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Diagenesis effects on specific carbon isotope composition of plant *n*-alkanes

T.T. Nguyen Tu[a,*], S. Derenne[b], C. Largeau[b], G. Bardoux[a], A. Mariotti[a]

[a]*Laboratoire de Biogéochimie des Milieux Continentaux, UMR 7618 UPMC-CNRS-INRA, Centre INRA de Versailles-Grignon, Bâtiment EGER, 78 850 Thiverval-Grignon, France*

[b]*Laboratoire de Chimie Bioorganique et Organique Physique, Ecole Nationale Supérieure de Chimie de Paris, CNRS UMR 7573, 11 Rue Pierre et Marie Curie, 75 231 Paris Cédex 05, France*

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Abstract

The stable carbon isotope compositions of individual *n*-alkanes extracted from leaves of (1) *Ginkgo biloba* degrading in a well-drained soil and (2) *Eretmophyllum*, a fossil Ginkgoale, were investigated in a first approach to refining the estimate of potential diagenetic effects on the isotopic composition of sedimentary alkanes derived from higher plants; *n*-alkanes extracted from fresh leaves of *G. biloba* appeared ^{13}C-depleted when compared with bulk leaves. Within a given extract they exhibited characteristic variability in specific isotope composition with respect to chain length. Although the isotope composition of bulk leaves remained unchanged through senescence and litter formation, the specific *n*-alkane isotopic composition varied in two respects: (i) general ^{13}C-enrichment in all *n*-alkanes and (ii) homogenisation of specific isotopic composition of all the *n*-alkanes of a given extract. As for fresh and degrading leaves of *G. biloba, n*-alkanes extracted from the fossil *Eretmophyllum* appeared ^{13}C-depleted when compared with bulk leaves. Their isotope distribution patterns suggested that the isotope composition of fossil alkanes may have been slightly affected by diagenesis. Results for degrading leaves and fossil leaves (1) indicated that the isotope composition of individual alkanes can be more affected by degradation than that of bulk leaves and (2) confirmed that the isotope signal in fossils, and sedimentary organic matter in general, may be less altered than in the organic matter of well-drained soil.

1. Introduction

1.1. Stable carbon isotope ratios in organic geochemistry

Stable carbon isotope ratios can differ among living organisms and may vary according to environmental conditions (e.g. reviews by Deines, 1980; Gannes et al., 1998; Dawson et al., 2002). The isotope composition of sedimentary organic carbon is thus often used, in combination with other tools, to infer the biological sources of organic matter and palaeoenvironmental conditions (e.g. Fogel and Cifuentes, 1993; Popp et al., 1997). However, diagenetic processes can lead to (i) carbon loss by various chemical transformations such as defunctionalisation or depolymerisation of biomolecules (e.g. Macko et al., 1993; Czimczik et al., 2002) and/or (ii) selective preservation of some resistant constituents of organic matter relative to more degradable compounds (e.g. Derenne et al., 1990; Hedges and Prahl, 1993). Therefore, stable carbon isotope ratios can be altered in bulk organic matter, if the carbon which is lost upon diagenesis by microbial/chemical degradation

* Corresponding author at present address: Laboratoire de Paléobotanique et Paléoécologie, CEB- EA 3496, Université Paris VI, 12 Rue Cuvier, 75 005 Paris, France. Fax: +33-1-44-27-65-72.
E-mail address: ttnguyen@snv.jussieu.fr (T.T. Nguyen Tu).

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has $\delta^{13}C$ values significantly different from the preserved carbon (e.g. Benner et al., 1987; Schleser et al., 1999; van Bergen and Poole, 2002). To avoid bias due to differential alteration of the various constituents of organic matter, several authors have suggested the use of compound specific isotope analysis of biomarkers that are stable over geological time (Hayes et al., 1990; Macko, 1994; Popp et al., 1997). Recent isotope geochemical studies are thus based on the individual isotopic composition of various biomarkers. Among these, isoprenoids and alkyl lipids, notably *n*-alkanes, are the most commonly used (e.g. Kennicutt and Brooks, 1990; Harada et al., 1995; Grice et al., 1996; Huang et al., 2000; Schouten et al., 2000).

1.2. Diagenesis effects on isotope composition of biomarkers

The utilization of the isotope composition of individual biomarkers is based on the assumption that diagenesis induces little fractionation at the molecular level. Hayes et al. (1990) have indeed demonstrated for Cretaceous sediments that geoporphyrins and acyclic isoprenoids retain their original isotope composition. Individual characterisation of long-chain fatty acids from recent sediments has also suggested that early diagenesis and bacterial reworking have not significantly affected the isotope composition (Naraoka and Ishiwarati, 2000). In addition, Grice et al. (1996), have shown for Permian sediments that sulfurisation, which can represent an important process during diagenesis, does not significantly bias the interpretation of the isotope composition of free lipids, in terms of biological sources. Nevertheless, experimental evidence for the absence of isotope fractionation during diagenesis is scarce for the biomarkers used in geochemistry. Grice et al. (1998) have demonstrated that zooplankton herbivory does not affect the isotope composition of long-chain alkenones and sterols. Except for that study, most of the experiments on the subject deal with molecules that are (1) known for their chemical sensitivity to degradation and (2) are seldom used as biomarkers. For example, Macko et al. (1994) and Fogel and Tuross (1999) showed that the isotopic composition of individual amino acids can be strongly affected by early diagenesis. In the same way, Harvey and Macko (1997) detected a ^{13}C-enrichment in residual short-chain fatty acids (C_{12}–C_{18}) after 40 days of biodegradation. Bioremediation studies have also frequently revealed a ^{13}C-enrichment trend through biodegradation of individual hydrocarbon pollutants (i.e. benzene, styrene and toluene; e.g. Stehmeier et al., 1999; Ahad et al., 2000), which is in agreement with the ^{13}C-depletion (vs. substrate) often observed for the inorganic carbon released during biodegradation of organic matter (e.g. Blair et al., 1985; Baedecker et al., 1993; Zyakun, 1996). However, it is not yet well established whether this fractionation effect, observed on small molecules, can significantly affect the isotope composition of molecules containing many more carbon atoms, such as the biomarkers generally used in geochemistry.

