatcher *et al.*, 1981, 1985, Hayes *et al.*, 1989b, Rice and MacCarthy, 1990, De Leeuw and Hatcher, 1992). For instance, the role of the selective preservation of resistant biopolymers vs. the condensation of small organic molecules, is still open to debate.

Although radiocarbon dating of soil organic matter has provided some information on the temporal sequence of humic substances formation (Paul, 1970, Martel and Paul, 1974, Scharpenseel and Schiffmann, 1977, Campbell, 1978), measurements of mean residence times can be biased by 1) the low precision of <sup>14</sup>C-dating over relatively short periods, e.g. tens of years, 2) the input of <sup>14</sup>C free carbon from fossil fuel consumption (Suess effect), 3) the input of fairly large concentrations

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<sup>#</sup> for *n*-alkanes see *Org. Geochem.* **22**, 349 (1994) and *Tetrahedron Lett.* **36**, 529 (1995).

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of  $^{14}\text{C}$  from bomb testing and by the nuclear industry 4) variations in the natural  $^{14}\text{C}$  content in different part of the world and 5) isotope discrimination (Paul, 1970, Scharpenseel and Schiffmann, 1977). Hence, turnover determined either by radiocarbon dating or by  $^{14}\text{C}$  incubation fails to distinguish clearly the most abundant components of soil organic matter, humin and humic acids (Campbell *et al.*, 1967, Paul, 1970, Martel and Paul, 1974, Scharpenseel and Schiffmann, 1977, Stevenson, 1985). In order to study the turnover of the main pools of soil organic carbon, we have used carbon-13 as a tracer in a long-term field experiment and a short-term laboratory experiment.

## EXPERIMENTAL

*Natural labelling:* Maize (*Zea mays*), a  $\text{C}_4$  plant, was grown in an "experimental" field at *La Minière*, France, and harvested once per year from a soil previously cultivated with  $\text{C}_3$  plants. Details on sample setting and mineral content are described elsewhere (Balabane and Balesdent, 1992). *Artificial labelling:* soil samples from a crop field (Grignon, France) were sterilized with  $\gamma$  rays, then incubated for 21 days in the dark at pF 2 at  $28^\circ\text{C}$  with water from the same field containing either D-glucose ( $\delta^{13}\text{C} = -10.60\text{‰}$ ) or " $^{13}\text{C}$ -D-glucose" ( $\delta^{13}\text{C} \sim +500\text{‰}$ ) (Lichtfouse *et al.*, 1995a).

Soil samples (100-120 g) were dried, sieved to 2 mm, finely ground and extracted with 0.1 M NaOH (48 h under  $\text{N}_2$ ) to give an insoluble fraction (humin and minerals) and a soluble fraction (humic and fulvic acids) from which humic acids were separated by precipitation at pH 1.5 with 2.4 M HCl (3x). They were further purified by centrifugation at pH 7 to remove most clay particles, dialysed 10 days against distilled water (molecular weight cut-off of dialysis tubes: 2000 Å) and freeze-dried at  $-20^\circ\text{C}$ . Typical concentrations of bulk organic carbon of soils from *La Minière* amount to about 9.5 mg of C per g of dry soil (e.g. Lichtfouse *et al.*, 1995b). Humin and humic acids represent respectively 66% and 6% by weight of the bulk soil carbon. Other organic components, such as fulvic acids ( $\sim 25\%$  of bulk C), were not studied here. Isotopic compositions were measured on a Carlo Erba NA 1500 elemental N and C analyser coupled to a VG Sira 10 mass spectrometer: precision 0.03‰, overall deviation 0.1‰ (3 replicates). Isotopic compositions are expressed in per mil. relative to the PDB standard:  $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}/{}^{13}\text{C}/{}^{12}\text{C}_{\text{std}}) - 1] \times 10^3$ , where  ${}^{13}\text{C}/{}^{12}\text{C}_{\text{std}} = 0.0112372$ .

