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Eric Lichtfouse. Heterogeneous turnover of molecular organic substances from crop soils as revealed by  $^{13}\text{C}$  labeling at natural abundance with *Zea mays*. *The Science of Nature Naturwissenschaften*, 1997, 84 (1), pp.23-25. <10.1007/s001140050342>. <hal-00193327>

**HAL Id: hal-00193327**

**<https://hal.science/hal-00193327v1>**

Submitted on 3 Dec 2007

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HAL Authorization

Revised version

Naturwissenschaften 84, 22-23, 1997.

doi: 10.1007/s001140050342

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## **Heterogeneous turnover of molecular organic substances from crop soils as revealed by $^{13}\text{C}$ labelling at natural abundance with *Zea mays***

Éric Lichtfouse

### **Abstract**

Understanding the dynamics of soil organic matter is of major importance for agronomical and environmental studies. In particular, soil organic matter holds soil physical stability and thus prevents erosion; soil organic matter stores pollutants; and soil organic carbon impacts climate change as either a source or sink of atmospheric  $\text{CO}_2$ . However, knowledge on the dynamics of soil carbon is still incomplete, particularly at the molecular level. Here, using a recently developed analytical technique that allows measuring the  $^{13}\text{C}$  isotope composition of individual substances within a complex organic mixture I show that plant-wax derived soil n-alkanes have a higher turnover than bulk soil organic matter. Soil samples were collected in a field experiment where maize was cropped 23 years. Since maize, a  $\text{C}_4$  plant, is  $^{13}\text{C}$ -enriched versus previous vegetation made of  $\text{C}_3$  plants, cropping maize introduces  $^{13}\text{C}$ -enriched carbon in the soil over time. This labelling at natural abundance thus allows to distinguish maize-derived carbon from initial  $\text{C}_3$  plant-derived carbon. The  $^{13}\text{C}$  composition of bulk soil carbon was measured by isotope ratio monitoring mass spectrometry (IRMS), whereas that of individual soil n-alkanes was measured by gas chromatography-IRMS. The results show that after 23 years of maize cropping the increase of  $^{13}\text{C}$  composition is faster for the  $\text{C}_{31}$  soil n-alkane (+7.6‰) than for bulk soil carbon (+5.6‰). This finding evidences the faster turnover of soil n-alkanes, which is rather unexpected because n-alkanes are chemically stable compounds. Using a first order kinetic model, it is estimated that the complete C turnover will last 192 years for bulk soil carbon and 148 years for the plant-derived soil n-alkane.

Keywords:  $^{13}\text{C}$ ; plant wax n-alkane; dynamics; soil organic matter; soil carbon; GC-IRMS; turnover

Note from the author: this abstract was written in 2007 for indexing in the HAL archive.

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## **Heterogeneous turnover of molecular organic substances from crop soils as revealed by $^{13}\text{C}$ labelling at natural abundance with *Zea mays***

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Because of the recent suggestion that the terrestrial biosphere may contain a 'missing sink' of atmospheric  $\text{CO}_2$  [1], studies on the dynamics of soil organic carbon are of particular interest to try to understand changes in the global carbon cycle [2]. Most investigations build models that consider soil carbon as the sum of bulk pools of various turnovers [2]. However, a simple consideration of the huge reactivity differences that exist between individual organic substances, such as lipids, amino acids and carbohydrates, strongly suggests that these compounds may also have significantly different dynamics in soils. If that is the case, models should take into account such heterogeneities *at the molecular level*. However, the dearth of knowledge on the dynamics of molecular species stems in large part from the extreme complexity of the soil medium and from the lack of suitable methods that allow long-term kinetics of organic species to be evaluated. Here, I use a recently developed molecular and isotopic method [3], namely gas chromatography-combustion-isotope ratio-mass spectrometry (GC-C-IRMS), to follow the fate of plant carbon into soil waxes during long-term field experiments [4].