1.3. Potential diagenesis effects on isotope composition of n-alkanes

Straight chain alkanes are among the most commonly used biomarkers for specific compound isotope analysis. Stahl (1980) detected a slight ^{13}C-enrichment in the bulk alkane fraction isolated from petroleum after 40 days of biodegradation. So far, only a few studies have experimentally investigated the effects of diagenesis on the specific carbon isotope composition of long-chain *n*-alkanes. Mansuy et al. (1997) noticed a slight but non-significant ^{13}C-enrichment in the *n*-alkanes of an Oklahoma crude oil remaining after two months of laboratory biodegradation within a sand and water environment. Huang et al. (1997) reported the absence of carbon isotope fractionation of individual *n*-alkanes from shoots of *Calluna vulgaris* after 23 years of decomposition within a peaty soil. Mazéas et al. (2002) also reported a remarkably constant alkane isotope composition during the degradation of an Arabian light crude oil by an enriched bacterial community. The experiment was run in a simulated marine aerobic environment and degradation appeared extensive after 11 days.

The above studies were undertaken under specific experimental conditions and further experiments, representative of the various environments existing on Earth, are necessary to validate fully the use of individual *n*-alkanes as an isotope record of palaeoenvironments. Moreover, the aforementioned studies were performed under either stagnant water or acid and anaerobic environments that are often considered as exhibiting slower rates of organic matter decomposition than well-drained, neutral and aerobic environments. These environmental differences in the general intensity of organic matter degradation are also valid for the evolution of isotope composition. Indeed, Nadelhoffer and Fry (1988) have shown, for example, that diagenesis can lead to significantly different evolution of the isotope composition of bulk organic carbon in well-drained soil and in stagnant water soil.

1.4. Aim of study

One of the most important sources of long-chain *n*-alkanes in sediments is terrestrial plants (e.g. Eglinton and Hamilton, 1967), although a variety of organisms such as microalgae, bacteria, dinoflagellates, diatoms and fungi can also produce long-chain *n*-alkanes (e.g. Nishimura and Baker, 1986; Volkman et al., 1998).

Higher plant detritus often undergoes a more or less long stay in soils before sedimentation. Moreover, early diagenesis appears to be a crucial step in the fractionation of bulk isotope ratios of plant debris after death (e.g. Benner et al., 1987; Schleser et al., 1999). Therefore, monitoring the evolution of the isotope composition of *n*-alkanes extracted from leaves undergoing degradation in a well-drained, aerobic and neutral soil, should significantly contribute to the knowledge of the isotope fractionation that specific hydrocarbons can undergo during diagenesis.

The isotope characterisation of *n*-alkanes extracted from fossil plants may provide additional information on the diagenesis of plant debris. Indeed, like sedimentary organic matter and contrary to soil debris, fossils undergo a long diagenetic evolution. Furthermore, geochemical analysis of morphologically well-defined fossils has an advantage over analysis of bulk organic matter (e.g. in sediments) because the problems associated with contributions derived from multiple sources are not encountered. Therefore, the study of *n*-alkanes from degrading leaves and *n*-alkanes extracted from fossil leaves should allow us to refine the estimate of potential diagenetic effects on the isotope composition of sedimentary hydrocarbons and thus lead to a better use of compound-specific isotope analysis for palaeoenvironmental reconstruction.

1.5. Methodology

This study represents a first approach to evaluating the effects of the first steps of early diagenesis on the isotope composition of individual *n*-alkanes in a well-drained, aerobic and neutral soil, by characterising the long-chain *n*-alkanes extracted from *Ginkgo biloba* leaves undergoing degradation in the above-ground litter of a brown soil. Leaves at various degradation stages (i.e. fresh, senescent, litter) were sampled at relatively short time intervals (i.e. July, August, December, January and March) in an attempt to estimate the timing of early diagenesis effects on the isotope composition of individual *n*-alkanes. The isotopic composition of individual alkanes was compared with that of bulk leaves, to weigh the timing of the potential diagenetic effects on individual alkanes against the well known effects on bulk leaves.

Palaeoflora from the Cenomanian (±90 m.y.) of France and the Czech Republic provide an exceptional opportunity for investigating the effects of diagenesis on specific compound isotope composition in fossils. Indeed, previous studies have shown that the bulk isotope signal of these fossils has not been significantly affected by diagenesis (Nguyen Tu et al., 1999, 2002). Moreover, the Cenomanian palaeoflora includes two fossil Ginkgoales, *Eretmophyllum andegavense* and *Eretmophyllum obtusum*. *Eretmophyllum* is a totally extinct genus and its closest living relative is *G. biloba*, which is the only living representative of the Ginkgoale order. Furthermore, *E. andegavense* lipid extracts were previously shown to include substantial amounts of *n*-alkanes (Nguyen Tu et al., 2003).

Therefore, in addition to the detailed study of degrading leaves of *G. biloba*, some fossil leaves of *Eretmophyllum* were investigated for specific *n*-alkane isotope determination. Rather than an exhaustive survey, this restricted sample set represents a first appraisal of the diagenetic variation that might be expected, in sediments, for the isotope composition of higher plant alkanes.

2. Material and methods

2.1. Samples

Each batch of samples (i.e. fresh, senescent, litter and fossil) corresponded approximately to 30 leaves in order to avoid bias linked to leaf morphotype and to obtain average isotope compositions representative of each batch.

2.1.1. Degrading leaves of G. biloba

Leaves of *G. biloba* were collected from a rural area where urban pollution is minimal (Massif Central), from a single tree growing on a brown soil with a mull-type humus. Reference samples of undegraded leaves (green leaves) were collected at the end of the growing season [i.e. July (sample "fresh 1") and August (sample "fresh 2") 1998] so as to limit bias linked to variation in chemical and isotope composition through the growing season (e.g. Gülz et al., 1991; Lockheart et al., 1997). One batch of senescent leaves (yellow) was collected in December 1998 and two batches of leaves from litters (brown) at different degradation stages were collected in January (sample "litter 1") and March (sample "litter 2") 1999. Leaves were collected from several representative branches of the same tree for the fresh and senescent samples, and at the base of this tree, all around the trunk on the topmost part of the soil, for the litter leaves.