Table 1.  $\delta^{13}\text{C}$  values and percentage of maize-derived carbon (in parentheses) for soil organic components at increasing times of maize cropping. All sampling and analysis have been repeated three times (overall deviation  $\pm 0.10\text{‰}$ ).  $\delta^{13}\text{C}$  values for  $\text{C}_4$  soil organic components have been calculated from  $\delta_{\text{m}} = \delta_{\text{mr}} - \delta_{\text{wr}} + \delta_0$ , where  $\delta_{\text{mr}}$  is the isotope values of maize roots ( $-12.20\text{‰}$ ) and  $\delta_{\text{wr}}$  is the isotope value of wheat roots ( $-27.79\text{‰}$ ).

Time (years)	Bulk carbon	Humin	Humic acids
0.00	-26.27 (0.00)	-25.59 (0.00)	-27.45 (0.00)
0.42	-26.27 (0.00)	-25.57 (0.13)	-27.35 (0.64)
1.00	-26.13 (0.90)	-25.40 (1.22)	-27.16 (1.86)
1.42	-25.92 (2.25)	-25.35 (1.54)	-26.99 (2.95)
1.75	-25.89 (2.44)	-25.11 (3.08)	-26.79 (4.23)
2.42	-25.66 (3.91)	-25.19 (2.57)	-26.46 (6.35)
3.42	-25.10 (7.50)	-24.76 (5.32)	-26.07 (8.85)
4.00	-25.07 (7.70)	-24.88 (4.55)	-25.66 (11.48)
4.33	-24.96 (8.40)	-24.66 (5.97)	-25.44 (12.89)

## RESULTS AND DISCUSSION

### *Maize field experiment*

Common terrestrial plants can be classified isotopically in two categories according to their mode of  $\text{CO}_2$  fixation (Smith and Epstein, 1971, Deines, 1980, O'Leary, 1981).  $\text{C}_3$  plants incorporate  $\text{CO}_2$  by ribulose biphosphate carboxylation (Calvin cycle) and show  $^{13}\text{C}/^{12}\text{C}$  isotopic compositions around  $-26\text{‰}$ .  $\text{C}_4$  plants fix  $\text{CO}_2$  by phosphoenol pyruvate carboxylation (Hatch-Slack cycle) and show heavier  $^{13}\text{C}/^{12}\text{C}$  isotopic compositions around  $-12\text{‰}$ . We report here  $^{13}\text{C}/^{12}\text{C}$  isotopic compositions of bulk organic carbon, humin and humic acids from soils cultivated with *Zea mays*, a  $\text{C}_4$  plant (Table 1). Before maize cultivation (time = 0), humic acids are significantly  $^{13}\text{C}$ -depleted relative to bulk carbon ( $-1.2\text{‰}$ ) and humin ( $-1.9\text{‰}$ ). These isotopic differences suggest that humic acids and humin have either different modes of formation or isotopically distinct molecular components, or both.

The soils had been planted previously with  $\text{C}_3$  plants only. Hence, the  $^{13}\text{C}/^{12}\text{C}$  isotopic compositions of the soil organic components increased with time of maize cultivation (Fig.1). Such variations are explained by the input of  $^{13}\text{C}$ -enriched carbon from maize into soil organic matter. From the isotopic trends, it is obvious that the three components studied incorporate maize-derived carbon at different rates that decrease in the following order: humic acids > bulk organic carbon > humin. These differences can be quantified in three ways.

First, the amount of isotopic increase after 5 crops of maize (4.33 years) shows (Table 1) clearly that humic acids ( $+2.0\text{‰}$ ) have incorporated more maize-derived carbon than bulk organic carbon ( $+1.3\text{‰}$ ) and humin ( $+0.9\text{‰}$ ). Secondly, we assume that isotopic trends follow a linear law over a short time period, though this might not be true over a longer time period. The slope, calculated by linear regression, thus represents the turnover of each component. It is indeed much higher for humic acids ( $0.47\text{‰yr}^{-1}$ ) than for bulk carbon ( $0.33\text{‰yr}^{-1}$ ) and for humin ( $0.21\text{‰yr}^{-1}$ ). Thirdly,

each organic component can be regarded as a mixture from two isotopically distinct C<sub>3</sub> and C<sub>4</sub> sources (Lichtfouse *et al.*, 1994, Lichtfouse, 1995). At any given time during cultivation, the weight percentage of maize-derived carbon, M, in each component can be calculated from

$$M = 100 (\delta - \delta_0) / (\delta_m - \delta_0) \text{ where}$$

$\delta$ : isotope composition of organic component at a given time of cultivation,

$\delta_0$ : isotope composition of organic component before maize cultivation (time = 0),

$\delta_m$ : isotope composition for C<sub>4</sub> soil organic component.

