$\delta\,^{13}$C of plant-derived n-alkanes in soil particle-size fractions

Christèle Cayet [a], Eric Lichtfouse [b],*

[a]*Laboratoire de Géologie, Géodynamique des rifts et des marges passives, Université du Maine, Avenue Olivier Messiaen, BP 535, 72017 Le Mans Cedex, France*
[b]*3, rue de l'Eglise, 54990 Xeuilley, France*

Abstract

Soil organic carbon can be labelled at natural abundance by the cultivation of C_4 plants, e.g. maize, on soils which have been previously cropped with isotopically distinct C_3 plants, e.g. wheat. Particle-size fractions from soils cultivated for 23 years either with maize or wheat have been analysed for C and N contents, and for $\delta\,^{13}$C values of bulk C and of plant-derived n-alkanes (C_{29}, C_{31}). The amount of maize-derived organic components has been calculated by isotope balance. After 23 years of maize cropping, $\delta\,^{13}$C values of n-alkanes from the same soil sample increase with particle size. The results have two implications. First, the input of leaf wax n-alkanes into the soil is occurring preferentially via large particles. Second, in a same soil sample, the C_{31} n-alkane from the 200–2000 μm particle-size fraction is younger than the C_{31} n-alkane from the 50–200 and 0–50 μm fractions. In this respect, ^{13}C analysis represents a method of relative dating of individual compounds. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Soil n-alkane; ^{13}C; Particle-size; C; N; *Zea mays*; *Triticum aestivum*; GC–C–IRMS

1. Introduction

Soil organic matter is a very complex medium composed of organic molecules derived from plants and from various soil organisms such as worms, bacteria and fungi. Although numerous studies have been undertaken to unravel this complex network, the molecular structure, the origin, the fate, the bioavailability and the pathways of transformation of soil organic substances are still a matter of debate (e.g. Schnitzer and Khan, 1978; Hayes et al., 1989; Schnitzer, 1991; De Leeuw and Hatcher, 1992; Amblès et al., 1994; Hedges and Oades, 1997; van Bergen et al., 1997, 1998a,b; Lichtfouse, 1997a; Bull et al., 1998, 2000a,b; Bol, 1999; Boutton et al., 1999; Gleixner et al., 1999; Schweitzer et al., 1999; Amelung et al., 1999; Lichtfouse and Lévêque, 1999; Derenne and Knicker, 2000; Kögel-Knabner, 2000).

These issues stem mainly from the lack of suitable methods to trace the flow of organic carbon at the molecular level. In recent investigations, we have used compound-specific isotope analysis (CSIA, Hayes et al.,

1990, reviewed by Lichtfouse, 2000) and biomarkers in order to study the sources and the formation of soil humic substances (Lichtfouse, 1998; Lichtfouse et al., 1997a,b, 1998a,b,c). More specifically, crop labelling experiments using ^{13}C-enriched carbon from maize have allowed one to calculate and to predict the turnover of individual soil n-alkanes (Lichtfouse, 1995, 1997b). Such models are supported by the absence of isotopic fractionation observed in long-term decomposition experiments (Huang et al., 1997). On the other hand, strong variations of bulk $\delta\,^{13}$C values have been observed within particle-size fractions of the same soil sample (Balesdent et al., 1987). Here, we report the $\delta\,^{13}$C values of individual n-alkanes from soil particle-size fractions, and discuss their implications for the flow of plant molecules into soil organic matter.

2. Experimental

2.1. Sampling

The soil is a hapludalf developed on loess (Balesdent et al., 1990). The soil was cultivated either with wheat (*Triticum aestivum*), called "wheat soil", or maize (*Zea*

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* Corresponding author.
E-mail address: Eric.Lichtfouse@ensaia.inpl-nancy.fr (E. Lichtfouse).

mays), called "maize soil", for 23 years at one crop per year (1970–1993). The soil was previously planted only with plants following the C_3 photosynthetic pathway. Soil samples were cored in 1993 through the 0–35 cm ploughed horizon at the experimental field of Boigneville, Essone, France. Nine cores, taken at various locations, were well-mixed, dried at $+20°C$ then sieved to 2000 µm. Maize and wheat plants were sampled in 1993.

