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Molecular, ^{13}C and ^{14}C evidence for the allochthonous and ancient origin of $\text{C}_{16}\text{-C}_{18}$ *n*-alkanes in modern soils.

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Abstract - The heterogeneous isotopic composition of C_3 and C_4 plants can be used to follow the fate of plant carbon into soil organic molecules. Thus, after 23 years of cropping of maize (C_4) on a soil which was previously under C_3 vegetation, $\text{C}_{25}\text{-C}_{33}$ soil *n*-alkanes are ^{13}C -enriched up to 9‰ relatively to the initial C_3 soil, reflecting the input of ^{13}C -enriched *n*-alkanes from maize waxes. In sharp contrast, $\text{C}_{16}\text{-C}_{18}$ soil *n*-alkanes do not show any significant $^{13}\text{C}/^{12}\text{C}$ variation over the same time interval. This absence of isotopic variation, along with consideration of their relative concentration, absolute concentration and biodegradability, demonstrate that these substances must represent a regular input from an external source. Evidence of a large contribution of an ancient source, amounting to more than 65% of the alkane fraction, is given by a ^{14}C -age of 8510 yrs BP. Moreover, short-chain *n*-alkanes from soils, diesel fuel, diesel automobile exhaust and petroleum products exhibit similar distributions and $\delta^{13}\text{C}$ values. These findings suggest that $\text{C}_{16}\text{-C}_{18}$ soil *n*-alkanes represent a non-point source pollution of ancient hydrocarbons either carried by aerosols or entering the soil *via* continuous hydrocarbon seepage from the deep sedimentary rocks of the Paris basin.

INTRODUCTION

n-Alkanes are widely occurring in geological samples such as soils, sediments, coals and petroleums. Like most molecular fossils, the precise origin of these substances, their role within the biogeochemical carbon cycle and the nature of processes involved in their transformation are still under speculation (KISSIN, 1987, GRIMALT and ALBAIGÉS, 1987, TEGELAAR *et al.*, 1989, SICRE *et al.*, 1990, DINEL *et al.*, 1990, LICHTFOUSE and COLLISTER, 1992, de LEEUW and LARGEAU, 1993, FREEMAN *et al.*, 1994, COLLISTER *et al.*, 1994, SPOONER *et al.*, 1994, AMBLÈS *et al.*, 1994). In recent studies we have shown that plants of distinct $^{13}\text{C}/^{12}\text{C}$ compositions, the so-called C_3 and C_4 plants, can be used as natural tracers in field experiments to study the origin and the dynamics of organic carbon from soil substances (BALESSENT *et al.*, 1987, LICHTFOUSE *et al.*, 1994a, LICHTFOUSE and BUDZINSKI, 1995, LICHTFOUSE, 1995, 1996). Indeed, those studies were based on isotopic variations observed after a change from C_3 to C_4 vegetation. Here, we show that the absence of isotopic variation of individual *n*-alkanes in such experiments can also provide some information on their source.

EXPERIMENTAL

Soil analysis

Zea mays was grown in two experimental fields at one crop per year on soils which have previously been cultivated only with C_3 plants. Soils were cropped during 5 years at *La Minière* and 23 years at *Boigneville*, France. Geological information on these soils is available elsewhere (BALABANE and BALESSENT, 1992, BALESSENT *et al.*, 1990).

Vertical cores through the 0-35 cm ploughed horizon were taken at nine random locations, well-mixed, dried at 20°C then sieved to 2 mm.

