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Unexpected ^{13}C -enrichment of organic components from wheat crop soils: evidence for the *in situ* origin of soil organic matter

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Abstract- Various organic constituents extracted from wheat and from soil organic matter have been analysed for their carbon content, their absolute concentration and their stable carbon isotope ratios. Most organic subfractions from plants, or soil, are ^{13}C -depleted by up to 9.4‰ relative to bulk organic matter, mainly as a result of their higher lipid content. Furthermore, soil organic constituents are unexpectedly ^{13}C -enriched by +1.5‰ to +4.3‰ relative to homologous plant constituents. Indeed, the selective preservation of plant lignin and lipids, following incorporation into the soil biomass, should have led to the accumulation of ^{13}C -depleted compounds. Hence, these results favour the *in situ* formation of soil organic matter either by recondensation of small molecules or by selective preservation of biopolymers from soil microorganisms.

Key words - carbon-13, humic acids, humin, soil lipids, plant alkanes, soil alkanes, pyrolysis of humic acids.

INTRODUCTION

The decay of plants leads to the preservation of a few percents only of plant carbon into soils, the bulk being rapidly biodegraded then returned to the atmosphere as CO_2 . Although many bulk chemical properties of soil organic matter are well-known, there is still a lack of understanding of the precise origin, and pathways, of transformation of soil organic substances, notably at the molecular level (Schnitzer and Khan, 1978). The formation of humic substances is believed to occur by two major processes (Hayes *et al.*, 1989): 1) the partial degradation and selective preservation of resistant macromolecules from plants or soil organisms and 2) the polycondensation of small molecules resulting from biomass degradation, e.g. the reaction of amino acids with carbohydrates (Maillard, 1916, 1917). Carbon isotope ratios have been used to define the origin and transformation of organic substances from soils and sediments (Nissenbaum and Kaplan, 1972, Collister *et al.*, 1992, Lichtfouse and Collister, 1992, Macko *et al.*, 1993, Collister *et al.*, 1994a, Lichtfouse *et al.*, 1994ab, Lichtfouse, 1995). In this note we show, using isotopic comparisons of various soil and plant organic substances, that the major part of soil organic matter where wheat has been grown is unlikely to be derived by the selective preservation of the plant's lipids or lignin, and thus must have been formed *within* the soil.

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EXPERIMENTAL

Plant and soil sampling

Soils, cropped solely with plants following the C₃ photosynthetic pathway (O'Leary, 1981), were cored through the 0-35cm ploughed horizon in September 1987 at *La Minière*, near Versailles, France, after 3 years of wheat cropping (1984-1986). The soil is a dystic eutrochrept, silty loam (Balabane and Balesdent, 1992). The 0-35 cm ploughed horizon comprises 11.4% of sand (50-2000 μm), 73.1% of silt (2-50 μm) and 15.5% of clay (0-50 μm). Two additional soil samples were cored at *Boigneville*, *Essonne*, France, in 1990 and 1993 after respectively 20 and 23 years of wheat cropping. These soils have very similar characteristics to the soils from the *La Minière* field (Balesdent *et al.*, 1990). Nine cores, taken at various locations, were well-mixed, dried at 20°C then sieved to 2000 μm . Wheat leaves, stems and roots (*Triticum aestivum*) were washed with distilled water then freeze-dried at -20°C. All samplings and further analyses were repeated three times.

Organic matter fractionation

Dried soils, leaves, stems and roots were finely ground and extracted ultrasonically (30 min., 30°C, 3x) with CHCl₃-MeOH (3/1 v/v). Extracts were fractionated into neutral and acid fractions by passage through silica gel impregnated with KOH followed by HCO₂H acidification (McCarthy and Duthie, 1962, Collister *et al.*, 1994a). Neutral fractions were fractionated into hydrocarbon-ester, ketone, alcohol and polar fractions by silica-gel thin layer chromatography, using CH₂Cl₂ as developer and 1,2:3,4-dibenzanthracene, friedelin and cholesterol as reference compounds (modified after Lichtfouse *et al.*, 1994c). Neutral fractions were fractionated into alkane-alkene, aromatic and ester fractions by silica-gel thin layer chromatography, with *n*-hexane as developer and using *n*-octacosane, 1-phenyldodecane, 2-methylphenanthrene and 1,2:3,4-dibenzanthracene as reference compounds. Individual *n*-alkanes from the alkane-alkene fractions were identified by gas chromatography-mass spectrometry, and co-elution with pure standards, and quantified by gas chromatography fitted with a flame ionization detector and using 5 α -androstane as internal standard.