Common plants can be classified in two main categories according to their photosynthetic mode [5]:  $\text{C}_3$  plants incorporate  $\text{CO}_2$  by ribulose biphosphate carboxylation (Calvin cycle) and show bulk  $^{13}\text{C}/^{12}\text{C}$  compositions [6] ranging from -34‰ to -24‰, whereas  $\text{C}_4$  plants fix  $\text{CO}_2$  by phosphoenol pyruvate carboxylation (Hatch-Slack cycle) and show isotope values of -23‰ to -12‰. This isotope heterogeneity can thus be used as a natural label to follow the fate of plant carbon into various media such as soil [7] and food [8]. Because most plants from temperate areas follow the  $\text{C}_3$  photosynthetic mode, the organic matter in underlying soils have isotope values around -26‰. In several experimental crop fields in France, soils previously planted solely with  $\text{C}_3$  plants were cultivated with *Zea mays*, a  $\text{C}_4$  plant of bulk carbon isotope value of -12.5‰. As a result, the bulk soil organic carbon becomes progressively enriched in  $^{13}\text{C}$  due to the addition of maize carbon, reaching a carbon isotopic difference ( $\Delta\delta$  value) of +5.6‰ after 23 years (Table 1, Fig. 1). A simple calculation of isotope balance between the ' $\text{C}_3$ ' initial pool and the added ' $\text{C}_4$ ' pool allows the percentage M of maize-derived C in each soil organic compartment to be calculated [4] (Table 1). M thus amounts to 41.7‰ for total organic C after 23 years of cultivation.

Table 1.  $^{13}\text{C}/^{12}\text{C}$  Isotopic compositions ( $\delta$ ), isotopic difference ( $\Delta\delta$ ),  $\text{C}_4$  carbon content (M) and  $\text{C}_3$  carbon content (B) of total organic carbon (TOC) and  $n$ -hentriacontane ( $\text{C}_{31}\text{H}_{64}$ ) from crop soil cultivated during 0-23 years with maize (*Zea mays*).

Time (yrs)	$\delta_{\text{TOC}}$ (‰)	$\delta_{\text{C}_{31}}$ (‰)	$\Delta\delta_{\text{TOC}}$ (‰)	$\Delta\delta_{\text{C}_{31}}$ (‰)	$\text{M}_{\text{TOC}}$ (%)	$\text{M}_{\text{C}_{31}}$ (%)	$\text{B}_{\text{TOC}}$ (%)	$\text{B}_{\text{C}_{31}}$ (%)
0 <sup>a</sup>	-26.28	-35.7	0	0	0	0	100	100
0 <sup>b</sup>	-25.92	-35.7	0	0	0	0	100	100
0 <sup>c</sup>	-26.20		0		0		100	
3 <sup>c</sup>	-25.20		+1.00		7.31		92.69	
4.3 <sup>a</sup>	-24.96	-33.7	+1.32	+2.0	9.59	13.25	90.41	86.75
13 <sup>c</sup>	-23.00		+3.20		23.38		76.62	
20 <sup>b</sup>	-20.74	-28.7	+5.18	+7.0	38.63	46.36	61.36	53.64
23 <sup>b</sup>	-20.33	-28.1	+5.59	+7.6	41.69	50.33	58.31	49.67
Maize	-12.51	-20.6						

Isotopic compositions are expressed as  $\delta^{13}\text{C}$  values [6]. a, b and c refer to French experimental fields at La Minière, Boigneville and Auzeville, respectively (Auzeville data from [12]). Time 0 refers to soils from the same fields cultivated only with  $\text{C}_3$  plants.  $\Delta\delta = \delta - \delta_0$ , where  $\delta$  and  $\delta_0$  are the values of the organic compartments at times t and 0, respectively. M is the percentage of ' $\text{C}_4$ ' maize-derived C in each organic compartment :  $M = 100 \cdot \Delta\delta / (\delta_m - \delta_0)$ , where  $\delta_m$  is the value of maize [4]. B is the percentage of initial ' $\text{C}_3$ ' carbon remaining in each organic compartment :  $B = 100 - M$ .