2.1.2. Fossil leaves

Fossil leaves of *E. andegavense* and *E. obtusum* originated from Cenomanian deposits of France ("Argiles du Baugeois" member, "Le Gué de Moré" locality, north of Angers) and the Czech Republic (unit 3 of "Peruc" member, Pecínov quarry, west of Praha), respectively. The geological setting for these sites has been studied in detail previously: the two deposits similarly consist of grey and finely laminated clays deposited under anoxic conditions in lagoonal environments during the Cenomanian transgression in Europe (Louail,

1984; Uličný et al., 1997). Both deposits were located at close palaeolatitudes, 35±5°N and 37±5°N for France and the Czech Republic, respectively (Philip et al., 2000) and in the same semi-arid climatic zone (Parish et al., 1982). The sediments yielded a rich and abundant flora with excellent morphological preservation as shown by leaf aspect: they look like falling leaves during autumn. Their good morphological preservation suggests that burial occurred near the vegetation zone (Louail, 1984; Uličný et al., 1997).

The fossil flora is similar in both deposits and very diversified; it includes leaves, wood and reproductive organs of pteridophytes, angiosperms and numerous gymnosperms (Pons et al., 1980; Uličný et al., 1997). Among the gymnosperms, either *E. andegavense* or *E. obtusum* is one of the most abundant species of the French and Czech floras, respectively. *E. andegavense* and *E. obtusum* exhibit a number of similar morphological and anatomical characteristics, leading some authors to include them in the same species (Hluštík, 1986; Pons, personal communication). Several isotope and biogeochemical patterns showed that the isotope signal of the bulk leaves has not been significantly affected by diagenesis (Nguyen Tu et al., 1999, 2002). Multidisciplinary characterisation of the fossiliferous deposits demonstrated that the various plants of the palaeoflora were distributed along salinity gradients in marine-influenced environments, with both *Eretmophyllum* species occupying among the most salt-influenced habitats in the environment (Uličný et al., 1997; Nguyen Tu et al., 1999, 2002). Significant and correlated variations in $\delta^{13}C$ and leaf-width demonstrated that *E. andegavense* and *E. obtusum* were both able to thrive in habitats of contrasting salinity (Nguyen Tu et al., 1999, 2002).

Fossil leaves of *E. andegavense and E. obtusum* were collected in May 1996 and July 1998, respectively. In each of the aforementioned sites, several sediment blocks were sampled at the same level from different parts of the outcrop. Two batches of *E. andegavense* (samples "fossil 1" and "fossil 2") and one batch of *E. obtusum* (sample "fossil 3") leaves were used for investigating the isotope composition of individual *n*-alkanes. Each batch consisted of leaves of similar bulk isotope composition (s.d. 0.6‰), grouped together after preliminary isotope measurements. The two batches of *E. andegavense* leaves respectively corresponded to leaves of significantly different bulk isotope composition and leaf width (i.e. leaves originating from contrasting salinity habitats).

2.2. Experimental

Blocks of sediments containing fossil leaves were taken from the field and leaves were separated from the embedding sediment in the laboratory by taking off the leaves shrivelled up after drying overnight at 50 °C. After collection, fossil and modern leaves were dusted free of any adhering particles using pre-extracted cotton wool, dried overnight at 50 °C and stored in aluminium foil in the dark at ambient temperature until analysis. Before analysis, all the leaves in a given batch were combined in an agate mortar and ground to a fine homogeneous powder.

2.2.1. Bulk isotope analysis

Stable isotope ratios were measured on fossil and modern leaves using an automatic unit that combined an elemental analyser with an isotope ratio mass spectrometer. The samples were combusted in a Carlo-Erba CHN elemental analyser connected to a VG-SIRA 10 isotope ratio mass spectrometer. After flash combustion at 1000 °C with copper oxide, CO_2 was cryogenically distilled, purified and introduced on-line into the mass spectrometer to determine carbon isotope ratios (Girardin and Mariotti, 1991; Bocherens and Mariotti, 1999). The $\delta^{13}C$ values were measured with instrumental standard deviation of 0.02‰ and repeated measurements on the same sample showed a precision (1σ) of 0.1‰. Each sample was run in triplicate and tyrosine standards were run frequently between samples to obtain reliable mean values.

2.2.2. Lipid extraction and n-alkane isolation

Powdered fossil and modern leaves were extracted with 30 ml of CH_2Cl_2/CH_3OH (2/1, v/v) by stirring overnight at room temperature, the extract being recovered by 10 min centrifugation at 4000 rpm. Total lipids were fractionated by micro-column chromatography in a Pasteur pipette filled with approximately 500 mg of Al_20_3 (activity I). For the fossil samples, fine copper turnings previously washed in acid and solvent were added above the alumina to remove elemental sulfur. Before fractionation of the lipid extract, the entire micro-column device was cleaned by elution with 10 ml heptane; alkanes were recovered in the hydrocarbon fraction after elution with 3 ml heptane. Hydrocarbon fractions were concentrated to dryness under nitrogen and redissolved in a small volume of heptane prior to analysis.

2.2.3. Isotope analysis of individual n-alkanes

Identification of *n*-alkanes was carried out by gas chromatography and gas chromatography/mass spectrometry using a Hewlett-Packard 5890 series II gas chromatograph equipped with a split injector fitted with a fused silica capillary column, coated with CP-SIL-5CB (25 m × 0.32 mm i.d., 0.12 μm film thickness) and a Hewlett-Packard 5989A quadrupole mass spectrometer, scanning from m/z 40 to 800 (electron energy 70 eV, scan time 1.3 s). The temperature programme was 100–300 °C at 4 °C min^{-1} and then 300 °C isothermal for 20 min, using helium as carrier gas. Identification of *n*-alkanes and other lipid constituents of fresh, senescent

and litter leaves of *G. biloba*, as well as fossil leaves of *E. andegavense*, were previously achieved on other samples collected at similar time periods. The results are discussed in detail elsewhere (Nguyen Tu et al., 2001, 2003). Briefly, *G. biloba n*-alkanes were dominated by odd-carbon-numbered homologues maximising at C_{27} while *E. andegavense n*-alkanes maximised at C_{29} without any strong odd/even predominance (Nguyen Tu et al., 2001, 2003). Compound specific $\delta^{13}C$ values of the dominant alkanes were determined with a gas chromatograph/isotope ratio mass spectrometer coupling a Micromass Isochrom III, including a Hewlett-Packard 5890 series II gas chromatograph (50 m BPX 5 capillary column, i.d. 0.32mm, film thickness 0.50 μm, heating programme 100–300 °C at 3 °C min^{-1}, splitless injector at 320 °C) to an Isochron isotope ratio mass spectrometer via a Micromass combustion interface (CuO combustion furnace at 850 °C and cryogenic trap at −100 °C). The $\delta^{13}C$ values were measured with instrumental standard deviation of the measurement being 0.1‰ and repeated measurements on a single sample showed a precision (1σ) of 0.5‰. Each sample was run in triplicate. Specific isotope composition of *n*-alkanes containing 21–27 carbon atoms are reported in Table 1 except when nearly coeluting unidentified compounds were present in the chromatogram. The isotope composition of homologues containing more than 27 carbon atoms was not investigated due to the presence of unresolved compounds around the *n*-alkanes in the chromatogram.