A similar fractionation procedure is described by LICHTFOUSE *et al.* (1994b). Drastic precautions were taken to prevent contamination. Typically, 100 g of sieved dried soil (total organic carbon ~ 1 %) were finely ground and extracted three times by sonication at 30°C with CHCl₃-MeOH (3:1 v/v) to give about 18.0 mg of extract. The extract was fractionated into neutral (~9.0 mg) and acid (~3.6 mg) fractions by column chromatography on KOH impregnated silica gel (McCARTHY and DUTHIE, 1962). The neutral fraction was then fractionated by thin layer chromatography (TLC) on fluorescein impregnated silica gel (0.25 or 0.5 mm thickness, CH₂Cl₂), with 1,2:3,4-dibenzanthracene, cholesterol and friedelin as side-references, to give a hydrocarbon-ester fraction (~3.2 mg, $R_F > \sim 0.85$), a ketone fraction (~2.0 mg, $R_F \sim 0.28-0.85$), an alcohol fraction (~2.0 mg, $R_F \sim 0.06-0.28$) and a polar fraction (~1.8 mg, $R_F < \sim 0.06$). The hydrocarbon-ester fraction was then fractionated by TLC on fluorescein impregnated silica gel (0.25 mm thickness, *n*-hexane), with *n*-octacosane, 1-phenyldodecane, 2-methylphenanthrene and 1,2:3,4-dibenzanthracene as side-references, to give an alkane fraction (~1.5 mg, $R_F > \sim 0.87$), an aromatic fraction (~0.9 mg, $R_F \sim 0.15-0.87$), and an ester fraction (~0.8 mg, $R_F < \sim 0.15$).

Gas chromatography (GC) analyses of *n*-alkanes from alkane fractions were performed using a Hewlett Packard GC 5990 fitted with an on-column injector and, either a flame ionization detector (FID) or a HP 5989A quadrupole mass spectrometer. Relative concentrations were obtained by integration of FID peak areas. Absolute concentrations were calculated by comparison with the area of an internal standard, 5 α -androstane. Isotopic analyses were carried out under continuous helium flow using an HP 5990 GC coupled to a CuO furnace (850°C), and a cryogenic trap (-100°C) coupled to a VG Optima mass spectrometer, monitoring continuously ion currents at $m/z = 44, 45$ and 46 . Isotopic compositions are expressed in per mil. relative to the Pee Dee Belemnite standard: $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C} \text{ sample} - {}^{13}\text{C}/{}^{12}\text{C} \text{ std})/({}^{13}\text{C}/{}^{12}\text{C} \text{ std})] \times 10^3$, where ${}^{13}\text{C}/{}^{12}\text{C} \text{ std} = 0.0112372$. The $\delta^{13}\text{C}$ values of maize wax *n*-alkanes are -19.0‰ (C₂₅), -19.1‰ (C₂₇), -18.4‰ (C₂₉), -20.6‰ (C₃₁) and -22.2‰ (C₃₃) (LICHTFOUSE *et al.*, 1994c).

An alkane fraction from a soil cultivated 4 years at La Minière field was ¹⁴C-dated as described elsewhere (EGLINTON *et al.*, 1996, LICHTFOUSE and EGLINTON, 1995). Accelerator Mass Spectrometry dating indicated a radiocarbon age of 8510 ± 120 yrs before the present (BP) corresponding to a fraction Modern (fM) of ~ 0.35.

Diesel analysis

A sample of diesel from an automobile as well as a sample of exhaust from the same vehicle were obtained. Particulate exhaust was collected on an ashed (550°C, 1 hour) glass fiber filter using a low volume (60-100 L/min) air sampler. The filter was immediately immersed in dichloromethane and stored in a sealed vial at 4°C until analysis.

Approximately 1 mL of diesel fuel was charged to an ash glass column fitted with a teflon stopcock and containing 2.5 g silica (J. T. Baker, 40-140 mesh) and 1.6 g alumina (Fisher Scientific, 80-200 mesh) in a hexane slurry. Both adsorbents were

activated at 200°C for 4 hours and stored under desiccation prior to use (DURAND, 1970). The alkanes were isolated from the aromatic components by elution from the column using 18 mL hexane and were concentrated under vacuum desiccation prior to instrumental analysis. The exhaust aerosol filter was extracted in 150 mL dichloromethane for 16 hours using a Soxhlet procedure. The dichloromethane in which the aerosol filter was immersed was filtered gravimetrically to remove soot particles and was combined with the aerosol filter extract. The combined extract was concentrated by rotoevaporation at < 30°C and the alkanes were isolated from the aromatic fraction using the same procedure as described above.