Ground dry soils were extracted 48 h. at 20°C under N₂ with 0.1 M NaOH to give an insoluble fraction (humins and minerals) and a soluble fraction (humic and fulvic acids). Humic acids were separated from the soluble fraction by precipitation at pH 1.5 with 2.4 M HCl (3x). Humic acids were further purified by centrifugation at pH 7 to remove most clay particles, dialysed for 10 days against distilled water then freeze-dried at -20°C. Humic acids were pyrolysed at 550°C in an open gold tube under continuous argon flow to give pyrolysates and residues.

Isotope analysis

Isotopic compositions, and carbon contents, were measured on a Carlo Erba NA 1500 elemental C and N analyser coupled to a VG Sira 10 mass spectrometer: precision 0.03‰, overall deviation 0.1‰ (3 replicates). Isotopic analyses of individual *n*-alkanes were carried out under a continuous helium flow using an HP 5890 gas chromatograph coupled to a CuO furnace (850°C) and a cryogenic trap (-100°C): this system was coupled to a VG Optima mass spectrometer. Ion currents were monitored continuously ($m/z = 44, 45$ and 46 : precision 0.1‰, overall deviation 0.3‰, 3 replicates). Carbon isotope compositions are expressed in per mil. relative to the Pee Dee Belemnite

Table 1. Chemical composition of wheat and wheat crop soil organic components from the *La Minière* field. $\delta^{13}\text{C}$ values of bulk carbon from the *Boigneville* field are -25.92‰ and -25.78‰ after wheat cropping during 20 and 23 years, respectively.

WHEAT	YIELD ($\mu\text{g/g}$ dry weight)	CARBON (weight %)	CARBON ($\text{mgC/gC}_{\text{total}}$)	$\delta^{13}\text{C}$ (‰)	$\Delta\delta^{\text{a}}$ (‰)
Bulk leaf		44.99	1000	-29.61	0
Bulk stem		42.85	1000	-28.47	0
Bulk root		34.36	1000	-27.79	0
Leaf organic extract	26480	73.20	43.08	-33.19	-3.58
Stem organic extract	7900	52.30	9.64	-30.95	-2.48
Root organic extract	8568	72.90	18.18	-31.64	-3.85
Leaf C ₂₅ <i>n</i> -alkane	0.8	85.23	0.002	n.d.	
Leaf C ₂₇ <i>n</i> -alkane	4.6	85.26	0.009	-35.9	-6.3
Leaf C ₂₉ <i>n</i> -alkane	16.5	85.29	0.031	-36.5	-6.9
Leaf C ₃₁ <i>n</i> -alkane	21.7	85.32	0.041	-36.7	-7.1
Leaf C ₃₃ <i>n</i> -alkane	7.6	85.34	0.015	-36.7	-7.1
<hr/>					
SOIL	YIELD ($\mu\text{g/g}$ soil dry weight)	CARBON (weight %)	CARBON ($\text{mgC/gC}_{\text{total}}$)	$\delta^{13}\text{C}$ (‰)	$\Delta\delta^{\text{a}}$ (‰)
Bulk soil		0.95 (0.09) ^c	1000	-26.28	0
Humin ^b		0.63 (0.06) ^c	663.6	-25.59	+0.69
Humic acids	1812	32.78 (3.89) ^c	62.52	-27.45	-1.17
Humic acids pyrolysate	89.8	62.15	5.87	-26.04	+0.24
Humic acids pyr. residue	1028	37.14	40.19	-27.64	-1.36
Organic extract	161.2	61.00	10.35	-28.89	-2.61
Acid fraction	38.0	n.d.		-29.24	-2.96
Neutral fraction	57.0	n.d.		-29.40	-3.12
C ₂₅ <i>n</i> -alkane	0.02	85.23	0.002	-34.6	-8.3
C ₂₇ <i>n</i> -alkane	0.05	85.26	0.005	-34.2	-7.9
C ₂₉ <i>n</i> -alkane	0.16	85.29	0.014	-35.7	-9.4
C ₃₁ <i>n</i> -alkane	0.21	85.32	0.019	-35.7	-9.4
C ₃₃ <i>n</i> -alkane	0.06	85.34	0.005	-35.7	-9.4

^afraction minus bulk. ^bAnalyses were carried out on humin including mineral matrix. ^cNitrogen content in parenthesis. n.d.: not determined

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 ${}^{13}\text{C}/{}^{12}\text{C}_{\text{std}} = 0.0112372$.