3. Results and discussion

3.1. Fresh leaves of G. biloba

Two batches of fresh leaves of *G. biloba* were investigated as reference samples for non-degraded leaves. The C_{22}–C_{27} *n*-alkanes were investigated for compound specific isotope analysis; their $\delta^{13}C$ values are similar for both batches and range from −33.0 to −30.0‰ (Table 1). In agreement with literature data (e.g. Rieley et al., 1993; Collister et al., 1994; Lichtfouse et al., 1994; Huang et al., 1997; Lockheart et al., 1997, 1998), individual alkanes appeared ^{13}C-depleted when compared with bulk leaves (Table 1 and Fig. 1). The ^{13}C-depletion of lipids with respect to bulk biomass is mainly due to a kinetic isotope effect associated with the oxidative decarboxylation of pyruvate into acetyl-CoA during lipid synthesis by the fatty acid synthase complex (O'Leary, 1976; DeNiro and Epstein, 1977; Monson and Hayes, 1982; Melzer and Schmidt, 1987; Hayes, 2001). The ^{13}C-depletion measured for *G. biloba* alkanes varies from 2.3 to 5.3‰ (mean ^{13}C-depletion of 3.9‰; Table 1 and Fig. 1). This depletion falls within the range previously reported for *n*-alkanes of higher plants using the C_3 photosynthetic pathway (i.e. 1.5–10.0‰). Nevertheless, the ^{13}C-depletion measured here is among the smallest of this literature range (Rieley et al., 1993; Collister et al., 1994; Lichtfouse et al., 1994; Huang et al., 1997; Lockheart et al., 1997, 1998). DeNiro and Epstein (1977) pointed out that the magnitude of ^{13}C-depletion of lipids may be species-specific. This probably accounts for (1) the large range of *n*-alkane ^{13}C-depletion noted upon compilation of literature data on higher plants and (2) the rather weak ^{13}C-depletion detected here for *G. biloba n*-alkanes.

Specific *n*-alkanes of fresh leaves of *G. biloba* tend to be more ^{13}C-depleted with increasing chain-length (Table 1 and Fig. 2). Such a trend has been previously observed in 10 of the 35 higher plants investigated previously (i.e. Rieley et al., 1993; Collister et al., 1994; Lichtfouse et al., 1994; Huang et al., 1995; Grossi et al., 2003). However, it is not systematic; some plants exhibit an increasing $\delta^{13}C$ value with increasing alkane length (4/35) or show no particular trend related to chain

Table 1
Isotope composition of bulk samples and individual *n*-alkanes extracted from leaves of modern and fossil Ginkgoales[a]

Sample	bulk	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	*n*-alkane average
G. biloba									
Fresh 1 (July)	−27.7	–	−30.0 (2.3)	−31.5 (3.8)	−30.5 (2.8)	−32.5 (4.8)	−32.2 (4.5)	−33.0 (5.3)	−31.6 (3.9)
Fresh 2 (August)	−27.8	–	−30.3 (2.5)	−31.2 (3.4)	−30.0 (2.2)	−32.3 (4.5)	−32.5 (4.7)	−32.7 (4.9)	−31.5 (3.7)
Senescent (December)	−27.5	−29.3 (1.8)	−30.0 (2.5)	−30.5 (3.0)	−31.9 (4.4)	−31.1 (3.6)	–	–	−30.6 (3.1)
Litter 1 (January)	−27.9	–	−29.2 (1.3)	−30.0 (2.1)	−29.8 (1.9)	−30.0 (2.1)	−30.5 (2.6)	−30.5 (2.6)	−30.0 (2.1)
Litter 2 (March)	−27.7	–	–	−28.5 (0.8)	–	−29.5 (1.8)	−28.7 (1.0)	−28.6 (0.9)	−28.8 (1.1)
E. andegavense									
Fossil 1	−25.5	−27.6 (2.1)	−27.0 (1.5)	−27.7 (2.2)	−27.7 (2.2)	–	–	–	−27.5 (2.0)
Fossil 2	−22.5	−24.8 (2.3)	−26.0 (3.5)	−25.5 (3.0)	−24.5 (2.0)	–	–	–	−25.2 (2.7)
E. obtusum									
Fossil 3	−24.7	−27.3 (2.6)	−27.4 (2.7)	−27.1 (2.4)	−26.8 (2.1)	−27.0 (2.3)	−26.8 (2.1)	−26.3 (1.6)	−27.0 (2.3)

[a] Expressed as $\delta^{13}C$ values in per mil (‰), measurement precision being 0.5‰. Bracketed values correspond to the ^{13}C-depletion for *n*-alkanes with respect to bulk leaves.

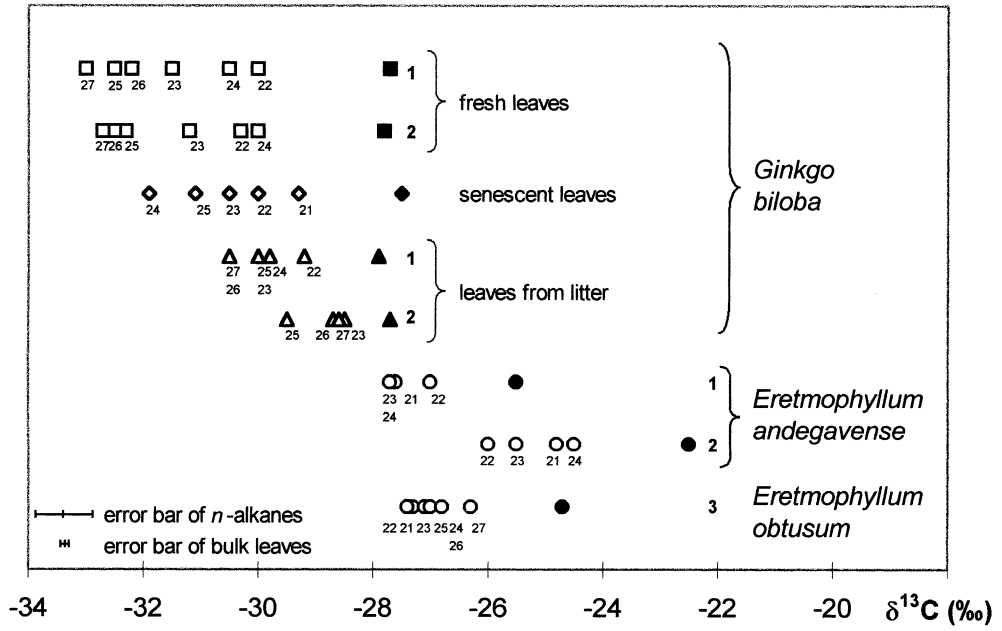

Fig. 1. Isotope composition of bulk samples (filled symbols) and individual *n*-alkanes (empty symbols) extracted from leaves of modern and fossil Ginkgoales.