Table 1. Carbon isotopic composition of soil *n*-alkanes

TIME (years)	C ₁₆ (‰)	C ₁₇ (‰)	C ₁₈ (‰)	C ₂₅ (‰)	C ₂₇ (‰)	C ₂₉ (‰)	C ₃₁ (‰)	C ₃₃ (‰)
<i>La Minière field experiment</i>								
0.00	-29.0 (0.8)	-30.2 (0.8)	-29.2 (0.6)	-34.6 (0.3)	-34.2 (0.3)	-35.7 (0.3)	-35.7 (0.3)	-35.7 (0.3)
0.42	-29.6 (0.7)	-29.8 (0.7)	-29.7 (0.8)	-32.3 (0.3)	-32.5 (0.3)	-35.3 (0.5)	-35.2 (0.5)	n.d.
1.00	-28.9 (0.5)	-29.6 (0.7)	-28.4 (0.7)	-31.6 (0.3)	-32.7 (0.3)	-35.5 (0.3)	-35.8 (0.3)	-35.7 (0.3)
1.42	-29.5 (0.6)	-29.4 (0.8)	-28.7 (0.6)	-31.7 (0.3)	-32.1 (0.3)	-35.4 (0.3)	-35.6 (0.3)	-35.7 (0.3)
1.75	-29.5 (0.5)	-30.1 (0.5)	-29.0 (0.5)	-31.6 (0.3)	-32.3 (0.3)	-35.5 (0.3)	-35.5 (0.3)	-35.4 (0.3)
2.42	-29.7 (0.5)	-30.3 (0.5)	-29.5 (0.8)	-30.6 (0.5)	-31.1 (0.3)	-35.1 (0.3)	-35.0 (0.3)	-35.2 (0.3)
3.42	-29.5 (0.5)	-30.4 (0.5)	-29.1 (0.6)	-29.9 (0.3)	-29.5 (0.3)	-34.7 (0.3)	-34.0 (0.4)	-33.9 (0.4)
4.00	-29.4 (0.8)	-30.3 (0.5)	-29.6 (0.6)	n.d.	-29.7 (0.3)	-34.5 (0.3)	-34.4 (0.3)	-33.6 (0.5)
4.33	-29.3 (0.5)	-30.0 (0.6)	-29.5 (0.6)	-30.2 (0.3)	-29.5 (0.3)	-34.3 (0.3)	-33.7 (0.3)	-33.4 (0.3)
Average [†]	-29.4 (0.3)	-30.0 (0.3)	-29.2 (0.4)					
<i>Boigneville field experiment</i>								
Wheat [*]	-29.7 (0.6)	-31.6 (0.7)	-29.8 (0.6)	n.d.	-33.0 (0.3)	-35.7 (0.3)	-35.7 (0.3)	-33.7 (0.3)
20.0	-29.7 (0.8)	-31.5 (0.8)	-29.8 (0.8)	n.d.	-24.1 (0.3)	-28.8 (0.3)	-28.7 (0.3)	-26.7 (0.3)
23.0	-28.3 (0.5)	-28.9 (0.5)	-28.1 (0.5)	n.d.	-24.0 (0.3)	-28.3 (0.3)	-28.1 (0.3)	-25.6 (0.3)

Note: $\delta^{13}\text{C}$ values of soil *n*-alkanes versus time of maize cultivation. ^{*} Soil cultivated 23 years with wheat. The average deviation of 3-4 replicates are given in parenthesis. [†] Calculated mean deviation in parenthesis. n. d. = not determined.

The alkanes were characterized with a Hewlett Packard (HP) 5890 series II gas chromatograph equipped with an HP-1 (12m x 0.2mm i.d.) column which was interfaced to a HP 5971 mass selective detector (GC/MS). The GC temperature program was as follows : hold at 70°C for 2 min, increase at a rate of 6°C/min to 280°C, hold at 280°C for 20 min. Compound specific carbon isotope data were obtained using a VG Prism series II isotope ratio mass spectrometer (IRMS) and a Hewlett Packard 5890 series II GC equipped with the same column as above and coupled through a combustor furnace and water trap to the VG Prism ratio mass spectrometer (GC/C/IRMS).