2.2. Particle size fractionation

Typically, 100 g of soil was gently shaken for 16 h using a home-made "bicycle-wheel" apparatus with 20 small glass balls in 250 ml distilled water, then sieved (200 µm). The non-sieved solids were separated by density in water to yield (1) the 200–2000 µm soil fraction (0.08% dry-weight) including visually recognisable plant remains, and (2) heavier mineral particles (3.07%). The 200 µm-sieved mixture was sieved at 50 µm to give (1) the 50–200 µm fraction (0.53%) and (2) heavy mineral particles (5.78%) in the same way. The two heavy mineral fractions were found to contain no C within the detection limit (0.03%), and were thus not analysed further. The 50 µm-sieved mixture was treated with $CaCl_2$ (1 g/l) to precipitate the 0–50 µm fraction which was recovered by centrifugation (90.54%). The soil particle size fractions were then freeze-dried and finely crushed 1 min in a steel-ball mortar.

2.3. Bulk and molecular analysis

Fractionation of $CHCl_3$-MeOH (3/1 v/v) extracts, analysis of bulk C, N and *n*-alkanes are described elsewhere (Lichtfouse et al., 1995, 1997a). Vigorous procedures were used to avoid contamination e.g. solvent distillation, aluminium foil burning, TLC plate washing, etc. (e.g. Henner et al., 1997). Carbon isotope compositions are expressed in per mil. relative to the Pee Dee Belemnite standard where $^{13}C/^{12}Cstd = 0.0112372$. IRMS analytical deviation was 0.03‰ and GC–C–IRMS analytical deviation 0.3‰.

3. Results and discussion

3.1. Bulk C and N content

C and N contents of maize and of the soil cultivated during 23 years with maize are reported in Table 1. The highest percentage of soil organic C is found in the 0–50 µm fraction (86.8%), whereas larger particle-size fractions contain only 6.2% (50–200 µm) and 2.5% of total soil C (200–2000 µm). On the other hand, organic C, N and C/N values increase with soil particle size, toward those of maize (Fig. 1). These results suggests that maize carbon is flowing into the soil via large particles. Such a

Table 1
Carbon and nitrogen contents of particle-size fractions from soil and of maize. The soil has been cultivated 23 years with maize

	Weight (%)	C (%)	C/total C[a] (%)	N (%)	C/N
Total soil	100	0.97	100	0.10	9.7
0–50 µm	90.54	0.93	86.8	0.11	8.5
50–200 µm	0.53	11.4	6.2	0.68	16.8
200–2000 µm	0.08	30.1	2.5	1.43	21.0
Maize[b]		40.4		1.50	26.9

[a] 4.5% of C has been lost during the particle-size fractionation, most probably as soluble or suspended material which has not precipitated after $CaCl_2$ treatment of the 0–50 µm sieved fraction.
[b] Average of leaves, shoots and roots.

hypothesis is strengthened by the microscopic observation of recognisable plant remains in the 50–200 µm- and 200–2000 µm-fractions. Moreover, Amelung et al. (1999) observed a similar increase of C/N ratios with particle size in soils amended with dung. Further insights on this incorporation pathway are given by consideration of carbon isotope ratios, as follows.

3.2. Bulk ^{13}C isotopic composition

$δ$ ^{13}C values of bulk carbon of plants, *Zea mays L.* (maize) and *Triticum aestivum L.* (wheat), and of soil particle-size fractions are reported in Table 2 and Fig. 2. The soils from the *Boigneville* experimental field have been cultivated during 23 years either with maize or wheat. The bulk carbon from the wheat soil (−25.78‰) is ^{13}C-enriched relative to wheat (−28.62‰). As suggested earlier by Lichtfouse et al. (1995), this enrichment could be explained, at least partly, by in situ formation of soil organic matter, rather than by selective preservation of plant lipids and lignin. Furthermore, the 50–200 and 200–2000 µm-particle size fractions from the wheat soil show $δ$ ^{13}C values (−27.14‰, −27,19‰) intermediate between wheat (−28.62‰) and total soil (−25.78‰). These results strengthen the hypothesis that large particle-size fractions are intermediates in the stabilisation of soil organic matter. Further evidence using ^{13}C-enriched carbon from maize as a tracer of carbon flow is given below.