length (Rieley et al., 1991, 1993; Collister et al., 1994; Lichtfouse et al., 1994; Huang et al., 1995, 1997; Ficken et al., 1998). Moreover, the isotope distribution pattern of *n*-alkanes with respect to chain length may vary through the growing season (Lockheart et al., 1997). Nevertheless, increasing ^{13}C-depletion with increasing chain length is the most frequently reported trend for specific *n*-alkanes within a given plant sample so it might be of general significance (i.e. Rieley et al., 1993; Collister et al., 1994; Lichtfouse et al., 1994; Huang et al., 1995; Grossi et al., 2003). This trend probably accounts, at least partially, for the relatively weak ^{13}C-depletion, relative to bulk leaves, detected here for *G. biloba* alkanes. Indeed, the range of chain length investigated (C_{22}–C_{27}) includes relatively short chains when compared with those generally studied (C_{25}–C_{33}). Collister et al. (1994) suggested that this decrease in δ^{13}C value of alkanes with increasing chain length may be due to the combined effects of the two following factors: (1) δ^{13}C values of plants depend on environmental conditions (e.g. O'Leary, 1981; Farquhar et al., 1982) and (2) different homologues of a given series can be produced in different proportions at the various stages of the growing season (e.g. El-Otmani and Coggins, 1985; Gülz et al., 1991). For example, short-chain fatty lipids are generally synthesized earlier in the growing season than long-chain homologues (e.g. El-Otmani and Coggins, 1985; Gülz et al., 1991). Moreover, light intensity, for example, can be lower in spring than in summer. Since increasing light intensity induces a decrease in δ^{13}C values (e.g. Francey et al., 1985; Zimmerman and Ehleringer, 1990), the mentioned factors can lead to lower δ^{13}C values for long-chain alkanes than for short-chain alkanes. However, the trend towards ^{13}C-depletion with increasing chain length has been observed in a wide variety of situations: open air and glasshouse, C_3 and CAM photosynthetic pathways, herbaceous and arborescent plants, evergreen and deciduous leaves, monocotyledonous and dicotyledonous taxa, modern and fossil plants (Rieley et al., 1993; Collister et al., 1994; Huang et al., 1995, 1996; Lichtfouse et al., 1995). Such a diversity could suggest an additional cause for the decreasing trend observed for *n*-alkane isotope composition with increasing chain length. Long-chain alkyl lipids are derived from palmitic and stearic acids by successive additions of two-carbon atom units (e.g. Garrett and Grisham, 2000). A weak fractionation associated with each addition of two-carbons units may also contribute to the observed decrease. A weak fractionation at each step of chain elongation would explain why the effect could not be detected for short-chain components (DeNiro and Epstein, 1977) although it is detected for long chains. Such a fractionation associated with chain elongation has been suggested previously by Monson and Hayes (1980).

The *n*-alkanes from fresh leaves of *G. biloba* exhibit a marked odd/even predominance (Nguyen Tu et al., 2003). Nevertheless, the abundance of even homologues in the hydrocarbon fraction was sufficient to allow precise measurement of the carbon isotope composition. In the two studied batches of fresh leaves of *G. biloba*, even-carbon-numbered alkanes tend to exhibit δ^{13}C values slightly higher than odd-carbon-numbered

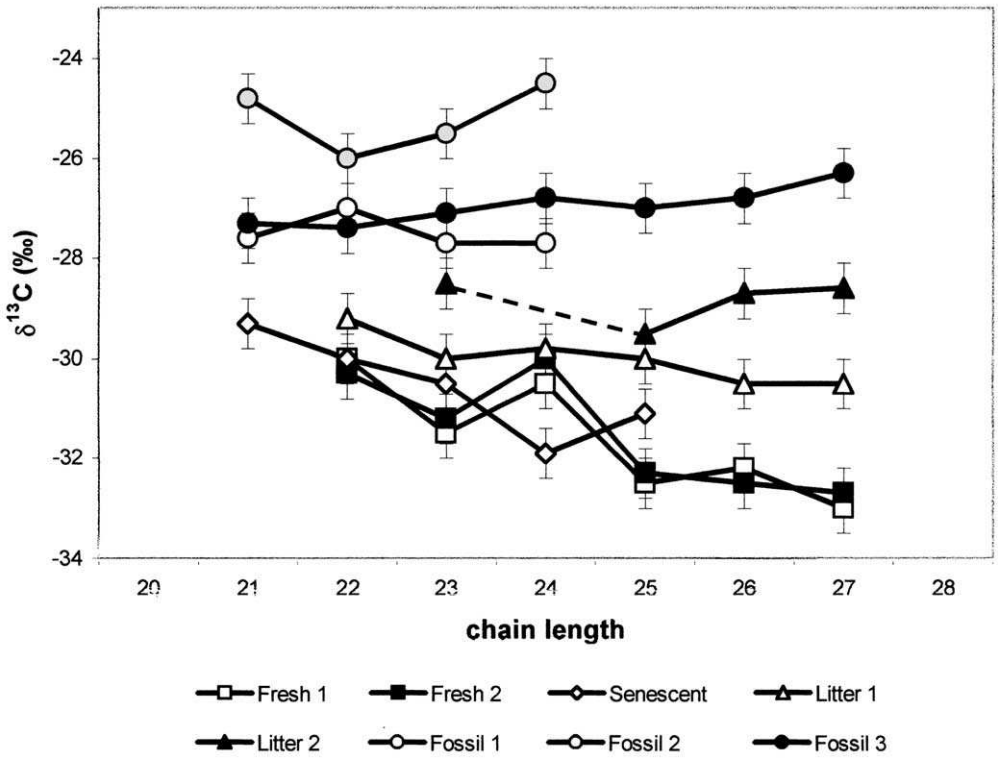

Fig. 2. Distribution of *n*-alkane isotope composition with respect to chain length.