RESULTS AND DISCUSSION

¹³C Labelling of Soil Organic Molecules at Natural Abundance

A labelling experiment with carbon 13 at natural abundance has been designed to follow the fate of plant carbon into soil organic matter. Thus, maize, a C₄ plant, has been cultivated at two sites on soils which were previously cultivated with isotopically distinct C₃ plants. Maize has been cultivated at one crop per year over a 5-year period at the *La Minière* site and over a 23-year period at the *Boigneville* site. The carbon isotopic composition of *n*-alkanes from those soils are reported in Table 1.

The isotopic compositions of C₂₅-C₃₃ *n*-alkanes from soils increase with increasing time of maize cultivation (Figure 1). It has been shown that this increase resulted from the input of ¹³C-enriched *n*-alkanes from maize waxes into soil organic matter (LICHTFOUSE *et al.*, 1994a, LICHTFOUSE, 1995, 1996). It is even possible to calculate the contribution of maize *n*-alkane to each soil *n*-alkane at any time of cultivation, using the following equation:

$$M = 100 \cdot (\delta - \delta_0) / (\delta_m - \delta_0)$$

where M is the carbon percentage (or mass percentage) of maize *n*-alkane within soil *n*-alkane, δ is the isotopic composition of soil *n*-alkane at a given time of cultivation, δ_0 is the isotopic composition of soil *n*-alkane before maize cultivation and δ_m is the isotopic composition of leaf wax *n*-alkane. For instance, after 23 years of maize cultivation, M reaches 50.3 % for the C₃₁ *n*-alkane. These experiments under natural conditions can therefore give access to the dynamics of individual organic substances.

Isotopic Compositions of Soil C₁₆-C₁₈ *n*-alkanes

In contrast, C₁₆, C₁₇ and C₁₈ soil *n*-alkanes show, within the experimental error, constant δ ¹³C values over the 5-year experiment at *La Minière*, averaging at -29.4‰, -30.0‰ and -29.2‰ respectively (Table 1, Figure 1). Moreover, even after 23 years of maize cultivation at the Boigneville field, no isotopic increase is apparent for C₁₆-C₁₈ *n*-alkanes (~ -28.4‰). This absence of isotopic variation on these long-term experiments demonstrates that these *n*-alkanes must derive from an external source, as explained below.

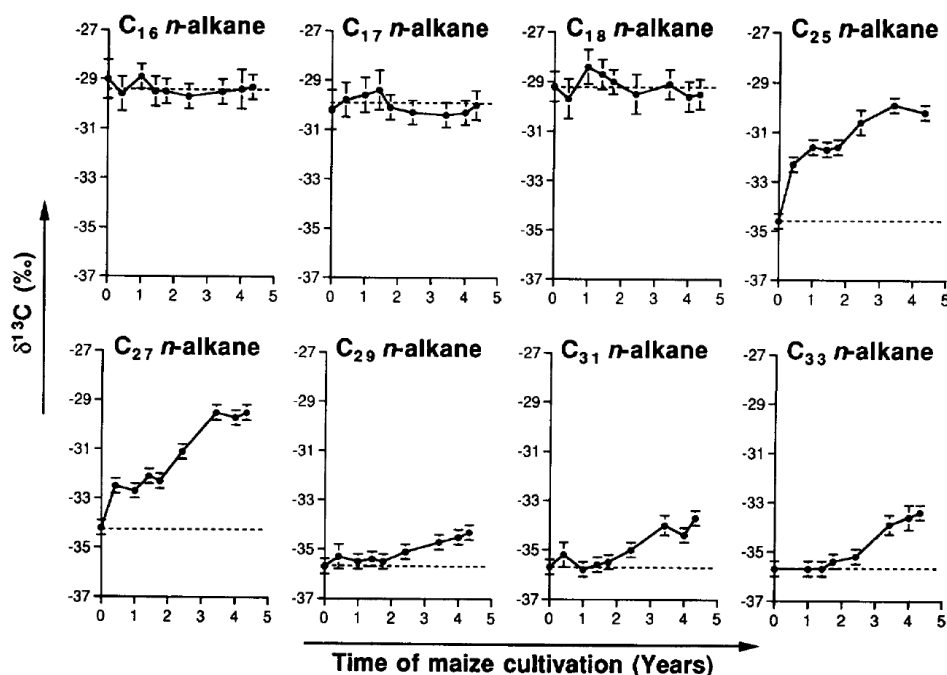


Figure 1. $\delta^{13}\text{C}$ isotopic compositions of *n*-alkanes from soils cultivated maize. Note the absence of significant variations of C₁₆-C₁₈ *n*-alkanes $\delta^{13}\text{C}$ values over the 5-year period.

