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### Isotope and molecular evidence for direct input of maize leaf wax *n*-alkanes into crop soils

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**Abstract-** The contribution of plant carbon to crop soils can be followed by isotope labelling at natural abundance, such as growing a C<sub>4</sub> plant on a soil which was previously under C<sub>3</sub> vegetation. For this purpose, carbon isotope compositions and relative abundances of *n*-alkanes of maize leaf waxes and maize crop soils were compared. Isotope values of soil *n*-alkanes increased with time of maize cultivation as the result of maize carbon integration into soil organic matter. With increasing time of cultivation, the increase in isotopic difference between *n*-heptacosane (C<sub>27</sub>) and *n*-nonacosane (C<sub>29</sub>) is explained, at least partly, by a *direct* input of maize leaf *n*-alkanes. The amount of maize-derived carbon within each *n*-alkane has been calculated by isotopic means.

*Key words* - *n*-alkane, soil, plant, waxes, *Zea mays*, <sup>13</sup>C.

#### INTRODUCTION

Distributions of sedimentary *n*-alkanes have been widely used for source identification in sediments. However, the specificity of *n*-alkanes as source parameters might be limited because our knowledge of biological *n*-alkanes, or their possible precursors, is relatively poor, especially among microorganisms, as reviewed by Collister *et al.*, 1994a. For example, the distribution of *n*-alkenes from the alga *Botryococcus Braunii* (A race) with a strong predominance of odd-carbon number homologues in the range C<sub>27</sub> to C<sub>33</sub>, is very close to *n*-alkane distributions in terrestrial plants (Lichtfouse *et al.*, 1994a). Moreover, recent isotope studies have shown that extracted sedimentary *n*-alkanes can derive not only from higher plants *n*-alkanes but also from *n*-alkanoic acids (Lichtfouse and Collister, 1992) or from algal *n*-alkenes (Freeman *et al.*, 1994, Collister *et al.*, 1994a, Spooner *et al.*, 1994, Lichtfouse *et al.*, 1994a).

In soils, studies of possible sources of organic components seem more straightforward, as compared to sediments, because the input of phytoplankton is minor relative to the input of higher plants. However, numerous soil organisms are able to biosynthesize *n*-alkanes or their precursors (Kolattukudy, 1976, Diné *et al.*, 1990). For this reason, and also because food chain flows from plants to soil biomass are far from being understood, the *direct* input of leaf wax *n*-alkanes into soil is still to be proved. The fate of plant carbon into a soil can be followed by isotopic labelling in natural conditions. Hence, growing a C<sub>4</sub> plant, maize (bulk δ<sup>13</sup>C -12‰) on a soil which was previously under C<sub>3</sub> vegetation (bulk δ<sup>13</sup>C -26‰) can cause the isotopic composition of bulk soil organic matter to increase from -26‰ toward -13‰ (Balesdent *et al.*, 1987). Because leaf wax *n*-alkanes from C<sub>3</sub> plants and C<sub>4</sub> plants are also isotopically different (Rieley *et al.*, 1991, 1993, Collister *et al.*, 1994b, Lichtfouse *et al.*, 1994a), the isotopic composition of soil *n*-alkanes should be related to their source. In this preliminary report, we

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have evaluated the contribution of leaf wax *n*-alkanes to soil *n*-alkanes via molecular and isotope analysis.

### EXPERIMENTAL

Detailed procedures will be described elsewhere (Lichtfouse, 1994). *Zea mays* was grown in an experimental field at La Minière, France, at one crop per year on a soil which has previously been cultivated only with C<sub>3</sub> plants. Soils and *Z. mays* leaves were extracted with CHCl<sub>3</sub>-MeOH 3/1 v/v. Alkane fractions were obtained from extracts by silica gel chromatography (Lichtfouse *et al.*, 1994b). *n*-Alkanes were identified by gas chromatography-mass spectrometry (GC-MS) and by co-elution of pure standards. Their purity (>99.9%) was carefully checked by GC-MS and gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) in order to give accurate isotopic values (Lichtfouse *et al.*, 1991). The overall deviation was  $\pm 0.3\%$ . *n*-Alkane relative abundances (area/area) were determined by gas chromatography using a flame ionization detector. GC-C-IRMS conditions are given in Lichtfouse *et al.* (1994a). Carbon isotopic compositions are expressed in per mil. relative to the PDB standard:  $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C} \text{ sample} - {}^{13}\text{C}/{}^{12}\text{C} \text{ std})/({}^{13}\text{C}/{}^{12}\text{C} \text{ std})] \times 10^3$ , where  ${}^{13}\text{C}/{}^{12}\text{C} \text{ std} = 0.0112372$ .