RESULTS AND DISCUSSION

Mass balance

Yields, carbon contents and isotope values of various organic fractions from wheat and wheat crop soils from the *La Minière* field are reported in Table 1. Humin and humic acids amount respectively to 664 and 63 mg carbon per g of soil organic carbon, and are thus, quantitatively, the most abundant organic components of the soil. Soil and wheat organic extracts, and their subfractions, occur in minor amounts. Nevertheless, they are probably highly involved in soil transformation processes because they represent the "free" part of the organic matter. Moreover, due to their high lipid content, they should be selectively preserved in soils because lipids are more resistant to biodegradation than carbohydrates and amino acids. However, the carbon contents of organic extracts from wheat, averaging 23.6 mg C per g of bulk C, are higher than that from soil (10.4 mg C/g bulk C). Carbon concentrations of individual *n*-alkanes are also higher in wheat leaf than in soil organic matter: 0.041 vs. 0.019 mg of carbon per g of bulk carbon, respectively, for the C_{31} *n*-alkane. Also, the decrease of soil- vs. plant carbon concentration of organic extracts, and *n*-alkanes, suggests that the selective preservation of lipids is probably not a major pathway in the formation of soil organic matter because a higher content of lipids should have remained in the soils. This view is strengthened by consideration of carbon isotope ratios, as discussed in the following section.

Figure 1. Carbon isotope ratios of various organic components from wheat and wheat crop soils. All data are from the *La Minière* field, except values of bulk soil carbon from the *Boigneville* field (-25.92‰, -25.78‰). Note the ${}^{13}\text{C}$ -depletion of most soil subfractions versus bulk soil carbon. Note also the ${}^{13}\text{C}$ -enrichment of soil components versus wheat components.

Carbon isotopic compositions

Carbon isotope ratios of wheat and soil organic components range respectively from -36.7‰ to -27.79‰ and from -35.7 ‰ to -25.59‰ (Table 1, Fig. 1). These values are typical of the organic carbon from plants fixing CO₂ by the C₃ photosynthetic pathway (Nissenbaum and Schallinger, 1974, Deines, 1980, O'Leary, 1981, Collister *et al.*, 1994b, and refs. therein). With the exception of humin and humic acid pyrolysates, all organic subfractions are depleted in ¹³C, reaching a value of -9.4‰ relative to bulk organic carbon. This ¹³C-depletion can be attributed mainly to the higher lipid content of those organic subfractions relative to the bulk organic matter (Deines, 1980).

Plant lipids and lignin are ¹³C-depleted relative to bulk tissue, whereas amino acids, cellulose and hemicellulose are usually ¹³C-enriched (Deines, 1980, Benner *et al.*, 1987, Schleser, 1992). Since lipids and lignin are more resistant to biodegradation relative to other plant constituents, and since the major part of plant carbon is rapidly recycled to the atmosphere, then the accumulation of plant matter into soil by selective preservation of plant lipids, or lignin, versus polysaccharides should have led to a notable shift of the isotope compositions of soil organic components toward ¹³C-depleted values. For instance, the selective preservation of aliphatic plant components, as a major process, should have given δ ¹³C values as low as -36‰ for soil organic matter, as suggested by the δ ¹³C values of *n*-alkanes (Fig. 1).

However, we do not observe a depletion but rather a ¹³C-enrichment of +1.5‰ to +4.3‰ for soil versus wheat organic components: +1.5-3.8‰ (average +2.6‰) for wheat parts vs. bulk soils, +2.1-4.3‰ (average +3.0‰) for wheat part extracts vs. soil extract. Humin also shows a notable ¹³C-enrichment of +2.2‰ to +4.0‰ (average +3.0‰) versus bulk wheat parts. This result is of great significance because humin is the major soil fraction, amounting here to 66% of soil organic C and, most important, humin is considered to be the *most stable* organic component of soils (Schnitzer and Khan, 1978, Hayes *et al.*, 1989, and refs. therein). Moreover, it should be noted that a ¹³C-enrichment* of about +2‰, in deeper horizons versus surface litter, is commonly observed with increasing depth in forest soil profiles (Volkoff and Cerri, 1987, Melillo *et al.*, 1989, Martin *et al.*, 1990, Balesdent *et al.*, 1993). Hence, all these lines of evidence demonstrate that the selective preservation of plant lipids and lignin is not a major pathway of organic matter accumulation into soils. Two other pathways can thus be considered.

Firstly, soil organic matter may be formed by *condensation* of small molecules from plant degradation products or from soil biomass. The rapid biodegradation of ¹³C-enriched plant polysaccharides (Benner *et al.*, 1987) followed by condensation of carbohydrate monomers with amino acids (Maillard, 1916), which should be ¹³C-enriched, is in good agreement with this hypothesis.

