¹³C Labelling of Soil *n*-Hentriacontane (C₃₁) by Maize Cultivation

Éric Lichtfouse

Laboratoire de Biogéochimie Isotopique, associé à l'Institut National de la Recherche Agronomique,
Université Pierre et Marie Curie, Case 120, 75252 Paris Cedex 05, France.

Abstract: The fate of plant carbon into molecular organic substances from soils can be followed by stable carbon analysis. Soil organic matter has thus been progressively labelled with ¹³C at natural abundance by cultivation of *Zea mays*, a C₄ plant, on a soil which was previously under isotopically distinct C₃ vegetation. The molar carbon percentage of maize *n*-hentriacontane within soil *n*-hentriacontane has been calculated by isotopic means and amounts to around 50% after 23 years of maize cultivation.

Soil organic matter is a complex mixture of humic substances inherited from plants and soil biomass such as algae, worms, fungi and bacteria. It is also partly polymeric and partly bound to minerals. Though bulk chemical properties of humic substances have been extensively studied,¹ our knowledge of the precise biological origin and the transformation pathways of soil organic molecules is poor. The recent availability of gas chromatography-combustion-isotope ratio monitoring mass spectrometry (GC-C-IRMS)² now allows ¹³C/¹²C determination of organic substances occurring at trace levels in complex mixtures. It is thus possible to use ¹³C as a tracer to evaluate the contribution of isotopically distinct carbon sources within *each* molecule. I wish to report here the use of two carbon sources of different isotopic compositions, C₃ and C₄ plants, to follow the fate of *n*-hentriacontane from plant waxes into soil organic matter **1**.

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Common higher plants can be classified into two isotopic categories according to their mode of CO₂ fixation.³ C₄ plants, e.g. *Zea mays* (maize), fix CO₂ by phosphoenol pyruvate carboxylation (Hatch-Slack cycle) and are ¹³C-enriched relative to C₃ plants, e.g. *Triticum aestivum* (wheat), which incorporate CO₂ by ribulose biphosphate carboxylation (Calvin cycle). Alkane fractions from plant leaves and crop soils have been isolated by silica gel thin layer chromatography then analysed by GC-C-IRMS.^{4,5} As expected for C₄ and C₃ plants,⁶ the *n*-hentriacontane of leaf wax from maize shows a heavier isotopic composition⁷ (δ¹³C = -20.6‰) than the *n*-hentriacontane of leaf wax from wheat (-36.7‰). Maize cultivation on a soil which was previously cultivated with C₃ plants thus increases the isotope value of soil *n*-hentriacontane, as a result of progressive inputs of ¹³C-enriched *n*-hentriacontane of leaf wax from maize (Fig. 2). The following data were obtained (years, ‰): (0, -35.7), (4.3, -33.7), (20, -28.7), (23, -28.1).

Fig. 2. Isotopic composition of soil *n*-hentriacontane (left) and molar carbon percentage M of *n*-hentriacontane from maize in soil *n*-hentriacontane (right) versus time of maize cultivation.

Soil *n*-hentriacontane is mainly a mixture of *n*-hentriacontane from maize and *n*-hentriacontane from previous C_3 vegetation. Also, the molar carbon percentage M of maize *n*-hentriacontane within soil *n*-hentriacontane can be calculated⁵ by $M = 100 \cdot (\delta - \delta_0) / (\delta_m - \delta_0)$, where δ is the isotope composition of soil *n*-hentriacontane at a given time of cultivation, δ_0 is the isotope composition of soil *n*-hentriacontane before maize cultivation (-35.7‰) and δ_m is the isotope composition of *n*-hentriacontane of leaf wax from maize (-20.6‰). After 23 years of maize cultivation, M reaches 50.3 %: about half of the carbon of soil *n*-hentriacontane thus comes from maize waxes at that time. This low turnover is probably due to the resistance of long chain *n*-alkanes to biodegradation, in comparison with more functionalized substances such as amino acids and carbohydrates.

REFERENCES AND NOTES

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7. Isotopic compositions are expressed relative to the PDB standard: $\delta^{13}\text{C} (\text{‰}) = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{std}}) / ({}^{13}\text{C}/{}^{12}\text{C}_{\text{std}})] \times 10^3$, where ${}^{13}\text{C}/{}^{12}\text{C}_{\text{std}} = 0.0112372$.