### RESULTS AND DISCUSSION

#### *Initial isotope ratios*

Carbon isotope compositions and relative abundances of selected *n*-alkanes from maize leaf waxes and maize crop soils are reported in Table 1. Before maize cropping, the soil was cultivated with C<sub>3</sub> plants. Distributions of soil *n*-alkanes in the range C<sub>25</sub> to C<sub>35</sub> were very similar to those of higher plants, e.g. they display a strong odd-carbon number predominance (Lichtfouse, 1994). Before cultivation, the isotope compositions for *n*-heptacosane (C<sub>27</sub>) and *n*-nonacosane (C<sub>29</sub>) were  $-34.2\%$  and  $-35.7\%$ , respectively (Table 1, Figure 1). These values are well within the range of those reported for C<sub>3</sub> plants (Rieley *et al.*, 1991, 1993, Collister *et al.*, 1994b, Lichtfouse *et al.*, 1994a). Also, observation of distributions together with isotope values strongly suggests a direct contribution of C<sub>3</sub> plants waxes from previous C<sub>3</sub> vegetation, although a contribution from soil organisms which biosynthesize those *n*-alkanes on C<sub>3</sub> carbon is not excluded.

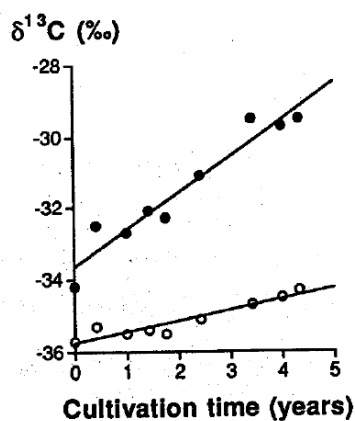


Fig. 1. Carbon isotope composition of soil *n*-alkanes versus time of maize cultivation. Note the higher isotopic increase of *n*-heptacosane (C<sub>27</sub>) versus *n*-nonacosane (C<sub>29</sub>). Linear regression gives  $\delta_{27} = 1.028 \cdot \text{time} - 33.66$  ( $r = 0.96$ ) and  $\delta_{29} = 0.300 \cdot \text{time} - 35.74$  ( $r = 0.94$ ).

Table 1. Carbon isotope composition and relative abundance of selected *n*-alkanes from crop soils and maize leaf waxes.

Cultivation time (years)	Carbon number		C <sub>27</sub> /C <sub>29</sub>
	27	29	
0*	-34.2	-35.7	0.321
0.42	-32.5	-35.3	0.332
1.00	-32.7	-35.5	0.335
1.42	-32.1	-35.4	0.355
1.75	-32.3	-35.5	0.338
2.42	-31.1	-35.1	0.378
3.42	-29.5	-34.7	0.389
4.00	-29.7	-34.5	0.403
4.33	-29.5	-34.3	0.411
Maize	-19.1	-18.4	0.916

\* Uncultivated soil previously under C<sub>3</sub> vegetation.

#### ***Isotopic variations with increasing time of cultivation***

Isotope compositions of *n*-alkanes increased with cultivation time (Figure 1). Such variations are explained by the input of <sup>13</sup>C enriched carbon from maize into soil organic components. A *direct* input of maize leaf waxes into soil is possible since isotope compositions of maize *n*-alkanes are <sup>13</sup>C enriched: -19.1 ‰ for *n*-heptacosane (C<sub>27</sub>) and -18.4‰ for *n*-nonacosane (C<sub>29</sub>). Also the progressive addition of maize *n*-alkanes during cultivation should increase the isotope values of the soil *n*-alkanes, as shown in Fig. 1.

From the isotopic trends, it is obvious that the amount of maize-derived carbon in *n*-heptacosane (C<sub>27</sub>) increased faster than in *n*-nonacosane (C<sub>29</sub>). This difference can be quantitated by two means. Firstly, it can be assumed that isotopic trends versus cultivation time follow a linear law on a short time period, though this is probably not true for longer experiments. The slope calculated by linear regression, which is representative of the isotopic increase versus cultivation time for each component, is indeed much higher for *n*-heptacosane (1.028 ‰.yr<sup>-1</sup>) than for *n*-nonacosane (0.300 ‰.yr<sup>-1</sup>). Secondly, each soil *n*-alkane can be regarded as a mixture of two distinct isotopic sources: "C<sub>3</sub>" *n*-alkane from the initial soil and "C<sub>4</sub>" *n*-alkane from maize waxes. At a given time of cultivation, the molar percentage of maize-wax derived carbon M in each soil *n*-alkane can be calculated by

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

$\delta$ : isotope composition of soil *n*-alkane at a given time of cultivation.