Detailed experimental procedures have been described elsewhere [13]. All soils from the three sites have close physico-chemical properties and have been cropped under similar practices (J. Balesdent, pers. commun.). At La Minière, the soil is an Eutrochrept containing 16% of clay (<2  $\mu\text{m}$ ), 73% of silt (2-50  $\mu\text{m}$ ), 11 % of sand (50-2000  $\mu\text{m}$ ), 0.9 % of organic carbon and 0.1 % of organic nitrogen [14]. At Boigneville, the soil is a Hapludalf developed on loess containing 22 % of clay, 1 % of organic carbon and 0.1 % of organic nitrogen [15]. At Auzeville, the soil is an Eutrochrept containing 27 % of clay, 41 % of silt, 32 % of sand and 0.9% of organic carbon [12]. All soils have pH ~ 6.5-6.7 and average bulk density of 1.4  $\text{g}/\text{cm}^3$ .

Typically, 0-30 cm cores, corresponding to the ploughed horizon, were sampled at random positions in the fields, mixed, dried at 20°C, sieved to 2 mm to remove coarse debris, then finely ground. 100 g were thoroughly extracted with  $\text{CHCl}_3$ -MeOH 3/1 v/v. The extract was fractionated into acid and neutral fractions by passage through silica gel impregnated with KOH followed by  $\text{HCO}_2\text{H}$  acidification. The neutral fraction was fractionated into polar, alcohol, ketone and hydrocarbon-ester fractions by silica-gel thin layer chromatography using  $\text{CH}_2\text{Cl}_2$  as developer and 1,2:3,4-dibenzanthracene, friedelin and cholesterol as reference compounds. Hydrocarbon-ester fractions were fractionated into alkane-alkene, aromatic and ester fractions by silica-gel thin layer chromatography using  $n$ -hexane as developer and 1-phenyldodecane, 2-methylphenanthrene and 1,2:3,4-dibenzanthracene as reference compounds.

$\delta^{13}\text{C}$  values of total organic carbon 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 from alkane-alkene fractions 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).

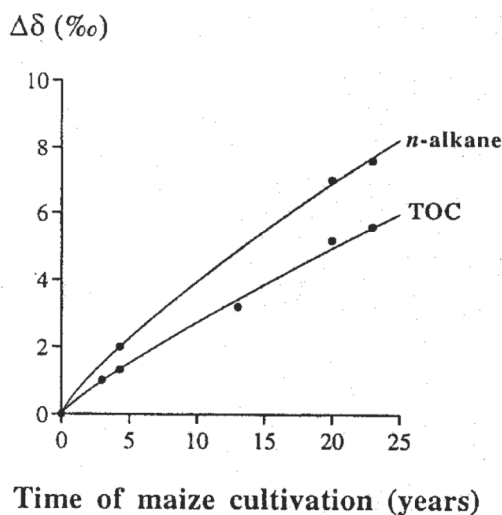


Figure 1. Isotope variations of total organic carbon (TOC) and of *n*-hentriacontane ( $C_{31}$ ) from soils cultivated with *Zea mays* (see also Table 1). Note the faster increase of the individual *n*-alkane isotope variations.