The absence of isotopic variation shows that C₁₆-C₁₈ *n*-alkanes are not derived from soil biomass. Indeed, if those substances were to derive from certain soil organisms, they should have integrated at least a small portion of heavy maize-derived carbon. This is especially true after 23 years of cultivation, where the maize carbon content of C₃₁ *n*-alkane is 50.3%. Moreover, because short-chain *n*-alkanes are more biodegradable, more volatile and more susceptible to weathering and water-washing than higher homologues (VOLKMAN *et al.*, 1983, BOSSERT and BARTHA, 1984, PETERS and MOLDOWAN, 1993), one would expect a higher turnover and thus a higher maize-derived carbon content for C₁₆-C₁₈ soil *n*-alkanes. This rapid degradation of short-chain *n*-alkanes is well supported by an incubation of C₂₀ *n*-alkane into a rendzina soil, which show that 25% of this component is mineralized only after 8 weeks (AMBLÈS *et al.*, 1994). On the contrary, the lack of isotopic variation of C₁₆-C₁₈ soil *n*-alkanes in our experiments indicates that their carbon does not derive from the soil biomass. These results are also supported by incubation of soils from Grignon, France, with ¹³C-labelled glucose, showing that all *n*-alkanes in the C₁₆-C₃₅ range do not incorporate heavy carbon from glucose (LICHTFOUSE *et al.*, 1995).

The distribution of C₁₅-C₁₈ soil *n*-alkanes do not show any strong predominance of odd (or even) carbon-number homologues (Figure 2). This feature is typical of hydrocarbons occurring in mature, ancient sedimentary organic matter such as fossil fuels (TISSOT *et al.*, 1971, TISSOT and WELTE, 1984). To check this possible contribution of ancient carbon, a soil alkane fraction was analysed for ¹⁴C. AMS dating indicated a radiocarbon age of 8510 ± 120 yrs before the present (BP) corresponding to a fraction Modern (fM) of ~ 0.35 . If we assume that this fraction is a mixture of modern C and fossil fuel C (no ¹⁴C), then the fraction Modern indicates that there is 35 % of modern C and 65% of fossil-fuel C. In fact, 65% represents a minimum value for fossil-

fuel carbon because incorporation of some bomb-derived ^{14}C into the biomass gives rise to overestimation of $\delta^{13}\text{C}$ values (LICHTFOUSE and EGLINTON, 1995). This additional isotopic evidence therefore demonstrates unambiguously the occurrence of ancient carbon in the soil alkane fraction.