Common higher plants can be classified into two isotopic categories according to their mode of CO_2 fixation (Bender, 1971; Smith and Epstein, 1971; Deines, 1980, O'Leary, 1981). C_4 plants, e.g. maize with a bulk $δ$ ^{13}C of −12.5‰, fix CO_2 by phosphoenol pyruvate carboxylation (Hatch-Slack cycle) and are ^{13}C-enriched relative to C_3 plants, e.g. wheat with a bulk $δ$ ^{13}C of −28.6‰, which incorporate CO_2 by ribulose bisphosphate carboxylation (Calvin cycle). Hence, growing
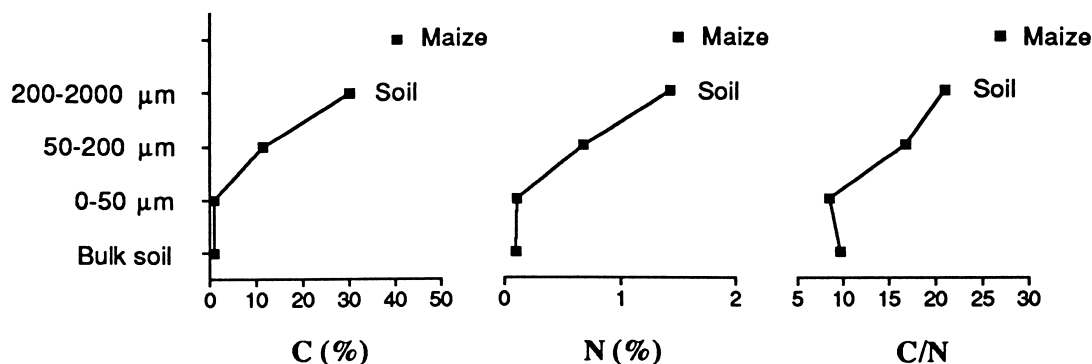
Fig. 1. Organic C, N and C/N values of maize and of soil particle-size fractions. The soil was cultivated for 23 years with maize. Note the increases of C, N and C/N values with particle size, toward maize values.

Table 2
δ ^{13}C values of bulk soil C, soil particle-size fraction C, and soil n-alkanes from fields cropped 23 years with wheat (δ_0) or maize (δ_{23})[a]

	Bulk C			C_{29} n-alkane			C_{31} n-alkane		
	δ_0 (‰)	δ_{23} (‰)	M (%)	δ_0 (‰)	δ_{23} (‰)	M (%)	δ_0 (‰)	δ_{23} (‰)	M (%)
Total soil	−25.78	−20.33	41	−35.6	−28.3	42	−36.2	−28.1	52
0–50 μm	−25.52	−21.33	32	−36.6	−28.0	47	−37.0	−27.3	59
50–200 μm	−27.14	−16.61	72	−36.7	−23.3	73	−37.1	−24.3	78
200–2000 μm	−27.19	−13.88	91	−36.6	−23.8	70	−36.9	−24.0	79

[a] The percentage of maize-derived carbon M has been calculated by isotope balance (see text). Since all plants and soils have been sampled in 1993, M values could be slightly underestimated because the δ ^{13}C of atmospheric CO_2 is decreasing due to fossil fuel burning (Marino and McElroy, 1991) and, in turn, the δ ^{13}C of maize was slighly higher in 1970 at the beginning of the experiment. Nonetheless, this phenomenon is assumed to be negligible because the δ ^{13}C shift of atmospheric CO_2 should have also decreased the δ ^{13}C of wheat and wheat soils used as the second isotopic end-member. δ ^{13}C value of bulk maize carbon (δ_m) is −12.51‰, calculated by averaging leaves (−13.18‰), shoots (−12.15‰) and roots (−12.20‰). δ ^{13}C values of n-alkanes (δ_m) from maize leaves are −18.4‰ and −20.6‰ for C_{29} and C_{31} n-alkanes, respectively. δ ^{13}C value of bulk wheat carbon is −28.62‰, calculated by averaging leaves (−29.61‰), shoots (−28.47‰) and roots (−27.79‰). δ ^{13}C values of n-alkanes from wheat leaves are −36.5‰ and −36.7‰ for C_{29} and C_{31} n-alkanes, respectively.

maize on a soil which was previously under C_3 vegetation provokes the increase of the isotopic composition of soil organic matter from −26‰ toward −12‰ (Balesdent et al., 1987; Lichtfouse, 1997b). The percentage M of maize-derived carbon in any soil organic component can thus be calculated by:

$$M = 100.(\delta_t - \delta_0)/(\delta_m - \delta_0)$$

where δ_t refers to the δ ^{13}C value of the organic component at a time t of maize cultivation, δ_0 to the organic component before maize cultivation (under C_3 vegetation), and δ_m to the organic component from maize. For instance, the δ ^{13}C value of the organic matter from the maize soil reaches −20.33‰ after 23 years of maize cultivation, thus giving a global content of maize-derived carbon of 41% (Table 2, Fig. 2). Remarkably, the δ ^{13}C values of particle-size fractions from the same soil show high variations. Indeed, the 50–200 and 200–2000 μm-

fractions (−16.61‰, −13.88‰) are ^{13}C-enriched relative to the 0–50 μm fraction (−21.33‰). Therefore, these large particle-size fractions do contain a larger amount of maize-derived carbon, reaching 91% for the 200–2000 μm fraction. Large particle-size fractions thus contain the youngest carbon. These results confirm the hypothesis by which large particle-size fractions are intermediary in the incorporation of plant organic matter into soil. Nevertheless, other incorporation pathways such as root exudation cannot be excluded.