The isotopic composition of a soil *n*-alkane, *n*-hentriacontane ( $C_{31}H_{64}$ ), a soil substance derived from plant waxes [9], was also monitored. Before maize cultivation, at time 0, the soil *n*-alkane represents the contribution of waxes from previously grown  $C_3$  plants, e.g. wheat. Later, introduction of  $^{13}C$ -enriched maize waxes during cultivation induces the  $^{13}C$ -enrichment of the soil *n*-alkane (Fig. 1). During maize cultivation, the soil *n*-alkane is thus a mixture of - previously grown -  $C_3$  plant *n*-alkane ( $\delta$  -35.7‰) and of - newly added - maize *n*-alkane ( $\delta$  -20.6 ‰). Remarkably, this molecular component displays a much faster increase than total organic carbon, and reaches an M value of 50.3% after 23 years of cultivation. This notable difference between the bulk carbon and the molecular component clearly demonstrates the heterogeneous turnover of individual organic species. The higher turnover of the alkane is somewhat surprising given the relative stability of this class of chemical substances. Nonetheless, the occurrence of this compound in the organic extract, a soil organic compartment regarded as a 'free' or 'labile', could explain its higher turnover relative to the bulk soil C, which is mainly present as macromolecular, and thus less labile, humic substances [10]. Moreover, linear alkanes are also structurally more biodegradable than polycondensed and aromatic moieties.

In order to estimate the long-term fate of soil carbon, it is hypothesised that the decrease of the percentage B of  $C_3$  carbon in each soil compartment (Fig. 2) follows a first-order kinetic law,  $-dB/dt = kB$ , which gives by integration :

$$\ln (B/B_0) = -kt \quad \text{or} \quad B = B_0 e^{-kt}$$

Calculation of kinetic constants k by linear regression of  $\ln (B/B_0)$  versus -t gives values of 0.023 and 0.031 for total organic carbon and *n*-hentriacontane, respectively (correlation coefficients > 0.99). Corresponding half-lives of those compartments are therefore 28.8 yrs and 22.4 yrs ( $t_{0.5} = \ln 2/k$ ). The half-life value of the plant-derived *n*-alkane (22.4 yrs) contrasts sharply with that of decomposable plant material (0.165 yrs) and of resistant plant material (2.31 yrs) observed in the  $^{14}C$  Rothamsted

experiments [2]. Such a discrepancy again shows that molecular components may have strikingly different turnover behaviour than bulk compartments, strengthening the need to take their individual dynamics into account in further modelling of the global carbon cycle. Furthermore, if we assume that the complete replacement\* of C<sub>3</sub> carbon by C<sub>4</sub> maize-derived carbon occurs when B reaches a value of 1%, then the time needed for completion will be 192 yrs for total organic carbon and 148 yrs for *n*-hentriacontane.

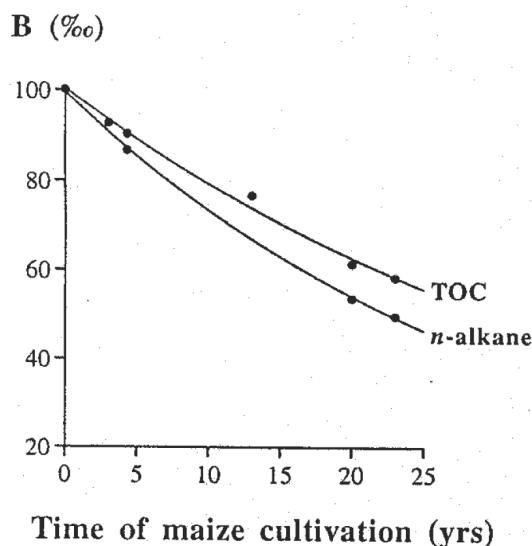


Figure 2. Percentage B of initial 'C<sub>3</sub>' carbon remaining in each soil organic compartment versus time of maize cultivation. *n*-Alkane values decrease faster than total organic carbon values.

The results of this isotopic study at the molecular level imply that the dynamics of natural substances are more complex than previously recognised. Indeed, the identification of individual substances having very different long-term dynamics may have considerable geochemical and environmental implications. So far, it should be stressed that kinetic investigations on individual substances occurring in complex media, such as soils and recent sediments, is a widely unexplored field [11]. Therefore, this approach using both molecular and stable isotope tools represents a novel means to investigate the long-term transformations of natural substances in the environment.

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\* Noteworthy, the mechanisms of degradation of most organic molecules in soil are so far poorly understood.

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