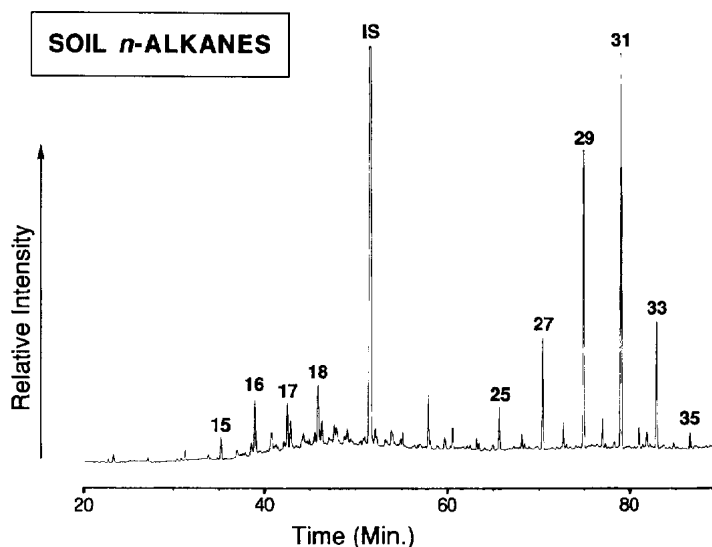


Figure 2. Typical distribution of *n*-alkanes from alkane fractions of soil organic extracts (this chromatogram from the La Minière field experiment after 4 years of maize cultivation). IS: Internal Standard, 5α -androstane. Carbon number of *n*-alkanes are drawn on top of peaks. Conditions: gas chromatography fitted with a flame ionization detector; fused-silica capillary column HP Ultra 1 (25 m x 0.20 mm); 100% methylsilicone phase (0.11 μm); helium carrier gas (1 ml/min). Temperature: 50°C (2 min), 50-320°C at 3°/min, 320°C (60 min).

Diesel *n*-alkanes

The *n*-alkanes from diesel fuel and diesel automobile exhaust range from C_{12} to C_{24} with no even or odd predominance (Figure 3 and 4). The *n*-alkanes from the fuel showed an abundance maximum at C_{15} , whereas those from the exhaust showed a maximum at C_{20} , suggesting a preferential loss during combustion and sampling. ^{13}C values of diesel *n*-alkanes range from -29.1‰ to -26.5‰ (Table 2, Figure 5). Pristane and phytane show lighter values averaging at -29.8‰ and -31.0‰, respectively.

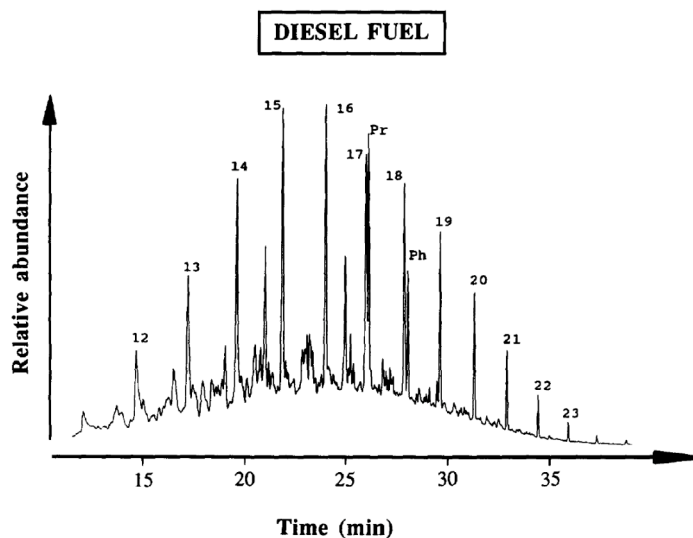


Figure 3. Gas chromatogram of alkanes extracted from the diesel fuel. Pr : pristane, Ph : phytane.

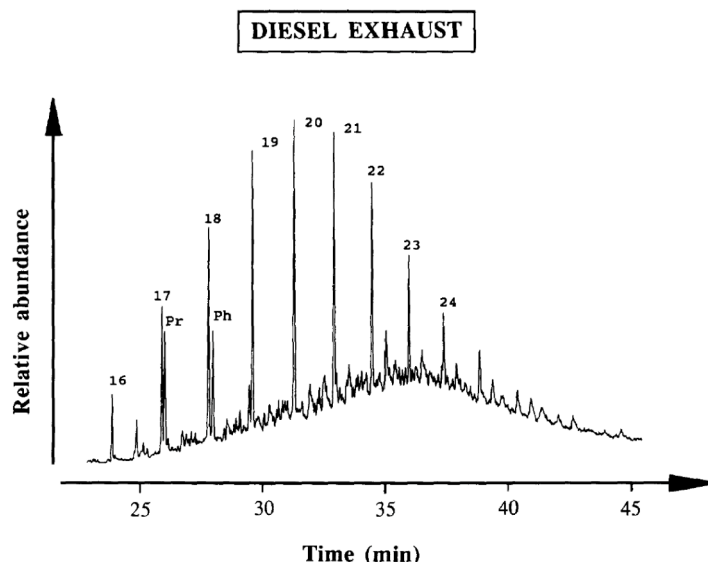


Figure 4. Gas chromatogram of alkanes extracted from the diesel exhaust. Pr : pristane, Ph : phytane.