3.3. n-Alkanes ^{13}C isotopic composition

The δ ^{13}C values of plant-derived C_{29} and C_{31} n-alkanes are reported in Table 2. n-Alkanes from the wheat soil have average δ ^{13}C values at −36.6‰ which are similar to wheat values (Fig. 3). Further, δ ^{13}C values of n-alkanes from the maize soil increase with particle size toward maize values, e.g. from −28.1‰ to
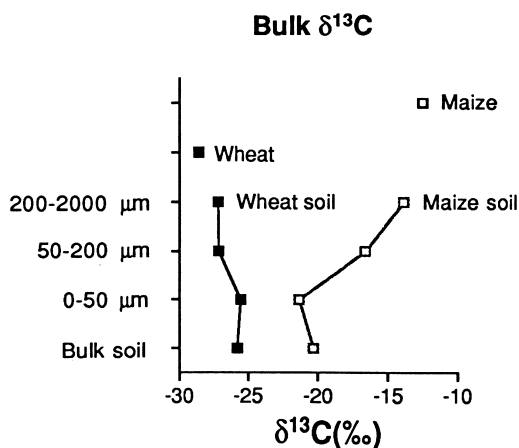
Bulk δ¹³C

Fig. 2. δ ¹³C values of bulk carbon from plants and from particle-size fractions of soils. "Wheat soil" and "Maize soil" refer to soils cultivated during 23 years with wheat and maize, respectively. Note the ¹³C-enrichment of large particles from the "Maize Soil".

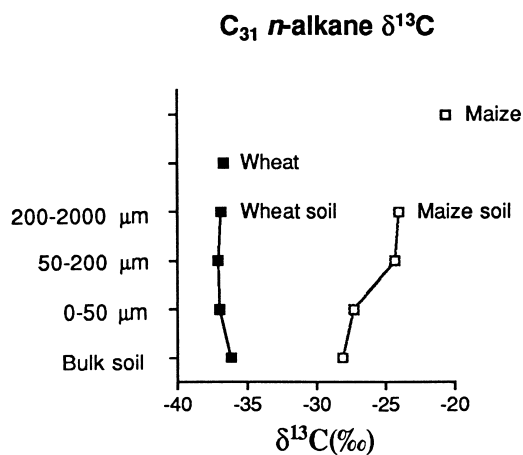## C₃₁ *n*-alkane δ¹³C

Fig. 3. δ ¹³C values of the C_{31} *n*-alkane from plants and from particle size fractions of soils. "Wheat soil" and "Maize soil" refer to soils cultivated during 23 years with wheat and maize, respectively. Note the ¹³C-enrichment of the C_{31} *n*-alkane from large particles of the maize soil.

−24.0‰ for the C_{31} *n*-alkane. Calculation by isotope balance clearly shows a larger amount of maize carbon in *n*-alkanes from large particles. For instance, the C_{31} *n*-alkane from the whole soil, and from the 200–2000 mm fraction, contains respectively 52 and 79% of maize carbon. These findings have two implications. First, plant *n*-alkanes are mainly incorporated into the soil via large particle-size fractions. Second, the incorporation of plant molecules into the soil is not homogeneous in time. Consequently, a molecular substance can occur in the same soil sample in various compartments of different biogeochemical behaviour. For example, in this

study, the C_{31} *n*-alkane is younger in the large particle-size fraction. Such molecular compartmentalisation is in agreement with recent investigations showing the occurrence of temporal pools of *n*-alkane in soil and humin (Lichtfouse et al., 1998b; Lichtfouse, 1999). In this respect, ¹³C analysis represents a method of relative dating of individual compounds, besides ¹⁴C-dating (Eglinton et al., 1996, 1997)

4. Conclusion

Elemental and isotopic studies have shown that bulk plant carbon and plant wax *n*-alkanes are incorporated into the soil, at least partly, via large particle-size fractions. Nonetheless, other possible processes of incorporation are not excluded. Isotopic analysis also provides evidence for the compartmentalisation of individual compounds into various soil pools of different age.

Acknowledgements

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