Soil organic matter may alternatively be stored by *selective preservation of resistant biopolymers from soil microorganisms* consuming ¹³C-enriched plant polysaccharides. Indeed, it has been shown recently that various microorganisms, such

* Note that the ¹³C-depletion sometimes observed in C₄ grassland soils can be attributed to organic matter inherited from paleo C₃ vegetation (e.g. Volkoff and Cerri, 1987, Cerling *et al.*, 1989, Mariotti and Peterschmitt, 1994)

as microalgae, cyanobacteria and bacteria, are able to biosynthesize cell wall aliphatic biopolymers which are extremely resistant to acid and alkaline hydrolysis (Chalansonnet *et al.*, 1988, Le Berre *et al.*, 1991, Derenne *et al.*, 1992, de Leeuw and Largeau, 1993). Moreover, the occurrence of these resistant biopolymers in soil is strongly supported by the detection of notable amounts of aliphatic structures within humic acids and humin by NMR studies (Hatcher *et al.*, 1981, 1985, Buddrus and Lambert, 1995).

Whatever pathway is followed during soil organic matter formation, we have shown, on both isotopic and quantitative grounds, that the major part of crop soil humic substances must be formed *in situ*, either by recondensation of relatively small molecules or by selective preservation of soil biopolymers. Though the formation of soil humic substances by selective preservation of plant lignin and lipids is not excluded, this process is likely to give rise to minor amounts only of crop soil organic carbon.

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REFERENCES

- Balabane M. and Balesdent J. (1992) Input of fertilizer-derived labelled N to soil organic matter during a growing season of maize in the field. *Soil Biol. Biochem.* **24**, 89-96.
- Balesdent J., Girardin C. and Mariotti A. (1993) Site-related $\delta^{13}\text{C}$ of tree leaves and soil organic matter in a temperate forest. *Ecology* **74**, 1713-1721.
- Balesdent J., Mariotti A. and Boissongotier D. (1990) Effect of tillage on soil organic carbon mineralization estimated from ^{13}C abundance in maize fields. *J. Soil Sci.* **41**, 587-596.
- Benner R., Fogel M. L., Sprague E. K. and Hodson R. E. (1987) Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature* **329**, 708-710.
- Buddrus J. and Lambert J. (1995) Isolated paraffinic methyl groups in humic substances. *Org. Geochem.* **23**, 269-271.
- Cerling T. E., Quade J., Wang Y. and Bowman J. R. (1989) Carbon isotopes in soils and palaeosols as ecology and palaeoecology indicators. *Nature* **341**, 138-139.
- Chalansonnet S., Largeau C., Casadevall E., Berkaloff C., Peniguel G. and Couderc R. (1988) Cyanobacterial resistant biopolymers. Geochemical implications of the properties of *Schizothrix sp.* resistant material. *Org. Geochem.* **13**, 1003-1010.
- Collister J. W., Lichtfouse É., Hieshima G. and Hayes J. M. (1994a) Partial resolution of sources of *n*-alkanes of the Parachute Creek Member, Green River Formation (Piceance Creek Basin, Colorado). *Org. Geochem.* **21**, 645-659.
- Collister J. W., Rieley G., Stern B., Eglinton G. and Fry B. (1994b) Compound-specific $\delta^{13}\text{C}$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. *Org. Geochem.* **21**, 619-627.
- Collister J. W., Summons R. E., Lichtfouse É. and Hayes J. M. (1992) An isotopic biogeochemical study of the Green River oil shale. *Org. Geochem.* **19**, 265-276.