Table 2. Carbon isotopic composition (‰) of alkanes isolated from diesel fuel and automobile exhaust.

Alkane	Fuel	Exhaust
C ₁₂	-28.3	n.d.
C ₁₃	-27.9	n.d.
C ₁₄	-27.0	n.d.
C ₁₅	-26.9	n.d.
C ₁₆	-27.2	-27.4
C ₁₇	-26.5	-28.0
C ₁₈	-27.0	-29.1
C ₁₉	-28.3	-26.8
C ₂₀	-28.5	-27.4
C ₂₁	-28.9	-27.6
C ₂₂	-27.7	-28.0
C ₂₃	-27.6	-27.5
C ₂₄	n.d.	-27.2
Pristane	-29.6	-30.1
Phytane	-30.7	-31.3

Short-chain soil *n*-alkanes and *n*-alkanes from diesel products show close distributions and ¹³C values. These findings suggest that fuel products, like those emitted by vehicles, are a likely source of soil *n*-alkanes through aeolian transport then deposition onto the soil surface. Removal of pollutants and particulate matter from the atmosphere by adsorption on waxy surfaces of plants (SMITH, 1977, SIMONICH and HITES, 1994) could also play a major role because cropping practices induce a high flux of plant debris into the soil. Crop fields could therefore act as a major sink of pollutants carried by aerosols.

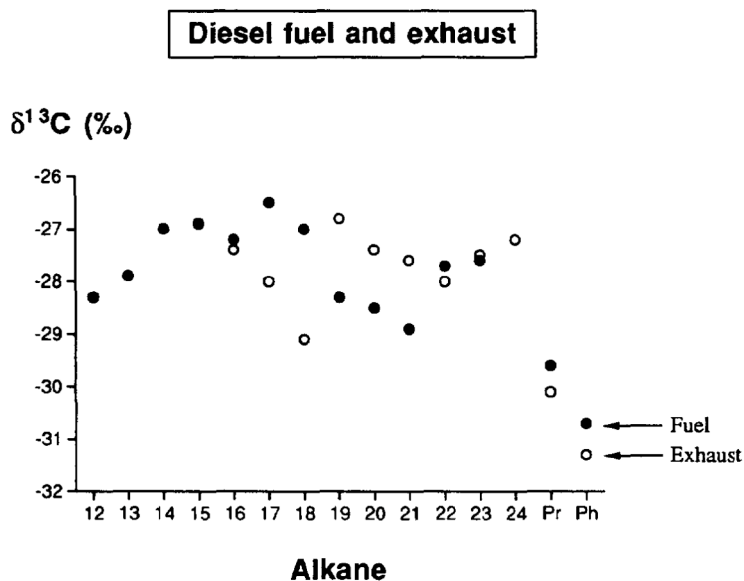


Figure 5. Carbon isotopic data for alkanes extracted from diesel fuel and exhaust. Pr : pristane, Ph : phytane.

Origin of Soil C₁₆-C₁₈ *n*-Alkanes

Several external sources can be considered. First, C₁₆-C₁₈ *n*-alkanes may be derived from the pre-existing sedimentary rock. However, they should have been biodegraded, or water-washed rapidly after the soil formation, especially after the beginning of tillage where water-washing and biodegradation are strongly enhanced by cropping practices. This view is strengthened by consideration of *n*-alkane absolute concentrations (Table 3). Indeed, at *La Minière*, absolute concentrations of C₁₆, C₁₇ and C₁₈ *n*-alkanes average at 33, 27 and 49 ng per g of soil, respectively, and do not show any systematic decrease nor increase with increasing time of cultivation. They must therefore represent a rather regular input in order to sustain their absolute concentrations in soils. Noteworthy, the absolute concentrations of C₁₆-C₁₈ soil *n*-alkanes (27-49 ng/g) is in the same concentration level as C₂₅-C₃₃ *n*-alkanes (25-232 ng/g). This suggests that, given their higher degradability, the amount of C₁₆-C₁₈ soil *n*-alkanes introduced into the soil over a fixed time period should be even higher than the amount of C₂₅-C₃₃ soil *n*-alkanes derived from maize waxes.