homologues (Table 1 and Fig. 2). This trend is not significant for all *n*-alkanes since the difference between the specific isotope composition of a given C_n homologue and its C_{n-1} and C_{n+1} neighbours is sometimes only slightly higher than the analytical error. However, a majority of even-carbon-numbered alkanes exhibit $\delta^{13}C$ values significantly higher than their neighbours. Although far from systematic, it has also been reported for a few plants that, within a given series of fatty lipids, even and odd homologues exhibit significantly different $\delta^{13}C$ values (Rieley et al., 1993; Collister et al., 1994; Lichtfouse et al., 1995; Grossi et al., 2003). The specific isotope compositions of even-carbon-numbered *n*-alkanes and of the biosynthetically related odd-carbon-numbered *n*-acids and *n*-alcohols are rarely investigated due to their low abundance in plant extracts. Nevertheless, due to the low abundance of these components in plant extracts the same pattern has also been noted in some fossil plants (Huang et al., 1995), soil organic matter (Lichtfouse et al., 1995) and terrestrially-derived sedimentary organic matter (e.g. Kennicutt and Brooks, 1990; Ishiwarati et al., 1994; Schouten et al., 2001). The difference in specific isotope composition of odd and even *n*-alkanes reveals different origins. In the case of sedimentary and soil organic matter, this is generally attributed to two distinct sources of *n*-alkanes, i.e. higher plants for the odd homologues, and algae, bacteria, diagenesis or else pollution for the even homologues (e.g. Kennicutt and Brooks, 1990; Ishiwarati et al., 1994; Schouten et al., 2001). In the case of *n*-alkanes directly extracted from higher plants, the difference in specific isotope composition of even and odd homologues may be explained by three main hypotheses.

(1) The *n*-alkanes may originate from two distinct sources, i.e. plant lipids may have been contaminated by alkanes from other organisms, such as fungi or bacteria, of different isotope composition. Indeed, these microorganisms can synthesize long-chain fatty lipids (e.g. Jones, 1969; Welch and Burlingame, 1973; Volkman et al., 1998) and can exhibit $\delta^{13}C$ values significantly higher than those of terrestrial plants (e.g. Deines, 1980; Kohzu et al., 1999; Henn et al., 2002). However, less than 2% of the surface of healthy leaves is generally colonized by fungi or bacteria (Juniper and Jeffree, 1983) and scanning electron microscopy of the fresh leaves of *G. biloba* revealed no trace of microbial invasion (Nguyen Tu et al., 2001). Therefore, contamination by microbial lipids is unlikely to be the main factor accounting for the small ^{13}C-enrichement noted for the even-carbon-numbered *n*-alkanes of *G. biloba* fresh leaves.

(2) Odd and even homologues may have been synthesized at different periods, under different environmental conditions which would have affected their specific isotope composition. However, to the best of our

knowledge, such a differential synthesis of odd and even homologues has never been reported, perhaps because of the scarcity of studies dealing with even-carbon-numbered *n*-alkanes and the biosynthetically related odd-carbon-numbered *n*-acids and *n*-alcohols.

(3) Odd and even homologues may have been synthesized *via* different pathways, leading to different isotope fractionations and/or from two distinct pools of precursors of distinct isotope composition. Contrary to odd-carbon-numbered-alkanes, which were demonstrated to derive from acetyl-CoA after elongation by the fatty acid synthase complex, the biosynthetic origin of even-carbon numbered *n*-alkanes is not well documented (e.g. Garrett and Grisham, 2000). Even alkanes were suggested to derive from the fatty acid synthase complex after either α-oxidation of long-chain even-numbered fatty acids (e.g. Martin and Stumpf, 1959; Khan and Kolattukudy, 1974) or elongation of odd-carbon-numbered precursors such as propionyl-CoA (e.g. Garrett and Grisham, 2000). Another minor pathway for the synthesis of fatty lipids was also detected in *Nicotiana tabacum*, *Petunia hybrida* and *Licopersicon pennellii*: the α-ketoacid elongation pathway that uses threonine or pyruvate as precursor (Kandra et al., 1990; Kroumova et al., 1994). This pathway proceeds by successive additions of one-carbon units and thus leads to the formation of equivalent amounts of odd and even homologues (Kandra et al., 1990; Kroumova et al., 1994). Since they use either a different enzymatic complex and/or different precursors than for odd alkanes, the two last mentioned hypotheses for the biosynthetic origin of even *n*-alkanes might thus have played a role in the slight ^{13}C-enrichment detected here for even-carbon-numbered-alkanes of *G. biloba*.

The isotope composition of specific *n*-alkanes extracted from fresh leaves of *G. biloba* thus appears rather variable (i.e. within a 3‰ range). As noted by previous authors, this emphasizes the need for caution when interpreting small variations in the isotope compositions of specific biomarkers from modern and ancient sediments (e.g. Collister et al., 1994: Lockheart et al., 1997). Although the origins of these variations are not yet clearly established, the isotope distribution patterns of alkanes with respect to chain length appears rather reproducible for the two batches of *G. biloba* leaves studied: alkane specific isotope composition decreases with increasing chain length and even homologues tend to exhibit slightly higher δ^{13}C values than odd homologues.

3.2. *Degrading leaves of* G. biloba

Three batches of *G. biloba* leaves were analysed to study the evolution of the isotope signal during the first steps of early diagenesis: yellow senescent leaves (December) and two degradation stages of brown litter leaves (January "litter 1" and March "litter 2"). The δ^{13}C values of bulk leaves appeared virtually constant through the early stages of diagenesis (−27.5, −27.9 and −27.7‰ for senescent, litter 1 and litter 2 samples, respectively) and almost identical to those measured for fresh leaves (−27.7 and −27.8‰; Table 1 and Fig. 1). Previous work on the isotope effects of diagenesis have generally reported decreasing or increasing δ^{13}C values for bulk organic matter, depending on the environment. A decrease in bulk isotope composition of degrading plant material was documented in an anaerobic environment and was attributed to selective preservation of ^{13}C-depleted lignin (Benner et al., 1987). In contrast, an inverse trend is generally detected in soils (e.g. Nadelhoffer and Fry, 1988; Melillo et al., 1989; Balesdent et al., 1993). The stability of bulk δ^{13}C values observed here for degrading leaves is probably related to the short timescale investigated.