$\delta_0$ : isotope composition of soil *n*-alkane before maize cultivation (time = 0).

$\delta_m$ : isotope composition of *n*-alkane for maize waxes.

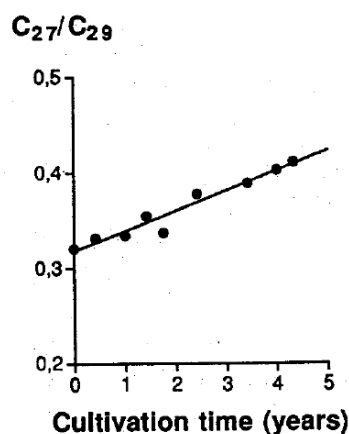


Fig. 2. *n*-Heptacosane (C<sub>27</sub>)/*n*-nonacosane (C<sub>29</sub>) ratios in crop soils versus time of maize cultivation. Linear regression gives  $C_{27}/C_{29} = 0.021 \cdot \text{time} + 0.319$  ( $r = 0.97$ ).

Calculated M values after 5 crops of maize (4.33 yrs.) are 31.1‰ for *n*-heptacosane and 8.1‰ for *n*-nonacosane. Those values show again that *n*-heptacosane incorporated more maize-derived carbon than *n*-nonacosane, probably *via* direct input of leaf waxes. Indeed, this hypothesis is strengthened by consideration of *n*-alkane relative abundances, as discussed below.

#### *n*-Alkane ratios in soils and maize

In order to get more insight on the mode of integration of maize carbon into crop soils, *n*-alkane relative abundances from maize leaves and soils were compared using C<sub>27</sub>/C<sub>29</sub> ratios (Table 1). Maize waxes had a much higher C<sub>27</sub>/C<sub>29</sub> ratio (0.916) than did the soil before maize cultivation (0.321). It is thus expected that the C<sub>27</sub>/C<sub>29</sub> ratio would increase with time of cultivation if a direct input were happening. C<sub>27</sub>/C<sub>29</sub> ratios indeed showed a regular increase from 0.321 to 0.411 during 5 years of maize cropping (Fig. 2), thus demonstrating that soil *n*-alkanes were derived, at least partly, by *direct* input of maize leaf waxes. Further evidence is given by comparison of C<sub>27</sub>/C<sub>29</sub> ratios with δ<sub>27</sub>-δ<sub>29</sub> in soils (Figure 3). The correlation observed confirms that variations of isotopic differences between *n*-heptacosane and *n*-nonacosane resulted, at least partially, from a higher input of *n*-heptacosane from maize leaf waxes.

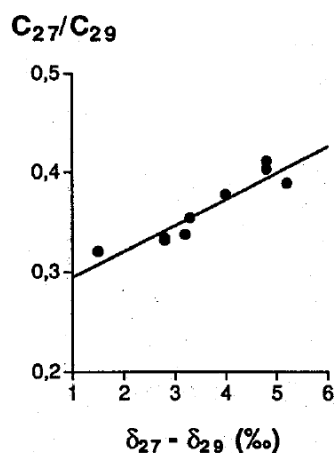


Fig. 3. Abundance ratios versus isotopic difference for *n*-heptacosane (C<sub>27</sub>) and *n*-nonacosane (C<sub>29</sub>) in maize crop soils. Linear regression gives  $\delta_{27} - \delta_{29} = 0.026 \cdot C_{27}/C_{29} + 0.269$  ( $r = 0.93$ ).

## CONCLUSION

The contribution of plant waxes to crop soils has been evaluated by *in situ* labelling under natural conditions. A simple calculation has been used to evaluate the contribution of isotopically distinct sources within each *n*-alkane. Furthermore, soil *n*-alkanes have been shown to derive, at least partly, from the *direct* input of plant leaf *n*-alkanes. Isotope studies of other *n*-alkanes and organic fractions will be published elsewhere (Lichtfouse, 1994). Further work on soil alcohols and fatty acids is in progress.

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