Table 3. Absolute concentration of soil *n*-alkanes

TIME (years)	C ₁₆	C ₁₇	C ₁₈	C ₂₅	C ₂₇	C ₂₉	C ₃₁	C ₃₃
(ng/g dried soil)								
<i>La Minière field experiment</i>								
0.00	35	40	39	21	50	155	214	59
0.42	28	25	n.d.	24	66	198	284	77
1.00	17	16	26	18	36	108	153	42
1.42	44	29	96	22	50	139	198	53
1.75	19	18	34	19	41	121	174	50
2.42	62	39	68	31	72	191	280	79
3.42	24	21	40	29	67	173	262	71
4.00	33	26	41	22	61	150	212	64
4.33	31	25	49	37	88	213	312	89
Average [†]	33	27	49	25	59	161	232	65
	(13)	(8)	(21)	(6)	(15)	(34)	(51)	(14)
<i>Boigneville field experiment</i>								
Wheat [*]	24	11	39	14	41	140	188	61
20.0	10	5	6	10	30	61	101	46
23.0	28	28	53	15	30	58	97	40

Note: ^{*} Soil cultivated 23 years with wheat. n. d. = not determined. [†] Calculated mean deviation in parenthesis.

Secondly, C₁₆-C₁₈ soil *n*-alkanes could be derived from algae growing at the soil surface, because atmospheric CO₂ represents a carbon source of relatively constant isotopic composition. There are indeed several reports of C₁₅-C₂₃ *n*-alkanes and *n*-alkenes in microalgae (GELPI *et al.*, 1968, 1970, WEETE, 1976). However, those algal substances show a strong predominance of odd-carbon number homologues, e.g. at C₁₇, which should be retained, at least partly, when algal constituents are transferred to soil organic matter. Such a predominance is not apparent in the C₁₆-C₁₈ *n*-alkanes distributions (Figure 2, Table 3). Moreover, soil algae fix both atmospheric CO₂ and CO₂ produced by the degradation of soil organic matter. This soil CO₂ must show a significant isotopic increase with increasing time of cultivation due to the input of maize matter. Therefore algal constituents should also show an isotopic shift toward heavier values with increasing time of maize cultivation. A contribution of C₁₆-C₁₈ soil *n*-alkanes can thus be reasonably excluded here.

The most probable origin of C₁₆-C₁₈ soil *n*-alkanes is an input from fossil fuels, either by aeolian input, by cropping practices, e.g. fumes from exhausts, or by continuous hydrocarbon seepage from the subsurface. Indeed, their weak even carbon number predominance is usually encountered in petroleums and sediments of medium to high maturity, whereas soil *n*-alkanes in the C₂₅-C₃₅ range show a strong predominance of odd carbon number homologues, indicative of an immature contribution (Figure 2, TISSOT and WELTE, 1984). Moreover, the isotopic values of C₁₆-C₁₈ soil *n*-alkanes range from -28.1 to -31.6 (Table 1), which is in good agreement,

with only few exceptions, with isotopic ranges reported in various mature sedimentary rocks and petroleum (CLAYTON and BJORØY, 1994, WILHEMS *et al.*, 1994, BJORØY *et al.*, 1994, LICHTFOUSE *et al.*, 1994b), and in diesel fuel products (this study). On the other hand, the two crop fields studied are both lying in the Paris basin, which is well known for the occurrence of mature Liassic organic matter and petroleum in deep formations (TISSOT *et al.*, 1971, LICHTFOUSE *et al.*, 1994b). Continuous hydrocarbon seepage from the subsurface may thus alternatively account for the persistence of short-chain alkanes in soils at low but detectable levels.

CONCLUSION

Molecular, ^{13}C and ^{14}C information has been used to decipher the origin of non-point source pollutants occurring in soil. These findings show that short-chain *n*-alkanes from crop soils represent a regular input of fossil fuel hydrocarbons either from aerosols, e.g. vehicle exhausts, or from deep sedimentary rocks. Furthermore, the absence of isotopic variation during long term labelling experiments can be used to assess the indigenous origin of individual organic substances. This principle may apply to any media that has recorded a well-marked isotopic change : sediments, soils, tree rings, peat, ice, shells, etc.

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