Depending on the batch, compound isotope compositions were measured on four to six homologues ranging from C_{21} to C_{27}; their δ^{13}C values vary between −31.9 and −28,5‰ (Table 1 and Fig. 1). As in fresh samples, all the *n*-alkanes examined in *G. biloba* leaves undergoing degradation are ^{13}C-depleted when compared with bulk leaves. The mean ^{13}C-depletion for senescent, litter 1 and litter 2 samples are −3.1‰ (C_{21}–C_{25} measured), −2.1‰ (C_{22}–C_{27} measured) and −1.1‰ (C_{23}, C_{25}–C_{27} measured), respectively. The ^{13}C-depletion in *n*-alkanes with respect to bulk leaves is similar in senescent leaves to that calculated for fresh leaves. However, there is a marked decrease in this depletion as litter formation progresses. Comparing alkanes with different ranges of carbon number may bias the interpretation of isotope composition since *n*-alkanes of fresh leaves of *G. biloba* exhibited decreasing δ^{13}C values with increasing chain length. However, the trend is also observed when only the C_{23} and C_{25} homologues are considered (i.e. the two homologues measured in all five batches of *G. biloba* leaves). Indeed, the mean ^{13}C-depletion for these two alkanes progressively decreases from July to March: 4.3‰ (fresh 1), 4.0‰ (fresh 2), 3.3‰ (senescent), 2.1‰ (litter 1) and 1.3‰ (litter 2). There is thus a rather regular ^{13}C-enrichment for specific alkanes during the first steps of early diagenesis (Fig. 1). This trend is significant since it is higher than the analytical error. Moreover, specific isotope composition of all the *n*-alkanes of a given extract tended to become homogeneous with progressive litter formation. Indeed, the decrease in δ^{13}C values with increasing chain length that was detected in fresh leaves is no longer detectable in litter leaves (Table 1 and Fig. 2). Similarly, even-numbered-alkanes no longer seem ^{13}C-enriched when compared with odd homologues.

The origins of these alterations of the isotope composition of individual *n*-alkanes extracted from degrading *G. biloba* leaves can be multiple; the two most probable explanations are discussed as follows.

(1) A contribution from microbial *n*-alkanes that can exhibit (i) long chain length and (ii) a ^{13}C-enriched

isotope signal when compared with higher plants, may induce an enrichment and a homogenisation of the isotope composition of all the *n*-alkanes in a given sample.

(2) Otherwise, an isotope fractionation associated with the degradation of the alkanes of *G. biloba* leaves could also lead to the alteration pattern detected here for the isotope composition of individual *n*-alkanes.

Abiotic degradation processes, such as evaporation and water washing, might have partially influenced alkane $\delta^{13}C$ values. However, these processes are among the less intense for hydrocarbons and are not likely to result in substantial alteration of specific alkane isotope composition (e.g. Mansuy et al., 1997). In contrast, biodegradation is one of the most active degradative processes for long-chain *n*-alkanes (e.g. Mansuy et al., 1997). As mentioned in the Introduction section, low molecular weight hydrocarbon pollutants often exhibit increasing $\delta^{13}C$ values with biodegradation (e.g. Stehmeier et al., 1999; Ahad et al., 2000). So far, no significant fractionation has been reported for the degradation of higher molecular weight hydrocarbons. However, metabolism of carbohydrates, which are high molecular weight molecules, by bacteria and fungi can lead to significant fractionation effects (e.g. Blair et al., 1985; Will et al., 1989; Gleixner et al., 1993; Henn and Chapela, 2000). These effects are detectable at the bulk level as well as at the individual molecular level (e.g. *n*-acids, chitin) and can be positive or negative depending on substrate, microbe species and microbe metabolism (Blair et al., 1985; Gleixner et al., 1993; Abraham et al., 1998; Henn and Chapela, 2000). Depending on authors, such a fractionation would take place at different stages of microbial metabolism: respiration, various branching points during catabolism and anabolism pathways, substrate transport and uptake (Will et al., 1989; Gleixner et al., 1993; Abraham et al., 1998; Henn and Chapela, 2000). Fractionation during substrate uptake is likely to lead to a substantial alteration of the isotope composition of the remaining substrate. The preservation potential of long-chain *n*-alkanes is much higher than that of carbohydrates. Nevertheless, a number of bacteria and fungi are able to efficiently degrade long-chain hydrocarbons (e.g. Singer and Finnerty, 1984; Leahy and Colwell, 1990). Moreover, the surfaces of senescent and litter leaves are more likely than those of fresh leaves to be invaded by various degrading microorganisms such as bacteria and fungi. Furthermore, a decrease in *n*-alkane absolute abundance was previously noted through litter formation from *G. biloba* leaves (Nguyen Tu et al., 2003). Therefore, preferential biodegradation of ^{13}C-depleted *n*-alkanes might have contributed to the ^{13}C-enrichment detected here for the alkanes remaining in degrading leaves.

In summary, with senescence and litter formation a general ^{13}C-enrichment was detected for individual *n*-alkanes extracted from *G. biloba* leaves. Moreover, the specific isotope composition of all the *n*-alkanes in a given extract tended to become homogeneous as litter formation progressed, while in fresh leaves they exhibited characteristic isotope distribution patterns with respect to chain length. These findings suggest that early diagenesis has significantly affected the isotope composition of individual *n*-alkanes in *G. biloba* leaves. The origins of these diagenetic effects are not clear. Nevertheless, it would be interesting to test whether the effects detected here could be confirmed on *G. biloba* and other species in various environments. If so, homogeneous isotope compositions for all the specific *n*-alkanes of a given sample could be used as a marker of diagenetic alteration of the isotope signal. The sample set studied here is too restricted to allow immediate generalisation. Nonetheless, a diagenetic effect was detected although the precautions generally suggested to avoid problems related to the diagenetic alteration of isotope signal were applied: (1) study of a morphologically well-defined object and (2) measurements on individual biomarkers stable through geological time. Moreover, alteration patterns were detected for the isotope composition of individual *n*-alkanes although the isotope composition of bulk leaves remained constant. These results thus suggest that, in well-drained soils, diagenesis might affect the isotope composition of individual molecules, such as *n*-alkanes, more intensively and/or more rapidly than that of bulk plant material.