From the calculation of M for soil organic components at various times of maize cropping (Table 1, Fig.1), it is obvious that the relative amount of maize-derived carbon increases faster for humic acids (3.0%yr<sup>-1</sup>) than for bulk carbon (2.1%yr<sup>-1</sup>) and for humin (1.4%yr<sup>-1</sup>).

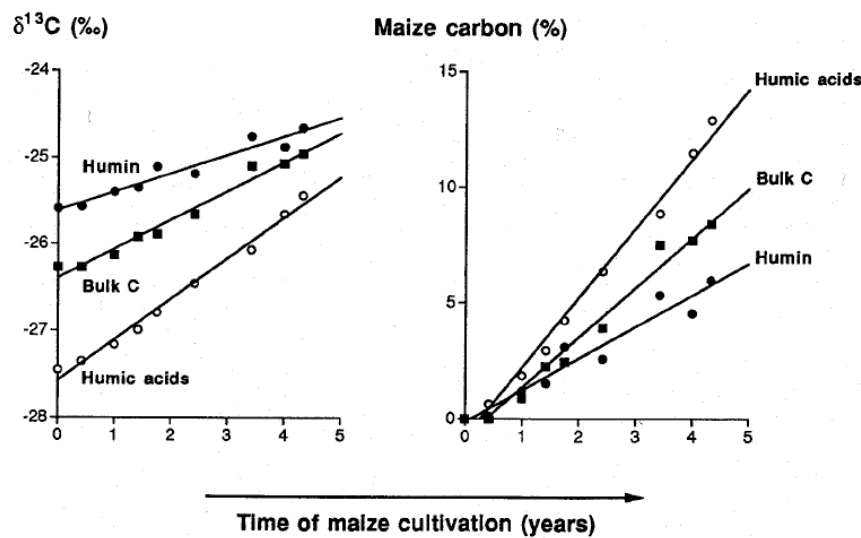


Figure 1.  $\delta^{13}\text{C}$  values (left) and percentage of maize-derived carbon M (right) for organic components from maize crop soils.  $\delta^{13}\text{C}$  linear regression gives (slope, initial value, r): humin (0.212‰yr<sup>-1</sup>, -25.61‰, 0.97), bulk C (0.334‰yr<sup>-1</sup>, -26.39‰, 0.99) and humic acids (0.468‰yr<sup>-1</sup>, -27.57‰, 0.99). M linear regression gives: humin (1.362%yr<sup>-1</sup>, -0.130%, 0.97), bulk C (2.141%yr<sup>-1</sup>, -0.786%, 0.99) and humic acids (3.004%yr<sup>-1</sup>, -0.789%, 0.99).

### *<sup>13</sup>C-Glucose laboratory experiment*

The fate of carbon incorporated into soil organic components has also been followed by a 3-week experiment in which soil was incubated with added <sup>13</sup>C-labelled glucose (Table 2). From the isotope difference between unlabelled glucose and <sup>13</sup>C-labelled glucose incubation, the decreasing rate of carbon flow is in the order: humic acids (+ 6.44‰) > bulk carbon (+2.31‰) > humin (+1.88‰), as observed previously in the maize field experiment.

Table 2.  $\delta^{13}\text{C}$  values of organic components for maize crop soils incubated with D-glucose.

	<b>Bulk carbon</b> (‰)	<b>Humin</b> (‰)	<b>Humic acids</b> (‰)
Glucose	-22.55	-24.52	-24.62
<sup>13</sup> C-Glucose	-20.24	-22.64	-18.18
$\delta$ difference	+2.31	+1.88	+6.44

## CONCLUSION

Two tracer experiments using carbon-13 at either natural or artificial abundance levels have measured the relative turnover rates of the main pools of soil organic carbon. These rates decrease in the order: humic acids > bulk organic carbon > humin. The use of isotopically distinct pools of organic carbon during the evolution of soil organic matter thus represent a fruitful way to study the dynamics of the degradation of organic matter.

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