- Deines P. (1980) The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry* (Edited by Fritz P. and Fontes J. Ch.), pp. 329-406. Elsevier, Amsterdam.
- Derenne S., Largeau C., Berkaloﬀ C., Rousseau B., Wilhem C. and Hatcher P. (1992) Non-hydrolysable macromolecular constituents from outer walls of *Chlorella fusca* and *Nanochlorum eucaryotum*. *Phytochem.* **31**, 1923-1929.
- Hatcher P. G., Breger I. A., Maciel G. E. and Szeverenyi N. M. (1985) Geochemistry of humin. In *Humic Substances in Soil, Sediment, and Water* (Edited by Aiken G. R. *et al.*), pp. 275-302. Wiley, New York.
- Hatcher P. G., Maciel G. E. and Dennis L. W. (1981) Aliphatic structure of humic acids; a clue to their origin. *Org. Geochem.* **3**, 43-48.
- Hayes M. H. B., MacCarthy P., Malcolm R. L. and Swift R. S. (1989) Structures of Humic Substances: the Emergence of "forms". In *Humic Substances II, In Search of Structure* (Edited by Hayes M. H. B., MacCarthy P., Malcolm R. L. and Swift R. S.), pp. 689-733. Wiley, Chichester.
- Le Berre F., Derenne S., Largeau C., Connan J. and Berkaloﬀ C. (1991) Occurrence of non-hydrolysable, macromolecular, wall constituents in bacteria. Geochemical implications. In *Organic Geochemistry. Advances and Applications in Energy and the Natural Environment* (Edited by Manning D. A. C.), pp. 428-431. University Press, Manchester.
- Leeuw J. W. de and Largeau C. (1993) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation. In *Organic Geochemistry, Principles and Applications* (Edited by Engel M. H. and Macko S. A.), pp. 23-72. Plenum, New York.
- Lichtfouse É. (1995) ^{13}C labelling of soil *n*-hentriacontane (C_{31}) by maize cultivation. *Tetrahedron Lett.* **36**, 529-530.
- Lichtfouse É., Albrecht P., Behar F. and Hayes J. M. (1994c) A molecular and isotopic study of the organic matter from the Paris Basin, France. *Geochim. Cosmochim. Acta* **58**, 209-221.
- Lichtfouse É. and Collister J. W. (1992) Tracing biogenic links of natural organic substances at the molecular level with stable carbon isotopes: *n*-alkanes and *n*-alkanoic acids from sediments. *Tetrahedron Lett.* **33**, 8093-8094.
- Lichtfouse É., Derenne S., Mariotti A. and Largeau C. (1994a) Possible algal origin of long chain odd *n*-alkanes in immature sediments as revealed by distributions and carbon isotope ratios. *Org. Geochem.* **22**, 1023-1027.
- Lichtfouse É., Elbisser B., Balesdent J., Mariotti M. and Bardoux G. (1994b) Isotope and molecular evidence for direct input of maize leaf wax *n*-alkanes into crop soils. *Org. Geochem.* **22**, 349-351.
- Macko S. A., Engel M. H. and Parker P. L. (1993) Early diagenesis of organic matter in sediments. Assessment of mechanisms and preservation by the use of isotopic molecular approaches. In *Organic Geochemistry, Principles and Applications* (Edited by Engel M. H. and Macko S. A.), pp. 211-224. Plenum, New York.
- Maillard L. C. (1916) Synthèse des matières humiques par action des acides aminés sur les sucres réducteurs. *Ann. Chimie (Paris)* **5**, 258-317.
- Maillard L. C. (1917) Identité des matières humiques de synthèse avec les matières humiques naturelles. *Ann. Chimie (Paris)* **7**, 113-152.
- Mariotti A. and Peterschmitt E. (1994) Forest savanna ecotone dynamics in India as revealed by carbon isotope ratios of soil organic matter. *Oecologia* **97**, 475-480.
- Martin A., Mariotti A., Balesdent J., Lavelle P. and Vuattoux R. (1990) Estimate of organic matter turnover rate in a savanna soil by ^{13}C natural abundance.

- measurements. *Soil Biol. Biochem.* **22**, 517-523.
- McCarthy R. D. and Duthie A. H. (1962) A rapid quantitative method for the separation of free fatty acids from other lipids. *J. Lipid Res.* **3**, 117-119.
- Melillo J. M., Aber J. D., Linkins A. E., Ricca A., Fry B. and Nadelhoffer K. J. (1989) Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. *Plant and Soil* **115**, 189-198.
- Nissenbaum A. and Kaplan I. R. (1972) Chemical and isotopic evidence for the *in situ* origin of marine humic substances. *Limnol. Oceanogr.* **17**, 570-582.
- Nissenbaum A. and Schallinger K. M. (1974) The distribution of the stable carbon isotope ($^{13}\text{C}/^{12}\text{C}$) in fractions of soil organic matter. *Geoderma* **11**, 137-145.
- O'Leary M. H. (1981) Carbon isotope fractionation in plants. *Phytochem.* **20**, 553-567.
- Schleser G. H. (1992) $\delta^{13}\text{C}$ pattern in a forest tree as an indicator of carbon transfer in trees. *Ecology* **73**, 1922-1925.
- Schnitzer M. and Khan S. U. (Eds.) (1978) *Soil Organic Matter*. Elsevier, Amsterdam.
- Volkoff B. and Cerri C. C. (1987) Carbon isotopic fractionation in subtropical Brazilian grassland soils. Comparison with tropical forest soils. *Plant and Soil* **102**, 27-31.