3.3. *Fossil leaves of* E. andegavense *and* E. obtusum

Two batches of *E. andegavense* (samples "fossil 1" and "fossil 2") and one batch of *E. obtusum* leaves (sample "fossil 3") were extracted for isotope measurements of individual *n*-alkanes. Previous studies have shown that the bulk isotope composition of these fossil leaves has not been significantly affected by diagenesis (Nguyen Tu et al., 1999, 2002). The two batches of *E. andegavense* studied exhibit substantially different bulk $\delta^{13}C$ values due to differences in the salinity of the soil on which the plants grew (Nguyen Tu et al., 1999). The *n*-alkanes extracted from leaves of the fossil Ginkgoales exhibited specific isotope composition ranging from −27.7 to −24.5‰ (Table 1 and Fig. 1). *n*-Alkanes extracted from the fossil leaves batches 1, 2 and 3 were ^{13}C-depleted when compared with bulk leaves, by 2.0, 2.7 and 2.3‰, respectively. Within a given extract, all the measured *n*-alkanes exhibited rather homogeneous specific $\delta^{13}C$ values (Fig. 2).

Plant carbon isotopic composition is dependent on a number of factors that were probably different for the Cenomanian *Eretmophyllum* and the present-day *Ginkgo*. Therefore, direct comparison of specific alkane isotope composition between both Ginkgoales must be considered cautiously. Keeping in mind these limitations, it is possible to propose a tentative discussion of

the individual $\delta^{13}C$ values of *Eretmophyllum* *n*-alkanes. Fossil alkanes are ^{13}C-depleted with respect to bulk leaves, in agreement with results on fresh and degrading leaves of *G. biloba,* and other published work on present day plants (e.g. Rieley et al., 1993; Collister et al., 1994; Lichtfouse et al., 1994; Huang et al., 1997; Lockheart et al., 1997, 1998). The ^{13}C depletions calculated for *Eretmophyllum* alkanes are similar to those calculated for senescent leaves and January litter of *G. biloba*. This finding, as well as the absence of any trend with respect to chain length in the isotope composition of fossil alkanes, may be due to the two hypotheses discussed below.

(1) The observed isotope patterns may reflect physiological differences between both Ginkgoales. Indeed, the isotope pattern of individual alkanes may be species-specific (e.g. Rieley et al., 1991; Collister et al., 1994; Ficken et al., 1998). Moreover, although *Ginkgo* is the closest living relative of *Eretmophyllum*, it does not belong to the same genus and therefore could exhibit different characteristics in alkane isotope composition.

(2) The observed isotope patterns may also reflect a slight diagenetic alteration for *Eretmophyllum* alkanes. Such a diagenetic effect on individual molecules, with the isotope composition of bulk fossil leaves not being significantly affected by diagenesis, is in agreement with the results found for degrading leaves of *G. biloba*. Nevertheless, the slight alteration detected here for *n*-alkane individual $\delta^{13}C$ values does not preclude a palaeoenvironmental interpretation. Indeed, specific *n*-alkane isotope composition, which varies in the same direction as bulk leaf isotope composition, which was shown to be related to local environment. These findings tend to confirm that fossilisation can lead to a less altered geochemical signal than degradation occurring in well-drained soils.

4. Conclusions

The isotope compositions of individual *n*-alkanes extracted from fresh and degrading leaves of *Ginkgo biloba* and fossil leaves of *Eretmophyllum* were investigated in a first approach to refining the estimate of potential diagenetic effects on the isotope composition of sedimentary alkanes derived from higher plants.

The *n*-alkanes extracted from fresh leaves of *G. biloba* appeared to be ^{13}C-depleted when compared with bulk leaves. They exhibited characteristic isotope patterns with respect to chain length: their individual $\delta^{13}C$ value decreased with increasing chain length and odd homologues tended to be ^{13}C-depleted when compared with even homologues. These variations occurred within a 3‰ range, emphasizing the need for caution when interpreting small variations in the isotope compositions of specific sedimentary biomarkers.

Two trends could be detected for the specific $\delta^{13}C$ value of *n*-alkanes through leaf senescence and litter formation: (i) general ^{13}C-enrichment in all homologues and (ii) homogenisation of specific isotope composition of all the *n*-alkanes of a given extract. The origin of these trends could not be unequivocally established. Nevertheless, they might constitute indicators of the level of diagenetic alteration of the isotope signal in alkanes, provided that such trends could be confirmed for other samples within other environments. These molecular changes were detected even though the isotope composition of bulk leaves remained unchanged, which suggested that diagenesis might affect the isotope composition of individual molecules, such as *n*-alkanes, more intensively and/or more rapidly than that of bulk plant material. Moreover, diagenesis is apparently able to affect the isotope composition of individual *n*-alkanes very rapidly over a few months after leaf senescence and fall (i.e. March); alkanes extracted from litter leaves appeared ^{13}C-enriched by about 3‰ when compared with those from fresh leaves.

The *n*-alkanes extracted from the fossil Ginkgoales, *Eretmophyllum andegavense* and *Eretmophyllum obtusum*, also appeared ^{13}C-depleted when compared with bulk leaves. The isotope distribution patterns of individual *n*-alkanes of *Eretmophyllum* suggested that the isotope composition of fossil alkanes may have been slightly affected by diagenesis. It should be noted that this diagenetic alteration of the isotope signal could be detected although the precautions generally suggested to avoid problems related to diagenesis of the isotope signal were applied, i.e. (1) study of a morphologically well-defined object and (2) measurements on individual biomarkers that are thought to be stable over geological time. This slight alteration does not seem to have erased the palaeoenvironmental information recorded by individual *n*-alkanes of *Eretmophyllum*. For the fossils, the diagenetic alteration undergone by the specific isotope composition of *n*-alkanes seemed much weaker than that expected after 3 months degradation in the above-ground litter of a well-drained soil. These findings thus suggest that the isotope signal in fossils, and sedimentary organic matter in general, can be better preserved than in soil organic matter. Nevertheless, the intensity of the alteration detected in the litter shows that the specific *n*-alkane isotope composition can potentially be strongly affected by diagenesis (i.e. if the residence time of plant debris in soil, before burial, is long enough), here again calling for circumspection when interpreting the specific isotope composition of sedimentary biomarkers such as alkanes.

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